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Overcoming tetracycline pollution in soils through the addition of a mycorrhizal fungal species *Funneliformis mosseae*

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ABSTRACT

The presence of residual antibiotics in the black soils of northeastern China poses a significant threat to food safety. This study investigated the potential of *Funneliformis mosseae*, one of the predominant biocontrol fungi in northeastern China, to mitigate the negative effects of tetracycline contamination (40 mg kg^{-1}) in soil. Advanced biotechnological methods were employed to assess plant growth, soil microbial antioxidant enzyme activity, and soil fertility. Additionally, changes in microbial diversity, composition, and abundance at both the phylum and genus levels were analyzed through high-throughput sequencing of the 16S rRNA and ITS gene regions of soil microorganisms. The results demonstrated that *F. mosseae* colonization in tetracycline-contaminated soils significantly improved soybean growth. Enhanced antioxidant enzyme activity in the soybean plants further contributed to increased resistance against tetracycline stress. Notably, *F. mosseae* colonization was associated with lower tetracycline levels, elevated total nitrogen (TN) content in the soil, alongside a shift in microbial diversity and abundance favoring nitrogen-fixing bacteria. This indicated that *F. mosseae* colonization influenced the bacterial and fungal community composition, altering the relative abundance of dominant microbial taxa and modulating the overall soil microenvironment. In conclusion, the application of *F. mosseae* effectively mitigated tetracycline-induced stress, improved soil health, and provided a promising strategy for the bioremediation of antibiotic-contaminated agricultural soils.

1. Introduction

Tetracycline (TC) is one of the most commonly used antibiotics for the treatment of human diseases and bacterial infections in the farming industry, and it has been detected in different environments (Chen et al., 2022). Although antibiotics can treat human and animal diseases, very little is actually consumed in the body, the vast majority of antibiotics are excreted in urine and feces (Lyu et al., 2020). These excess antibiotics are discharged into the soil through wastewater treatment plants (WWTPs), medical wastewater, household wastewater, and livestock waste (Gros et al., 2019). Accumulation of TC increases the ecotoxicity of the soil system and seriously threatens the soil microbial community, which has a huge impact on human dietary safety (Cycoń et al., 2019). Therefore, the soil environmental behavior of TCs has become a topic of focus in recent studies.

The importance of the black soil in Northeast China, which is known as the "giant panda" of arable land, for national food production and security is well recognized (Hou and Wang, 2022; Zhang et al., 2024). However, in recent years, these northeastern black soils have been negatively affected by various anthropogenic activities; associated with high levels of antibiotic and microbial resistance gene contamination (Li et al., 2022). The effect of antibiotics on soil microbial community structure cannot be ignored, high concentrations of antibiotics alter soil microflora abundance, microbial activity, enzyme activity, and nitrogen

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cycling processes (Li et al., 2021; Xu et al., 2016). Prolonged exposure to high concentrations of antibiotics promotes the proliferation of antibiotic resistance and leading to altered antibiotic susceptibility within the microbial community (Wang et al., 2024). Therefore, there is an urgent need to find an efficient, safe and stable method for mitigating the environmental issues caused by the soil antibiotics.

Arbuscular mycorrhizal fungi (AMF) can form symbiotic relationships with 80 % of terrestrial plants and provide important help in mitigating environmental stresses (Hnini et al., 2024; Ma et al., 2022). AMF enhances the uptake of water and mineral elements (N, P, K, etc.) by the host root system (Pang et al., 2023; Wei et al., 2014), accelerates the formation of chloroplasts, and increases photosynthetic rates and plant biomass (Zhang et al., 2014). Studies have shown that under drought stress, the root system of *Hovenia dulcis* exhibits elevated antioxidant enzyme activities following inoculation with AMF, along with increased net H_2O_2 efflux and Ca^{2+} influx (Li et al., 2017). In addition, inoculation of *Funneliformis mosseae* was able to increase the content of phenylpropanes, ferulic acid and p-hydroxycinnamic acid (Lu et al., 2020), which are important raw materials for the synthesis of lignin, which in turn is a key substance for the degradation of tetracycline (Olusegun et al., 2023).

In recent years, AMF-plant symbiosis system has been widely used in soil remediation (Sun et al., 2025; Zhou et al., 2023). For example, Cao et al. (2015) demonstrated that constructing a symbiotic system involving earthworms, arbuscular mycorrhizal (AM) fungi and maize effectively enhanced the degradation of oxytetracycline in soil. Similarly, Sabeti et al. (2023) confirmed that the symbiotic fungi *F. mosseae* and *Serendipita indica* may regulate rhizosphere enzymatic activities, thereby influencing the growth of lettuce and the accumulation of antibiotics in its shoots. However, prior research has not clearly demonstrated whether *F. mosseae* alone can degrade tetracycline. This study is thus designed to specifically address that question. The current experiment was conducted in the Heilongjiang region of Northeastern China, where soybeans are the main crop. The experiment will assess *F. mosseae*'s ability to degrade antibiotics and the potential of this AMF to be developed into a high-quality biofertilizer for soybeans. In addition, studies investigating how *F. mosseae* affects microbial community dynamics in tetracycline-contaminated soils remain limited. We hypothesize that the symbiosis between *F. mosseae* and soybeans may play a key role in tetracycline degradation by reshaping the soil microbial community structure. To enhance the practical relevance of the study, we had selected black soil from Northeast China and soybean, a major crop, as experimental subjects. Based on this hypothesis, we compared tetracycline degradation efficiency, plant biomass, rhizosphere soil enzyme activities and nutrient contents, and microbial community structure and abundance between *F. mosseae*-inoculated and uninoculated treatments; in order to verify the potential mechanisms by which *F. mosseae* regulates microbially mediated tetracycline degradation. The results of this study will provide scientific evidence and data support for the development of antibiotic pollution mitigation strategies in black soil farmlands of Northeastern China.

2. Materials and methodology

2.1. Plant material and growing conditions

In this study, an important cash crop soybean (*Glycine max* [L.] Merr.), which is widely cultivated in Northeast China and has high nutritional value, was selected as our host species. Soybean (HN48) was supplied by Heilongjiang Provincial Academy of Agricultural Sciences. Seeds of uniform size and clean surface were selected and soaked in 75 % ethanol for 2 min, then rinsed with sterile water and set aside. *F. mosseae* (BGC HLJ02A) was purchased from the Germplasm Bank of *Glomeromycota* in China, Institute of Plant Nutrition and Resources, Beijing Academy of Agricultural Sciences (BGC).

The experiment was conducted from May 22 to August 28, 2023, at the Experimental Station of the Sugar Industry Research Institute, Harbin Institute of Technology, Heilongjiang Province, China (126°36′18″E, 49°35′42″N). Although the study employed a pot-culture approach, the management practices closely followed those of conventional farmland cultivation. Soil moisture was monitored daily throughout the experimental period, and irrigation was carried out in the evening as needed. The environmental temperature during the cultivation period was maintained at approximately 22 °C/30 °C (night/day), with a photoperiod of 16 h/8 h (light/dark). The region is characterized by a temperate continental monsoon climate, with an average annual precipitation of 569.1 mm, most of which occurs between June and September. Prior to planting, soil was collected from the cultivated layer (<20 cm depth) of a local farmland. The soil was passed through a 1 cm mesh to remove coarse particles larger than 1 cm in diameter. The basic physicochemical properties of the soil are presented in Table S1.

2.2. Experimental design and sampling methods

The field potting treatment was used in this experiment. Each pot was filled with 4 kg of air-dried soil (natural age tillage soil). According to the preliminary investigation, it was found that the levels of tetracycline in the soils of contaminated farmland in Heilongjiang Province were generally between 37.5 and 52.3 mg/kg. Therefore, the chosen concentration of this experiment was applied at 40 mg/kg of tetracycline, and the *F. mosseae* fungus was inoculated at 10 g/kg (each gram containing approximately 20 spores). The experiment was set up with four treatment groups: (i) CK (soil with soybean only), (ii) SF (soil with soybean and *F. mosseae*), (iii) ST (soil with soybean and tetracyline), and (iv) FT (soil with soybean and *F. mosseae* and tetracycline); In addition to this a C0 group (soil with tetracycline only) was set up to assess the trace element content of the soil in its natural state, and the experimental design groupings are shown in Table_S2. Five plants were initially planted per pot (ensuring >3 germination per pot), and only three plants were retained for continual growth after germination. Each treatment was replicated three times, with a total of 63 pots arranged in a completely randomized design.

Starting on the 30th day after soybean planting, samples were collected at 15-day intervals. Sampling was conducted at five growth stages: seedling stage (30 days), flower bud differentiation stage (45 days), flowering and pod-setting stage (60 days), pod-filling stage (75 days) and maturity stage (90 days). At each stage, three pots were randomly selected for sampling, with a total of nine soybean plants (three plants per pot). During sampling, surface debris was first removed from the pots and entire plants were carefully uprooted. Based on soil adherence, samples were separated into aboveground and belowground parts. The rhizospheric soil was gently brushed from within approximately 5 mm of the root surface using sterilized brushes. The collected rhizosphere soil was immediately frozen in liquid nitrogen and subsequently stored at –80 °C for further analysis.

2.3. Determination of AM fungal colonization and plant biomass

AM fungal infection was determined by alkaline digestion-acid fuchsin staining. In each group of root samples, 50 whiskers were randomly selected, treated with 10 % KOH for 90 min, neutralized with 2 % HCl for 10 min, and finally stained with acid fuchsin to observe the AM fungal infection.

$$Infectionrate(\%) = \frac{\sum_{i=1}^{n} P_i \times N_i}{N_{total}} \tag{1}$$

P_i is the infection percentage level of the i^{th} category (e.g., 0 %, 10 %, 20 %, …, 100 %); N_i is the number of root segments at each infection level; N_{total} is the total number of observed root segments.

Plant dry weight was determined by the drying method, where fresh soybean plants and roots were dried in an oven at 60°C for 48 h and the weight was measured using a pallet balance. A meterstick was used to measure plant height (measured from the bottom of the plant above ground to the functional leaf) and root length. A root scanner (IN-GX02, Shandong, China) was used to calculate the root volume.

2.4. Determination of tetracycline, antioxidant enzyme activities and nutrient contents in soil samples

The collected soil samples were divided into individual sample bags and stored in an ultra-low temperature refrigerator. The antibiotic content in the rhizosphere soil was analyzed using an Agilent 1290–6495 series HPLC system. The analytical procedure was based on the method described by Cao et al. (2015), with minor modifications. Superoxide dismutase (SOD) was determined by NBT reduction method (Huehne et al., 2020); Peroxidase (POD) was determined spectrophotometrically (Li et al., 2023); Catalase (CAT) was determined by hydrogen peroxide titration (Anilkumara et al., 2024); Soil total nitrogen (TN) content was determined by Kjeldahl method using sulfuric acid-accelerator digestion(Ministry of Forestry of the People's Republic of China, 2015); Soil total phosphorus (TP) content was determined by NaOH alkali fusion and molybdenum-antimony antimony spectrophotometry; Soil total potassium (TK) content was determined by NaOH alkali fusion and flame photometer method (Ministry of Agriculture of the People's Republic of China et al., 1988).

2.5. DNA extraction, 16 s rRNA sequencing and ITS sequencing

The rhizosphere soil (5 mm) of each sample was gently brushed down using a disposable brush, poured with liquid nitrogen to freeze it, divided and placed in an ultra-low temperature refrigerator to await testing. Microbial DNA was extracted from the soil using CTAB (Yu et al., 2021) and DNA integrity and purity were examined using 1 % agarose gel electrophoresis. 16S rRNA amplification of the V4-V5 hypervariable region of soil bacteria was performed using PCR amplification primers 515 F (5′-GTGCCAGCMGCCGCGGGTAA –3′) and 907 R (5′-GGACTACHVGGGTWTCTAAT-3′). ITS amplification of the ITS1–1 highly variable region of soil fungi was performed using PCR amplification primers ITS5–1737F (5′-GGAAGTAAAAGTCGTAACAAGG –3′) and ITS2–2043R (5′-GCTGCGTTCTTCATCGATGC –3′). The NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA) standard procedure for library construction. Constructed amplicon libraries were subjected to PE250 sequencing using the Illumina Nova 6000 platform. To obtain the final feature sequence list, the fastp (an ultra-fast all-in-one FASTQ preprocessor, version 0.14.1, https://github.com/OpenGene/fastp) plugin was utilized for quality filtering, pruning, denoising, merging and removing the chimeric sequences. The UPARSE method was chosen for OUT clustering and species annotation.

2.6. Statistical analysis

Calculation of the significance of soybean biomass and soil basic indicators was performed using Tukey HSD and Duncan in IBM SPSS (version 20.0) and SAS JMP (version 13.2) ($P < 0.05$), and the visualization analysis was applied with the chiplot platform (https://www.chiplot.online/). OUT denoising (filtering, de-duplication, chimera filtering, splicing of reads) was performed using QIIME DADA2 (version v2020.11.0), while USEARCH (v10.0.240) performed OUT species annotation and calculation of alpha diversity indices (Shannon_2 and Chao1). NMDS and PCoA analyses were performed using R software (version 4.3.3) to visualize differences in soil rhizosphere microbial composition between treatment samples. The 16S rRNA and ITS species annotation databases were used Greengenes (version v13.8) and Unite (version v8.0), respectively. Microbial Rank Abundance was calculated using Python (version v2.7). Finally, visualization of inter- and intra-species correlation network prediction plots was performed using the ggplot2 package of R software (version 4.3.3), and correlation analysis (RDA) between environmental factors and microorganisms was also performed.

3. Results

3.1. Plant biomass, fungal colonization and tetracycline degradation trends

Soybean plant and root development were significantly affected by tetracycline stress; however, the negative effect was mitigated by the addition of *F. mosseae*. The colonization rate of *F. mosseae* throughout soybean growth and development was determined using the colonization rate formula. By day 75, the SF and FT treatment groups exhibited colonization rates of 95 % and 93 %, respectively, marking the first inflection point (Fig. 1C). Further analysis of plant biomass during this period revealed that under tetracycline stress, *F. mosseae* inoculation in the FT treatment group led to increases of 51.47 % in root dry weight, 6.51 % in root length, and 13.35 % in root volume compared to the ST treatment group ($P < 0.05$; Fig. 1A, E, F). Although there were no significant differences in the dry weight of aboveground plant parts, plant height, or root dry weight, all showed slight increases of 9.15 %, 2.15 %, and 1.74 %, respectively (Fig. 1A, D, G). Additionally, the SF treatment group exhibited significantly higher plant dry weight, root dry weight, plant height, root length, root volume, and nodule dry weight compared to the CK control group, with increases of 18.08 %, 57.24 %, 12.57 %, 9.76 %, 37.25 %, and 10.52 %, respectively ($P < 0.05$; Fig. 1), and likewise outperformed the other treatment groups. In conclusion, *F. mosseae* significantly enhanced plant biomass under both normal and tetracycline-stressed conditions, promoting plant height, improving leaf health, and stabilizing root structure.

We measured tetracycline concentrations in the rhizosphere soil at five key growth stages of soybean, and the results are shown in Fig. 1B. As the growth period progressed, tetracycline levels in the ST, FT, and C0 groups showed a continuous decline. By day 90, the FT group exhibited the greatest reduction (62.75 %), followed by the ST group (51.5 %) and the C0 group (43.3 %). During the first 50 days, no significant differences in tetracycline concentrations were observed among the treatment groups. However, after day 50, the degradation efficiency in the FT group was significantly higher than that in the ST and C0 groups, by 21.8 % and 45.1 %, respectively, indicating that colonization by *F. mosseae* may enhance tetracycline degradation. Notably, the degradation rate slowed around day 75. Considering the colonization dynamics of *F. mosseae* (Fig. 1C), we further analyzed the soil microbial community status at this stage.

3.2. Antioxidant defense systems of soil microorganisms and environmental effects

The activities of three key antioxidant enzymes (SOD, POD and CAT) were measured to assess microbial defense responses in the soil. The FT treatment group exhibited significantly higher SOD, POD, and CAT activities ($P < 0.05$; Fig. 1A–C), with increases of 1.62-fold, 0.44-fold, and 0.10-fold compared to the ST treatment group, and 1.25-fold, 1.31-fold, and 1.35-fold compared to the SF treatment group, respectively. In the SF group, SOD and POD activities were significantly higher than those in the CK control group, increasing by 12.33-fold and 0.18-fold, respectively ($P < 0.05$; Fig. 1A–C), while CAT activity was significantly reduced. Additionally, the ST treatment group showed significantly higher SOD, POD, and CAT activities than the CK control group, with increases of 10.42-fold, 0.95-fold, and 0.08-fold, respectively. Overall, both tetracycline and *F. mosseae* stimulated the antioxidant defense system to some extent, enhancing antioxidant enzyme activities. Furthermore, under tetracycline stress, *F. mosseae* treatment significantly increased the enzymatic activity of soil microorganisms.

TN, TP, and TK are essential soil elements and key indicators of soil fertility and microbial activity. The contents of N, P, and K were significantly higher in all treatment groups compared to the C0 no-

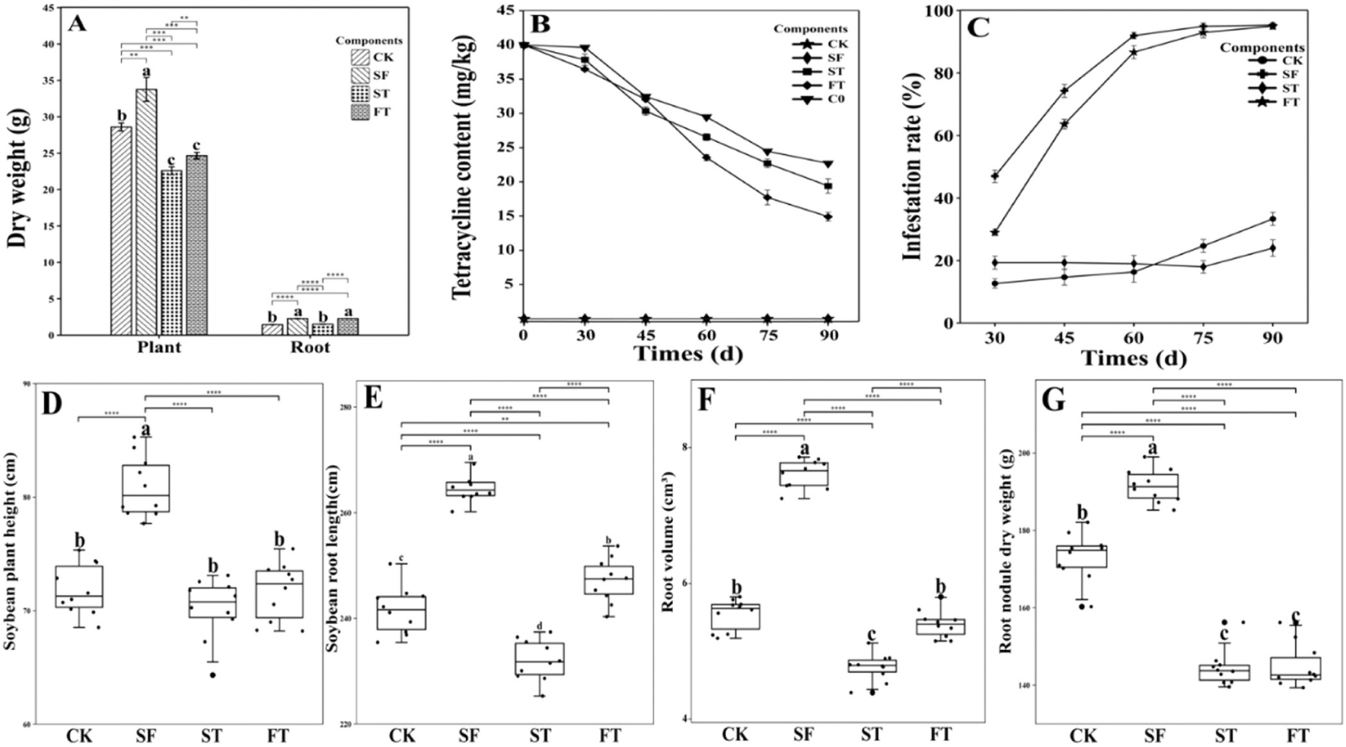

Fig. 1. Effects of *F. mosseae* on soybean dry weight (A), tetracycline content (B), colonization rate (C), plant height (D), root biomass (E–F) and nodules (G). Graphs a, b, c, and d represent significant differences ($P < 0.05$) and Tukey HSD test were applied to determine significance (sample number n = 9). *Indicates the significance of the difference between the two groups, with more representing a more significant difference (*t*-test). C0: Soil only with tetracycline; CK: soil only with soyabean; SF: soil with soyabean and *F. mosseae*; ST: soil with soyabean and tetracycline; FT: soil with soyabean and *F. mosseae* and tetracycline.

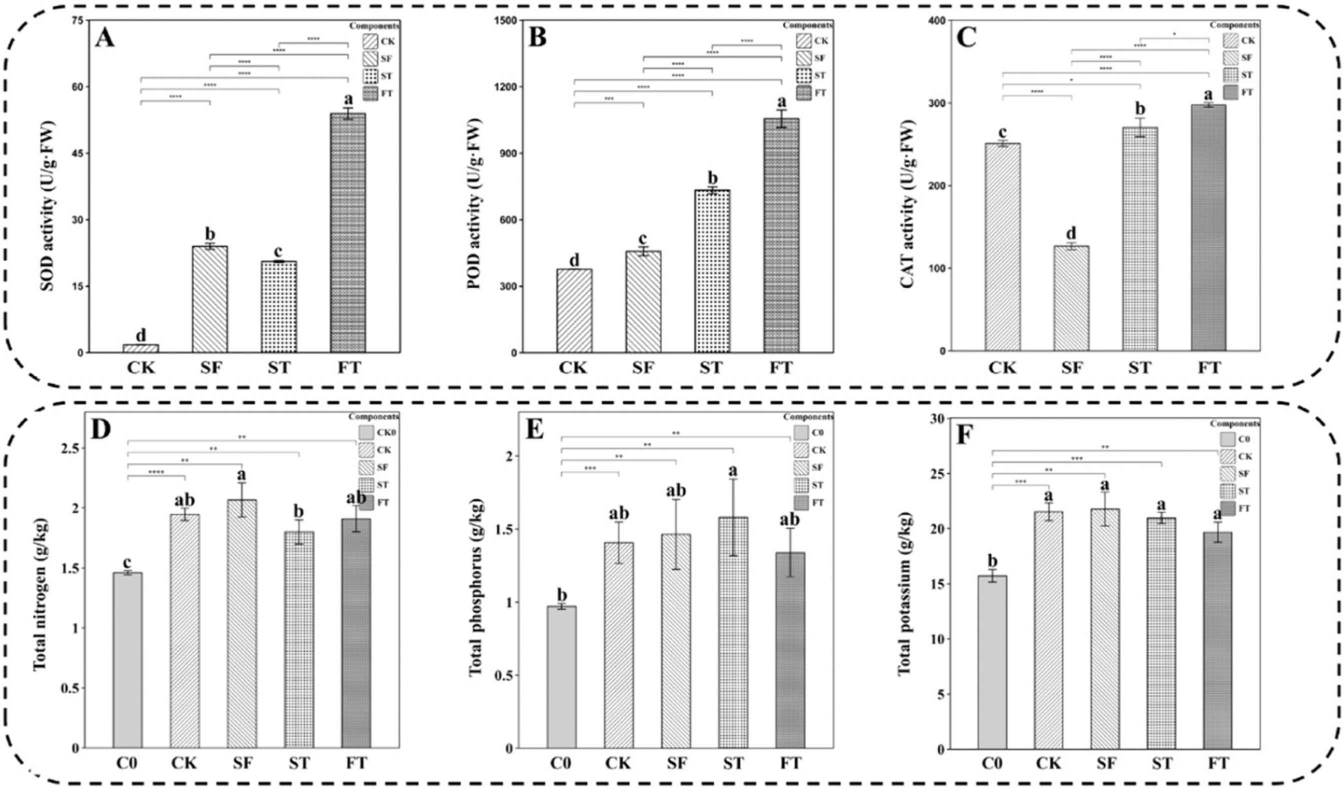

Fig. 2. Changes in soil antioxidant enzyme activities and nutrient contents. Panels A–C show the activities of soil microbial antioxidant enzymes, while panels D–F show the contents of major soil nutrients. Statistical significance was determined using Tukey's test ($n = 3$). Different lowercase letters indicate significant differences ($P < 0.05$). C0: Soil only with tetracycline; CK: soil only with soyabean; SF: soil with soyabean and *F. mosseae*; ST: soil with soyabean and tetracycline; FT: soil with soyabean and *F. mosseae* and tetracycline.

treatment group ($P < 0.05$; Fig. 2D–F). Although no significant differences in soil N content were observed among the treatment groups, the trend followed SF > FT > CK > ST (Fig. 2D). The P content in the ST treatment group increased by 15.3 % compared to the FT group, while the SF group showed almost no difference from CK within the same category (Fig. 2E). Soil K content did not vary significantly; however, treatment groups without tetracycline stress exhibited significantly higher K levels than those under stress (Fig. 2F). These findings indicate that the soybean–microbial symbiotic system significantly ($P < 0.05$) enhanced soil fertility, although tetracycline stress slightly reduced soil physicochemical properties. Furthermore, based on the soybean nodule dry weight results (Fig. 1G), it was evident that *F. mosseae* colonization enhanced nitrogen fixation capacity in both plants and soil microorganisms.

3.3. *Analysis and prediction of rhizosphere bacterial diversity under tetracycline stress*

Sequencing of the collected soil bacterial samples yielded over 1.4 million clean tags and identified 8594 OTUs across 12 samples (Table S3a). Alpha diversity indices, including Shannon_2 and Chao1, showed no significant differences ($P > 0.05$), indicating that rhizosphere soil α-diversity was not significantly affected (Fig. 3A, B). PCoA based on Bray–Curtis distance also revealed a low but nonsignificant correlation (Fig. 3C, 95 % confidence interval). NMDS demonstrated structural similarity in species composition among samples, with good overall sample clustering (Fig. 3D, STRESS = 0.1). According to the bacterial community composition histogram (Fig. 3E), Proteobacteria was the dominant bacterial phylum, accounting for 93 %, 90 %, 74 %, and 75 % of the CK, SF, ST, and FT treatment groups, respectively, followed by Acidobacteria, Actinobacteria, Chloroflexi, and Bacteroidetes, which were also present in relatively high abundance. Under *F. mosseae* colonization, the relative abundance of Proteobacteria and *Azotobacter* increased by 1.27 % and 1.1 %, respectively, while Acidobacteria abundance decreased by 1.17 % in the FT group compared to the ST group (Fig. 3E). Clustering heatmap analysis at the phylum level (Figure S1) showed that *Proteobacteria* were more abundant in the CK and SF treatment groups, whereas the other 14 bacterial taxa exhibited higher abundance in the ST and FT groups. Similarly, genus-level clustering (Fig. 3F) revealed significant differences in the relative abundances of *Sphingomonas*, MND1, *Haliangium*, and *Nitrospira* between the FT and ST groups.

To further investigate how *F. mosseae* influenced the rhizosphere soil bacterial community structure under tetracycline stress, the FT and ST treatment groups were analyzed at the genus level. Differential bacterial genera in these groups were identified using linear discriminant analysis effect size (LEfSe), with linear discriminant analysis (LDA) values serving as a criterion for significant differences in abundance between groups (Table S4a; Figure S2a). In the ST treatment group, *Azotobacter* (3.56), *Sphingomonas* (3.13), and *Phreatobacter* (2.11) were the most significantly enriched genera, whereas in the FT treatment group, *Geobacter* (3.36), *Dechloromonas* (3.31), *Dechlorosoma* (2.68), *Aeromonas* (2.16) and *Polyangium* (2.00) were the dominant differentially abundant bacteria. These findings suggest that *F. mosseae* treatment altered the soil bacterial community structure and increased the relative abundance of dominant species.

Furthermore, based on the relative abundance of OTUs across samples, Spearman correlation coefficients were used to construct a covariance network diagram, illustrating species interactions such as cooperation, competition, antagonism, and predation. Proteobacteria were the most abundant rhizosphere soil bacteria, contributing more than 70 % of the relative abundance in each sample and playing a crucial role in the overall environmental system, followed by Acidobacteria and Actinobacteria (Fig. 4A). The species interaction network

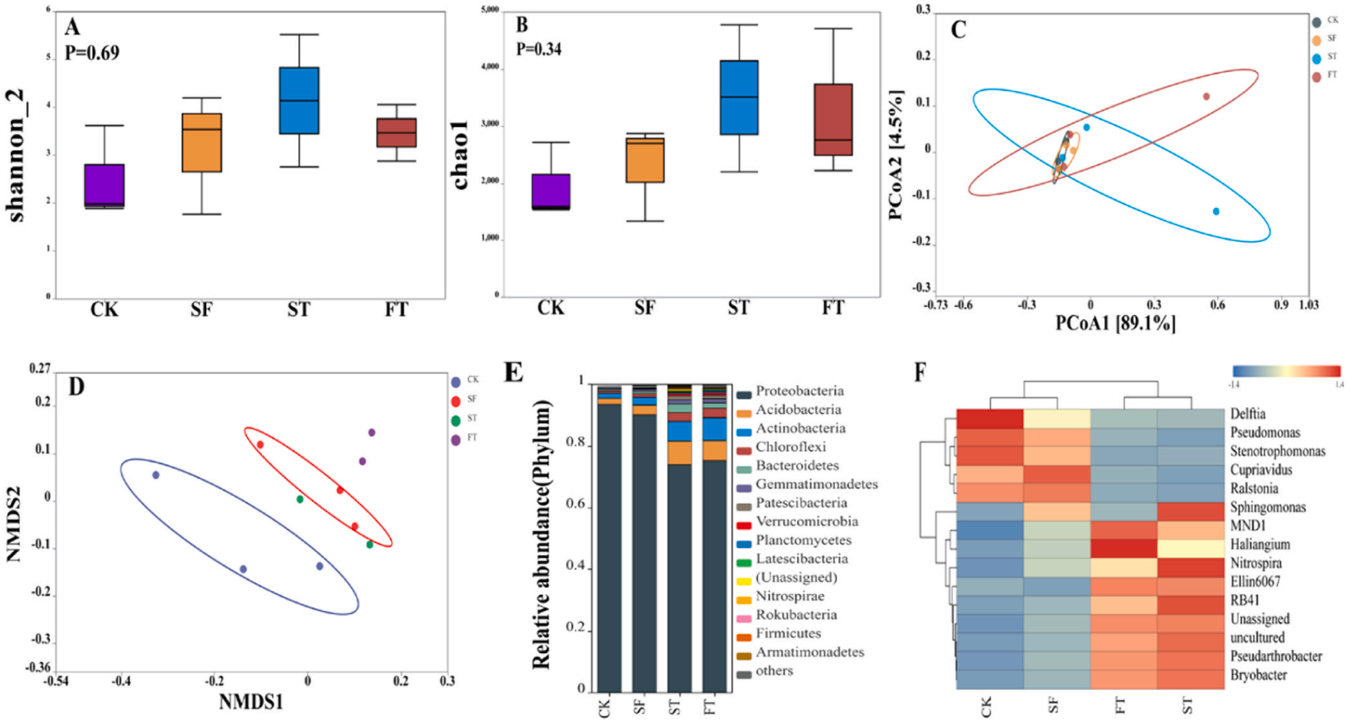

Fig. 3. The rhizosphere soil bacterial diversity and community structure analyses. (A-B) shannon_2 and chao1 represent alpha diversity analysis. (C) PCoA (Principal coordinate analysis) simplifies the structure of the data to show the natural distribution of samples at some particular distance scale. (D) Overall distribution pattern of OTU-based bacterial community variability across treatment groups (based on nonmetric multidimensional sealing (NMDS). (E) Selection of the top 15 species in abundance (Phylum level) based on information from OUT taxonomic level sequences. (F) Heat map of the top 15 relative abundances of rhizosphere bacteria (Genus level). CK: soil only with soyabean; SF: soil with soyabean and *F. mosseae*; ST: soil with soyabean and tetracycline; FT: soil with soyabean and *F. mosseae* and tetracycline.

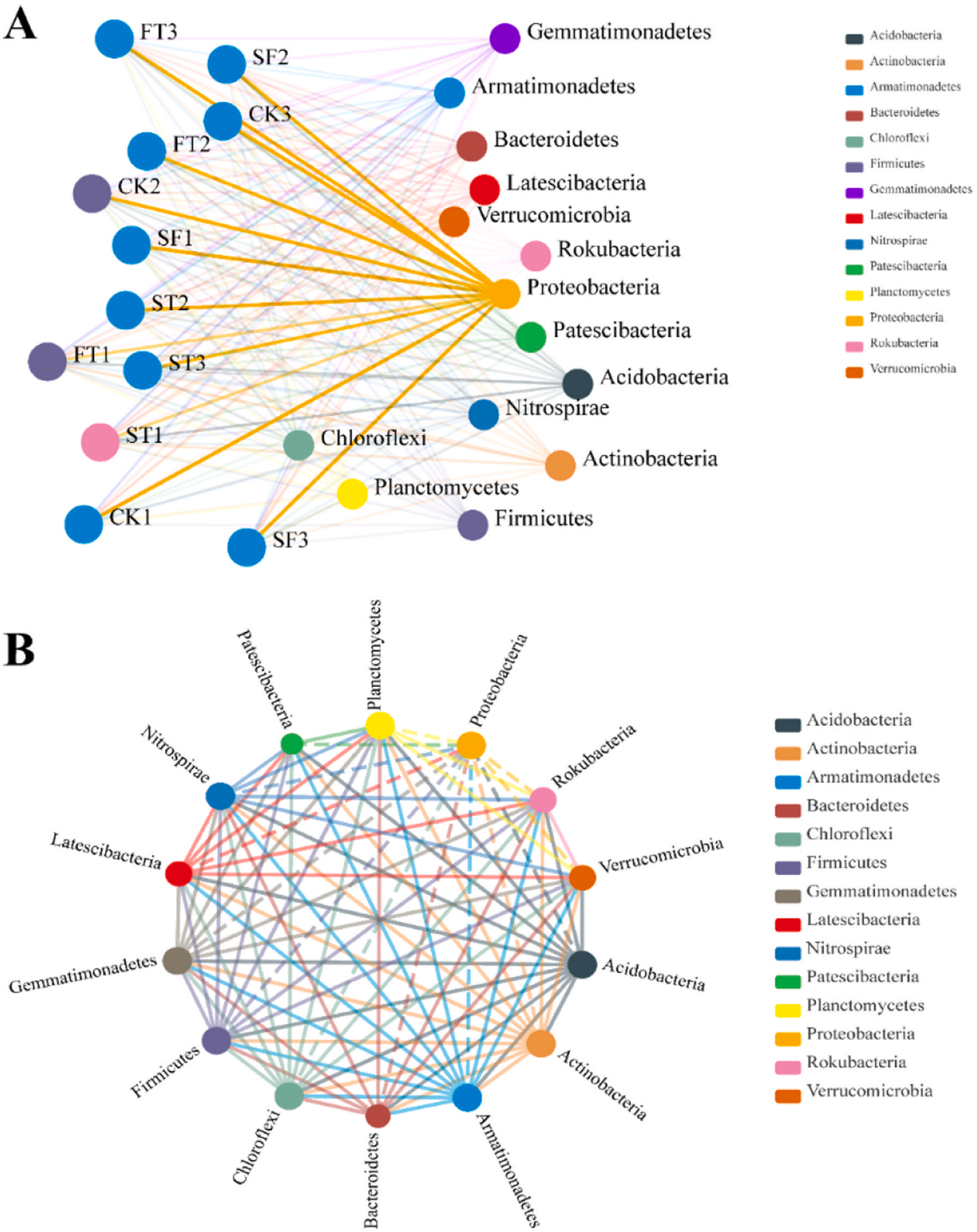

Fig. 4. Network and predictive analysis plot for rhizosphere soil bacteria. (A) Covariance network graph analysis can be used to show the distribution between samples and species, with a connecting line between a species and a sample if that species occurs in a sample; the higher the species' percentage abundance, the thicker the line, and the color of the line for whichever species is the color of the species, which is the species with the strongest influence. (B) Species correlation network diagram to obtain interrelationships between species within a sample, with solid lines representing positive correlations and dashed lines representing negative correlations. CK (1–3): soil only with soyabean; SF (1–3): soil with soyabean and *F. mosseae*; ST (1–3): soil with soyabean and tetracycline; FT (1–3): soil with soyabean and *F. mosseae* and tetracycline.

(Fig. 4B) revealed a predominantly positive correlation among bacterial taxa, suggesting potential cooperative interactions, whereas Proteobacteria appeared to exhibit a competitive relationship with other bacterial groups.

3.4. *Analysis and prediction of rhizosphere fungal diversity under tetracycline stress*

Similarly, sequencing of the 12 soil fungal samples yielded 0.95 million clean tags, identifying 1229 OTUs (Table S3b). No significant differences were observed in the α-diversity of rhizosphere fungal communities, as indicated by the Chao1 and Shannon_2 indices ($P > 0.05$; Fig. 5A–B). PCoA based on Bray–Curtis distance showed that neither tetracycline nor *F. mosseae* had a significant effect on rhizosphere soil fungal communities (Fig. 5 C). Similarly, NMDS revealed structural similarity in species composition across samples, with close clustering among groups (Fig. 5D, STRESS = 0.1). Further analysis of soil fungal community composition showed that Ascomycota was the most dominant fungal phylum across all treatment groups, accounting for 36.8 %, 48.8 %, 43.8 % and 41.6 % in CK, SF, ST, and FT, respectively. Other predominant fungal phyla included Mortierellomycota (8.4 %, 6.2 %, 7.6 %, and 7.0 %), Basidiomycota (3.8 %, 2.9 %, 2.8 %, and 3.7 %), and Chytridiomycota (0.6 %, 0.7 %, 0.5 %, and 1.0 %) (Fig. 5E). A correlation heatmap at the phylum level (Figure S1b) indicated that Chytridiomycota and Basidiomycota were more abundant in the FT treatment than in the ST treatment, whereas Basidiobolomycota, Rozellomycota, and Monoblepharomycota showed significantly lower relative abundances.

At the genus level, the relative abundances of *Cladorrhinum*, *Humicola*, and *Tausonia* were significantly higher in the FT treatment than in the ST treatment (Fig. 5 F). The clustering tree on the left side further revealed significant differences in the relative abundance of these genera among CK, SF, and FT, indicating that tetracycline and *F. mosseae* treatments altered the rhizosphere soil fungal community structure. Differential genus analysis using LEfSe (LDA > 2; Table S4b, Figure S2b) identified Unidentified (3.94), Rozellomycota (2.95), Chaetomiaceae (2.75), *Verticillium* (2.45) and Sebacinales (2.34) as significantly enriched in the ST treatment. While 13 fungal genera, including Auriculariales (3.50), *Cladorrhinum* (3.36), *Operculomyces* (3.11), *Leptosphaeria* (2.95) and *Trichoderma* (2.78), were significantly enriched in the FT treatment. These findings suggest that *F. mosseae* altered the abundance and composition of rhizosphere soil fungi under tetracycline stress.

Network and predictive analyses of rhizosphere fungal communities showed that Ascomycota (40.1 %), Mortierellomycota (9.2 %), Basidiomycota (3.31 %), Chytridiomycota (1.09 %), and Glomeromycota (1.44 %) played key roles in shaping the rhizosphere fungal community (Fig. 6A). The species correlation network (Fig. 6B) indicated that Mortierellomycota and Glomeromycota had a collaborative relationship, whereas all other fungi exhibited competitive interactions, with Ascomycota exerting the strongest competitive influence.

3.5. Correlation analysis between rhizosphere soil microorganisms and environmental factors under tetracycline stress

The RDA score plot illustrates the relationships among soil rhizosphere microorganisms, antioxidant defense enzymes and soil physicochemical properties (Fig. 7). Specifically, Proteobacteria (*Pseudomonas*, *Cupriavidus*, *Ralstonia* and *Stenotrophomonas*) exhibited a negative correlation with antioxidant defense enzymes (SOD, POD, and CAT) and a positive correlation with soil physicochemical properties (TN, TP, and TK), while other bacterial groups showed the opposite trend (Fig. 7A-B). Although fungal communities displayed weaker correlations than bacterial flora, Basidiomycota (*Tausonia*) and Ascomycota (*Thermomyces* and *Humicola*) were negatively correlated with antioxidant defense enzymes and positively correlated with soil physicochemical properties. In contrast, Ascomycota (*Aspergillus*) exhibited the opposite trend, while other fungal taxa showed minimal correlation.

4. Discussion

Tetracycline (TC) stress indeed affected the growth performance of soybean plants. Interestingly, this adverse effect was effectively alleviated by the addition of *F. mosseae*. In this study, monitoring tetracycline concentrations in soil across five growth stages (Fig. 1B) revealed a significant decline, with the degradation rate following the order FT > ST > C0. This indicated that inoculation with *F. mosseae* facilitated

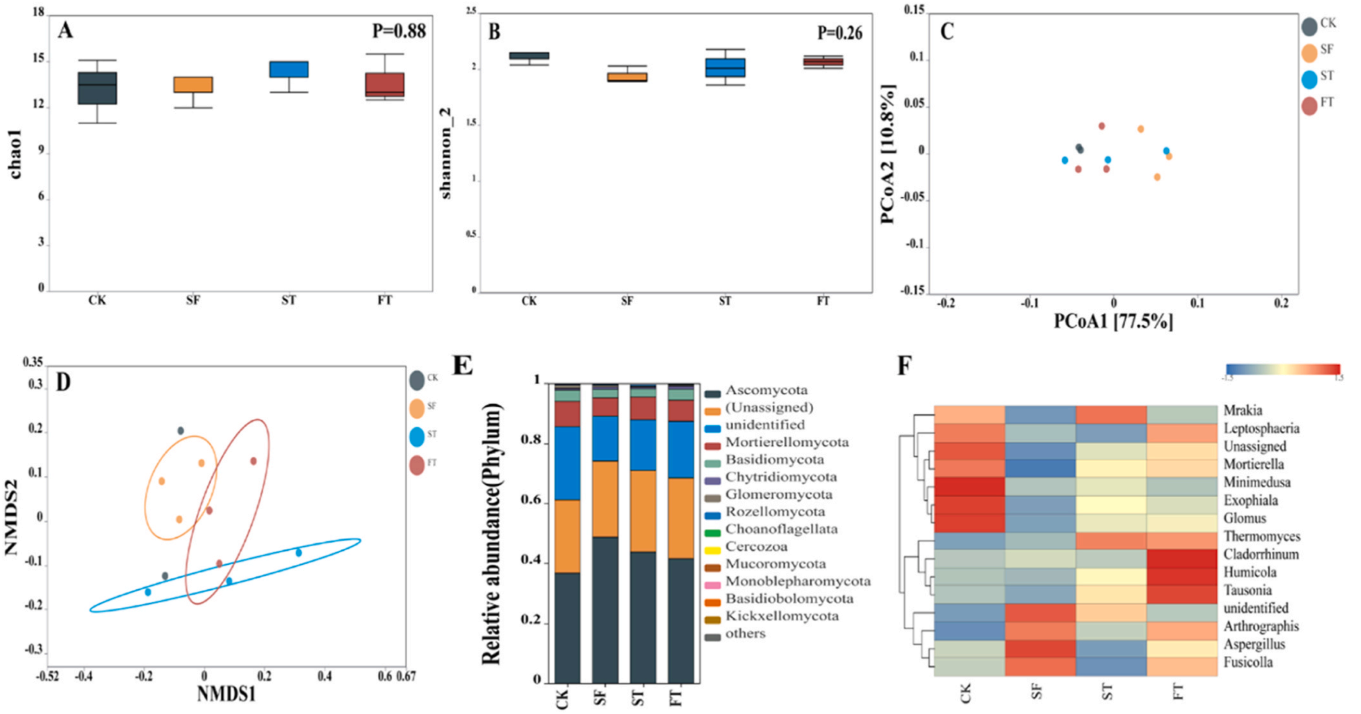

Fig. 5. Analysis of rhizosphere soil fungal diversity and community structure. (A-B) shannon_2 and chao1 represent alpha diversity analysis. (C) PCoA simplifies the structure of the data by showing the natural distribution of samples at some particular distance scale. (D) Overall distribution pattern of OTU-based bacterial community variability across treatment groups (based on nonmetric multidimensional sealing (NMDS). (E) Selection of the top 15 species in abundance (Phylum level) based on information from OUT et al. taxonomic level sequences. (F) Top 15 heat map of relative abundance of rhizosphere bacteria (genus level). CK: soil only with soyabean; SF: soil with soyabean and *F. mosseae*; ST: soil with soyabean and tetracycline; FT: soil with soyabean and *F. mosseae* and tetracycline.

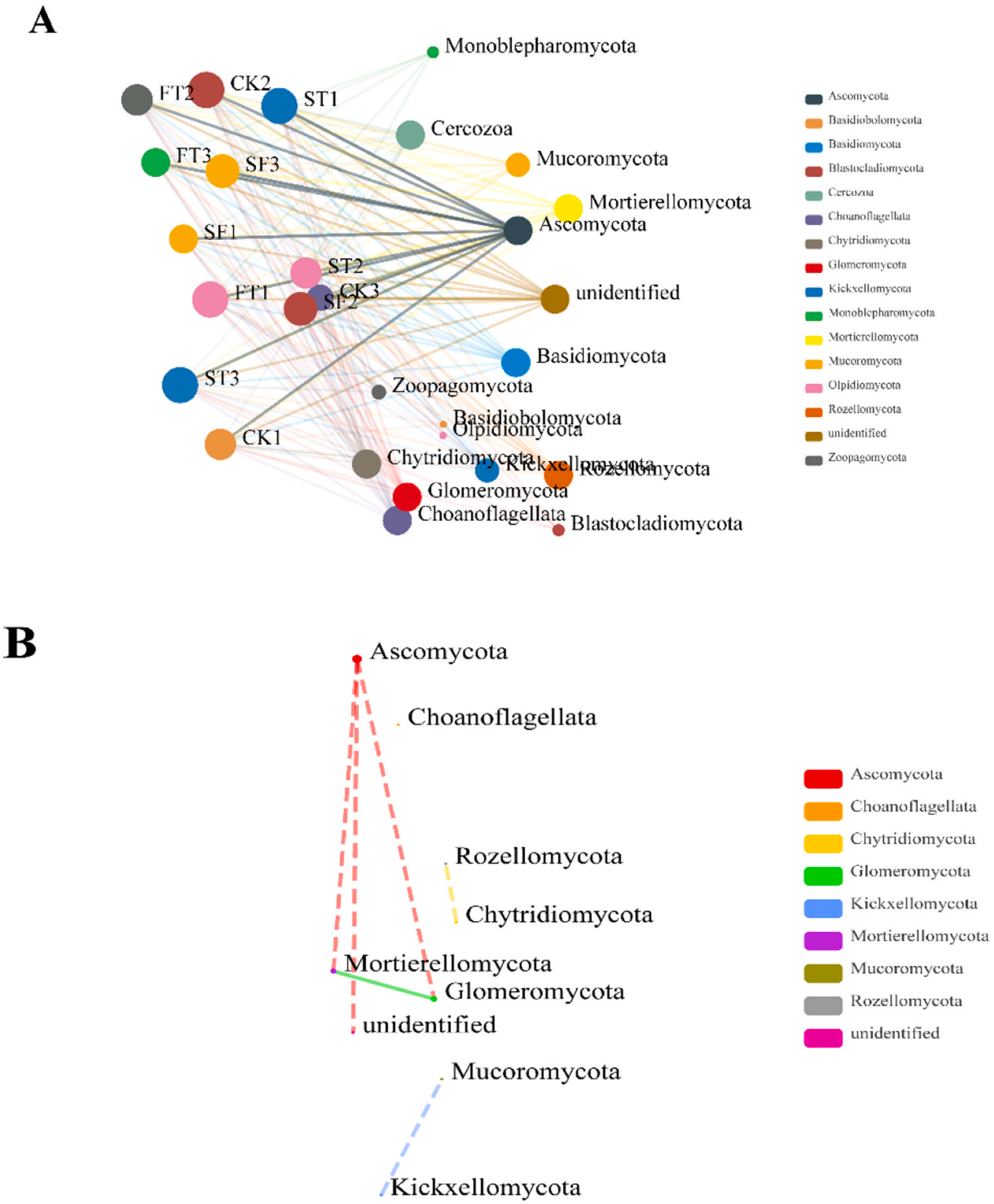

Fig. 6. Network and predictive analysis plot of rhizosphere soil bacteria. (A) Covariance network plot analysis can be used to show the distribution of samples to species, with a connecting line between a species and a sample if that species occurs in a sample, with the higher the species percentage abundance, the thicker the line. (B) Species correlation network diagrams obtain the interrelationships between species within a sample, with solid lines representing positive correlations, dashed lines representing negative correlations, and lines colored to indicate which species is highly influential. CK: soil only with soyabean; SF: soil with soyabean and *F. mosseae*; ST: soil with soyabean and tetracycline; FT: soil with soyabean and *F. mosseae* and tetracycline.

tetracycline degradation, consistent with the earlier findings of Cao et al. (2015). Moreover, inoculation with *F. mosseae* alone significantly improved plant height, root length, dry weight, root volume and the number of root nodules (Fig. 1; $p < 0.05$), in agreement with the results reported by Shao et al. (2021), further confirming that arbuscular mycorrhizal fungi (AMF) having a vital role in regulating host plant growth (Pang et al., 2023; Sun et al., 2025).

Under tetracycline stress, root dry weight, root length and root volume were significantly higher in the *F. mosseae* (FT) treatment added than in the non-inoculated (ST) treatment (Figure_1A, E, F; $P < 0.05$), which did not show significant differences in plant dry weight, height and root dry weight, but increased in mean and content (Figure_1A, D, G), which is in agreement with the results of many publication reports (Meng et al., 2022; Yang et al., 2023). *Rhizobium* is an important symbiotic partner to legumes; plays an important role in reducing soil acidification by increasing soil nitrogen by symbiotic nitrogen fixation with plants (Yong et al., 2014; Zhang et al., 2024). In this study, the soybean nodules dry weight of *F. mosseae* treatment was significantly

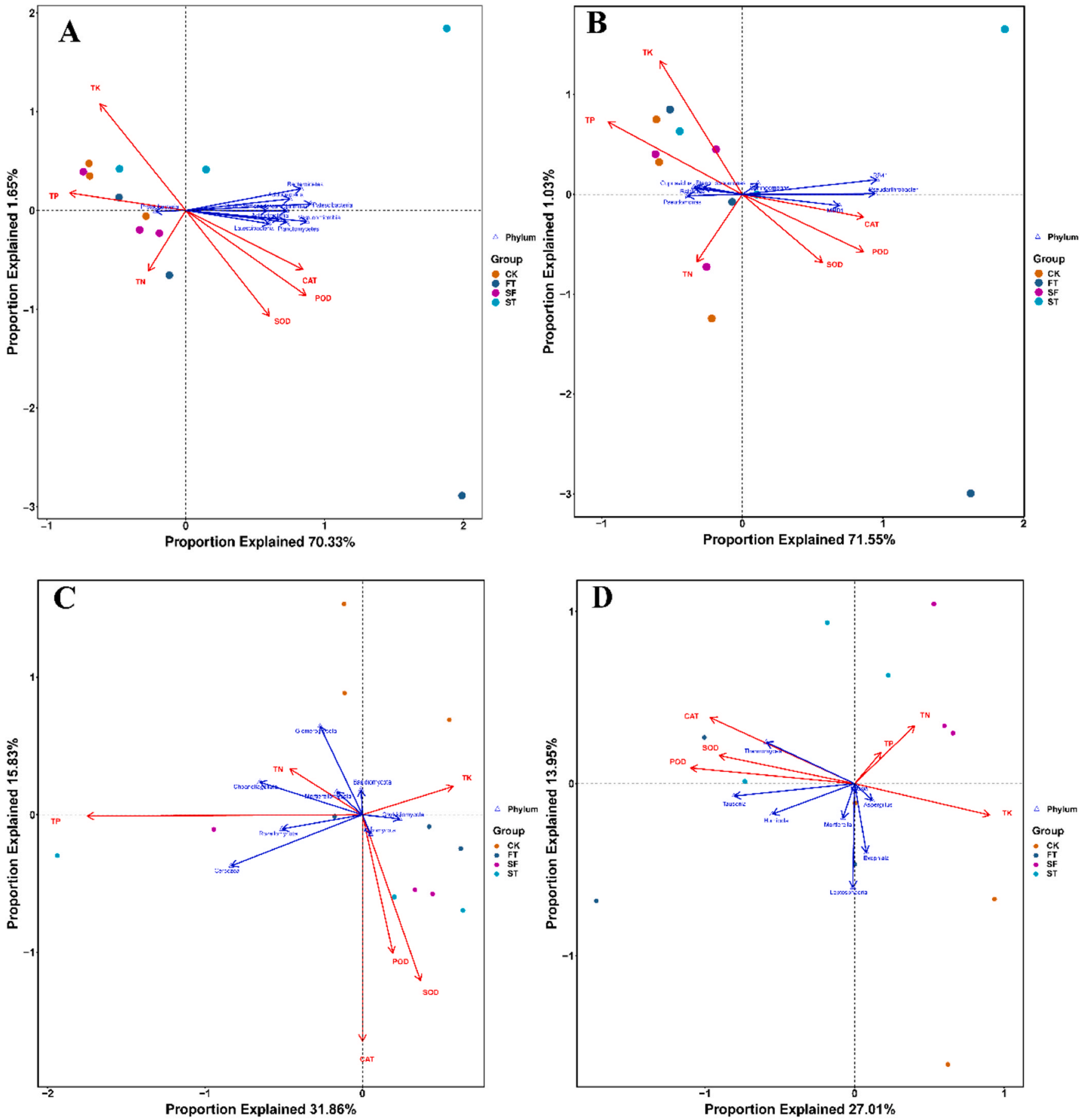

Fig. 7. The RDA score plots for soil microbes and environmental factors in the rhizosphere. (A-B) are at the soil bacterial phylum level and genus level, respectively, and (C-D) are at the fungal phylum level and genus level, respectively. Arrows indicate the microbial community and environmental media, length indicates the percentage of that score, and the angle between two arrows indicates the correlation between the two components, with an acute angle being a positive correlation and an obtuse angle being a negative correlation. Scatter is for each test sample. CK: soil only with soyabean; SF: soil with soyabean and *F. mosseae*; ST: soil with soyabean and tetracycline; FT: soil with soyabean and *F. mosseae* and tetracycline.

higher than that of the untreated soybeans (Figure_1G). Specifically, plant growth status is an important criterion for evaluating the condition of the soil environment (Pang et al., 2023; Yong et al., 2014), and *F. mosseae* under tetracycline contamination apparently improved the plant growth indexes and plant root system and in some degree of reaction AMF can improve the soil environment, increase soil fertility and possibly, enhancing the uptake capacity of the plant itself.

Normally under stress conditions, organisms produce reactive oxygen species (ROS) free radicals, which accumulate to a certain level and cause damage to cellular structures (Roubalová et al., 2018). Antioxidant enzymes can eliminate harmful substances produced during the metabolism of living organisms; for example, they play an important role in the removal of ROS, H_2O_2 and benzene toxic substances (Tian et al., 2024). Therefore, the degree of plant stress can be determined by assessing the activities of antioxidant enzymes such as SOD, POD and CAT (Zhu et al., 2020). In this study, the FT treatment group had high levels ($p < 0.05$) of the three antioxidant enzyme activities compared to the other treatment groups; due to the fact that the stress of tetracycline caused the microbiome to develop a stress response that activated the antioxidant defence system to produce more antioxidant enzymes. Whereas, *F. mosseae*-plant-microbe symbiosis provided a synergistic defense and also enhanced antioxidant enzyme activity, which was used to defend against tetracycline contamination (Figure_2A-C). Our results were in agreement with Zhang et al. (2024); they reported earlier that when an organism is invaded by tetracycline, it will rapidly produce a large amount of SOD to remove excess -O^{2-} through a positive feedback mechanism, thereby delivering a protective role. Our study demonstrated similar results, and it was certain that *F. mosseae* assisted the microbial community to stimulate the antioxidant activity system and strengthening the defense mechanisms to some extent (Song et al., 2012).

The soil microenvironment influences the biological functionality of microbes and plants. In this study, we found soil TN, TP, and TK contents were significantly higher ($p < 0.05$) in the treatment group than in the pre-planting soil but did not show big significant differences between treatment groups (Figure_2D-F). The TN content of the *F. mosseae* treatment was significantly higher than that of the uninoculated group, and the dry weight of the roots was also significantly higher, indicating that AMF increased the nitrogen-fixing capacity of the microorganisms. Although no statistically significant differences were observed ($p > 0.05$), the variation in total nitrogen (TN) content suggests that both plants and soil microorganisms may consume soil nitrogen sources as energy during tetracycline degradation, potentially explaining the observed differences (Zhang et al., 2021). The TP and TK contents of the FT treatment were lower than those of the ST treatment, probably because the AMF colonization increased the plant's ability to absorb soil nutrients used to resist external stresses.

In earlier studies, it was unclear whether AMF degrades antibiotics by altering the structure of the microbial community (Guo et al., 2021). In this study, we demonstrated that AMF altered the structure of the associated soil microbial community; thereby alleviating the environmental stress upon soybeans caused by antibiotics. The results showed that AMF colonization increased bacterial abundance but not the diversity (Chao1, Shannon_2, and PCoA; Figure_3A-C) and altered community composition in the treatment groups. Tetracycline contamination altered the soil bacterial community and abundance (Figure_3E), which was similar to the results reported by Zhang et al. (2024) and is directly related to Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and Bacteroidetes, among others. For the bacterial phyla with higher abundance, we made correlation network prediction plots showing that Proteobacteria are in competition with other bacterial phyla, and the vast majority of other bacterial phyla are in synergistic cooperation (Figure_4). Further examination of FT treatment and ST treatment at the genus level revealed that under tetracycline contamination, AMF significantly favored *Geobacter*, *Dechloromonas*, *Aeromonas* and *Polyangium*, which were is differential bacteria, although the levels were not the most dominant (Table_S4a; Figure_S2a). *Geobacter* has been reported to anaerobically oxidize aromatic hydrocarbons and play an important role in the defense of soils contaminated with aromatic hydrocarbons (Lovley et al., 2011), whereas tetracyclines are organic compounds with benzene rings as their carbon skeleton. *Dechloromonas* is able to degrade man-made organic pollutants by regulating the involvement of key enzymes (xenobiotics) (Zhang et al., 2021). *Polyangium* is associated with the lysis of dead microbes and the degradation of cellulose (Wang et al., 2021). These bacteria play an important role in the degradation of antibiotics.

We also examined fungal abundance, diversity, and structural changes in the rhizospheric soils. Colonization of *F. mosseae* did not significantly increase the diversity of soil fungi (Chao1 & Shannon_2; Figure_5A-B). However, it did increase their abundance. The NMDS results revealed a clear separation between fungal communities based on the colonization of *F. mosseae* with or without tetracycline stress, as shown in Figure_5D (Liu et al., 2023). The rhizosphere soil fungi with high relative abundance in this study were Ascomycota, Mortierellomycota, Basidiomycota, and Chytridiomycota. This finding is consistent with the results of previous studies conducted by Gui et al. (2020). After *F. mosseae* colonization, the FT treatment significantly reduced the relative abundance of Ascomycota compared to the ST treatment. However, it increased the relative abundance of Basidiomycota and Chytridiomycota, which are saprophytic fungi involved in degrading lignin-related materials in the soil (Wen et al., 2009). This process is crucial for the breakdown of tetracycline. Mortierellomycota is a phylum of fungi that has a positive effect on soil phosphorus bioavailability and promotes the production of phytohormones, such as cytokinins, gibberellins and indoleacetic acid, serving as biostimulants to promote plant growth (Vedenicheva and Kosakivska, 2023; Zhu et al., 2022). According to Figure_6B, Mortierellomycota and Glomeromycota have a synergistic effect. It is noteworthy that the addition of *F. mosseae*, a genus of Glomeromycota, may contribute to their competitive effect against other saprophytic fungi. *Cladorrhinum* and *Humicola* are fungi that show promise in both biological control of plant pathogens and promotion of plant growth (Ibrahim et al., 2021). The relative abundance of these two fungi was significantly higher in the FT treatment compared to the ST treatment. Under tetracycline stress, colonization of *F. mosseae* increased the diversity of different species of the fungal genera (Figure_S2b) and showed significant differences (LDA > 2, $p < 0.05$). It is noteworthy that the two groups had significantly different fungal diversity, with *Trichoderma* and *Glomeraceae* (likely due to inoculation with *F. mosseae*) being present. These fungi are important biocontrol agents in Northeastern agricultural fields and concomitantly, having essential regulatory role on soil elemental phosphorus content (He et al., 2014).

Previous studies have shown that under long-term fertilization with inorganic nutrients, the relative abundance of Proteobacteria significantly increases, while the abundance of Chloroflexi decreases; other bacterial phyla show relatively minor changes, and the relative abundance of fungi remains largely unaffected (Yang et al., 2024), which is consistent with our findings. In a study using the *Camellia oleifera* agroforestry system, soil CAT activity was significantly negatively correlated with Proteobacteria, but positively correlated with other bacterial groups such as Chloroflexi and Acidobacteriota (Bajiu et al., 2024); similar pattern also observed in our research. Moreover, Proteobacteria represent a major bacterial phylum that includes a variety of pathogenic microorganisms (Umeda et al., 2023); thus, the negative correlation between certain pathogenic members of Proteobacteria and antioxidant enzymes (such as SOD, POD, and CAT) is not surprising (Fig. 7A–B). Conversely, the other bacterial taxa were generally positively correlated with antioxidant enzyme activities and negatively correlated with Proteobacteria, further supporting the outcomes predicted by our network-based model. For fungal communities, the influence of environmental factors appeared to be less pronounced, although Ascomycota and Basidiomycota exhibited responses associated with changes in soil conditions (Fig. 7C–D). It has been reported that in subtropical evergreen broadleaf forests, soil CAT activity is correlated with the abundance of Basidiomycota, while no statistically significant relationship has been observed with Ascomycota (Fu et al., 2021). This discrepancy may be attributed to variations in environmental factors such as temperature and humidity. Nevertheless, it is undeniable that both groups are likely to be the dominant fungal contributors to soil ecosystems under different environmental conditions, a conclusion that aligns with many previous studies (Liu et al., 2023). Taken together, these findings suggested that bacterial communities may play a more critical role than fungi in soil responses to external stressors.

5. Conclusions

It was observed that the inoculation with *F. mosseae* significantly reduced the concentration of tetracycline in the soybean rhizosphere soil, showing a continuous downward trend with a degradation efficiency of 61.18 %. Tetracycline stress altered the phenotypic traits of soybean plants, which may be closely related to changes in the soil nutrient environment. Interestingly, successful colonization by *F. mosseae* notably enhanced root biomass, plant height, and leaf development, effectively reinforcing the reticular structure of the root system. The concomitant higher activity of soil antioxidant enzymes also contributed to alleviating tetracycline toxicity and promoting its degradation. Although the overall physicochemical properties of the soil did not exhibit significant changes among the treatment groups, TN content increased remarkably, which may serve as a plausible nutritional driver for the synergistic degradation of tetracycline by plant–microbe interactions. In terms of microbial communities, the abundance of nitrogen-fixing microorganisms increased. Among soil bacteria, Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and Bacteroidetes were dominant, with high relative abundances and critical ecological functions. The colonization of *F. mosseae* altered the composition of beneficial bacterial genera, with *Geobacter*, *Dechloromonas*, *Aeromonas* and *Polyangium* showing significant differences. Conversely, the influence of *F. mosseae* on fungal community structure was relatively limited. The dominant fungal phyla in the rhizosphere soil were Ascomycota, Mortierellomycota, Basidiomycota, and Chytridiomycota, while *Trichoderma* and Glomeraceae were significantly different fungal genera and are also considered important biocontrol agents in soil. Collectively, these findings suggested that bacterial communities may play a more crucial role in tetracycline degradation. This study provided scientific evidence to support the use of *F. mosseae* to remediate antibiotic-contaminated black soils in the cold regions of Northeast China.

CRediT authorship contribution statement

Baiyan Cai: Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Lye Harry Hin Chong:** Writing – review & editing, Resources. **Chao Yang:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Khim Phin Chong:** Writing – review & editing, Conceptualization. **Linghui Li:** Visualization, Investigation. **Jean Wan Hong Yong:** Writing – review & editing, Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118973.

Data availability

Data will be made available on request.

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