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Effects of *Trichoderma harzianum* and *Arthrobacter ureafaciens* on control of Fusarium crown rot and microbial communities in wheat root-zone soil

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Abstract

Biocontrol by inoculation with beneficial microbes is a proven strategy for reducing the negative effect of soil-borne pathogens. The effects of *Trichoderma harzianum* LTR-2 and *Arthrobacter ureafaciens* DnL1-1 on reducing Fusarium crown rot (FCR) disease and influencing microbial community structure in wheat root-zone were evaluated by a plot experiment. The experimental design consisted of four treatments: (1) control, (2) *Fusarium pseudograminearum* Fp (FP), (3) *F. pseudograminearum* + LTR-2 (LFP), and (4) *F. pseudograminearum* + LTR-2 + DnL1-1 (HFP). The results showed that wheat seeds coated with LTR-2 spore suspension and combination of LTR-2 and DnL1-1 had relative control efficacies of 50.77% and 67.73% on FCR disease, and increased wheat yield by 58.32% and 64.19%, respectively. Illumina MiSeq sequencing revealed that bacterial and fungal abundance and diversity were significantly higher ($P < 0.05$) in both treatment groups (HFP and LFP) than in FP and control groups. Principal coordinates analyses revealed that fungal and bacterial communities were distinctly separated among the treatment and control groups. Fungal community composition analysis demonstrated that the relative abundance of phytopathogenic fungi *Alternaria*, *Fusarium*, and *Cladosporium* decreased and that of beneficial fungi *Mortierella* and *Gamsia* was more enriched in HFP and LFP than in FP group. Bacterial community composition analysis revealed that the beneficial microbes, such as *Bacillus* and *Streptomyces* were more abundant in HFP and LFP than in FP group. LEfSe analysis indicated that the key different genera, e.g. *Tetracladium* (fungus), *Sphingomonas* and *Ramlibacter* (bacteria), which were significantly negatively correlated with TP in HFP treatment. It was concluded that application of LTR-2 and DnL1-1 may recruit a variety of phosphate-solubilizing microbes to promote wheat growth. Overall, these results confirm that the relative abundance of phytopathogenic fungi decreased significantly following application of LTR-2 alone and combined with DnL1-1 and beneficial microbes accumulated more easily in the wheat root-zone compared with that in FP and control groups.

Keywords *Fusarium pseudograminearum*, Synergistic biocontrol, High-throughput sequencing, Root-zone microbial community, LEfSe analysis, Phosphate-solubilizing microbes

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Background

The rhizosphere is a hot spot of microbial interactions, as exudates secreted by plant roots are the main food source for microorganisms and a driving force of their population density and activities. The root-associated microbial communities play an important role in the soil ecosystem, influencing many soil biochemical processes and impacting plant growth and health. Although a large number of studies have shown that beneficial microbes application result in changes in the rhizosphere soil microbial structure (Sui et al. 2022), very few studies have involved root-zone soil. Root-zone soil is not (or only lightly) affected by plants roots and root exudates, with a consequent lower level of microbial activity and soil fertility. Nonetheless, this part of the soil is necessary for the stability of soil aggregates and the resistance of soil to negative phenomena (e.g., soil erosion, leaching of nutrients) (Li et al. 2016). Research has also shown that the soil physicochemical environment play an important role in affecting microbe establishment (Ou et al. 2021). Nevertheless, the effects of microbial control strains on root-zone soil microbial communities and physicochemical property are rarely reported.

Wheat is one of the most important food crops, ranking third after rice and corn, for nearly a half of the global population. It is widely cultivated in China annually more than 24 million hectares, accounted for 17% and 16% of the total world wheat production and consumption, respectively (Wang et al. 2021). Fusarium crown rot (FCR) of wheat caused by *Fusarium pseudograminearum* Fp that occurs extensively across Huanghuai winter wheat region of China, including Henan, Hebei, Shandong, Shanxi, Anhui, Jiangsu provinces and so on. In China, FCR has posed serious threat to wheat production, resulting in 35%–70% losses (Xu et al. 2016). Meanwhile, FCR also produces deoxynivalenol (DON) to present the potential threat to wheat quality and production (Xie et al. 2023). Chemical control presented the most direct and effective method for controlling FCR disease; however, overuse of fungicides usually leaded to serious harm to the ecological environment and human healthy. Biological control has been proved to prevent FCR disease and promote wheat growth. *Bacillus siamensis* YB-1631, *Chaetomium globosum* 12XP1-2-3, *Paeniocollus polymyua*, and *Piriformospora indica* have been isolated and applied for control for FCR of wheat (Dong et al. 2023; Feng et al. 2023; Li et al. 2023a, b, c; Wang et al. 2023).

The application of exogenous beneficial microorganisms can enhance nutrient absorption, promote crop growth, change microbial community structure, and reduce soil-borne diseases. It has been proposed that *Trichoderma* inoculants, and combination of *Trichoderma* mixed with other strains can reduce wheat disease, improve the niche environment and increase wheat productivity (Hu et al. 2021; Stummer et al. 2022; Sui et al. 2022). The synergistic inhibition effect of *Trichoderma gamsii* and *Pseudomonas azotoformans* against FCR on wheat has been reported by Makhlouf et al. (2023). Our previous researches have demonstrated that *Arthrobacter ureafaciens* DnL1-1 not only significantly promoted plant root growth, possessed phosphate-mobilizing activity and motility, but also improve the yield and quality of wheat (Bazhanov et al. 2017; Li et al. 2019). *Trichoderma harzianum* LTR-2 has the significant control efficiency against wheat disease and yield increase (Wu et al. 2015). Effects of seed dressing treatment with *T. harzianum* LTR-2 on the growth of winter wheat seedlings, soil borne diseases, and rhizosphere fungal community have been reported by Hu et al. (2021). The synergistic effect of *T. harzianum* LTR-2 and *A. ureafaciens* DnL1-1 against Fp and effective growth-promoting activity on wheat by pot experiment has also been investigated (Yang et al. 2023). For microbial inoculants, the ability to establish and maintain sufficient population size within the rhizosphere is a critical prerequisite for the control of soil-borne wheat diseases. However, the reaction of the root-zone soil community by LTR-2 inoculation and combined LTR-2 and DnL1-1 applications on wheat after the whole growth period has not been investigated. In this context, plot experiment of wheat was carried out to determine the effects of LTR-2 and DnL1-1 against FP inoculation on soil by measurement of (i) the disease index in different treatments and the nutrient profile and physicochemical characteristics of wheat soil; and (ii) the root-zone fungal and bacterial communities of wheat in different treatments. The results of this study would explore and improve our understanding of the biocontrol mechanism of the microbial strain LTR-2 and DnL1-1 from a root-zone microbiota ecology perspective.

Results

Effects of *T. harzianum* LTR-2 and *A. ureafaciens* DnL1-1 on disease

The survey results of wheat FCR disease showed that both *T. harzianum* LTR-2 (LFP) and combined *T. harzianum* LTR-2 with *A. ureafaciens* DnL1-1 (HFP) treatments on wheat seed dressing significantly reduced the disease index of FCR ($P < 0.05$) (Table 1), with the values of 29.78% and 34.04%, respectively. In addition, both LFP and HFP treatments also significantly reduced the white-heads rate ($P < 0.05$). The control efficiencies of these two treatments (LFP and HFP) were 50.77% and 67.73%, respectively. Compared with the negative control of FP group, the wheat yield of both treatments increased by

58.32% and 64.19%, respectively ($P < 0.05$). The content of DON increased only 6.30 and 4.29 fold for LFP and HFP treatments compared with control group, respectively, which were notably lower than that of FP group (9.29 fold).

Soil physicochemical properties

The impact of different treatments of *T. harzianum* LTR-2 and *A. ureafaciens* DnL1-1 application on the soil physicochemical properties was shown in Table 2. In both treatments compared to control and FP groups, there were no obvious changes observed in the levels of pH, soil organic matter (SOM), total nitrogen (TN), total phosphorus (TP), total potassium (TK), available nitrogen (AN), available phosphorus (AP), and available potassium (AK) ($P > 0.05$).

Microbial community composition and alpha-diversity

After read-quality filtering, 11,075 high-quality fungal sequences and 69,474 high-quality bacterial sequences were obtained. Venn diagrams were constructed to analyze amplicon sequence variant (ASV) among different treatments samples (Fig. 1). A total of 2550 fungal ASVs and 16,977 bacterial ASVs were obtained in this study.

Table 1 Effect of *T. harzianum* LTR-2 and *A. ureafaciens* DnL1-1 on FCR, yield, and DON content of wheat

Treatment	Disease index	Whiteheads rate (%)	Control efficiency (%)	Yield (g/m^2)	Content of DON (mg/kg)
Control	5.00 ± 5.00c	0.23 ± 0.40c	–	498.22 ± 81.15a	2.03 ± 0.34d
FP	78.33 ± 2.89a	12.27 ± 1.21a	–	71.47 ± 28.97c	18.86 ± 9.39a
LFP	55.00 ± 8.66b	6.04 ± 1.63b	50.77	181.25 ± 49.39bc	12.78 ± 2.98ab
HFP	51.67 ± 7.64b	3.96 ± 1.81b	67.73	199.60 ± 69.54b	8.70 ± 4.06bc

Analysis of variance ($n = 3$) followed by Duncan's test where treatments in the same column with different letters are significantly different at the 95% confidence interval

Table 2 Chemical characteristics of root rhizosphere soil at harvest stage

Treatment	pH	SOM(g/kg)	TN(g/kg)	TP(g/kg)	TK(g/kg)	AN(mg/kg)	AP(mg/kg)	AK(mg/kg)
Control	8.26 ± 0.03a	16.40 ± 2.21a	1.10 ± 0.05a	1.09 ± 0.23a	17.45 ± 0.17a	84.94 ± 2.04a	114.40 ± 44.41a	135.44 ± 8.14a
FP	8.25 ± 0.18a	17.09 ± 2.14a	1.16 ± 0.12a	1.12 ± 0.07a	17.00 ± 0.97a	95.28 ± 9.21a	118.42 ± 25.48a	155.06 ± 11.54a
LFP	8.16 ± 0.13a	15.35 ± 2.24a	1.10 ± 0.05a	1.06 ± 0.18a	16.93 ± 0.56a	96.26 ± 22.95a	98.75 ± 51.79a	158.72 ± 24.49a
HFP	8.16 ± 0.05a	17.87 ± 1.24a	1.03 ± 0.09a	0.91 ± 0.14a	17.76 ± 0.19a	86.91 ± 8.99a	94.33 ± 32.44a	145.05 ± 13.62a

Values are presented as means ± SD ($n = 3$). Means within a column followed by various letters are significantly different ($P < 0.05$). SOM, soil organic matter; TN, total nitrogen; TP, total phosphorous; TK, total potassium; AN, available nitrogen; AP, available phosphorous; AK, available potassium

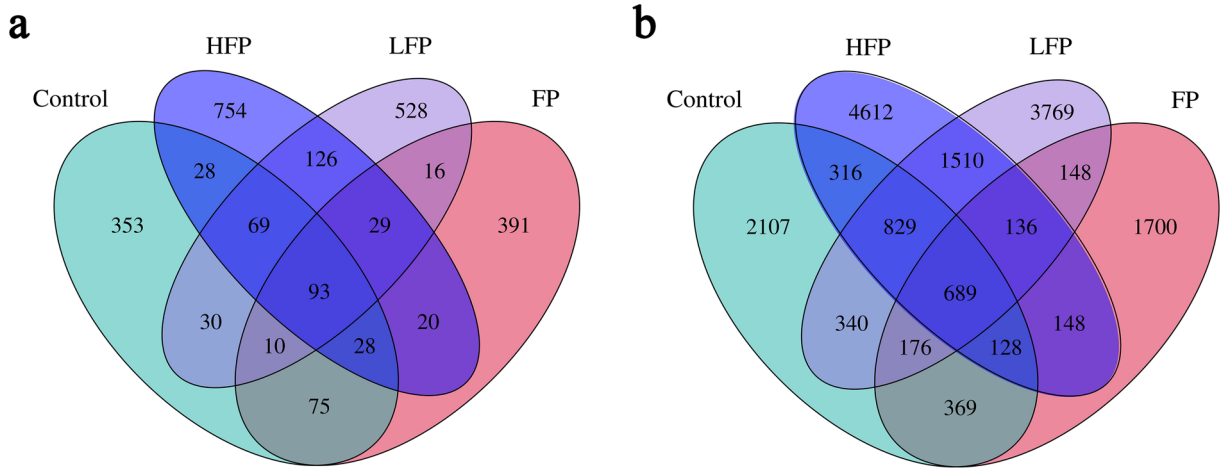

Fig. 1 Venn diagrams showing the unique and shared ASVs between the microbial inoculant treatments and control group. **a** A Venn diagram of fungi from the FP, LFP, HFP treatments and control group; **b** A Venn diagram of bacteria from the FP, LFP, HFP treatments and control group

Among the fungal ASVs, 93 ASVs were common to all samples, and 353, 391, 754, and 528 ASVs were unique obtained under control, FP, HFP, and LFP, respectively (Fig. 1a). Bacterial ASV analysis indicated that 689 ASVs were shared among the four samples, and 2107, 1700, 4612, and 3769 ASVs were unique to the control, FP, HFP, and LFP, respectively (Fig. 1b).

As shown in Fig. 2 and Additional file 2: Table S1, the ASVs, the Shannon and Chao 1 index and Simpson index were compared under various conditions in bacterial and fungal colonies, respectively. The ASVs, Shannon and Chao 1 index revealed that HFP exhibited the highest fungal richness compared to control and FP ($P < 0.05$), but no obvious changes were observed between LFP and control ($P > 0.05$). Despite this, differences in Simpson indexes were not detected from control, LFP, and HFP ranging between 0.92 and 0.95, while greater diversities were found compared to FP (0.86) ($P < 0.05$) (Fig. 2a–d). The ASVs, Shannon and Chao 1 index indicated that the diversity of soil bacteria exhibited the following trend: HFP > LFP > control > FP, indicating different bacterial diversity among the different treatments ($P < 0.05$), while no significant difference was observed between HFP and LFP ($P > 0.05$) (Fig. 2e–h).

Soil fungal community composition

The composition and relative abundance of the soil fungal community under various microbial treatments are presented in Fig. 3a, c. At the phylum level, Ascomycota, Basidiomycota, Mortierellomycota, and Glomeromycota were the dominant microorganisms, accounting for up to 55% of the total microorganisms, with a relative abundance of 30.90%–50.38%, 4.19%–21.15%, 1.89%–13.88%, and 0.05%–1.69%, respectively. Compared with FP group, HFP and LFP treatments significantly decreased the relative abundance of Ascomycota and Basidiomycota but significantly increased the relative abundance of Mortierellomycota and Glomeromycota ($P < 0.05$) (Fig. 3a). The predominant genera under the four treatments included *Alternaria* (6.31%–20.72%), *Fusarium* (0.67%–17.15%), *Filobasidium* (1.13%–17.32%), *Mortierella* (1.28%–11.65%), *Cladosporium* (2.05%–6.19%), *Cystofilobasidium* (0.08%–4.95%), *Papiliotrema* (0.086%–3.46%), and *Gamsia* (0.073%–1.21%) (Fig. 3c). Compared with FP group, HFP and LFP treatments significantly decreased the relative abundance of *Alternaria*, *Fusarium*, *Filobasidium*, *Cladosporium*, and *Cystofilobasidium*, but significantly enriched *Mortierella* and *Gamsia* ($P < 0.05$).

Soil bacterial community composition

The composition and relative abundance of the soil bacterial community under various microbial treatments are presented in Fig. 3b, d. At the phylum level, Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, and Firmicutes were the dominant microorganisms, accounting for up to 75% of the total microorganisms, with a relative abundance of 22.2%4–26.57%, 21.02%–31.81%,

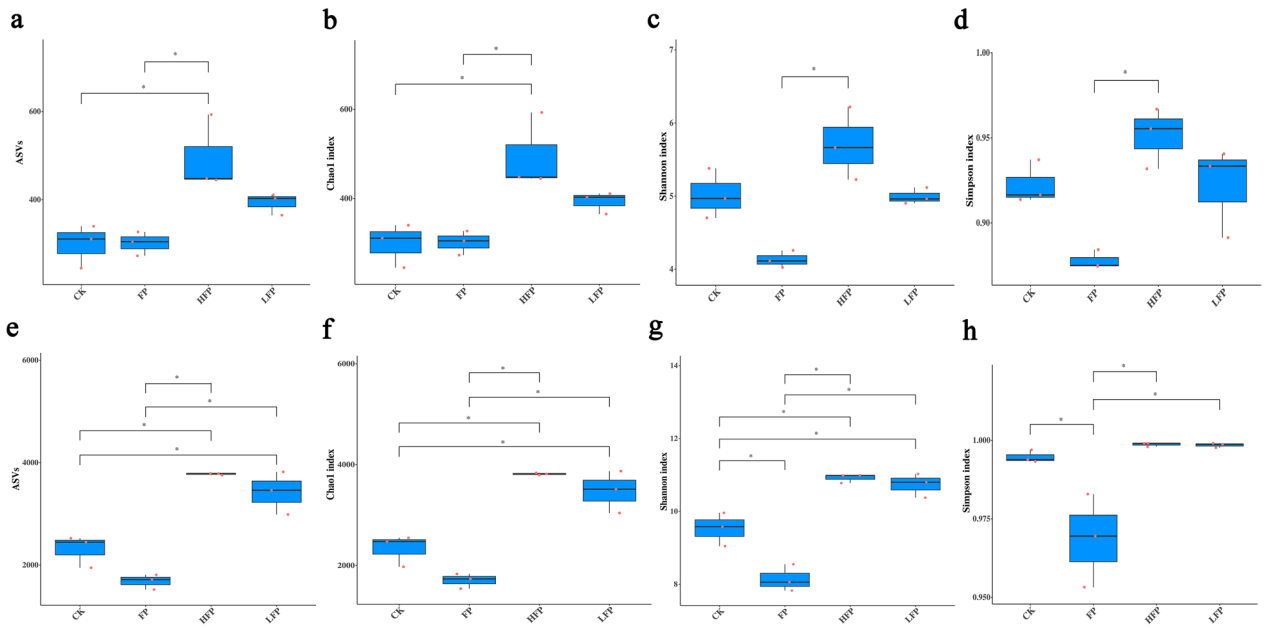

Fig. 2 Box plots showing the fungal (**a**–**d**) and bacterial (**e**–**h**) diversity (ASVs, Chao 1 index, Shannon index and Simpson index) in wheat root-zone soil. * indicates significant difference at $P < 0.05$ by nonparametric Wilcoxon test

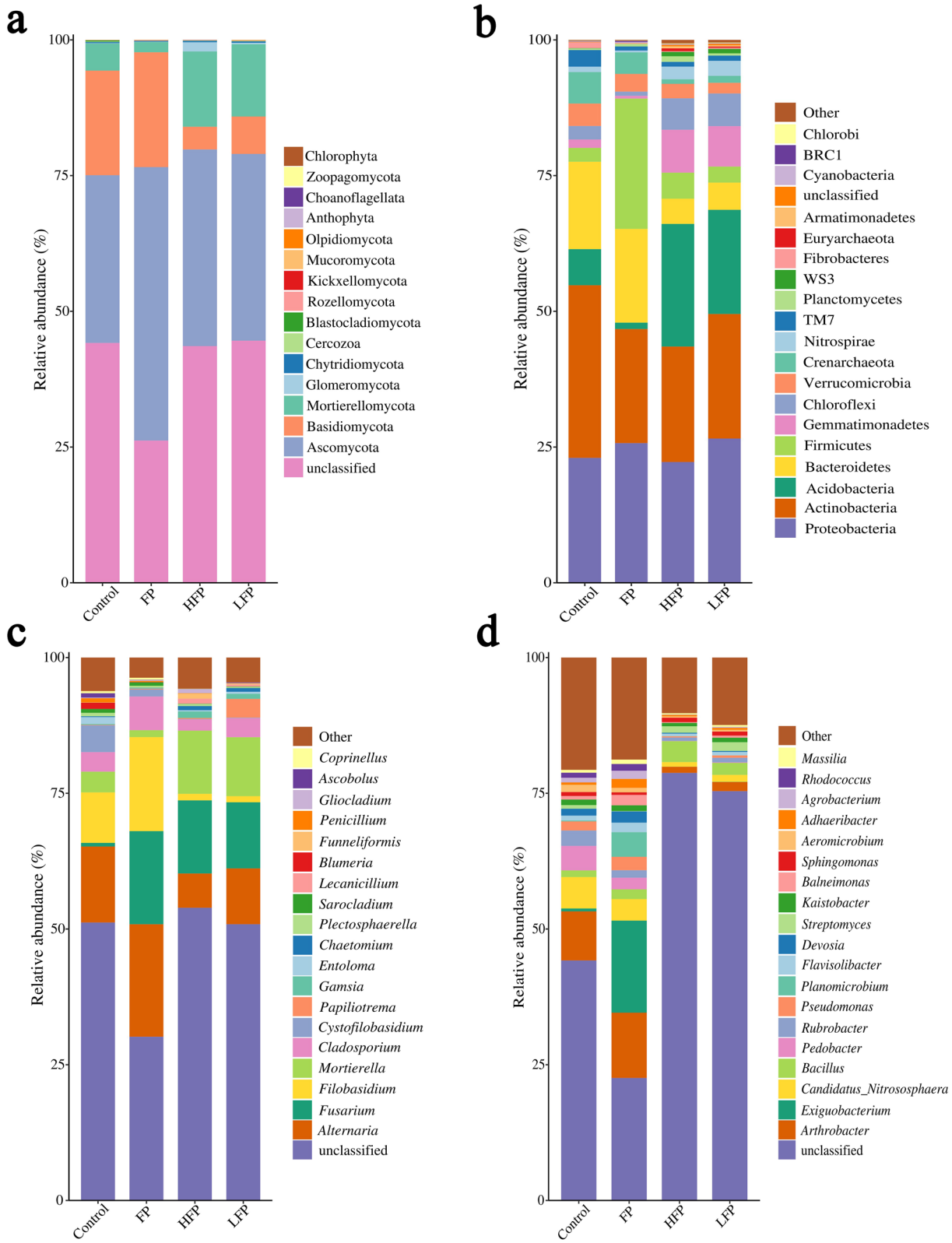

Fig. 3 The relative abundances of the top 20 taxa of fungi and bacteria at the phylum (**a**, **b**) and genus (**c**, **d**) level in wheat root-zone soil. **a**, **c** Fungi. **b**, **d** Bacteria

1.17%–22.59%, 4.64%–17.27%, and 2.55%–24.00%, respectively. In addition, Gemmatimonadetes (0.51%–7.90%), Chloroflexi (0.77%–6.02%), Verrucomicrobia (1.97%–4.13%), Crenarchaeota (0.85%–5.80%), Nitrospirae (0.33%–2.77%), and TM7 (0.76%–3.06%) all accounted for more than 1% of the total microorganisms (Fig. 3b). Compared with FP group, HFP and LFP treatments significantly increased the relative abundance of Acidobacteria, Gemmatimonadetes, Chloroflexi, and Nitrospirae but no significantly differences with Proteobacteria and Actinobacteria. In addition, HFP and LFP treatments significantly decreased the relative abundance of Bacteroidetes, Firmicutes, Verrucomicrobia, and Crenarchaeota compared with FP group ($P < 0.05$). At the genus level, most of microorganisms were unclassified, followed by *Arthrobacter*, *Exiguobacterium*, *Candidatus_Nitrososphaera*, *Bacillus*, *Pedobacter*, *Rubrobacter*, *Pseudomonas*, *Planomicrobium*, *Flavisolibacter*, *Devosia*, *Streptomyces*, and *Kaistobacter* were the dominant microorganisms. Compared with FP group, HFP and LFP treatments significantly decreased the relative abundance of *Arthrobacter*, *Exiguobacterium*, *Candidatus_Nitrososphaera*, *Pedobacter*, *Pseudomonas*, *Planomicrobium*, *Flavisolibacter*, and *Devosia*, but significantly enriched *Bacillus* and *Streptomyces* ($P < 0.05$) (Fig. 3d).

Comparison of microbial community between the different treatment groups

The richness and diversity of the fungal community in the root-zone soil were assessed by the alpha diversity. The average alpha diversity indexes (± SD) obtained from root-zone fungal and bacterial communities associated to wheat different treatments (control, FP, LFP, HFP) were showed in Additional file 2: Table S1.

Principal coordinates analysis (PCoA) was performed to analyze the differences or similarities (Bray–Curtis index) in root-zone microbial community between microbial inoculant treated groups and control groups. Both the fungal and bacterial community structures of the treatment groups (HFP and LFP) were clearly separated from the FP and control groups along the Axis.1 (43%) for fungi and Axis.1 (42.5%) for bacteria (Fig. 4). HFP and LFP resulted in higher PC1 values than FP and control groups. On the Axis.2, the control group was obviously separated from the FP group along the Axis.2 (17.3%) for fungi and Axis.2 (13.3%) for bacteria. The HFP and LFP treatments showed higher PC2 values than FP group and lower PC2 values than control group for fungi, while showed lower PC2 values than FP group and higher PC2 values than control group for bacteria. These results indicate that the first component was differentiated based on microbial inoculant treatment or no treatment, while the second component was differentiated based on the *F. pseudograminearum* Fp inoculant or no treatment.

Identification of keystone taxa under different treatment groups

Linear discriminant analysis Effect Size (LEfSe) analysis revealed that microbial communities were significantly distinct under the four treatments. Analysis by LEfSe showed that there were 107 bacterial and 32 fungal taxa

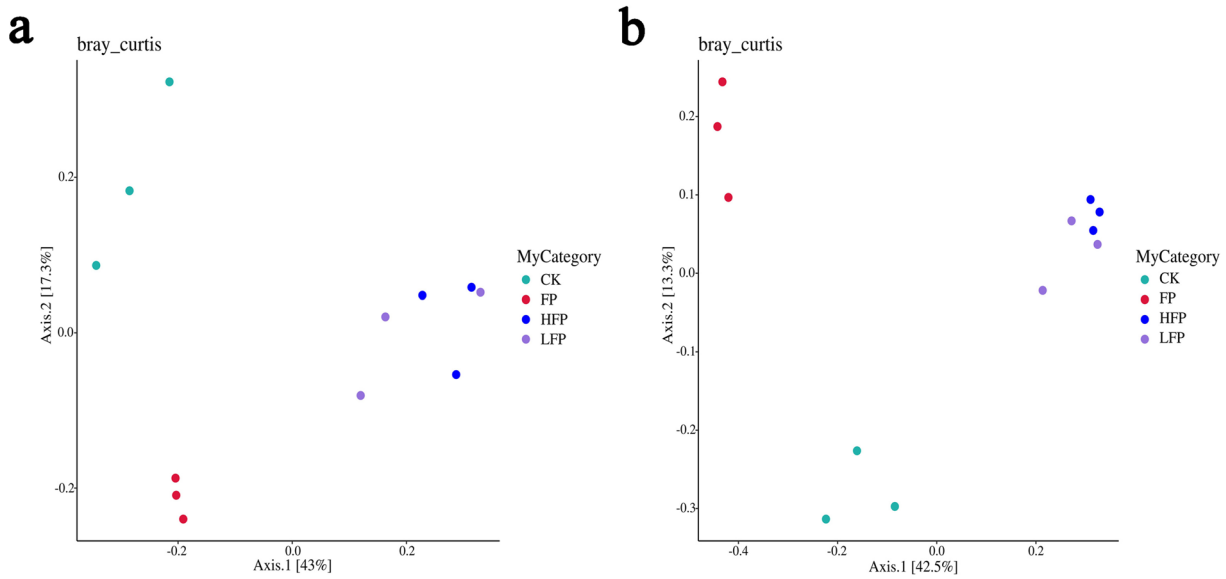

Fig. 4 Principal coordinate analysis of soil microbial community composition based on Bray–Curtis distance at the ASV level. **a** Fungi. **b** Bacteria

enriched ($P < 0.05$, linear discriminant analysis (LDA) score > 3.0) (Fig. 5). The four groups showed differentially abundant fungi from two phylum, five classes, seven orders, eight families, and ten genera (Fig. 5a, b). The abundances of phyla Basidiomycota and Mortierellomycota were statistically different and distinctive for control and HFP treatment, respectively. At the genus level, *Filobasidium* and *Alternaria* were enriched under FP; *Sarocladium*, *Cystofilobasidium*, *Naganishia*, *Dioszegia*, and *Ascobolus* were significantly under control; *Mortierella*, *Tetracladium*, and *Chaetomium* were significantly characteristic for HFP treatment. In addition, no discrimination fungal taxa could be found under LFP treatment. Meanwhile, the four groups displayed differentially abundant bacteria from six phylum, 12 classes, 15 orders, 28 families, and 46 genera (Fig. 5c, d). At the phylum level, Firmicutes was enriched under FP; Acidobacteria and Proteobacteria were significantly enriched under LFP; Armatimonadetes, Chloroflexi, Gemmatimonadetes were significantly enriched under HFP. At the genus level, *Arthrobacter*, *Pedobacter*, *Phyllobacterium*, *Janthinobacterium*, *Lentzea*, *Luteolibacter*, *Promicromospora*, and *Chthoniobacter* were enriched under control; *Exiguobacterium*, *Planomicrobium*, *Rhodococcus*, and *Dyadobacter* were significantly characteristic for treatment FP; *Streptomyces*, *Kaistobacter*, *Hyphomicrobium*, *Nocardioides*, *Reyranella*, *Lysobacter*, *Actinomadura*, *Cupriavidus*, *Candidatus_Solibacter*, *Virgisporangium*, *Pseudonocardia*, *Geodermatophilus*, and *Arenimonas* were significantly enriched under LFP. HFP treatment had the largest number of distinguishing taxa, including *Bacillus*, *Sphingomonas*, *Achromobacter*, *Steroidobacter*, *Rhodoplanes*, *Opitutus*, *Iamia*, *Ramlibacter*, *Phenylobacterium*, *Fimbriimonas*,

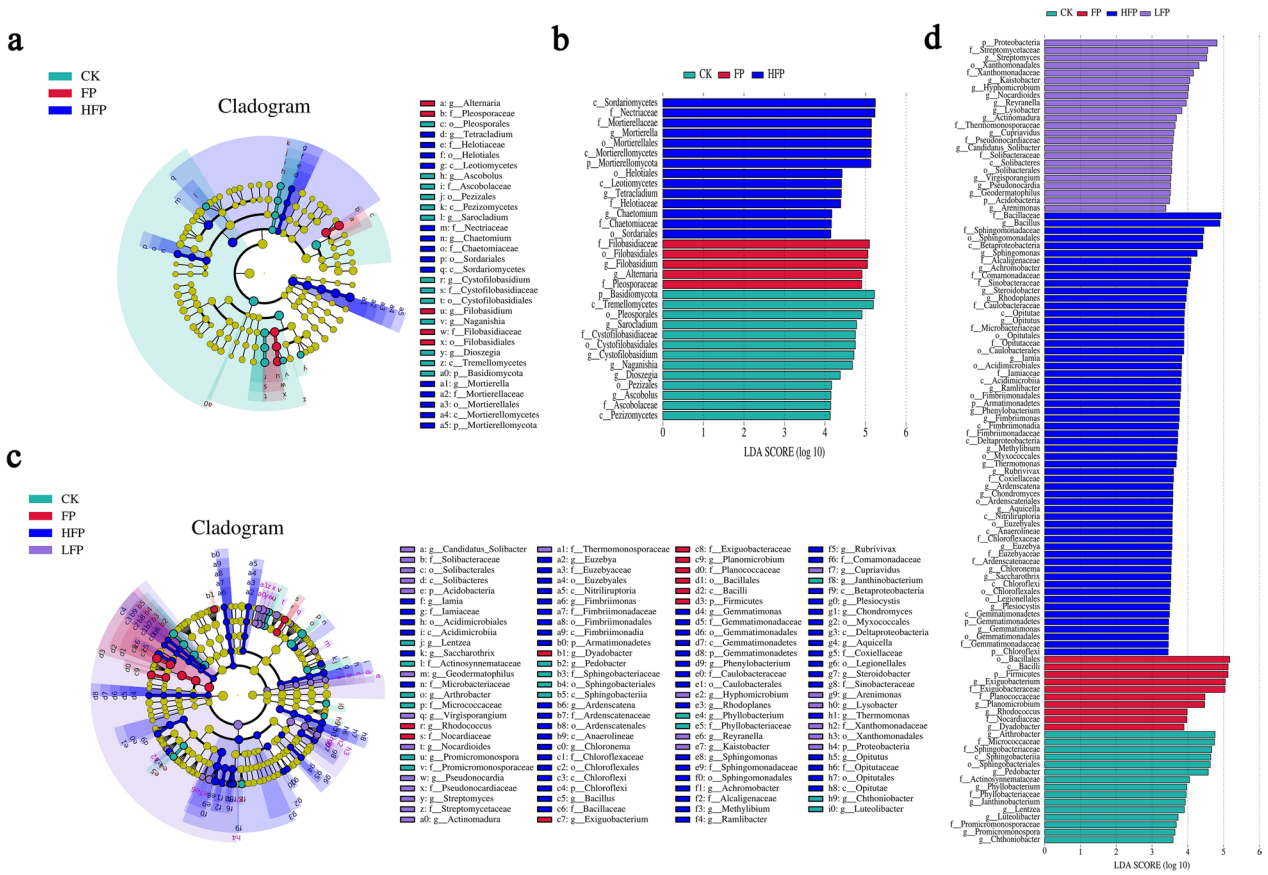

Fig. 5 Identification of wheat root-zone soil biomarker fungi and bacteria under different treatments. **a**, **c** Phylogenetic dendrogram of biomarkers in the wheat root-zone groups. **b**, **d** LDA scores of biomarker fungi (**b**) and bacteria (**d**) for each combination of wheat inoculated with different strains. The phylum and genus levels are listed in order from the inside to the outside of the cladogram with various colored dots. Taxa that were significantly abundant in control, FP, HFP, and LFP plots are indicated in cyan, red, blue, and purple, respectively. Species with the significant difference that have a higher LDA score than the estimated value. The length of the histogram represents the LDA score; i.e., the degree of influence of taxa with a significant difference between different treatments. (LDA score > 3.0, Kruskal–Wallis rank sum test, $P < 0.05$). Control, both seeds and soil were only applicated with water; FP, only soil were applicated with Fp; HFP, seeds were inoculated with LTR-2 and DnL1-1 and soil were applicated with Fp; LFP, seeds were inoculated with LTR-2 and soil were applicated with Fp

Methylibium, *Thermomonas*, *Rubrivivax*, *Ardenscatena*, *Chondromyces*, *Aquicella*, *Euzebya*, *Chloronema*, *Saccharothrix*, *Plesiocystis*, and *Gemmatimonas*.

Microbial network analysis

To identify potential interaction among fungal and bacterial community members associated with the root-zone soil, we constructed fungal and bacterial co-occurrence networks for different treatments by using the Cytoscape software (Fig. 6). Comparative analysis of the characteristic values of the four fungal correlation networks revealed (Fig. 6a–d) that the control, FP, LFP, HFP treatments contained 131, 146, 143, 176 network nodes and 1454, 1746, 1710, 2661 edges, respectively. Both network nodes and edges in control were smaller than in strain added treatments, and in FP and LFP were also smaller than in HFP treatment, while there is no significant difference between FP and LFP treatments.

While the characteristic values of the four bacterial correlation networks were compared and revealed (Fig. 6e–h) that the control, FP, LFP, HFP treatments contained 229, 229, 245, 256 network nodes and 5416, 5935, 6061, 6269 edges, respectively. The bacterial network had largest network nodes and edges in HFP treatment compared to other treatments. Thus, synergistic inoculation LTR-2 and DnL1-1 could induce the microbial community and enrich the fungal and bacterial network significantly.

Relationships between soil microbial communities and soil chemical properties

The redundancy analysis (RDA) was used to explain the impact of soil parameters on microbial populations at the genus level. These soil indicators elucidated 39.12% of the total variation in the soil fungal community (RDA1, 22.06%; RDA2, 17.06%; Fig. 7a). It was identified that soil TP, TN, and AP were the main factors to cause changes in fungal community variation. For the bacterial communities, RDA1 and RDA2 explained 29.83% and 14.26% of the total variation, respectively (Fig. 7b). Furthermore, soil AN, pH, and TP were the significant factors influencing the root-zone bacterial community.

Correlation analysis between soil parameters and fungal communities showed that *Ramicandelaber* was significant negatively associated with TN ($P<0.001$). *Tetracladium* was significant negatively associated with TP ($P<0.001$). *Leptosphaeria* was significant positively associated with pH ($P<0.05$), but negatively associated with AP and SOM ($P<0.05$). *Schizophyllum* and

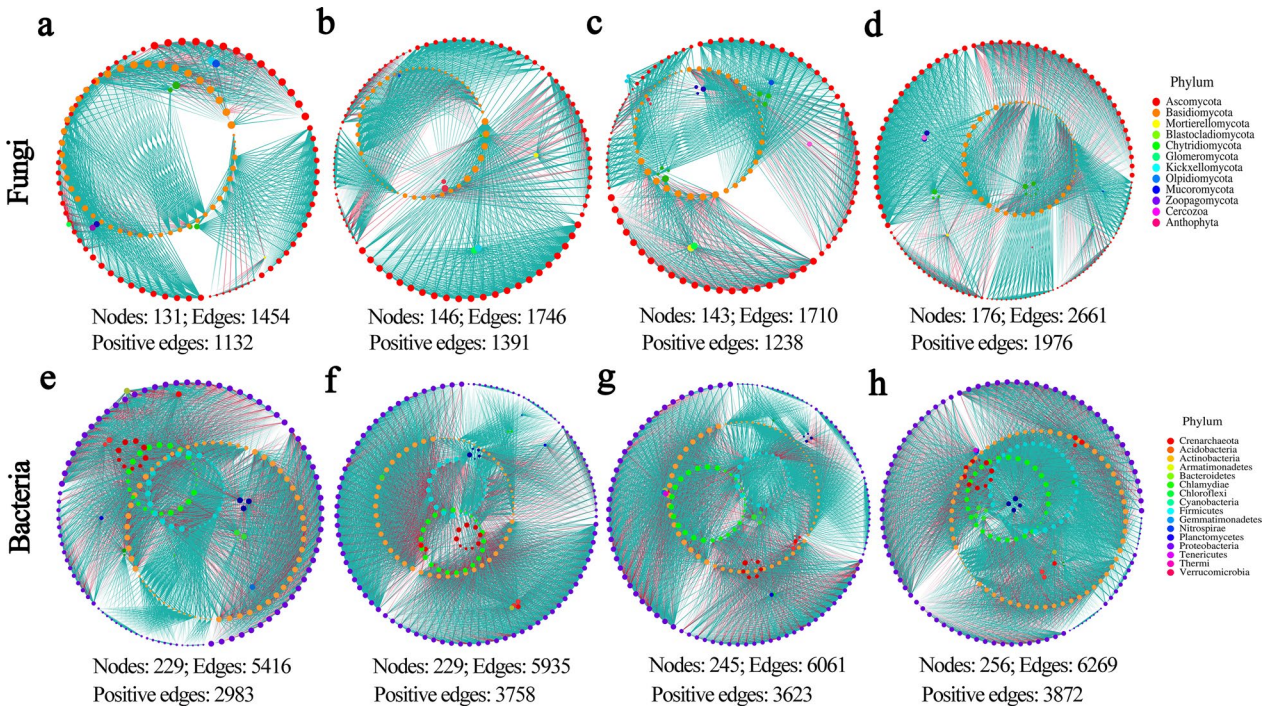

Fig. 6 Co-occurrence network of the fungal and bacterial communities associated with wheat root-zone soil in different treatments. **a**–**d** Fungal communities in control, FP, LFP, HFP treatments soil, respectively; **e**–**h** Bacterial communities in control, FP, LFP, HFP treatments soil, respectively. Different nodes are shown in different colors. Node size is proportional to the relative abundance; the larger node indicates the higher relative abundance. Links of different colors represent positive and negative interactions in the networks. Cyan respresents positive correlation, while red represents negative correlation

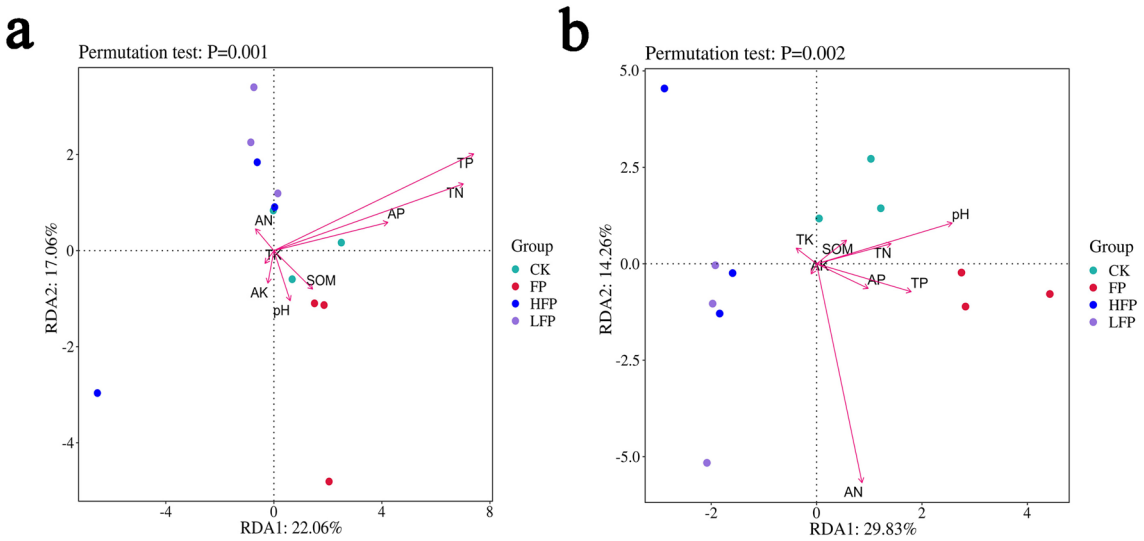

Fig. 7 Redundancy analysis (RDA) to test the soil properties influencing the fungal (**a**) and bacterial (**b**) ASV compositions in the root-zone of wheat

Eutypella were significant positively associated with AP ($P<0.05$), but negatively associated with pH ($P<0.05$). In addition, *Tomentella*, *Glomus*, *Cephaliophora*, *Microdiplodia*, and *Laetisaria* were significant negatively associated with TP ($P<0.05$) and AP ($P<0.05$) (Fig. 8a). Correlation analysis between soil parameters and bacterial communities showed that *Blastococcus* and *Myxococcus* were significant positively associated with TP ($P<0.05$) and AN ($P<0.05$). *Cellulosimicrobium* was positively associated with TK ($P<0.05$), but negatively associated with AN ($P<0.05$). *Kibdelosporangium* was negatively associated with TP ($P<0.05$) and AP ($P<0.05$). *Enterobacter* was positively associated with AK ($P<0.05$), but negatively associated with AP ($P<0.05$), respectively. *Solitalea* was significant negatively associated with TP ($P<0.01$), AP, SOM and TN ($P<0.05$). *Ramlibacter* was also significant negatively associated with TP, AP, and SOM ($P<0.01$). In addition, *Sphingomonas*, *Solimonas*, and *Asteroleplasma* were significant negatively associated with TP ($P<0.01$) and AP ($P<0.05$) (Fig. 8b).

Discussion

Effects of *T. harzianum* LTR-2 and *A. ureafaciens* DnL1-1 on wheat disease

Trichoderma harzianum have been widely used as commercial biocontrol agents against plant diseases. In this study, *T. harzianum* LTR-2 and *A. ureafaciens* DnL1-1 significantly decreased wheat FCR disease, increased wheat yield and reduced the content of DON. These results were in agreement with our previous finding that *T. harzianum* LTR-2 and *A. ureafaciens* DnL1-1 significantly increase wheat growth and control wheat crown rot efficiently in pot experiment (Yang et al. 2021, 2023).

Effects of *T. harzianum* LTR-2 and *A. ureafaciens* DnL1-1 on soil microbial community structure

Soil microbial community composition and diversity are crucial for soil quality and plant growth. In the present study, compared with the control and negative control, combination of *Trichoderma* and *Arthrobacter* inoculation significantly induced the fungi and bacterial richness and diversity and changed the bacterial taxonomic composition (Fig. 1, 5). Wang et al. (2021) reported that BIO-1 (*Paenibacillus jamilae* HS-26) and BIO-2 (*Bacillus amyloliquefaciens* subsp. *plantarum* XH-9) inoculation could effectively reduce the occurrence of disease caused by *F. graminearum* F0609, and significantly increase bacterial diversity and richness. Feng et al. (2023) reported that *C. globosum* 12XP1-2–3 exhibited a significant antagonistic effect against *F. pseudograminearum*, increased the accumulation of beneficial bacteria such as *Bacillus*, *Rhizobium* and *Sphingomonas* to contribute to health state of rhizosphere microorganisms and increased wheat yield. However, the effects of *T. harzianum* LTR-2 and *A. ureafaciens* DnL1-1 on root-zone soil community composition remain unclear.

In the present study, the predominant phyla of bacterial community in the wheat soil were Proteobacteria, Actinobacteria, Acidobacteria, and Bacteroidetes which was consistent with other findings (Meng et al. 2019). In comparison to FP group, the relative abundances of Acidobacteria, Gemmatimonadetes, Chloroflexi, and Nitrospirae were significantly increased under HFP and LFP

treatments. Earlier studies have shown that fertilization, management and biocontrol agents altered the relative abundance of Acidobacteria, Gemmatimonadetes, Chloroflexi, and Nitrospirae (Xu et al. 2020; Ma et al. 2021; Li et al. 2023a, b, c; Liu et al. 2023). These studies collectively suggest that the increased abundance of Acidobacteria, Gemmatimonadetes, Chloroflexi, and Nitrospirae in crop rhizosphere could be associated with the elevated potential for crops to defend against soil-borne diseases, making them pools of microbes with antimicrobial capabilities. It is explained by the related functions of these microflorae. For example, the majority of nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) clusters, which produce many metabolites such as many antibiotics, antifungals, siderophores, and immunosuppressants, occurred in a wide diversity of Acidobacteria (Crits-Christoph et al. 2018). Chloroflexi can fix inorganic CO_2 as well as aerobically oxidize carbon monoxide and nitrite and reduce ferric iron and nitrate, and act as primary degraders of polysaccharides (Ma et al. 2021). Nitrospirae can perform both ammonia and nitrite oxidation to produce nitrate (Shade and Gilbert 2015).

In comparison to FP group, the predominant genera of bacterial community in HFP and LFP treatments were *Bacillus* and *Streptomyces*. *Bacillus* species are commercially marketed as biopesticides, biofertilizers, and soil amendments (Chen et al. 2020; Lee et al. 2023). Most *Streptomyces* found in soil are non-pathogenic and have biocontrol activity and produce bioactive compounds to inhibit the growth of pathogen fungi (Moody and Loveridge 2014; Lee et al. 2023). Several studies have reported that higher soil bacterial community diversity might enhance resistance to pathogen invasion by improving the community's ability to efficiently exploit and compete for available resources in their environment (Mallon et al. 2015).

Gqozo et al. (2020) reported that fungal diversity along with species richness (OTU numbers) declined from the non-rhizosphere to the rhizosphere soils. Ascomycota and Basidiomycota were the dominant fungi in both the rhizosphere and non-rhizosphere soils. Moreover, in this study, Ascomycota is identified as the dominant fungal phylum in the root-zone soil, which is different from Zygomycota significantly affected by application of biochar reported by Meng et al. (2019), but consistent with wheat rhizosphere soil reported by Chen et al. (2021), Sui et al. (2022), and Li et al. (2023a, b, c). *Trichoderma* inoculation treatment affected the structure and diversity of the rhizosphere microbial community, such as increasing the content of Mucoromycota and Olpidiomycota as Sui et al. (2022) reported. However, in our study, the relative abundance of Mortierellomycota and Glomeromycota under HFP and LFP treatments was much higher than in the FP group, which can drive soil carbon cycling, P-dissolution and immobilization, lipid metabolism, and chitin degradation (Telagathoti et al. 2021; Liu et al. 2022). These reports indicate that different exogenous treatments show different effects on plant root microbial community structure.

According to the previous reports, the application of beneficial microbes significantly increased the relative abundance of some genera that can promote plant growth, while significantly decreasing some genera that hinder plant growth. In HFP and LFP treatments, the relative abundance of *Alternaria* spp. as the most dominant pathogen, which could cause various plant diseases was significantly decreased (Sui et al. 2022; Ali et al. 2023). *Fusarium* spp. including *F. pseudograminearum*, as the main pathogen causing wheat crown rot, was also significantly reduced. In addition, *Cladosporium* spp. causing leaf spot in various plant species such as tomato, canola crop and oat was also reduced (Idnurm et al. 2021; Razak and Abass 2023; Zhang et al. 2024). These results indicated that *T. harzianum* LTR-2 may also be effective in controlling plant diseases caused by them. In HFP and LFP treatments, *Mortierella* and *Gamsia* were significantly enriched, which is different from *Olpidium* and *Botryotrichum* reported by Sui et al. (2022). Arachidonic acid (ARA), produced by most *Mortierella* species, is an important component of biological cells, which can antagonize plant disease (Dedyukhina et al. 2014). Some species of *Mortierella* were proved to exhibit antagonistic activities against plant pathogens that cause root rot, potato scab, and vanilla *Fusarium* wilt disease and considered as indicator and enhancer of *Fusarium* wilt disease suppression in vanilla (Ma et al. 2021). *Mortierella* can improve the bioavailability of phosphorus and iron in the soil and improve the nutrient use efficiency of plants (Shi et al. 2022). Therefore, *Mortierella* might act as the key factor for soil-borne disease suppression properties of the soil.

Our study also found that after inoculation with *T. harzianum* LTR-2, the relative abundance of *Trichoderma* (< 0.1%) in the root-zone soil was lower than that in the rhizosphere soil (< 1%) (Sui et al. 2022). These researches indicated that even in the rhizosphere soil, the content of *Trichoderma* inoculation was still not absolutely dominant. However, whether in the rhizosphere or root-zone soil, the application of LTR-2 and DnL1-1 can increase the abundance of beneficial microorganisms, decrease the content of pathogenic microorganisms, and steer the composition of soil fungal and bacteria communities towards a heathy direction. This, in turn, reduces the occurrence of plant diseases and promotes plant growth and development.

Specific genera occur in the different treatment rhizosphere microbiota

According to the LEfSe method, unique microorganisms in each treatment were traced. 21 and 13 genera were significantly characteristic of the HFP and LFP treatments, respectively. Combined with microbial network analysis of different treatments, the combined application of LTR-2 and DnL1-1 on wheat probably supports a more complex soil microbial community than single application of LTR-2. Each strain treatment will enrich different bacterial taxa. Various PGPR, including *Bacillus*, *Sphingomonas*, *Rhodoplanes*, *Opitutus*, *Iamia*, *Ramlibacter*, *Methylibium*, *Thermomonas*, *Rubrivivax*, *Ardenscatena*, *Chondromyces*, *Chloronema*, *Saccharothrix*, and *Gemmatimonas* in HFP and *Streptomyces*, *Nocardioides*, *Lysobacter*, *Actinomadura*, *Candidatus_Solibacter*, *Virgisporangium*, *Pseudonocardia*, and *Geodermatophilus* in LFP as well as *Steroidobacter*, *Iamia*, *Phenylobacterium*, *Fimbriimonas*, and *Aquicella* in HFP and *Kaistobacter*, *Hyphomicrobium*, *Reyranella*, *Cupriavidus*, and *Arenimonas* in LFP as degrading bacterica.

Bacillus and *Achromobacter* have been reported to help suppress diseases and promote plant growth, and be related with P mineralization, as well as *Ramlibacter*, *Gemmatimonas*, and *Nocardioides* (Yin et al. 2022; Zhang et al. 2023). *Iamia* and *Nocardioides* are involved in nitrogen cycling (Liu et al. 2023; Zhang et al. 2023). *Nocardioides*, *Bacillus*, and *Streptomyces*, acted as PGPR, presented to produce a large amount of IAA, degrade toxic compounds (fungicide, herbicide and insecticide) and secrete actinomycin to antagonize the soil pathogens (Hayat et al. 2010; Liu et al. 2023). *Bacillus* sp., known as promising inoculants in agriculture, could increase biotic and abiotic stress resistance in plants by producing 1-aminocyclopropane-1-carboxylate (ACC) to inhibit the plant's production of stress-related hormones (Hayat et al. 2010). *Sphingomonas* and *Streptomyces* showed beneficial effects on enhancing biotic resistance by exciting sources of multiple biologically active metabolites, such as regulating the levels of ABA, SA, or secreting 1-aminocyclopropane-1-carboxylic acid deaminase (Singh and Dubey 2018; Lu et al. 2023). *Sphingomonas* spp. are known for their extraordinary ability to degrade recalcitrant environmental pollutants and can degrade the mycotoxin deoxynivalenol (DON) produced by *Fusarium*, thus showing potential for controlling *Fusarium* pathogens (He et al. 2017). *Saccharothrix* spp., a kind of actinobacterium, has the ability to produce numerous bioactive secondary metabolites such as dithiolopyrrolone derivatives with antifungal properties (Merrouche et al. 2017), and been correlated to ET/JA pathway to enhance disease resistance (Muzammil et al. 2014).

It is well known that wheat growth is closely related to phosphorus content, and the correlation between microbial communities and soil nutrients also showed that (Fig. 8). Like previous studies, Proteobacteria and Actinobacteria have been reported as the dominant phyla of phosphate-solubilizing bacteria (PSB) (Zhang et al. 2021). In our study, Proteobacteria (e.g. *Sphingomonas*, *Ramlibacter*, *Enterobacter*, *Solimonas*, *Myxococcus*), Actinobacteria (e.g. *Blastococcus*, *Kibdelosporangium*) showed the correlation with phosphorus content. In fact, *Sphingomonas* could secrete IAA, and has the ability of carbon and nitrogen fixation and iron production, which can effectively promote plant growth (Damo et al. 2022). An interesting finding was that *Sphingomonas* exhibited an extremely significant negative correlation with phosphorus content (TP and AP) ($P < 0.001$). In addition, phosphorus content (TP and AP) also showed significant negative correlation with *Ramlibacter*. These results showed that *Sphingomonas* and *Ramlibacter* may possess phosphorus-solubilizing properties, promoting the mineralization of phosphorus and accelerating the absorption of available phosphorus by plants, thereby promoting wheat growth.

Tetracladium has been reported to have a considerable ability to decay organic substrates and is correlated with variations in carbon resources in the soil (Li et al. 2023a, b, c). Lazar et al. (2022) have found that total phosphorus and extractable phosphorus were the most important environmental drivers of the relative abundance of *Tetracladium* spp. within plant roots. Among them, many species have the characteristics of phosphorus dissolution. In our study, TP presented an extremely significant negative correlation with *Tetracladium* ($P < 0.001$), as well as a significant negative relationship with *Cephaliophora*, *Glomus* and *Tomentella* ($P < 0.01$). *Glomus* and *Tomentella* have also been presented to have the ability to promote nutrients uptake (e.g. TP and AP) and promote the plant growth (Ramadhani et al. 2018; Wang et al. 2024). These results suggest that a variety of beneficial bacteria significantly promoted the absorption of phosphorus, thus promoting wheat growth and increasing yield.

The study found that the composition of the root-zone soil fungal community differed significantly among different treatments (control, FP, LFP, and HFP). Among them, *Mortierella*, *Tetracladium*, and *Chaetomium* were characteristical for HFP, which was also high relative abundance in the healthy rhizosphere soil fungi (Duan et al. 2022). According to previous reports, *Mortierella* and *Tetracladium* could effectively improve soil nutrient use efficiency, antagonize plant pathogenic bacteria and fungi, and promote plant growth (Duan et al. 2022; Shi et al. 2022). *Chaetomium* spp. have emerged as a source of multifarious bioactive natural compounds,

including chaetoglobosins, epipolythiodioxopiperazines, azaphilones, xanthones, anthraquinone, and terpenoids. These compounds can significantly inhibit the pathogenic fungi and bacteria (Jiang et al. 2017; Dwibedi et al. 2023). Nevertheless, only *Alternaria* and *Filobasidium,* which were soil-borne pathogenic fungi, were characteristic for FP. In fact, application of LTR-2 and DnL1-1 modulated the root-zone community composition and recruited the potential beneficial groups. These enriched beneficial microbes could protect plants against pathogens, improve soil physical and chemical composition and then to promote wheat's productivity and quality.

Conclusions

In summary, the combined application of LTR-2 and DnL1-1, as well as the single application of LTR-2, may create a healthier soil micro-ecological environment for the growth of wheat by reducing the abundance of plant pathogens in the fungal community of wheat root-zone soil, thereby providing evidence for the prevention and control of FCR and the improvement of wheat yield.

The root-zone populations of plant pathogens in HFP and LFP treatments were significantly lower than those in FP group, while more beneficial microorganisms were enriched in HFP and LFP treatments than in FP group ($P < 0.5$). Network analysis showed that the combined application of LTR-2 and DnL1-1 could significantly enrich the fungal and bacterial community networks. RDA results indicate that soil physicochemical factors, especially phosphorus, could also be closely correlated with microbial community. A variety of characteristic beneficial microorganisms enriched in LFP and HFP treatments possess the characteristics of phosphorus solubility, promoting the absorption of phosphorus, and thus promoting the growth of wheat. This observation could be attributed to the accumulation of plant-associated beneficial microorganisms by the microbial inoculants by producing secondary metabolites or affecting plant production of root exudates, thereby inhibiting proliferation of plant pathogens. Therefore, further studies need to assess and validate the role of secondary metabolites produced by microbial inoculants or root exudates in promoting the growth of beneficial microbes in root-zone soil. In addition, different effects of combined application of LTR-2 and DnL1-1 on microbial communities at different stages require further study.

Methods

Strain and culture medium

The plant-promoting strain *T. harzianum* (LTR-2) and atrazine-degrading strain *A. ureafaciens* (DnL1-1) were both deposited in the China General Microbiological Culture Collection Center (CGMCC). *T. harzianum* (LTR-2) was grown in potato dextrose agar (PDA) at 25°C for 7 days, following which the spores were washed with 20 mL sterile distilled water, filtered through degreasing cotton to prepare suspension and diluted to a final concentration of 1×10^6 CFU/mL. *A. ureafaciens* (DnL1-1) was prepared from 3 d cultures on salt-yeast medium (SYM) plates at 28°C and then was grown in Luria–Bertani culture (LB) at 28°C for 24 h with shaking and diluted to a final concentration of 1×10^8 CFU/mL in sterile distilled water.

F. pseudograminearum Fp, isolated from diseased wheat in Zhangqiu, Jinan, Shandong province and deposited in our laboratory, was used as the pathogen in this study. *F. pseudograminearum* Fp was prepared in PDA at 25°C for 7 days and then grown in corn meal medium (CMM) at 28°C for 7 days with shaking, following which the culture solution was filtered through degreasing cotton to prepare spores suspension.

Experimental design

An experimental plot system was established on the experimental fields of the innovation facility of Shandong Academy of Sciences (36.68N, 117.07E), Jinan, Shandong Province, China, from October 2022 to June 2023. This study used a completely randomized block design with three replicates per treatment. Four treatments were established, including control seeds without *F. pseudograminearum* inoculation (control), control seeds with *F. pseudograminearum* inoculation (FP), *T. harzianum* LTR-2 coated seeds plus *F. pseudograminearum* inoculation (LFP) and *T. harzianum* LTR-2 and *A. ureafaciens* DnL1-1 coated seeds with *F. pseudograminearum* inoculation (HFP). Fp spores suspension was diluted with water and dumped into the ground at 4×10^4 CFU/m^2 before sowing seeds. Then wheat seeds (Jimai 22) were surface-sterilized with 75% ethanol for 5 min, washed 5–8 times with sterile water, and soaked in 30 mL of the diluted LTR-2 (2×10^8 CFU/mL) and mixed suspensions of DnL1-1 (1×10^{10} CFU/mL) and LTR-2 (1×10^8 CFU/mL) in Petri dishes for 1 h, respectively. Control seeds were soaked in sterile water. Each replicate was consisted an area of 5 m^2 (5 m length×1 m width). Then the seeds were sown artificially on the plot at 1000 seeds per 5 m^2 on October 20, 2022. The average temperature is 15°C, and the soil relative humidity is 35%. The basal fertilizer (N_{14}-P_{16}-K_{15}, 375 g per 5 m^2) was applied prior to preplanting and the nitrogen fertilizer (urea, 125 g per 5 m^2) was applied during the green up period.

Effects of microbial inoculants on disease

At the jointing stage (180 days after sowing), wheat crown rot disease index was evaluated. 5 randomly selected wheat plants per plot were assessed for disease index. Assessment of Fusarium crown rot was performed by using disease

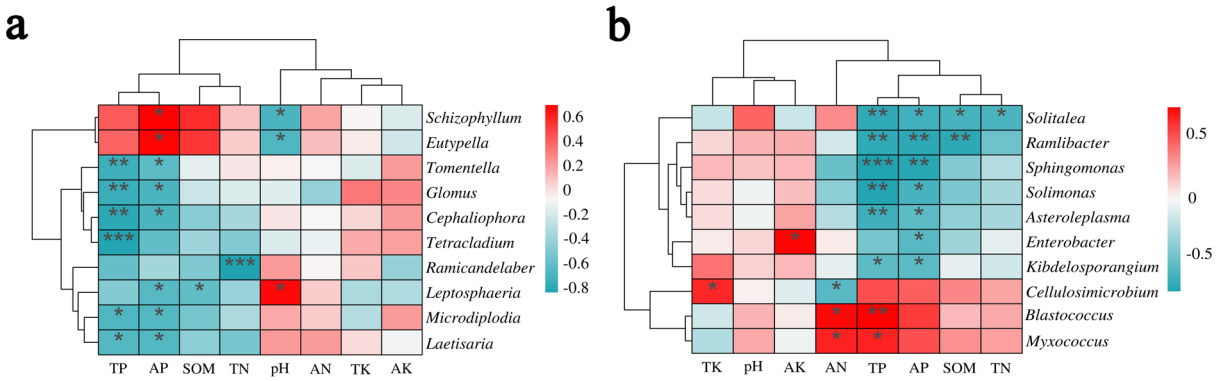

Fig. 8 Correlation heatmap between the top 10 fungal (**a**) or bacterial genera (**b**) and soil properties. The results were based on Spearman's correlation analysis. Positive relationships are represented in red, whereas negative relationships are represented in cyan. The significant correlations are presented as asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

classification standards (Moya-Elizondo et al. 2011) on the size of browning on the first internode as Grade 0: no browning; Grade 1: 1%–25%; Grade 2: 26%–50%; Grade 3: 51%–75%; and Grade 4: 76%–100%. The representative disease on wheat was shown at Additional file 1: Fig. S1. Disease indices were calculated as follows:

$$\text{Disease index} = \frac{\Sigma(\text{the number of diseased plants of each grade} \times \text{value of relative grade})}{(\text{total number of investigated plants} \times 4)} \times 100$$

where 'grade' means the level of disease severity, and 'value of relative grade' means the grade numbers.

White heads in wheat, the main disease characteristic, were investigated at the end of grouting (210 days after sowing). Each treatment had three replicates, five sampling points were selected at random for each replicate, and 100 wheat seedlings were surveyed for each sampling point. The number of white heads was counted, and the relative biocontrol efficacy was calculated based on white head rate in the plot using the following formulae.

$$\text{Whiteheads rate (\%)} = \frac{(\text{number of diseased plants})}{(\text{total number of plants under investigation})} \times 100$$

$$\text{Control efficiency (\%)} = \frac{\text{whiteheads rate of control group} - \text{whiteheads rate of treated group}}{\text{whiteheads rate of control group}} \times 100$$

Analysis of wheat yield and grain deoxynivalenol (DON) content

For wheat yield, grains were harvested by using manual harvest. Yield for each plot was measured and calculated according to the actual harvest of wheat yield to remove impurities, with a total of 3 replicates for each treatment (12 samples in total).

The DON content was determined by enzyme-linked immunosorbent assay (ELISA) kit for quantitative detection (Shanghai Youlong Biotech Co., Ltd). The limit of detection (LOD) in this test was 3 mg/kg. Extraction process, calibration and detection were performed according to the kit instructions (Tibola et al. 2019).

Soil sample collection and chemical analysis

Soil samples were taken from each plot 240 days after planting, which was the maturity stage of wheat in 2023. Five points on each plot were randomly selected for sampling. Soil was collected approximately 2 cm away from the sampled plants with a clean auger (washed and disinfected with 75% ethanol between sampling) inserted diagonally into wheat root from a soil depth of approximately 10 cm. Then soil was mixed thoroughly to form a composite sample. The 12 collected soil samples were ground and homogenized by sieving through a 1-mm stainless sieve after removing the stones and residual roots, and then portion stored in sealable bags at −80°C for DNA extraction and the remaining

part dried naturally and stored at room temperature for soil physicochemical analysis. Soil pH was measured with the glass electrode method. The SOM content was measured by the potassium dichromate oxidation—external heating method. The TN content was determined via the Kjeldahl method. The AN content was conducted using the Alkalolytic diffusion method. The TP and AP contents were extracted with NaOH and $NaHCO_3$ separately and measured using the molybdenum blue method. The TK and AK contents were extracted with NaOH and the ammonium acetate separately and conducted by the flame photometry method (Sun et al. 2023).

Genomic DNA preparation and Illumina miseq sequencing

Total soil genomic DNA was extracted using CTAB method, and then DNA was concentrated and purified with 1% agarose gels. Purified DNA was diluted to 1 ng/μL using sterile water and stored at −80°C prior to PCR amplication. 12 DNA samples (4 treatments × 3 replicate samples) were selected for bacterial and fungal community analysis, respectively. The bacterial 16S rRNA and fungal rDNA-ITS genes were amplified from the total soil genomic DNA using primers 16S rDNA V3-V4 genes (341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′)) and ITS2 region gene (ITS3-2024F (5′-GCATCGATGAAG AACGCAGC-3′) and ITS4-2409R (5′- TCCTCCGCT TATTGATATGC -3′)), respectively. All PCR reactions were carried out with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 1 μL of forward and reverse primers (2 μM), and 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, elongation at 72°C for 30 s, and a final extension at 72°C for 5 min. PCR amplicons were purified and quantified with Qubit2.0. Sequencing libraries were generated using NEB Next® Ultra DNA Library Prep Kit (Illumina, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Agilent 5400 (Agilent Technologies Co Ltd., USA) and then the library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated.

Bioinformatic analysis

The original data was uploaded to the CNGB (China National GeneBank) database to submit and save the original information for sequencing (accession number: CNP0006623). The analysis of sequencing data was conducted by following the 'Atacama soil microbiome tutorial' of Qiime2docs along with customized program scripts (https://docs.qiime2.org/2019.1/). Raw FASTO files were demultiplexed and quality-filtered using QIIME. Demultiplexed sequences from each sample were quality filtered and trimmed, de-noised, merged, and then chimeric sequences were identified and removed using the QIIME2 DADA2 plugin to obtain the feature table of amplicon sequence variant (ASV) (Callahan et al. 2016). The QIIME2 feature-classifier plugin was then used to align ASV sequences to a pre-trained GREENGENES 13.8 99% database (16S rRNA gene sequences trimmed to the V3V4 region bound by the 341F/806R primer pair and ITS rDNA genes sequences trimmed to the ITS2 region bound by the 2024F/2409R primer pair) to generate the taxonomy table (Bokulich et al. 2018). Diversity metrics were calculated using the core-diversity plugin within QIIME2. Feature level alpha diversity indices, such as observed Features, Chao1 richness estimator, Shannon diversity index, and Simpson diversity index were calculated to estimate the microbial diversity within an individual sample. Beta diversity distance measurements, including Bray Curtis, unweighted UniFrac, and weighted UniFrac were performed to investigate the structural variation of microbial communities across samples and then visualized via principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS). The linear discriminant analysis (LDA) and effect size (LEfSe) analyses were conducted using the Galaxy web application via the LEfSe algorithm, with LDA > 3.0 indicating important biomarkers. Redundancy analysis (RDA) was performed to reveal the association of microbial communities in relation to environmental factors based on relative abundances of microbial species at different taxa levels using the R package 'vegan.' Co-occurrence analysis was performed by calculating Spearman's rank correlations between predominant taxa and the network plot was used to display the associations among taxa. Unless specified above, parameters used in the analysis were set as default (Lozupone et al. 2011). The data processing was completed using the Wekemo Bioincloud (https:// www.bioincloud.tech) (Gao et al. 2024).

Statistical analysis

The wheat yield and soil properties under various treatments were compared using ANOVA followed by Duncan's test ($P < 0.05$). Spearman's correlation coefficient was used to determine the relationship between microbial parameters and soil characteristics. All statistical analyses were performed using the IBM version SPSS statistics 19.0 software (IBM Corporation, USA).

Abbreviations

ABA	Abscisic acid
ACC	1-Aminocyclopropane-1-carboxylate
AK	Available potassium
AN	Available nitrogen
ANOVA	Analysis of variance

AP	Available phosphorus
ASV	Amplicon sequence variant
CFU	Colony-forming units
CGMCC	China General Microbiological Culture Collection Center
CMM	Corn meal medium
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
ELISA	Enzyme-linked immunosorbent assay
ET	Ethylene
FCR	Fusarium crown rot
FP	*Fusarium pseudograminearum*
HFP	*Fusarium pseudograminearum* + LTR-2 + DnL1-1
IAA	Indoleacetic acid
JA	Jasmonic acid
LB	Luria–Bertani culture
LDA	Linear discriminant analysis
LEfSe	Linear effect size
LFP	*Fusarium pseudograminearum* + LTR-2
NMDS	Nonmetric multidimensional scaling
NRPS	Nonribosomal peptide synthetases
OTU	Operational taxonomic unit
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PGPR	Plant growth promoting rhinoacteria
PKS	Polyketide synthases
PSB	Phosphate-solubilizing bacteria
RDA	Redundancy analysis
SA	Salicylic acid
SYM	Salt-yeast medium
SOM	Soil organic matter
TN	Total nitrogen
TK	Total potassium
TP	Total phosphorus

Supplementary Information

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Additional file 1. Figure S1. The representative disease images on wheat.

Additional file 2. Table S1. Alpha diversity of bacterial and fungal communities in the wheat root-zone soil

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Author contributions
KY performed the majority of the experiments and wrote the paper. HW, ZZ, HL, YW (Yanli Wei), YW (Yilian Wang), JH, and YW (Yuanzheng Wu) performed part of the experiments and collected data. JL coordinated the research and revised the paper. All authors read and approved the final manuscript.

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Declarations

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Competing interests
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