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Soil Moisture Modulates Rhizosphere Microbiota and Suppresses Root Rot in Organic Panax ginseng Cultivated Under Pine Forests

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Abstract

Aims

The integration of agroforestry and understory cultivation offers a sustainable approach to producing high-value medicinal plants with ecological conservation. However, optimal irrigation management for organic *Panax ginseng* grown under pine forests (OPPF) remains poorly studied, particularly regarding its influence on root rot disease and rhizosphere microbial communities.

Methods

This study evaluated four soil moisture treatments—PTh1 (55–60% field capacity, FC), PTh2 (70–75% FC), PTh3 (80–85% FC), and PTh4 (95–100% FC)—on the growth, quality, and root rot incidence of OPPF. We analyzed agronomic traits, secondary metabolites, soil properties, enzymatic activities, and rhizosphere microbial composition via high-throughput sequencing and qPCR, and validated microbial isolates functionally through pot experiments.

Results

Moderate soil moisture (PTh2) significantly improved plant biomass, seedling survival, and the accumulation of flavonoids, polysaccharides, and ginsenosides. In contrast, high soil moisture (PTh4) increased the incidence of root rot and the abundance of pathogens such as *Fusarium solani* and *Ilyonectria mors-panacis*. Soil properties and enzyme activities were markedly influenced by moisture levels, with PTh2 maintaining a higher pH and beneficial nutrient profile. Rhizosphere microbiome analysis revealed that PTh2 enriched potential biocontrol agents, including *Trichoderma*, *Penicillium*, *Bacillus*, and *Streptomyces*, while reducing pathogenic taxa. Six antagonistic strains were isolated, with *Penicillium ortum* (F1) showing the strongest suppression of root rot and promotion of plant growth in pot trials.

Conclusions

Maintaining soil moisture at 70–75% FC optimizes the growth and health of OPPF by fostering beneficial microbial communities and inhibiting soil-borne pathogens. These findings provide science-based irrigation strategies to support the sustainable cultivation of organic ginseng in forest-based agroecosystems.

Introduction

Panax ginseng (*Panax ginseng* C.A. Meyer) is a valuable medicinal herb widely recognized for its health-promoting properties, such as anti-diabetic and anti-fatigue effects (Kim et al. 2023). The primary

cultivation methods include conventional farmland cultivation (cultivated ginseng) and understory cultivation (wild-simulated ginseng) (Bao et al. 2020). However, ginseng production faces significant constraints due to continuous cropping obstacles and the overuse of chemical pesticides, which contribute to a decline in crop quality. These unsustainable practices represent a common challenge in the cultivation of many medicinal plants.

Extensive studies on conventional cultivation have demonstrated that continuous cropping leads to soil microbial dysbiosis and aggravates soil-borne diseases, with root rot caused by *Fusarium* and *Ilyonectria* spp. being a key factor limiting sustainable production (Xia et al. 2016). In response, researchers both domestically and internationally have attempted various measures such as soil disinfection, crop rotation, and soil amendments, yet these approaches often show limited efficacy or introduce new environmental risks. In recent years, the ecologically friendly understory cultivation model has gained attention. Research indicates that this system can leverage forest biodiversity to suppress foliar diseases (e.g., *Alternaria panax*) and improve medicinal quality (He et al. 2022). However, existing studies have primarily focused on the effects of the forest environment on growth and foliar diseases, with systematic research on soil moisture management—a critical agronomic practice in this system—remaining notably scarce. Soil moisture not only directly affects plant physiology but also plays a key role in shaping rhizosphere microbial community structure and function, thereby influencing the incidence of soil-borne diseases (Patel et al. 2021;Berg et al. 2016). Although substantial knowledge exists on water management for grain crops and some commercial tree species, targeted studies on precise soil moisture regulation for understory medicinal plants, particularly ginseng, are virtually absent. Moreover, in organic *P. ginseng* cultivation under pine forests (OPPF) systems where rain shelters are used to control leaf diseases, the alteration of natural rainfall patterns adds complexity to soil moisture management, making the development of science-based irrigation strategies both necessary and underexplored.

To address these gaps, this study focuses on the OPPF system and is the first to systematically investigate the integrated effects of different soil moisture levels (55–60%, 70–75%, 80–85%, and 95–100% of field capacity) on ginseng growth, quality, root rot incidence, and rhizosphere microecology. We hypothesize that soil moisture influences root rot occurrence by altering the rhizosphere microbial community structure and that potentially exploitable biocontrol microbial resources may exist within this microbiome. Therefore, the objectives of this study are: (1) to identify the optimal soil moisture range for enhancing OPPF biomass and quality while reducing root rot incidence, and (2) to elucidate how soil moisture drives changes in rhizosphere microbial diversity—particularly in pathogenic and antagonistic populations—and its mechanistic association with root rot disease. The findings will provide a direct theoretical basis and technical support for establishing ecologically based water management protocols in OPPF systems, offering significant academic value and practical potential.

Materials and methods

Experimental design and treatments

The experiment was conducted in June 2023 at an organic *Panax ginseng* under pine forest (OPPF) test base located at the Lanheyu Forestry Center (41°09'56.98" N, 124°26'20.70" E) in Liaoning, China. The site experiences a mid-temperate monsoon humid climate, with a mean annual temperature of 7.5°C and annual precipitation of 1017.5 mm. Initial soil properties included pH 5.12, alkaline hydrolysis nitrogen (AHN) 441.83 mg/kg, electrical conductivity (EC) 281.63 μS/cm, and a field capacity (FC) of 48%. The starting soil moisture level was 68.52% FC.

A total of 240 pots (upper diameter: 184 mm; height: 164 mm; bottom diameter: 132 mm) were used, each filled with 1090 g of soil and planted with three three-year-old *P. ginseng* seedlings. Pots were assigned to four soil moisture treatments: PTh1 (55–60% FC), PTh2 (70–75% FC), PTh3 (80–85% FC), and PTh4 (95–100% FC). Soil moisture was maintained by weekly pot weighing and water adjustment. All pots were placed on artificial ridges under a pine canopy to simulate natural OPPF conditions.

Measurement of agronomic traits and chlorophyll content

After three months of moisture treatment, plants were harvested and transported to the laboratory. Soil was carefully removed from roots, and samples were air-dried before measuring fresh weight. Leaf length, leaf width, and fibrous root length were recorded, and leaf area was calculated based on leaf dimensions (Wang et al. 2016). Chlorophyll content was measured using a SPAD-502 handheld meter (Konica Minolta, Japan). Per treatment, 96 plants were randomly selected and grouped into six biological replicates for average fresh weight calculation.

Analysis of saponin, flavonoid, and polysaccharide quality, and total polysaccharides

Saponin content (including ginsenosides Rd, Re, Rc, Rg1, Rf, Rb1, Rb3, Rb2, Rg2, Rh1, Rh2, and Rg3) in ginseng roots was quantified using a modified UPLC method adapted from Li et al. (Li et al. 2013). Separation was performed on a Nexera X2 system (Shimadzu, Japan) equipped with a DAD detector and a Poroshell 120 EC-C18 column (Agilent; 4 μm, 100 × 2.1 mm). The mobile phase consisted of acetonitrile (solvent A) and 0.1% aqueous solution (solvent B) under gradient conditions (Supplementary Table S1). The injection volume was 10 μL, flow rate 1.0 mL/min, column temperature 30°C, and detection wavelength 203 nm. Identification and quantification were performed using ginsenoside standards (Supplementary Fig.S1). Flavonoid, polysaccharide, and total polysaccharide contents were determined using commercial assay kits (Michy Bio, China).

Root rot assessment and pathogen isolation

At harvest, 144 plants per treatment (6 replicates) were evaluated for root rot severity using the rating scale established by Li et al. (Li et al. 2020). Disease incidence and index were calculated as described by Yang et al. (Yang et al. 2022). Fungal pathogens were isolated from symptomatic root tissues

following Li et al. (Li et al. 2020). Genomic DNA was extracted using a 2×T5 Direct PCR kit (TSINGKE, China). The ITS region was amplified with primers ITS1/ITS4 (Hawa et al. 2017) and sequenced. Phylogenetic analysis was conducted in MEGA 7.0. Pathogenicity of isolates was confirmed using the method of Luo et al. (Luo et al. 2020).

Soil physicochemical properties and enzyme activities

Soil samples were collected and analyzed for physicochemical parameters according to Guo et al. (Guo et al. 2022). Activities of soil polyphenol oxidase (S-PPO), dehydrogenase (S-DHA), sucrase (S-SC), and cellulase (S-CL) were measured using specific assay kits (Michy Bio, China).

DNA extraction, sequencing, and bioinformatic

Total genomic DNA was extracted from soil samples, and amplification and library preparation were conducted following Edgar et al. (Edgar, 2013). High-throughput sequencing of bacterial 16S rRNA and fungal ITS regions was performed on the Illumina platform. Bioinformatic processing included quality filtering, OTU clustering, and taxonomic assignment using standard pipelines.

Quantification of pathogenic fungi and antagonistic via qPCR

DNA from soil samples was reverse-transcribed using a PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Japan). Quantitative real-time PCR (qPCR) was performed with strain-specific primers (Table S2)(Géry et al. 2021;Ye et al. 2019Gao et al. 2020Li et al. 2012Saravanakumar et al. 2017Reiter et al. 2011). Each 30 µL reaction contained 10 µL qPCR Mix, 0.5 µL each of forward and reverse primers (10 µM), 2 µL template DNA, and nuclease-free water. Amplification conditions followed Géry et al.(2021).

Isolation of culturable rhizosphere and functions validation

Rhizosphere microorganisms were isolated and purified as described by Guo et al. (2022). Functional validation was performed using pot assays under sterile soil conditions based on Fang et al. (2024). Three-year-old ginseng seedlings were planted in pots inoculated with 50 mL of a suspension containing pathogenic fungi (10^6 CFU/mL) and candidate antagonistic bacteria (OD_{600} = 0.5). Control groups received pathogen suspension only or sterile water. Each treatment included three replicates of five pots. Plants were grown in a greenhouse at 25 ± 2°C for 60 days, after which fresh weight and root rot incidence were recorded.

Statistical analysis

All data were analyzed using PASW Statistics 18 (SPSS Inc. USA). One-way ANOVA followed by Tukey's HSD test ($p < 0.05$) was used to determine significant differences among treatments. Data normality and

homogeneity of variance were verified beforehand. Correlation analyses were conducted using Spearman's rank correlation method (Fang et al. 2024).

Results

Optimal soil moisture level (PTh2) enhances growth and quality in OPPF-cultivated *P. ginseng*

Significant differences in agronomic traits and seedling survival rates were observed among *P. ginseng* plants subjected to varying soil moisture levels after three months of treatment (Fig. 1A–D). Plants under the PTh2 regime (70–75% FC) exhibited markedly greater fresh weight, fibrous root length, and chlorophyll content compared to those in PTh1, PTh3, and PTh4 (Fig. 1B). These results were consistent with field trial data from 2024 (Supplementary Table S3).

Analysis of secondary metabolites revealed that PTh2 also led to significantly higher flavonoid and total carbohydrate contents in fibrous roots relative to other treatments (Fig. 1C). Total polysaccharide content was notably elevated in PTh2 compared to the remaining moisture regimes. Although total saponin levels in roots increased with rising soil moisture (Fig. 1C), individual ginsenosides responded differently across tissue types and treatments. In fibrous roots, significant variations were detected in the contents of Rd, Rb1, Rb3, Rh2, and Rg3. In taproots, Rd, Rc, Rg1, Rf, Rb1, Rb3, Rb2, Rg2, Rh1, Rh2, and Rg3 levels differed significantly among treatments, with Rg2, Rb1, and Rh1 increasing with soil moisture. Notably, PTh3 resulted in higher Rc content in roots and elevated Rd in fibrous roots compared to other treatments (Supplementary Fig.S2). Overall, soil moisture strongly influenced ginseng growth and quality, particularly flavonoid and saponin accumulation (Supplementary Fig.S3).

Seedling survival was significantly higher in PTh2 than in PTh1 and PTh4 (Fig. 1D). Both root rot incidence and disease index were significantly lower in PTh1 and PTh2 than in PTh4, and a clear positive correlation was observed between soil moisture level and root rot severity (Fig. 1D). These findings were corroborated by 2024 field data (Supplementary Fig.S4), confirming the critical role of soil moisture in disease development under OPPF conditions.

Soil properties and enzyme activities are influenced by soil moisture regimes

Soil moisture levels significantly influenced various soil physicochemical properties and enzymatic activities (Fig. 2). Soil pH was significantly higher under the PTh2 treatment compared to PTh1, PTh3, and PTh4 (Fig. 2A). In contrast, soil electrical conductivity was markedly elevated in PTh3 relative to the other moisture regimes (Fig. 2B).

Soil alkaline hydrolysis nitrogen (AHN), dehydrogenase (S-DHA), sucrase (S-SC), and cellulase (S-CL) activities were highest under the PTh1 treatment, showing significant differences compared to PTh2, PTh3, and PTh4 (Fig. 2C–G). Redundancy analysis (RDA) further confirmed that soil properties and

enzyme activities varied systematically with soil moisture levels (Fig. 2H). Specifically, soil conductivity exhibited a negative correlation with S-DHA and S-SC activities, but was positively associated with moisture content in PTh3 and PTh4.

Rhizosphere microbial community structure shifts with soil moisture

Principal coordinate analysis (PCoA) and ANOSIM revealed significant differences in bacterial ($R = 0.5467$, $p = 0.001$) and fungal ($R = 0.4779$, $p = 0.001$) community structures among the four soil moisture treatments (Supplementary Fig.S5). Although alpha diversity indices (Chao1, Shannon, and observed species) did not differ significantly across treatments (Supplementary Fig.S4), bacterial diversity was generally higher in PTh2 and PTh3, while fungal diversity was notably elevated under the PTh2 regime.

Soil moisture alters the relative abundance of key microbial taxa

At the phylum level, *Ascomycota* dominated the fungal community under all soil moisture conditions, accompanied by *Basidiomycota*, *Mortierellomycota*, *Olpidiomycota*, *Mucoromycota*, *Glomeromycota*, *Rozellomycota*, *Chytridiomycota*, and *Zoopagomycota* (Fig. 3A). The relative abundance of *Ascomycota* was higher in PTh1 and PTh4 compared to PTh2, whereas *Basidiomycota* and *Mortierellomycota* were more abundant in PTh2 and PTh3.

Among bacteria, nine dominant phyla were identified: *Proteobacteria*, *Acidobacteria*, *Verrucomicrobiota*, *Chloroflexi*, *Actinobacteria*, *Myxococcota*, *Firmicutes*, and *Bacteroidota* (Fig. 3B). *Proteobacteria* and *Acidobacteria* were the most abundant (each > 1% relative abundance), with *Acidobacteria* increasing and *Actinobacteria* decreasing as soil moisture rose.

At the genus level, fungi such as *Humiicola*, *Trichoderma*, *Saitozyma*, *Pseudogymnoascus*, and *Mortierella* were more abundant under lower soil moisture but declined with increasing moisture (Fig. 3C). In contrast, known pathogenic genera including *Ilyonectria*, *Cylindrocarpon*, *Fusarium*, *Neonectria*, *Stagonosporopsis*, and *Chaetomium* were most prevalent in PTh3. Among bacteria, *Pseudomonas* increased with soil moisture, while *Arenimonas*, *Collimonas*, *Burkholderia-Caballeronia-Paraburkholderia* (B-C-P), and *Bacillus* decreased in abundance under higher moisture conditions (Fig. 3D).

Correlation analysis and abundance of potential beneficial microbiomes

Analysis of relationships between root rot incidence and microbial communities focused on taxa with relative abundances exceeding 0.1% (Fig. 4A). Within fungal communities, disease index (DI), incidence of rot disease (IRD), and moisture gradient (MG) showed positive correlations with *Fusarium*, but negative correlations with *Trichoderma*, *Chaetosphaeria*, *Chloridium*, *Mortierella*, and *Penicillium*. Among bacterial communities, DI, IRD, and MG correlated positively with *Aquicella*, but negatively with *Arenimonas* and the *Burkholderia-Caballeronia-Paraburkholderia* (B-C-P) complex.

Further analysis revealed significant correlations between dominant fungal and bacterial genera and key soil properties (Fig. 4B, C). Soil electrical conductivity (EC) correlated positively with *Fusarium* and *Ilyonectria*, but negatively with *Talaromyces*. In contrast, alkaline hydrolysis nitrogen (AHN), dehydrogenase (S-DHA), and cellulase (S-CL) activities were positively associated with *Talaromyces* and negatively associated with *Fusarium* and *Ilyonectria*. Soil pH and polyphenol oxidase (S-PPO) activity showed negative correlations with *Fusarium*, while soil moisture correlated positively with *Crossiella* and negatively with *Novosphingobium*, B-C-P complex, *Bradyrhizobium*, and *Arenimonas*. AHN, S-DHA, and S-CL exhibited opposite correlation patterns with these bacterial genera.

Quantitative PCR (qPCR) analysis of rhizosphere soil indicated that the copy numbers of the root rot pathogens *Fusarium solani* and *Ilyonectria mors-panacis* were significantly elevated under the PTh4 treatment. Conversely, antagonistic microbial populations were more abundant under both PTh1 and PTh4 conditions.

Functional validation of culturable microbial strains

Pathogens R3 and R6, isolated from symptomatic roots, were identified as *Ilyonectria mors-panacis* and *Fusarium solani*, respectively. Pathogenicity tests confirmed that both strains induced root rot symptoms in OPPF plants, whereas control plants remained healthy.

Using a dual-culture assay, three bacterial strains (L11, A1, G1) and three fungal strains (F1, F2, F3) were identified that inhibited the growth of R6 and R3 by more than 30%. Based on BLAST analysis, the bacterial strains were identified as *Bacillus mycoides* (G1), *Bacillus velezensis* (L11), and *Streptomyces drozdowiczii* (A1), and the fungal strains as *Penicillium ortum* (F1), *Talaromyces amestolkiae* (F2), and *Trichoderma koningii* (F3).

Pot experiments demonstrated that all six strains promoted ginseng growth and suppressed root rot. Among them, strain F1 (*Penicillium ortum*) exhibited the strongest disease suppression and most significantly enhanced plant biomass.

Discussion

Despite the potential of agroforestry systems to alleviate land-use pressures, research on the cultivation of Chinese medicinal plants—particularly organic ginseng—under forest canopy remains limited. Few studies have addressed soil moisture management specifically for organic Panax ginseng grown in understory environments. Our findings indicate that both drought (PTh1) and waterlogging (PTh4) impaired the healthy growth of understory ginseng, whereas moderate soil moisture (PTh2; 70–75% FC) promoted plant growth and increased the accumulation of polysaccharides, flavonoids, and saponins. PTh2 also resulted in superior biomass production and lower root rot incidence compared to the high-moisture PTh4 treatment. Furthermore, this study provides novel insights into how soil moisture influences rhizosphere microbial community structure and identifies several antagonistic

microorganisms, offering practical strategies for irrigation management in forest-based ginseng cultivation systems.

Soil moisture modulates growth and metabolite accumulation in *P. ginseng*

Plant phenotypic traits are strongly influenced by environmental cues, which in turn affect biomass and secondary metabolism (Loretta, 2014). Soil moisture plays a critical role in root development and overall plant health, particularly in medicinal species such as *Glycyrrhiza uralensis*, where drought stress significantly curtails growth (Yang et al. 2022;Sun et al. 2017). Conversely, excess moisture can also inhibit growth after an initial increase (Li et al. 2015). In our study, biomass peaked under moderate soil moisture (PTh2) and declined under both water-deficit and waterlogged conditions, consistent with reports that extreme moisture levels negatively affect ginseng biomass accumulation(Liu et al. 2016).

Notably, flavonoid and total carbohydrate contents were highest under PTh2, whereas polysaccharide and total saponin levels responded differently across treatments. Total flavonoids—commonly used as a quality marker—decreased significantly under severe water loss (Yang et al. 2020;Zhang, 2012), while polysaccharides increased under moderate stress. Total saponin content was positively correlated with soil moisture, rising significantly under high-water conditions (Puente-Garza et al. 2017). Individual ginsenosides (e.g., Rg1, Rf, Re, Rg3, Rb2, Rd, and Rh2) were lowest under optimal moisture and elevated under stress, underscoring how environmental conditions shape the phytochemical profile of medicinal plants (Zheng et al. 2021Lee and Mudge, 2014). These patterns align with observations in *P. notoginseng*, where saponin content varied under water stress(Zheng et al. 2021), highlighting species-specific metabolic plasticity.

Suboptimal soil moisture alters rhizosphere microbiome and promotes root rot

Soil moisture strongly influences the rhizosphere micro-environment and the prevalence of soil-borne diseases. In this study, higher soil moisture correlated with increased incidence and severity of ginseng root rot, consistent with earlier reports (Yang et al. 2022;Guo et al. 2022). Changes in moisture regimes also affected key soil properties such as pH and electrical conductivity, which in turn influenced pathogen behavior (Stover, 2011). We found negative correlations between root rot incidence and soil pH and alkaline hydrolysis nitrogen, whereas electrical conductivity showed a positive correlation with disease.

Sustained high moisture (PTh4; 95–100% FC) favored anaerobic microorganisms and known pathogens such as *Fusarium* and *Ilyonectria*, in line with studies showing that waterlogged conditions reduce microbial diversity and disrupt community balance (Harman et al. 2021). Such shifts likely suppressed the activity of beneficial enzymes and microbes, exacerbating disease incidence (Guo et al. 2022). Maintaining soil moisture at 60–75% FC helped minimize root rot, corroborating field observations (Yang

et al. 2022;Guo et al. 2022). Future studies should employ metagenomic and transcriptomic tools to unravel plant–microbe interactions under dynamic moisture conditions (Shi et al. 2019), offering deeper mechanistic insights into moisture-mediated disease regulation.

Moisture-driven microbial community shifts influence disease resistance in OPPF

High-throughput sequencing revealed that soil moisture significantly shaped the composition of bacterial and fungal communities in the ginseng rhizosphere. Dominant bacterial phyla included Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi, with genera such as *Streptomyces* and *Bacillus*—known bio-control agents—showing higher abundance under moderate moisture (Kobayashi et al. 2015;Moretti et al. 2021Tran, 2021). Pathogenic genera like *Aquicella* were positively correlated with root rot incidence.

Fungal communities were dominated by Ascomycota, Basidiomycota, and Mortierellomycota (Liu et al. 2020). Known pathogens including Fusarium and Ilyonectria were more abundant in high-moisture treatments, whereas antagonistic fungi such as *Trichoderma* and *Penicillium* were enriched under drier conditions (Zhang, 2012;Ozimek and Hanaka, 2020Tian et al. 2021). These patterns support the concept that plants under stress can recruit beneficial microbes for protection, as observed in *P. notoginseng* with *Arthrobacter* (Fang et al. 2024). Interestingly, qPCR results indicated higher abundance of antagonistic strains under both low and high moisture, suggesting complex, context-dependent microbial interactions (Lou et al. 2018Tettamanti Boshier et al. 2020).

Conclusions and implications

This study demonstrates that maintaining soil moisture at 70–75% FC optimizes the growth, quality, and health of organic *P. ginseng* under pine forests. We identified key microbial taxa and several culturable strains (e.g., *Bacillus velezensis*, *Trichoderma koningii*, and *Penicillium ortum*) with high biocontrol potential against root rot pathogens. These findings underscore the importance of irrigation management in balancing rhizosphere microbiota and suppressing disease in agroforestry systems. Future research would focus on the dynamic monitoring of microbial succession under fluctuating soil moisture, mechanistic studies on plant–microbe interactions using multi-omics approaches, field validation of optimal irrigation regimes and biocontrol agents, and economic and sustainability assessments of moisture management practices in ginseng agroforestry. Such efforts will advance our capacity to implement ecologically sound and productive cultivation systems for high-value medicinal plants.

Declarations

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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Author contributions

Huiling Wang and Xiahong He led the project. Huiling Wang and Rongshuang Sha handled field experiments and microbiom data analysis, with support from Wensong Sun and Lifu Sun in Liaoning Province. Tao Zhou, Qiongying Kang and Dan Li used HPLC-MS for data analysis. Huiling Wang, Zhenxing Wang and Baoyu Shen conducted microbial validation in pot experiments. Huiling Wang and Rongshuang Sha drafted the manuscript, which was revised by Rui Shi, Shu He and Xiahong He. All authors contributed and approved the final version.

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None.

Data availability

Data will be made available on request. All microbial genomic sequence data have been submitted to the National Center for Biotechnology Information (NCBI) database, with the accession number [PRJNA1031259].

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Figures

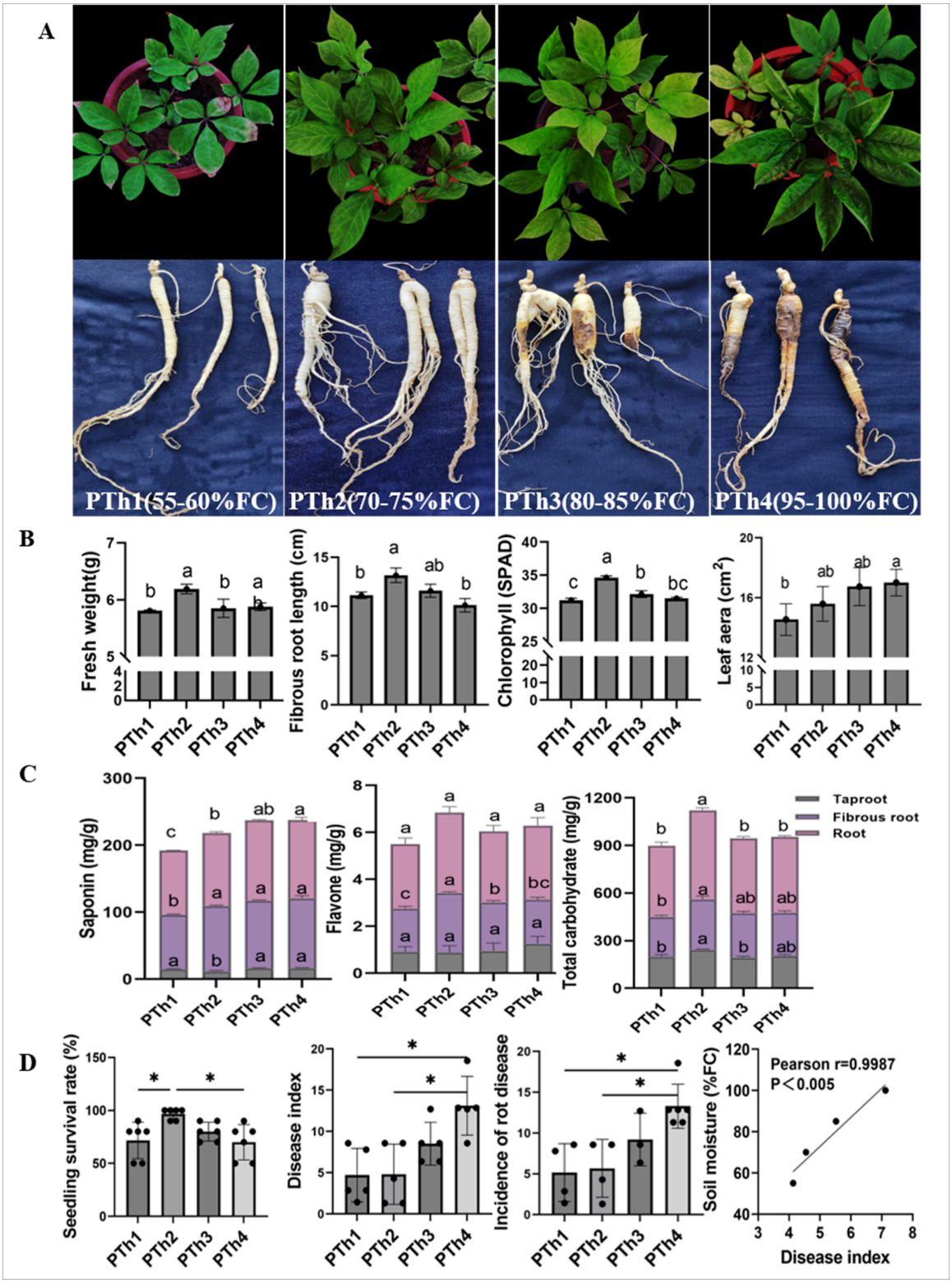


Figure 1

Effects of different soil moisture treatments on the agronomic traits and quality index of OPPF. (A) Growth status of OPPF. (B) Agronomic traits of OPPF. (C) Quality index of OPPF. (D) Seedling survival rate and root rot disease for different soil moisture contents ($p < 0.05$).

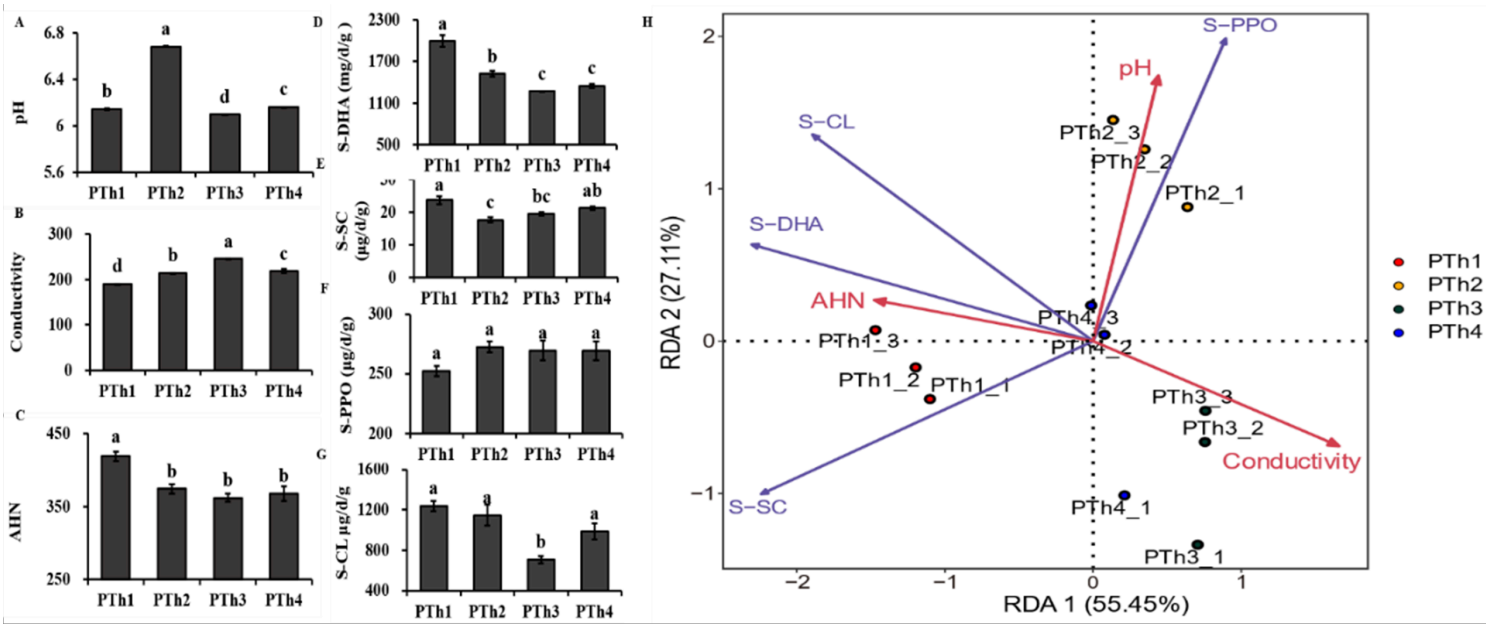


Figure 2

Changes in soil properties and enzyme activities under varying soil moisture contents: (A–C) Soil properties; (D–G) Enzyme activities; (H) RDA of soil moisture, properties, and enzyme activities. Different letters above plots indicate significant differences among treatments ($p < 0.05$).

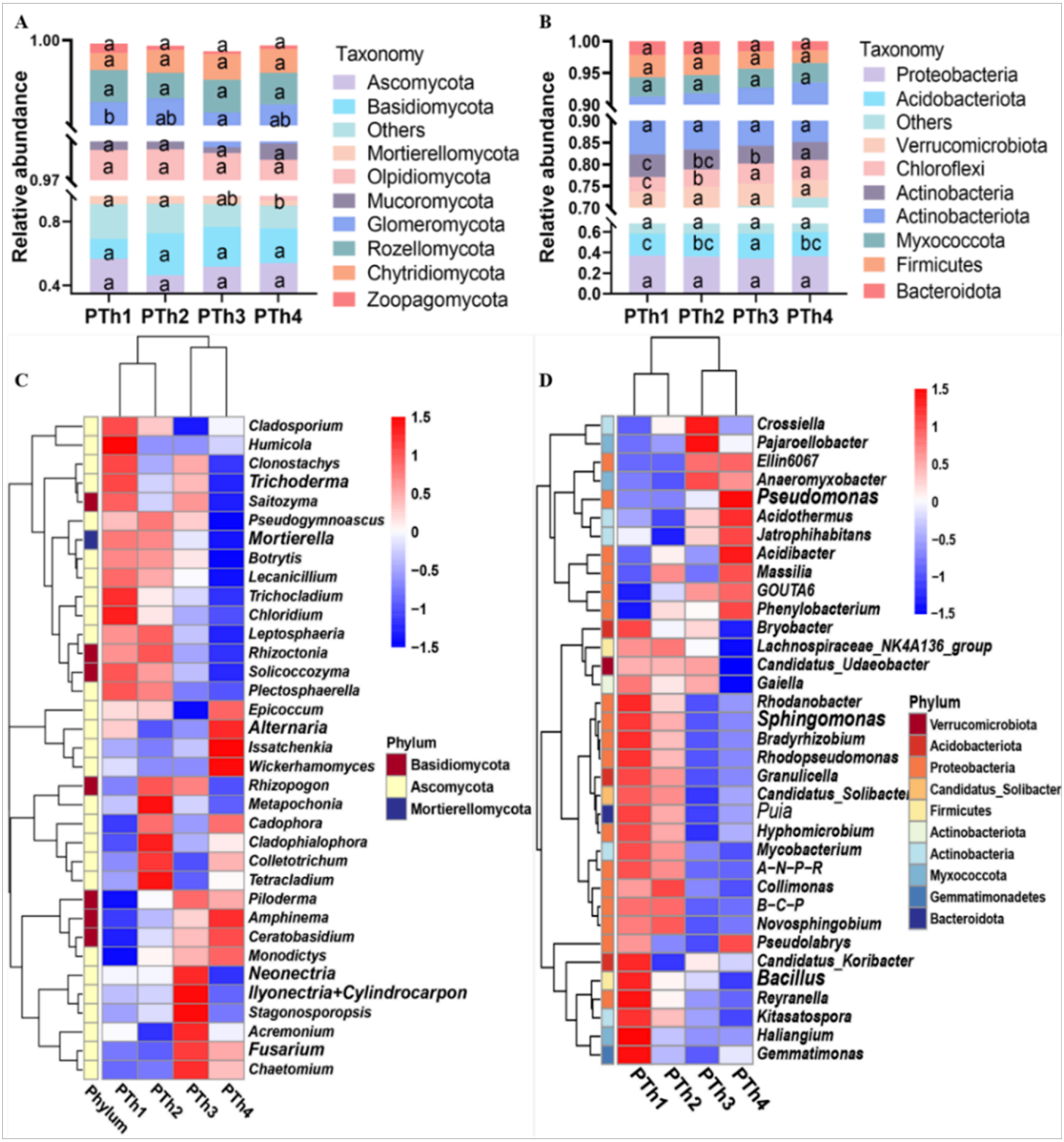


Figure 3

Relative abundance maps are generated at the phylum and genus taxonomic levels.

(A) Relative abundance of fungi; (B) relative abundance of bacteria; (C) heatmap of top 35 fungi genera; (D) heatmap of top 35 bacteria genera.

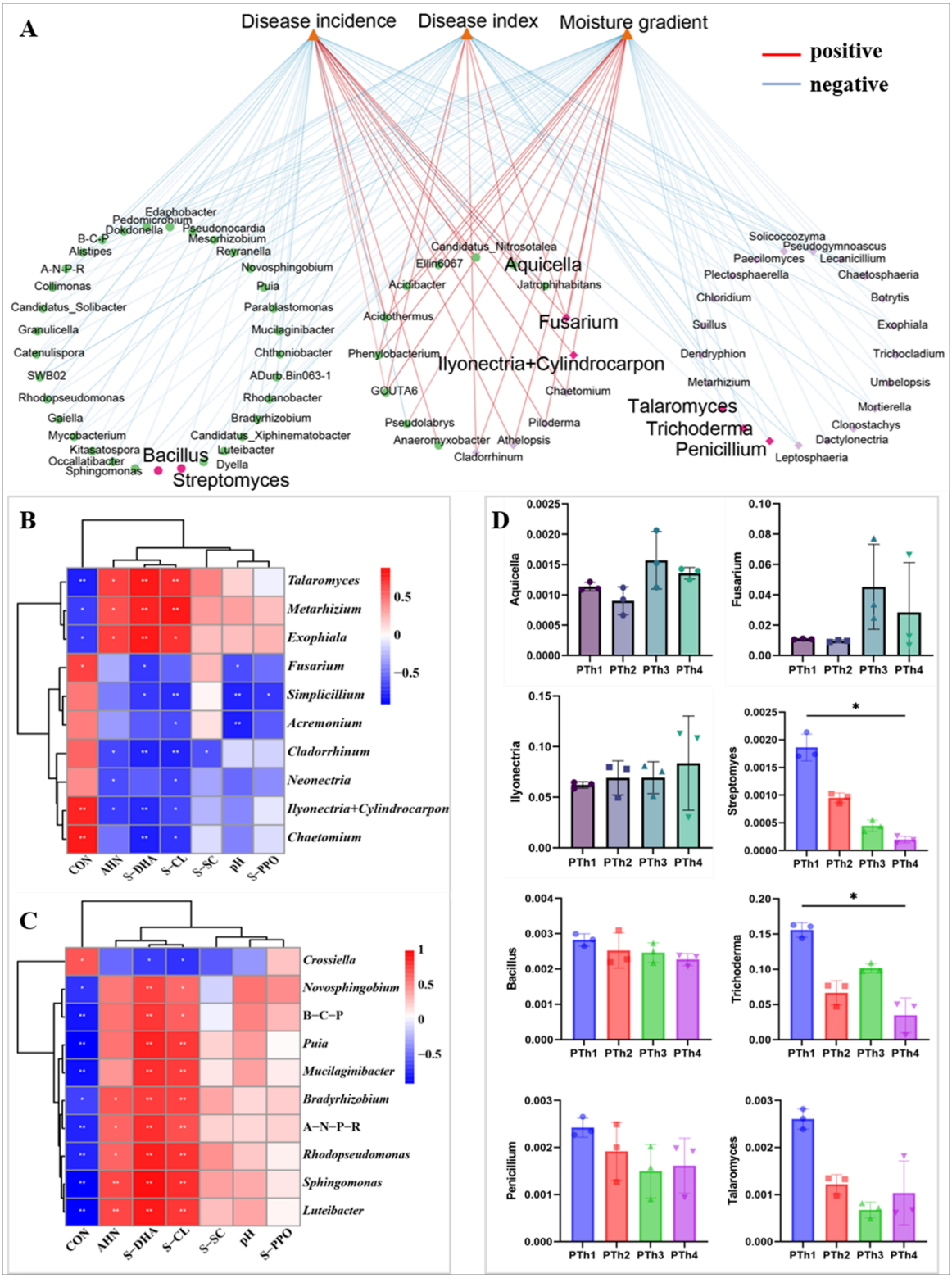


Figure 4

Correlation between environmental factors, root rot disease, and rhizosphere microbial communities analyzed using Spearman's correlation coefficient. (A) Correlations between root rot, soil moisture, and rhizosphere microbes, as well as the relationship between soil factors and the top 10 fungal genera; (B) correlations between soil factors and the top 10 fungal genera; (C) correlations between soil factors and the top 10 bacterial genera; (D) relative abundances of *Aquicella*, *Ilyonectria*, *Fusarium*, *Trichoderma*,

Talaromyces, *Penicillium*, *Streptomyces*, and *Bacillus*CON: soil conductivity; AHN: alkali-hydrolyzed nitrogen; S-DHA: soil dehydrogenase; S-CL: soil cellulase; S-SC: soil sucrase; S-PPO: soil polyphenol oxidase; MG: moisture gradient; DI: disease index; IRD: incidence of root rot disease.

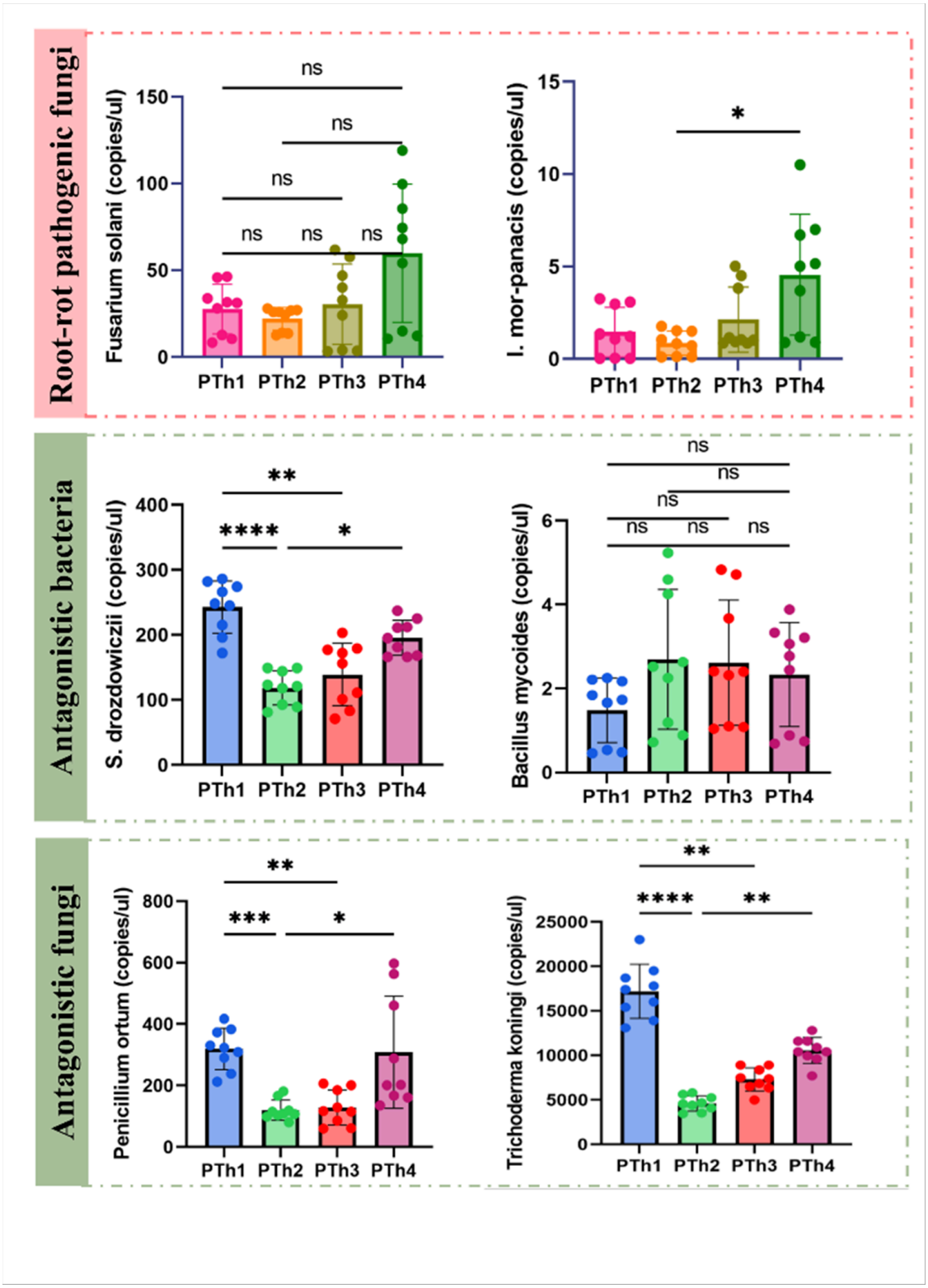


Figure 5

qPCR copy number of pathogens and antagonists in rhizosphere soil of OPPF.

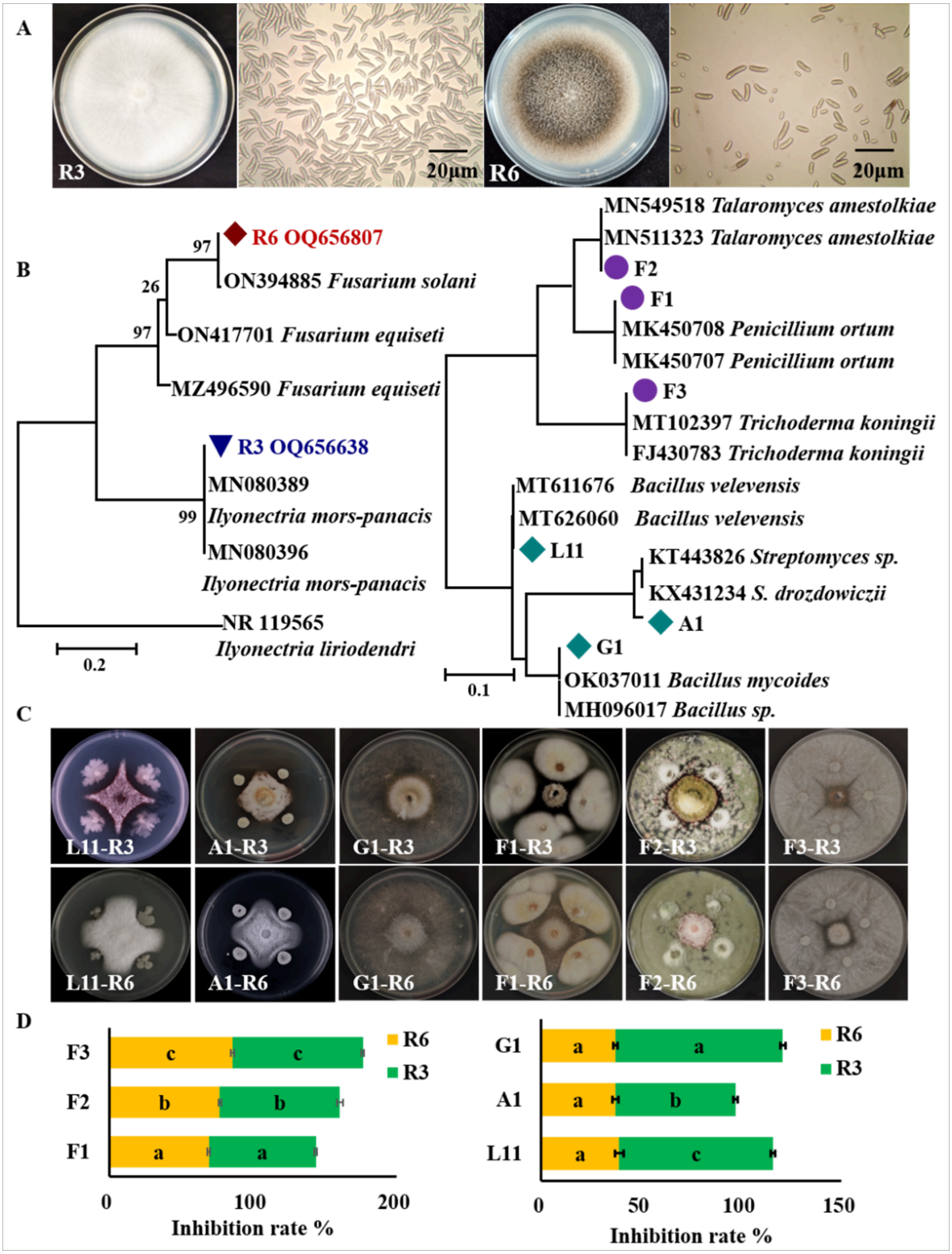


Figure 6

Antagonistic Effect of Rhizosphere Soil Strains. (A) Colony morphology and conidia of pathogens R3 and R6; (B) Phylogenetic tree based on internal transcribed spacer (ITS1) sequences (pathogens on the left, antagonists on the right); (C) Antagonistic effects of rhizosphere strains on R3 and R6; (D) Inhibition rates of rhizosphere strains against R3 and R6.

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