

The Influence of Rhizosphere Fungal Diversity and Complex Community Structure to Wheat Root Rot Disease

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Abstract

Background: Wheat root rot disease due to soil-borne fungal pathogens leads to tremendous yield losses worth billions of dollars worldwide every year. It is very important to study the relationship between rhizosphere fungal diversity and wheat roots to understand the occurrence and development of wheat root rot disease.

Results: A significant difference in fungal diversity was observed between the diseased and healthy groups in the heading stage, but the trend was the opposite in the filling stage. The abundance of most genera with high richness decreased significantly from the heading to the filling stage in the diseased groups; the richness of approximately one-third of all genera remained unchanged, and only a few low-richness genera, such as *Fusarium* and *Ceratobasidium*, had a very significant increase from the heading to the filling stage. In the healthy groups, the abundance of most genera increased significantly from the heading to the filling stage; the abundance of some genera did not change markedly, or the abundance of very few genera increased significantly. Physical and chemical soil indicators showed that low soil pH and density, increases in ammonium nitrogen, nitrate nitrogen and total nitrogen contributed to the occurrence of wheat root rot disease.

Conclusions: Our results revealed that in the early stages of disease, highly diverse rhizosphere fungi and a complex community structure can easily cause wheat root rot disease. The existence of pathogenic fungi is a necessary condition for wheat root rot disease, but the richness of pathogenic

fungi is not necessarily important. The increases in ammonium nitrogen, nitrate nitrogen and total nitrogen contributed to the occurrence of wheat root rot disease. Low soil pH and soil density are beneficial to the occurrence of wheat root rot disease.

Keywords: Rhizosphere, Fungal diversity, Community structure, Wheat root rot disease

Introduction

Soil microbial diversity is important to sustainable agriculture because microbes mediate many processes that are essential to the agricultural productivity of soil (1). However, to meet the food demand of an increasing population, intensive agricultural practices and the cultivation of exhaustive crops have destroyed soil structure and ignored the biological potential of roots or rhizospheres to efficiently mobilize and acquire soil nutrients (2-8). As such, soils have very low biological activity, and plants growing in these soils are predisposed to soil-borne pathogens (9-12).

With the destruction of soil structure, the degradation of soil and the increase in soil-borne pathogens, wheat, as one of the three major staple foods in the world, grown in Asia (China), Australia, Europe, North America, and South America, is vulnerable to attack by a complex of root pathogens, which results in tremendous yield losses (13). Annual losses in wheat industries due to soil-borne fungal pathogens amount to over billions of dollars worldwide (14-16). All cultivars of wheat are attacked by several soil-borne fungal pathogens that cause root diseases (15). The primary fungal pathogens include the following: *Fusarium culmorum*, *Fusarium pseudograminearum*, *Gaeumannomyces graminis* var. *Tritici*, *Bipolaris sorokiniana*, and *Alternaria* spp. in *Ascomycota*; *Rhizoctonia*, *Rhizopus oryzae*, *Rhizoctonia solani* and *Penicillium* spp. in *Basidiomycota*; *Pythium* spp. in *Oomycota*; and *Curvularia* spp. in *Deuteromycota* (10, 17). Root diseases are difficult to control because these soil-borne fungi are ubiquitous, the pathogens often occur as a complex (15, 18), and they can easily survive on infected plant debris or form durable chlamydospores in the soil with or in the absence of growing hosts and can outgrow or evade plant defenses (10, 19). There are no resistant varieties among adapted cultivars of wheat (20-21) and no chemical controls, although certain seed treatments can provide some early benefits to seedling health (21-22).

Therefore, it is very important to study the relationship between soil microbial diversity and plants to understand the occurrence and development of crop root rot. Plants depend on the ability of roots to communicate with rhizosphere microorganisms through signaling pathways, creating a connection between plants and microorganisms (23-25). The composition of the rhizosphere microbiota can negatively or positively influence plant traits such as stress tolerance, health,

development, and productivity (26-28). The plant, in turn, cultivates the structural and functional diversity of microbial communities in the rhizosphere by adjusting soil pH, releasing secondary metabolites (e.g., pyrones, sesquiterpenes) into the rhizosphere (25, 29-30), reducing competition for beneficial microbes, and providing an energy source, mostly in the carbon-rich rhizosphere (4, 21). Soil-plant-microbial health must remain in equilibrium to maintain sustainable agricultural practices (25, 31-32). Under unfavorable conditions, some fungi can cause plant diseases and sometimes even total loss of crop yields (28).

In addition to agrochemicals (33), fertilization (8, 34), soil types (35-36), tillage (1) and crop rotation (9-10), which can influence rhizosphere microorganisms, the structure and function of rhizosphere microflora may also be affected by the plant physiology of different plant genotypes (36-37) and may also fluctuate among the vegetation stages of the same plant genotype (36, 38). Plant growth stage influences root physiology and changes the quality and quantity of root exudates; consequently, these changes select for different root-associated microorganisms at different growth stages (39-41). The purpose of this study was to study the diversity of fungi, the variation in community structure and the trends in the microbial species in the rhizosphere of healthy and diseased wheat roots at different heading and filling stages of wheat growth. Additionally, by combining these results with the physical and chemical properties of soil, the possible causes of wheat root rot disease were revealed, providing an important theoretical basis and practice for the improved control of wheat root rot disease.

1 Materials and methods

1.1 Rhizosphere samples

From 2015 to 2016, we conducted a continuous follow-up survey on land planted with wheat at Xiangyang Original Farm in Hubei Province, China, and found that the wheat root rot disease at this farm was serious (Fig 1 A). In this study, wheat root samples were collected in early April (heading stage) and early May (filling stage) of 2017 (Fig 1 B). The sampling method consisted of first investigating the occurrence of wheat root rot, selecting weak seedlings and sampling the brown parts of the root as the diseased wheat rhizosphere. Areas with diseased wheat and healthy wheat (the diameter of each area was not more than 10 cm) were marked by inserting cards at fixed points at least 10 meters apart. Then, plants were sampled at 20 points, and 5 wheat plants were sampled at each point. The roots of the whole plant and the soil on the roots were collected. The majority of soil on the roots was shaken off, and samples were collected for the determination of physical and chemical soil properties. The roots of five wheat plants were placed in a sealed pocket and quickly stored in a dry-ice box for dry-ice preservation. After all the samples were collected, they were

immediately brought to the laboratory and stored in a cryogenic refrigerator for future use. In May, the samples were collected from the areas labeled in April. The ears of diseased wheat in this area had a clear white color, and these plants had markedly brown, black or yellow roots. Five soil samples from the diseased wheat root and five soil samples from the healthy wheat root were collected in May. The sampling method was the same as that for the previous collection. According to the cards inserted in the soil in April, the 20 samples collected in April, which were identical to the samples collected in May, were removed from the cryogenic refrigerator to analyze the diversity of fungi in the wheat rhizosphere.

1.2 Extraction and PCR amplification of total genomic DNA from rhizosphere fungi

The soil attached to the root was brushed off, a 0.1-g soil sample was accurately weighed, and the total genomic DNA from all samples was extracted according to the instructions provided with an E.Z.N.A.® Stool DNA Kit (Omega Biotek, Norcross, GA, U.S.). Total DNA was detected by 1% agarose gel electrophoresis, and the purity and concentration of DNA were determined by spectrophotometry. All DNA samples were stored in a refrigerator at -20 °C. Fungal diversity was determined by amplifying the ITS1 region using the CS1 ITS1F and CS2 ITS2R primer sets for fungi. The primer sequences were ITS1F 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS2R 5'-GCTGCGTTCTTCATCGATGC-3', and the amplification conditions were predenaturation at 95°C for 5 min, 27 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, and elongation at 72°C for 5 min. Three replicates of the PCR were performed, and a 20-μL reaction system (4 μL of 5× FastPfu buffer solution, 2 μL of 2.5 mM dNTPs, 0.8 μL of primer (5 μM), 0.4 μL of FastPfu polymerase and 10 ng of fungal total genomic DNA) was used.

1.3 Illumina HiSeq2500 sequencing

The PCR amplification product was recovered by 2% agarose gel electrophoresis followed by the use of an AxyPrep DNA gel Recovery Kit (Axygen Biosciences, Union City, CA, U.S.), and the quantitative analysis was carried out using a QuantiFluor®-ST (Promega, U.S.). Two-terminal sequencing was carried out using a HiSeq2500 platform after equally mixing the purified products. The original read data were deposited in the NCBI Sequence Read Archive (Accession number: SUB5829339).

1.4 Data processing

Using Perl script, the offline data were divided into different sample data groups according to

barcode sequences, and the barcode sequence and PCR-amplified primer sequence were truncated. Then, the divided data were spliced with FLASH to connect the reads of each sample, and the splicing sequence was the original tag data (raw tags). The raw tags obtained by splicing were strictly filtered to obtain high-quality tag data (clean tags). For tag quality control, the following operations in QIIME were used: a) tag intercept, truncation of raw tags from the first low-quality base site with consecutive low-quality bases (default mass threshold was ≤ 3) to the set length (default length was 3); b) tag length filtering, tag data sets obtained by tag intercepts were further filtered to remove tags with continuous high-quality bases of less than 75% of the tag length; c) chimera filtration. After the above processing steps, the tag sequences were compared to sequences in databases (Gold database, http://driver5.com/uchime_download.html; UCHIME algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html), the chimera sequence was detected, and the chimera sequence was finally removed to obtain the final valid data (effective tags). The sequencing depth was more than 30,000 original reads per library.

1.5 OTU and species community analysis

UPARSE software (Uparse v7.0.1001, (<http://drive5.com/uparse/>)) was used to cluster the effective tag sequences of all samples with 97% and 95% consistency to form operational taxonomic units (OTUs). The representative sequence of each OTU was identified, the `assign_taxonomy.py` script (http://qiime.org/scripts/assign_taxonomy.html) in QIIME was used, and RDP (16S, <http://rdp.come.msu.edu/>), Silva (18S, <http://www.arb-silva.de>) and Unite (ITS) were used to compare the data with the database to obtain species annotation information. Then, the annotated chloroplast or mitochondrial genes (16S amplifiers) and the OTUs and tags that could not be annotated at the boundary level were removed (confidence threshold was above 0.8 by default). Then, the OTUs and tags annotated as chloroplasts or mitochondria (16S amplifiers) and those that could not be annotated at the kingdom level were removed. Finally, the effective tag data, low-frequency tag data, annotated tag data, chloroplast and mitochondrial data and OTU data obtained from each sample were counted by a script. Additionally, we used R software to calculate the annotation ratio of OTUs and each taxonomic level and the relative abundance of the species in each taxonomic level.

Based on the above valid OTU data, the following evolutionary analysis was carried out: a) evolutionary relationships and relative abundance information of species systems based on OTU data in samples were determined, and species annotation results for a single sample were visualized using KRONA software (<http://sourceforge.net/projects/krona>); b) related genera were selected, a phylogenetic tree for the OTUs of these genera was constructed (QIIME software package: `make_phylogeny.py` (http://qiime.org/scripts/make_phylogeny.html)), and the systematic evolutionary relationship was displayed by combining the relative abundance of OTUs and the

reliability of annotation using a Perl script; and c) local Perl scripts were used to select the dedicated OTUs for intrasample and intersample phylogenetic analysis and to compare relative abundance.

To analyze the community structure of species, relative abundance thermograms were plotted at the OTU level and the genera level by R software. Cluster analysis and principal coordinate analysis (PCoA) were also carried out to compare samples.

2 Results

2.1 Fungal diversity in the rhizosphere

According to the diversity index, the community richness and diversity of the diseased groups were higher than those of the healthy groups in the heading stage, and there was a significant difference in community diversity between the diseased group and the healthy group (Shannon index: $P < 0.01$) (Supplemental table 1). The high community richness and diversity created conditions for subsequent diseases. However, the community richness and diversity of the disease group and the healthy group in the filling stage were higher than those of the disease group and the healthy group in the heading stage, and there was a significant difference between the disease group and the healthy group in the heading stage (Sobs index: $P < 0.05$; Shannon index: $P < 0.05$). The community richness and diversity of the healthy group in the filling stage were higher than those of the diseased group in the filling stage.

2.2 Community structure of fungi in the rhizosphere

There were 1393 OTUs distributed among 7 phyla, 346 genera and 549 species. The seven phyla were *Ascomycota*, *Basidiomycota*, *Zygomycota*, *Chytridiomycota*, *Glomeromycota*, *Blastocladiomycota* and unclassified fungi (Fig 2 A). Among these phyla, *Ascomycota* was the most abundant. The richness values were 65.48% and 67.61% and were 72.57% and 57.35% in healthy groups 4 and 5 (H4 and H5, respectively) and diseased groups 4 and 5 (D4 and D5, respectively), respectively, but there was no significant difference among the populations. The other three phyla showed very interesting results. *Basidiomycota* had the highest abundance in population H4 in the heading stage (25.3%). However, in the D4, D5 and H5 populations, the abundance was significantly decreased to 12.36%, 11.16% and 14.7%, respectively. Significant differences were found between populations H4 and D4 and populations D5 and H5 ($P < 0.01$). The abundance of *Zygomycota* in populations H4 and D4 was low in the heading stage (3.16% and 2.44%, respectively), but suddenly increased to 9.87% (H5) and 7.42% (D5) in the filling stage. However, the trends in the abundance of *Chytridiomycota* and *Zygomycota* were almost the opposite. The abundance of *Chytridiomycota* in the heading stage groups was 4.52% (H4) and 7.18% (D4), and

there was a significant difference between these two groups. The abundance of *Chytridiomycota* in population D4, a diseased group, was much higher than that in the healthy groups. By May, the abundance of *Chytridiomycota* in population H5 decreased sharply to 1.37%, while that of population D5 decreased to only 4.12%.

Excluding unclassified fungi, all fungi with richness greater than 1% in a single group were counted (Table 2). There are 30 genera in the four groups belonging to *Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Zygomycota*. Among these genera, *Chytridiomycota* and *Zygomycota* had only one genus, *Olpidiaster* and *Portierella*, respectively. *Basidiomycota* had seven genera, and *Ascomycota* had the largest distribution, with 21 genera (Fig 2 B and C, Supplemental table 2). In the heading stage, the abundance of the most highly abundant genera in population D4 was significantly decreased in population D5 at the filling stage; however, the abundance of *Mortierella* significantly increased, the abundance of one-third of the genera remained almost unchanged, and the abundance of a few low abundance genera, such as *Fusarium* and *Ceratobasidium*, significantly increased in the diseased population over time. Seven genera with high richness were found in the H4 population at the heading stage, and the richness of these genera was significantly increased in the H5 population at the grain filling stage; among these genera, the variation in the richness of *Microdochium*, *Mycosphaerella*, *Scytalidium*, *Acremonium* and *Olpidiaster* from the H4 population to the H5 population was the opposite of the change in abundance from the D4 population to the D5 population. The richness of 13 genera did not markedly change, and the richness of 4 low-richness genera was significantly increased at the grain filling stage compared with the heading stage. In general, the following six genera showed no change in richness from the heading stage to the grain filling stage, between the H5 and D5 populations and the H4 and D4 populations, respectively, *Alternaria*, *Sarocladium*, *Gibberella*, *Cladosporium*, *Neosetophoma* and *Cystofilobasidium*. The genera with significant or extremely significant differences in richness among the groups were *Alternaria*, *Mortierella*, *Cryptococcus*, *Apodus*, *Epicoccum*, *Scytalidium* and *Chaetomium*.

A Venn diagram of genera with richness greater than 1% was constructed (Fig 2 D). There were 153 shared genera, 35 endemic genera that appeared most frequently in the healthy group in the filling stage and 7 and 10 genera in the heading stage and filling stage, respectively. The results showed that the root exudates of diseased plants specifically promoted the growth of microbial fungi, while the rhizosphere of healthy plants was more suitable for the growth of various fungi. An increased diversity of soil microorganisms promoted healthy plant growth.

2.3 PCoA of populations

The PCoA of fungal community structure in different samples based on OTU revealed that the

populations could be divided into two groups: the healthy group and the diseased group (Figure 3 A and B). There was no overlap between the two groups, which indicated that the samples were consistent with the expectation and that the microbial community structures of the diseased group and the healthy group were quite different. The diseased group at the heading stage was significantly separated from the diseased group at the filling stage, and the microbial community structure was quite different. The healthy group at the heading stage and the healthy group at the filling stage were also clearly separated, and the microbial community structure was quite different. In addition, according to the flora classification, all rhizosphere fungi were divided into two types (Fig 3 C): type one included the diseased group, and type two included the healthy group. Only sample H5-5 was assigned to type one.

2.4 Effects of physical and chemical soil properties on rhizosphere fungi

The main physical and chemical properties of soil measured were total phosphorus (TP), ammonium nitrogen (NH_4), nitrate nitrogen (NO_3), total nitrogen (TN), pH, soil density (SD), total carbon (TC) and the soil dry-humidity ratio (DHR) (Table 3). TP and TC decreased and NH_4 increased from the heading stage to the filling stage. Moreover, the NO_3 and TN of the diseased group were higher than those of the healthy group, and the pH value and SD of the diseased group were lower than those of the healthy group. Heatmap cluster analysis based on Spearman correlation coefficients for the 30 genera with the most richness and these physical and chemical indicators was performed (Fig 4). The graph shows that pH and SD have the greatest influence on microbial abundance, and the influence of these factors is in the same direction. One-third of the genera were positively or significantly positively correlated with both pH and SD, and one-third were negatively or significantly negatively correlated with both pH and SD. The effects of NO_3 and TN on microbial richness were similar, but the impact of these factors on microbial species richness was almost the opposite of that of pH and SD. Soil DHR and TP had moderate effects on microbial richness. The physical and chemical properties of soil with the least influence on microbial abundance were NH_4 and TC.

Discussion

Microbial diversity in soil is an important factor that determines soil health and is considered one of the main contributors to soil suppressiveness (28, 42). The rhizosphere is one of the most complex environments; rhizospheres are influenced by plant roots and are an active microhabitat where plant roots and microbes interact (28, 43-44). Throughout the heading and filling stages of wheat, the fungi in the rhizosphere showed high diversity. The diversity of soil fungi at the grain filling stage was significantly higher than that at the heading stage. It is already well known that most crops can

significantly benefit from establishing associations with diverse soil microbes (25). Plants stimulate or inhibit the growth of specific rhizosphere microorganisms by releasing secondary metabolites into the rhizosphere (25, 29-30). In the rhizosphere of wheat with root rot disease, the interaction between wheat and microorganisms was intense, and the diversity and richness of the fungal community in the rhizosphere of the diseased group were significantly higher than that of the healthy group, creating conditions for the occurrence of wheat root rot. However, at the filling stage, wheat roots were clearly diseased due to infection by pathogenic fungi. During this process, the metabolites secreted by wheat roots tend to be monotonous. As a result, the fungi in the rhizosphere of diseased plants showed lower community diversity and richness than those in the rhizosphere of healthy plants. The interaction between rhizosphere fungi and wheat roots in the healthy group reached an optimal balance during the filling stage and then became mutualistic, which was favorable for the healthy growth of the fungi and wheat. Therefore, in the filling stage, the diversity and richness of fungi in the rhizosphere were higher in the healthy group than in the diseased group.

The richness change analysis at the phylum level revealed that the phylum with the highest richness was *Ascomycota*, which consisted of more than 60% of all healthy and diseased groups, and there was no significant difference among groups. However, the main pathogenic fungi that cause wheat root rot disease, such as *F. culmorum*, *F. pseudograminearum*, *G. graminis* var. *Tritici*, *B. sorokiniana* and *Alternaria* spp., are in this phylum. There are also some fungi that cause wheat root rot in the phylum *Basidiomycota*, such as *Rhizoctonia*, *R. oryzae*, *R. solani* and *Penicillium* spp. This phylum showed high richness in only the healthy group at the heading stage (25.3%), but the richness of this phylum was significantly decreased in the other groups (<14%). Analysis of the abundance of these two phyla shows that the impact of the fungi that cause wheat root rot disease is not due to phylum-level abundance. The richness of two other phyla, *Zygomycota* and *Chytridiomycota*, which contain almost no root rot fungi, showed notable differences between the heading and filling stages in the healthy group but showed no significant differences between these stages in the diseased groups. This change in abundance may contribute to the healthy growth of wheat roots or may be a cofactor in the occurrence of wheat root rot disease.

Relative to the changes at the phylum level, levels of richness at the genera level varied greatly. The abundance of most genera with high richness decreased significantly from the heading stage to the filling stage in the diseased groups; the richness of approximately one-third of all genera remained unchanged, and only very few low-richness genera, such as *Fusarium* and *Ceratobasidium*, had a very significant increase in richness over time. In the healthy group, the abundance of most genera increased significantly from the heading stage to the filling stage, except for some genera whose abundance did not markedly change or very few genera whose abundance increased significantly. This result also shows that the interactions between wheat roots and rhizosphere fungi must achieve a balance. If this balance is lost, the wheat roots will become diseased. From the analysis of the endemic genera in each group, we also found that there were 35 endemic genera in the healthy group,

which was many more than the approximately 10 endemic genera in the diseased group, indicating that the healthy growth of wheat roots can promote plant growth and suppress disease through various activities that prevent infection by pathogens. Therefore, the rhizosphere microorganisms show improved growth, and the microbial diversity and community richness are also significantly increased in the healthy plants compared with the diseased plants.

Fungi and fungus-like organisms form one of the most diverse groups of eukaryotes and represent an essential functional component of soil microbial communities (28, 45). Under unfavorable conditions, some fungi can cause plant diseases and sometimes even the total loss of crop yields. In many instances, these diseases are caused by a complex of fungal species (28). Only *Alternaria* and *Fusarium*, genera with richness values greater than one percent, have been documented to cause wheat root rot, but the abundance of these two genera is not high. Although the abundance of *Alternaria* in the diseased groups was significantly higher than that in the healthy groups, there was no significant change between the heading stage and filling stage. The abundance of *Fusarium* in the filling stage was significantly higher than that in the heading stage. Considering the abundance of these two pathogens, the richness level of a fungus does not indicate whether it can lead to wheat root rot. In addition, other pathogenic fungal genera mentioned in the literature were determined to have less than one percent abundance. Therefore, we can speculate that as long as these fungal genera exist in the rhizosphere, they can lead to wheat root rot disease, regardless of the abundance. However, whether a fungus can induce root rot disease depends on the result of the interaction between wheat root and rhizosphere microorganisms, which is also closely related to seasonal climatic conditions.

Differences in the fungal community structure among groups can clearly demonstrate the heterogeneity of each group, thus showing why there are so many differences in the rhizosphere fungi of the diseased groups and the healthy groups. PCoA clustering analysis clearly clustered the four groups into two groups: the healthy group and the diseased group. However, the healthy group and the diseased group were each clearly separated into the heading stage and grouting stage. In other words, the healthy group and the diseased group had significant differences in fungal community structure, and the difference in the fungal community structure of the heading stage and the grouting stage was significant. In addition, all rhizosphere fungi were divided into two types according to flora classification: one type included only the healthy group, and the other type included only the diseased group. When wheat roots were attacked by microorganisms and developed root disease, the structure of the rhizosphere fungal community was markedly changed. To differentiate between the fungi located very close to the epidermis in the root zone and to protect against the invasion of heterogeneous microbes, plants continuously secrete signaling molecules, which allows for the development of pathogenic, associative, symbiotic, or naturalistic relationships between microbes and the plant (25, 46).

At the heading stage, the root is slightly diseased, and the interaction between wheat roots and rhizosphere fungi is the most intense, resulting in marked heterogeneity of the soil environment and inducing a high level of rhizosphere fungal diversity and a complex community structure. However, at the filling stage, the wheat roots are completely diseased. At this time, the soil environment is stable, and the material secreted by wheat roots is relatively limited, which leads to a decrease in the microbial diversity of the rhizosphere and a relatively simple community structure. Soil-plant-microbial health must be maintained at an equilibrium to maintain sustainable agricultural practices (25, 31-32). At the heading stage, the antagonistic interaction between healthy wheat roots and rhizosphere fungi reaches a balance and promotes the healthy growth of wheat and fungi. By the filling stage, the ecological environment, such as temperature, humidity and other factors, is improved, the rhizosphere microbial diversity naturally significantly increases, and the community structure becomes more complex.

Biotic and abiotic factors are assumed to influence the structural and functional diversity of the microbial communities in the rhizosphere (47-48). Site properties, including soil type, climatic conditions and type of agricultural management, have been shown to strongly influence the relative composition of rhizosphere microbial communities (48-54). Changes in the physical and chemical properties of soil also have a significant impact on fungal diversity and community structure in crop rhizospheres. Soil physical and chemical properties are determined not only by the nature of the soil itself but also by the physical and chemical properties of the soil after the interaction between crop roots and rhizosphere fungi. The physical and chemical soil properties we measured were determined in soil collected around the rhizosphere of wheat. Organisms that are present in the rhizosphere microbiota can have profound effects on the growth, nutrition and health of plants in agroecosystems (25, 55-57). TP and TC decreased from the heading stage to the filling stage, indicating that the later the growth period was, the less TP and TC was needed; however, these metrics seemed unrelated to the occurrence of diseases. In addition, the levels of NH_4 , NO_3 and TN in the diseased group were higher than those in the healthy group at the heading and filling stages, indicating that their increase contributed to the occurrence of wheat root rot disease. In contrast, the pH and SD of the soils around diseased plants were lower than those of the soils around healthy plants at the heading and filling stages. This finding indicated that relatively low pH and SD values were beneficial to the occurrence of wheat root rot disease. The heatmap cluster analysis of the physical and chemical soil indicators and the 30 most abundant genera also showed that soil pH and SD affected fungal abundance and diversity in the same direction.

Conclusions

In the rhizosphere, many plant-microbial interactions occur that mediate soil processes (25). The occurrence of crop root rot disease is closely related to the interaction between rhizosphere microorganisms and crop roots, as well as the physical and chemical properties of soil. At present,

there are approximately 10 types of fungi that can cause wheat root rot disease alone or in a complex, according to the literature. By studying the diversity of fungi and the community structure of the rhizospheres of healthy and diseased wheat at different growth stages, the heading and filling stages, it was revealed that in the early stages of illness, the high diversity of rhizosphere fungi and a complex community structure can easily cause wheat root rot disease. Additionally, the existence of pathogenic fungi is a necessary condition for wheat root rot disease, but the richness of pathogenic fungi is not necessarily important. Based on the physical and chemical properties of the soil, an increase in NH_4 , NO_3 and TN contributes to the occurrence of wheat root rot disease. Soil pH and SD had the greatest influence on the abundance and diversity of rhizosphere fungi, and the influence was in the same direction; low soil pH and SD are beneficial to the occurrence of wheat root rot disease.

Additional files

Additional file 1: Supplemental figures (PDF 1838 kb)

Additional file 2: Supplemental tables (PDF 268 kb)

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Availability of data and materials

All data generated during this study is available at the Sequence Read Archive (SRA) under BioProject number PRJNA549031.

AUTHOR CONTRIBUTIONS

W. H., and Y.D.Z. designed research; Z.X.J., and W.H. performed research; Z.X.J., W.H.Y., and A.G. analyzed data; and Z.X.J., W.H., and W.H.Y. wrote the manuscript with input from all authors.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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FIGURES



Fig. 1 Sampling plots of wheat root. **a** The occurrence of wheat root rot in this wheat field in 2015 and 2016. **b** The soil sample collection of wheat root in this wheat field in 2017. Areas with diseased wheat and healthy wheat were marked by inserting white cards at fixed points. The samples, in April and May, were collected from the areas labeled in April.

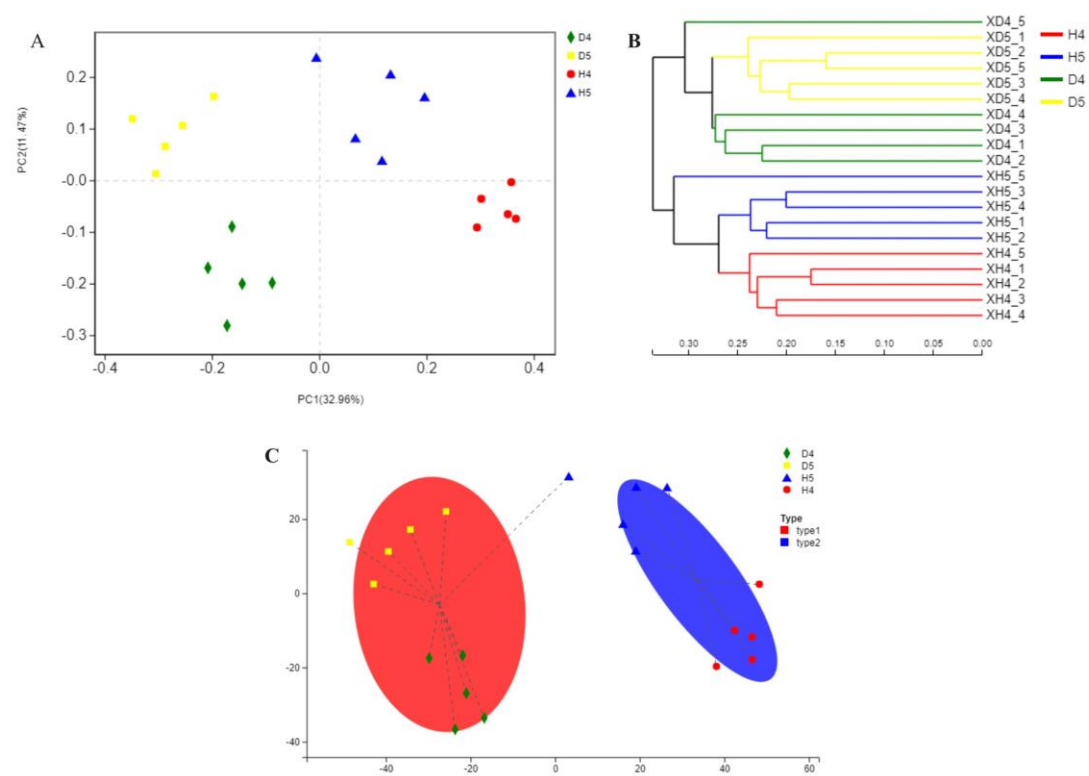


Fig. 2 Comparative analysis of OTUs and genus levels in each community. **a** Principal coordinate analysis (PCoA) at the OTU level in healthy and diseased groups at the heading stage and filling stage. Distance algorithm is based on bray_curtis. **b** Sample hierarchical clustering analysis based on OTU level. **c** Typing analysis of fungi at the genus level.

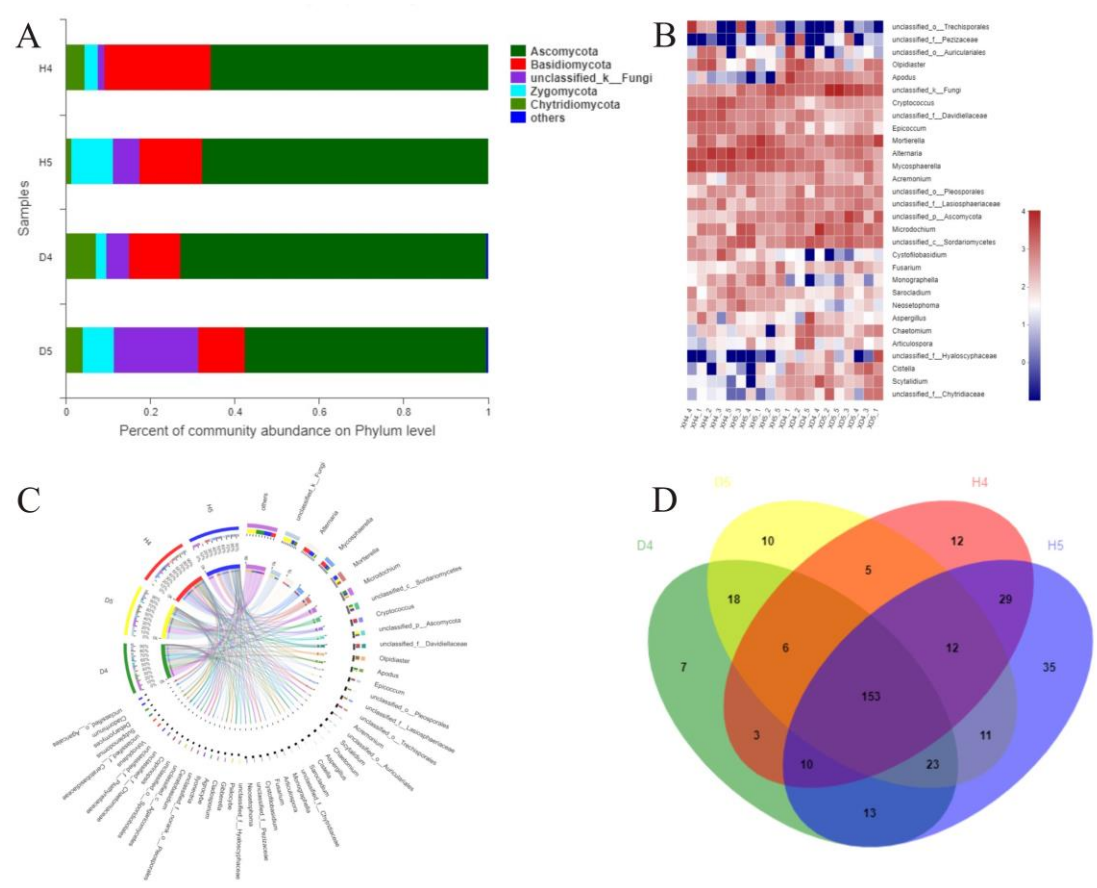


Fig. 3 Species composition analysis. **a** Percent community abundance at the phylum level in each groups. **b** Species abundance clustering at genus level in each sample. **c** Circos representation showing distribution of genus with significant difference in abundance in different groups. **d** Analysis of common and endemic genera in the different populations by Venn diagram.

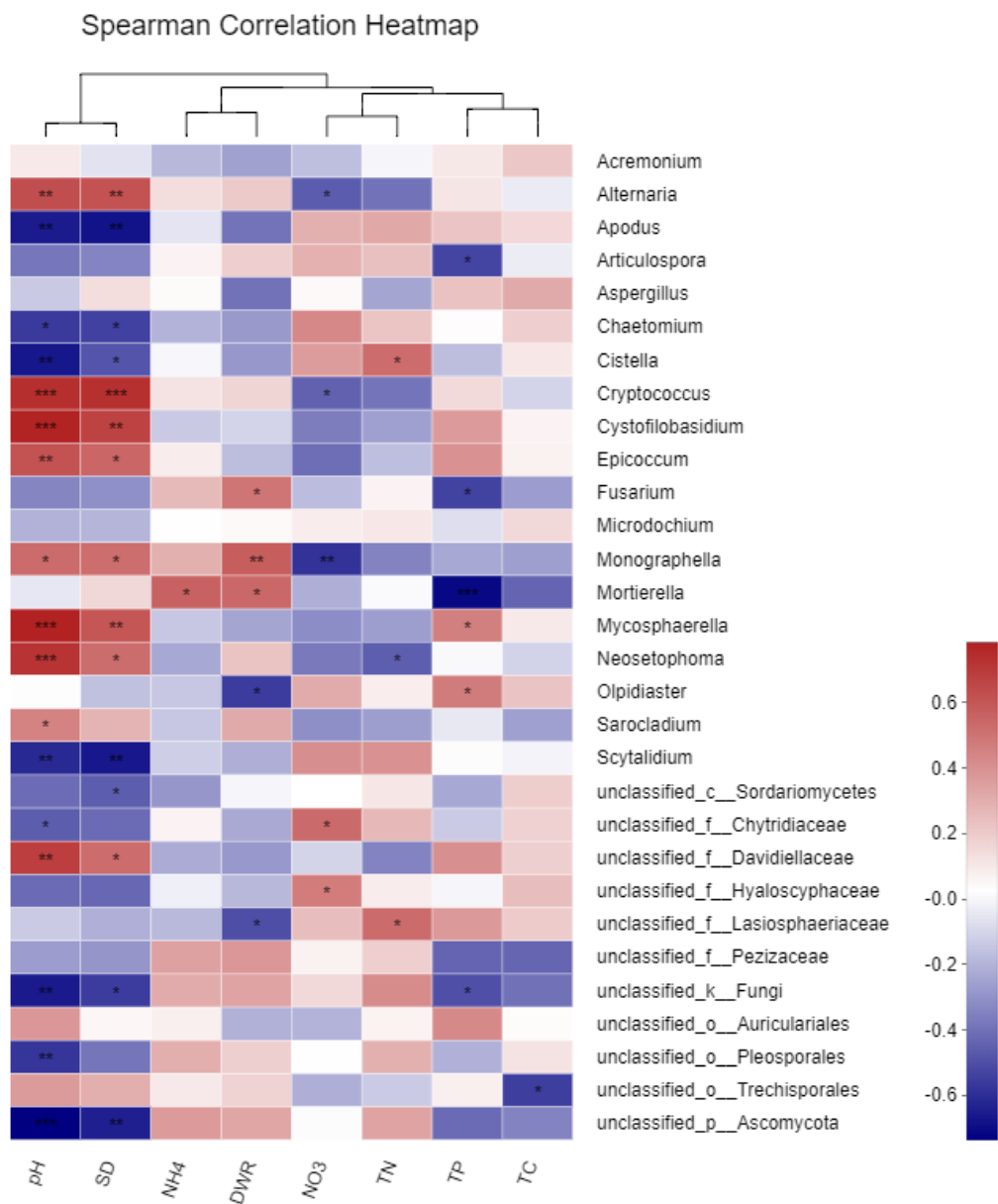


Fig. 4 Hierarchical clustering analysis at the OTU level between physical and chemical soil properties and the 30 most abundant genera.

TABLES

Table 1 The analysis of alpha diversity index in healthy and diseased groups.

Sample\Estimators	Sobs	Ace	Chao	Shannon	Coverage
H4	129.40	142.21	142.14	3.03	0.9994
D4	135.60	154.71	149.52	3.37	0.9995
H5	163.40	178.98	180.19	3.41	0.9993
D5	155.80	160.04	161.46	3.32	0.9997

Table 2 All fungi with a richness greater than one percent in each group. There are 30 genera in the four groups, which mainly belong to *Ascomycota*, *Basidiomycota*, and *Chytridiomycota*.

Phylum	Genus	H4	D4	H5	D5
<i>p__Ascomycota</i>	<i>g__Microdochium</i>	0.0184	0.0760	0.0455	0.0454
	<i>g__Apodus</i>	0.0010	0.0662	0.0035	0.0314
	<i>g__Mycosphaerella</i>	0.1291	0.0460	0.0609	0.0136
	<i>g__Scytalidium</i>	0.0005	0.0309	0.0027	0.0168
	<i>g__Chaetomium</i>	0.0028	0.0295	0.0029	0.0132
	<i>g__Alternaria</i>	0.1489	0.0284	0.1160	0.0288
	<i>g__Aspergillus</i>	0.0119	0.0274	0.0021	0.0035
	<i>g__Articulospora</i>	0.0012	0.0271	0.0044	0.0045
	<i>g__Cistella</i>	0.0010	0.0236	0.0015	0.0164
	<i>g__Acremonium</i>	0.0103	0.0213	0.0165	0.0081
	<i>g__Epicoccum</i>	0.0442	0.0160	0.0201	0.0057
	<i>g__Subplenodomus</i>	0.0005	0.0134	0.0009	0.0001
	<i>g__Debaryomyces</i>	0.0008	0.0108	0.0001	0.0003
	<i>g__Sarocladium</i>	0.0193	0.0055	0.0127	0.0042
	<i>g__Fusarium</i>	0.0021	0.0050	0.0197	0.0100
	<i>g__Gibberella</i>	0.0122	0.0036	0.0083	0.0038
	<i>g__Cladosporium</i>	0.0135	0.0027	0.0103	0.0013
	<i>g__Neosetophoma</i>	0.0106	0.0025	0.0195	0.0008
	<i>g__Monographella</i>	0.0114	0.0003	0.0259	0.0015
	<i>g__Ilyonectria</i>	0.0025	0.0001	0.0210	0.0005
	<i>g__Cladorrhinum</i>	0.0000	0.0000	0.0118	0.0000
<i>p__Basidiomycota</i>	<i>g__Agrocybe</i>	0.0000	0.0255	0	0.0006

	<i>g__Cryptococcus</i>	0.0726	0.0102	0	0.0161
	<i>g__Ceratobasidium</i>	0.0009	0.0022	0	0.0109
	<i>g__Psilocybe</i>	0.0049	0.0013	0	0.0214
	<i>g__Volvopluteus</i>	0.0156	0.0011	0	0.0001
	<i>g__Cystofilobasidium</i>	0.0319	0.0009	0	0.0003
	<i>g__Coprinopsis</i>	0.0113	0.0006	0	0.0001
<i>p__Chytridiomycota</i>	<i>g__Olpidiaster</i>	0.0432	0.0529	0	0.0128
	<i>g__Mortierella</i>	0.0315	0.0244	0	0.0737

Table 3 The physical and chemical properties of the rhizosphere.

Sample ID	TP	NH ₄	NO ₃	TN	pH	SD
CH4	4.16±0.13	16.02±3.27	26.38±6.03	204.1±30.94	7.18±0.06	2.68±0.06
CD4	4.13±0.21	14.04±2.62	30.97±5.95	251.48±21.09	6.95±0.08	2.42±0.05
CH5	3.82±0.18	17.45±4.20	23.48±2.23	217.65±49.43	7.15±0.07	2.5±0.03
CD5	3.84±0.16	18.71±2.41	29.38±3.82	237.39±52.74	6.76±0.15	2.48±0.03

*TP: total phosphorus, NH₄: ammonium nitrogen, NO₃: nitrate nitrogen, TN: total nitrogen, SD: soil density, TC: total carbon, DHR: soil dry-humidity ratio.