



OPEN Microbiome analysis for artificially establishing the symbiotic relationship between *Hebeloma hiemale* and *Quercus mongolica*

Hong-Bo Guo^{1,3,5}, Jia-Chen Zhao^{2,5}, Wei-Ye Liu², Yan-Di Bi¹, Lidiya Alekseevna Sibirina⁴ & Xiao-Dan Yu²✉

Ectomycorrhizae (ECM) play a critical role in enhancing plant growth and health. However, the influence of artificially established ectomycorrhizal symbioses on the structure and function of rhizosphere microbial communities remains inadequately understood. In this study, a symbiotic relationship between *Hebeloma hiemale* and *Quercus mongolica* was established to investigate the influence of ECM on soil microbial communities in the rhizosphere of the host plant. High-throughput sequencing revealed that *H. hiemale* inoculation altered the evenness of both the fungal and bacterial communities and reduced the diversity of the bacterial community relative to the blank control. In particular, several bacterial genera with an enhanced capacity for nutrient cycling, contaminant degradation, and host plant protection were enriched following *H. hiemale* inoculation. Shifts in fungal community structure suggest potential benefits for the host plant, including reduced cadmium uptake, enhanced mercury remediation, and increased protection against pathogens. Our results highlight the complex interactions between ECM and rhizosphere microbial communities to enable a better understanding of the importance of multi-species relationships in plant–microbe symbioses and their ecological implications.

Keywords Ectomycorrhizal fungi, Rhizosphere, Microbial community, Function prediction

Mycorrhizae are well-known mutualistic symbioses between soil fungi and host plant roots. They are commonly divided into two types: ectomycorrhizae (ECM) and endomycorrhizae^{1–3}. ECM symbioses are significant components of forest ecosystems and play essential ecological roles in enhancing plant growth and health^{4,5}. *Hebeloma hiemale* is an ECM-forming fungus that promotes plant growth^{6,7}. ECM acquire carbon from the host plant and enhances the uptake of essential nutrients (e.g., nitrogen and phosphorus) and water by the host plant^{8,9}. ECM symbioses are artificially established by the inoculation of host-beneficial ECM, offering a promising strategy for enhancing plant growth and resilience in degraded environments^{10,11}. This approach is a sustainable alternative to conventional agricultural and forestry practices¹². For instance, *Quercus mongolica*, an ecologically and economically valuable species^{13,14}, can successfully establish an artificial ECM symbiosis when inoculated with *Gomphidius viscidus*¹⁵. However, the influence of artificially established ECM symbioses on microbial communities in the rhizosphere of *Q. mongolica* remains inadequately understood.

The microbial community is an essential component of plant life^{16–18}. Based on their influence on plants, microbial communities are classified as beneficial, neutral, or harmful^{19,20}. Beneficial microbes can enhance plant growth and adaptation^{21,22}. On the other hand, harmful microbes, including plant pathogens, can adversely affect plant growth^{23–25}. ECM not only form mutualistic symbioses with the host plant but also influences the rhizosphere microbial community^{26,27}. For instance, *Tricholoma bakamatsutake* significantly alters the composition of the soil microbial community by suppressing the abundance of other fungal genera, such as *Russula* and *Penicillium*, while increasing the abundance of plant growth-promoting bacteria, such as *Solirubrobacter* and *Streptomyces*²⁸.

¹College of Life Engineering, Shenyang Institute of Technology, Fushun, China. ²College of Biological Science and Technology, Shenyang Agricultural University, Shenyang, China. ³Primorye State Agricultural Academy, Ussuriysk, Russia. ⁴Federal Scientific Center of the East Asia Terrestrial Biodiversity Far Eastern Branch of Russian Academy of Sciences, Vladivostok, Russia. ⁵These authors contributed equally: Hong-Bo Guo and Jia-Chen Zhao. ✉email: yuxd126@126.com

To investigate the influence of ECM on microbial communities in the rhizosphere of the host plant, *Q. mongolica* was artificially inoculated with *H. hiemale*. High-throughput sequencing was conducted to assess bacterial (16S rDNA) and fungal (ITS rDNA) sequences from the rhizosphere microbial community. This study aimed to explore the influence of ECM on fungal and bacterial communities in the rhizosphere of *Q. mongolica*.

Materials and methods

Description of ectomycorrhizal synthesis and sampling strategy

The experiment was conducted in a greenhouse at Shenyang Agricultural University in Shenyang, Liaoning Province, China (41°80'N 123°45'E). The method followed in this study was adopted from previous studies with some modifications^{29–32}. *Q. mongolica* seeds (obtained from the Laotudingzi National Nature Reserve, Benxi, China) were surface-sterilized with 30% H₂O₂ for 4 h and washed thrice with distilled water. The soil was autoclaved at 121 °C for 90 min. The treated seeds were sown in a plastic container filled with autoclaved soil. After 30 days, the seedlings were transplanted into pots containing 1 L of sterilized soil. Soil pH was adjusted to 6.5⁶. The experimental procedure is illustrated in Fig. 1. There were three experimental groups, each containing 20 seedling repeats. In the Hh1 and Hh2 groups, each seedling received 5 mL of *H. hiemale* at a concentration of 10⁷ and 10⁸ spores/mL, respectively. None of the seedlings in the CK group received *H. hiemale* spores. The three groups were maintained in a greenhouse under the same environmental conditions, watered every 3 days, and no fertilizer was added to the pots. After 150 days, the root tips of *Q. mongolica* were harvested and soil samples were obtained. Ectomycorrhizal synthesis was assessed using a Nikon SMZ25 stereomicroscope and via ITS rDNA sequence analysis (Fig. 2; GenBank accession number: PP897331). In the Hh1 and Hh2 groups, 14 and 18 seedlings, respectively, showed successfully ectomycorrhizal colonization. In the CK group, all seedlings remained uncolonized by ECM. The rhizospheres were carefully collected from each pot by gently brushing the root surfaces with a sterile brush after removing the loosely adhering soil by gentle shaking³¹. For each treatment group, we employed a partial pooling strategy to balance analytical depth with experimental feasibility^{33–35}. Within each group, samples from individual seedlings were randomly divided into three subgroups. Specifically, in the Hh1 group ($n = 14$ successful colonizations), samples were pooled into three analytical replicates with four to five individuals per pool. In the Hh2 group ($n = 18$ successful colonizations), samples were pooled into three analytical replicates, with six individuals per pool. In the CK group ($n = 20$), the samples were similarly pooled into three analytical replicates with six to seven individuals per pool. This pooling approach allowed us to focus on the treatment-level effects while maintaining sufficient biological representation for each group. Pooled samples were subjected to DNA extraction for high-throughput sequencing.

DNA extraction, PCR amplification, and illumina miseq sequencing

Total metagenomic DNA from the microbial community was extracted using the E.Z.N.A.[®] soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.) as per manufacturer's instructions. The quality of the extracted metagenomic DNA was determined using 1% agarose gel electrophoresis, and the concentration and purity of the DNA was determined using a NanoDrop 2000 (Thermo Scientific, USA).

For bacteria, 16S-specific primers 338 F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the 16S rRNA gene. For fungi, the primers ITS1 F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were used to

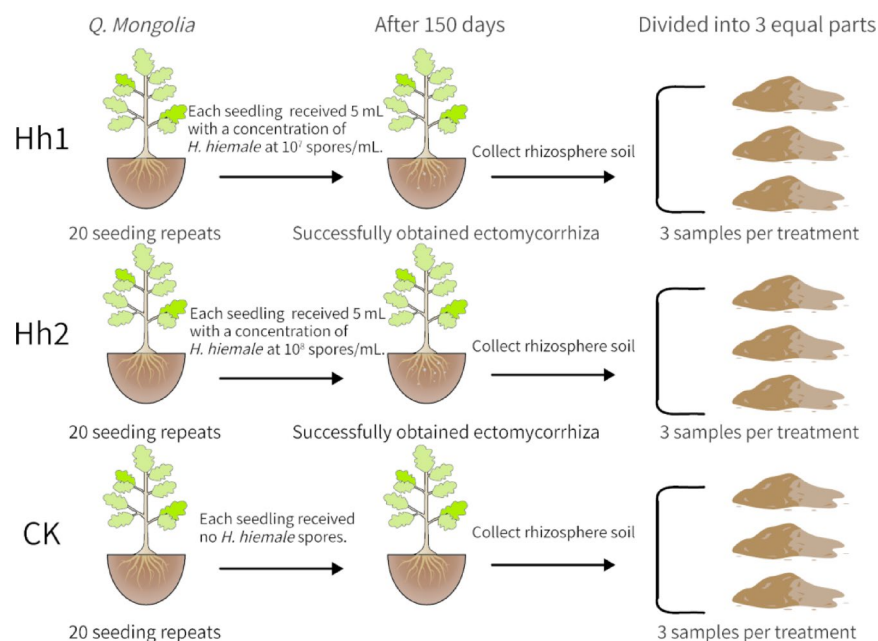


Fig. 1. Schematic representation of the experimental procedure.



Fig. 2. Root tips of *Q. mongolica* without *H. hiemale* (a) and with *H. hiemale* (b, c, d). Scale bars represent 1000 μm .

amplify the ITS gene. The PCR reaction mixture comprised 4 μL of $5 \times$ Fast Pfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of Fast Pfu polymerase, 10 ng of template DNA, and ddH₂O to a final volume of 20 μL . PCR amplification cycling conditions were as follows: initial denaturation at 95 $^{\circ}\text{C}$ for 3 min; followed by 27 cycles of denaturing at 95 $^{\circ}\text{C}$ for 30 s, annealing at 55 $^{\circ}\text{C}$ for 30 s, and extension at 72 $^{\circ}\text{C}$ for 45 s; single extension at 72 $^{\circ}\text{C}$ for 10 min; and end of the reaction at 4 $^{\circ}\text{C}$. All samples were amplified in triplicate. The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using a Quantus[™] Fluorometer (Promega, USA).

Purified amplicons were pooled in equimolar amounts and paired-end sequencing was performed on an Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA) according to the standard protocols of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw sequencing reads were deposited in the NCBI Sequence Read Archive database (BioProject: PRJNA1116842).

Data processing

Quality control of raw paired-end sequences was performed using fastp (version 0.19.6)³⁶. The filtered sequences were then merged using FLASH software (version 1.2.11)³⁷. Operational taxonomic units (OTUs) were clustered using UPARSE (version 7.1)^{38,39} at 97% similarity and chimeras were removed. To minimize the impact of sequencing depth on subsequent alpha and beta diversity analyses, the sequence number was rarefied to the minimum number of sequences across all samples. Taxonomic annotation of OTUs was performed using the RDP classifier (version 2.11)⁴⁰ against the Silva 16 S rRNA (Release 138)⁴¹ and Unite ITS databases (Release 8.0)⁴² with a confidence threshold of 70%. The community composition of each sample was summarized at different taxonomic levels. Functional predictions were performed using PICRUSt2 (version 2.2.0)⁴³.

Statistical analysis

Data analysis was performed using Meguiar's BioCloud platform (<https://cloud.majorbio.com>) and R software (version 4.3.3). Alpha diversity indices, including Chao and Shannon's indices, were calculated using the Mothur software⁴⁴. Intergroup variations in alpha diversity indices were assessed using the Wilcoxon rank-sum test. Non-metric multidimensional scaling (NMDS) analysis, based on the Bray-Curtis distance algorithm and the Anosim function, was employed to examine the similarity in microbial community structure between samples^{45–47}. To assess the relationships among fungal genera, we employed Spearman's rank correlation based on their relative abundances. To predict the ecological guilds of the fungal community, we used FUNGuild

(version 1.0) following the guidelines described by Nguyen et al.⁴⁸. Multiple- and two-group comparisons were conducted to compare the means of all samples within each group. Differential abundance analysis of microbial communities was performed using ALDEx2^{49–51}. The Kruskal-Wallis H test and Wilcoxon rank-sum test for differential abundance analysis were performed using the Majorbio Cloud platform.

Results

Sequencing results and microbial diversity

A total of 508,514 bacterial and 467,371 fungal sequences were obtained by paired-end sequence quality control and assembly (Tables S1 and S2). After rarefaction of sequence depth, bacterial community coverage ranged from 97.62 to 98.23%, and fungal community coverage ranged from 99.60 to 99.90% across all soil samples (Tables S3 and S4). The effective sequence counts and corresponding OTU numbers for each sample are listed in Tables S5 and S6. The results indicated that most bacterial and fungal taxa were detected, and that the sequencing depth was sufficient to capture the overall microbial composition across all soil samples.

Compared with those of the CK group, the Chao and Shannon indices of the bacterial community exhibited a decline in the Hh groups (Hh1 and Hh2), with the Hh2 group displaying the lowest values (Fig. 3a and b). Conversely, the Chao diversity index of the fungal community exhibited an increase in the Hh groups compared with the CK group, whereas the Shannon diversity index decreased, reaching its lowest value in the Hh2 group

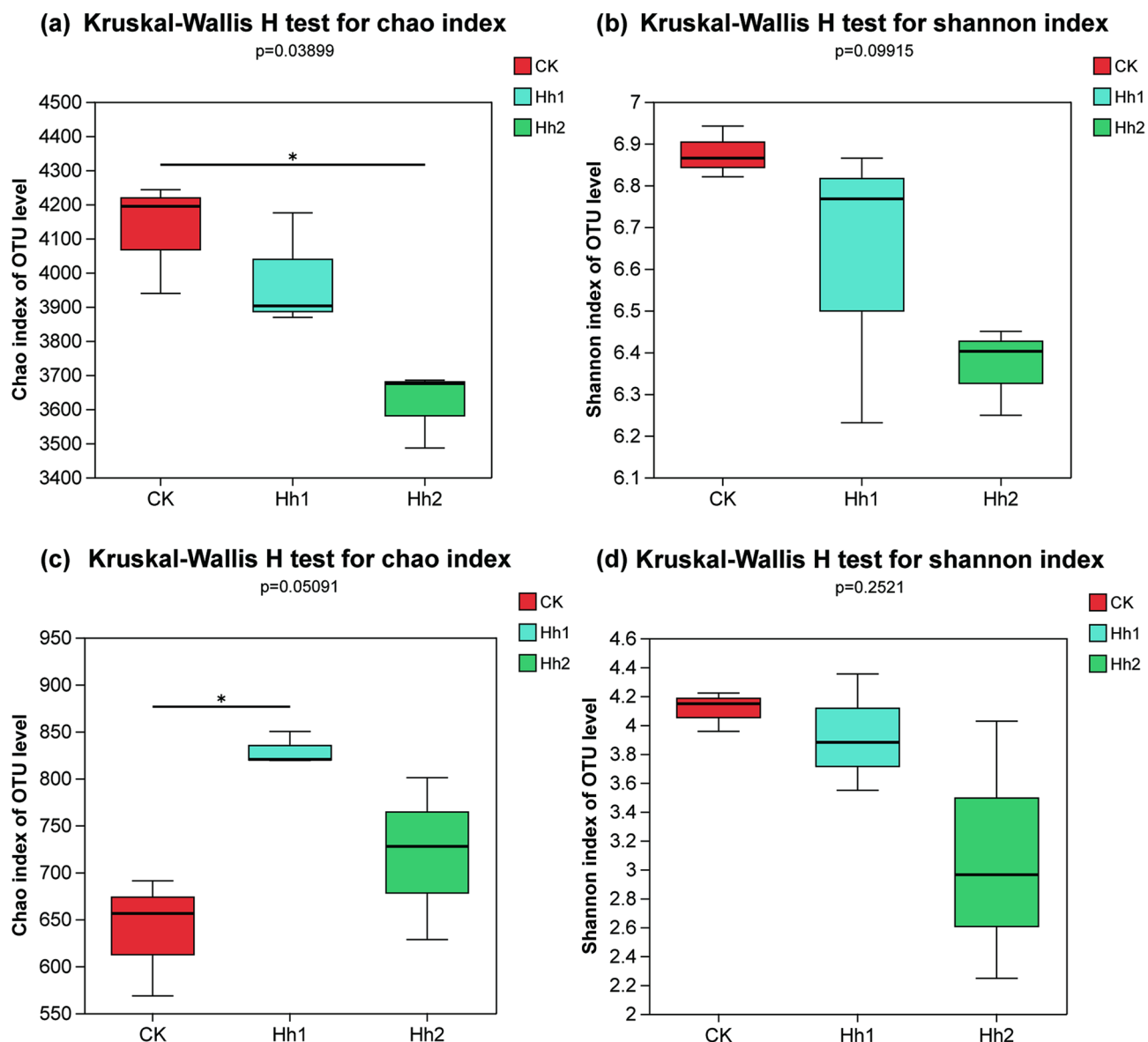


Fig. 3. Alpha diversity of bacterial and fungal communities among the three groups. The Chao (a) and Shannon (b) indices of the bacterial community; The Chao (c) and Shannon (d) indices of the fungal community. Asterisks indicate statistical significance levels between pairwise comparisons: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

(Fig. 3c and d). These results suggest that *H. hiemale* alters the evenness of the fungal and bacterial communities and reduces the diversity of the bacterial community.

Bacterial community composition

At the OTU level, the three groups shared 3,077 OTUs, with the Hh1 group having the highest number of unique OTUs (696) and the CK group having the lowest (533) (Fig. 4a). The Hh groups shared the highest number of OTUs (800). Pseudomonadota and Actinobacteriota were the dominant phyla in all three groups (Fig. S1). Acidobacteriota was less abundant in the Hh groups than in the CK group.

A ternary diagram reveals the distribution proportions of the dominant bacterial genera across the three groups (Fig. 4b). Notably, *Nostoc_PCC-7524*, *Sphingomonas*, and *Bacillus* spp. exhibited elevated distribution proportions in the Hh groups than in the CK group. Specifically, *Bacillus* displayed the highest distribution proportion in the Hh2 group, whereas *Nostoc_PCC-7524* was predominant in the Hh1 group. In the CK group, the proportions of dominant bacterial genera such as *RB41* was higher. Other genera showed similar proportional distribution across all three groups.

Fungal community composition

Overall, the three groups shared 529 OTUs. The CK group had the highest number of unique OTUs (242), whereas the Hh2 group had the lowest (126) (Fig. 5a). The Hh groups shared the highest number of OTUs (305). Ascomycota and Basidiomycota were the dominant phyla across the three groups (Fig. S2).

The ternary diagram displays the distribution proportions of dominant fungal genera in the three groups (Fig. 5b). *Peziza*, *Hebeloma*, *g__unclassified_p__Rozellomycota*, and *g__unclassified_k__Fungi* were present in higher proportions in the Hh groups than in the CK group. *Hebeloma* was the most abundant in the Hh2 group, and was not present in the CK group. *unclassified_f__Thelephoraceae*, *Penicillium*, *Mortierella*, *Fusarium*, *Chaetomium*, and *Talaromyces* were present in higher proportions in the CK group, with *unclassified_f__Thelephoraceae* being unique to this group.

Differences in structure and species in microbial communities

Variations in the bacterial and fungal communities among the three groups were analyzed using NMDS (Fig. 6). Notably, the bacterial community structure displayed greater divergence between the different groups compared with the fungal community structure. This indicated that the structure of the bacterial and fungal communities was altered by *H. hiemale*.

Analysis of the average relative abundance among the three groups revealed significant variations in the fungal and bacterial communities at the genus level (Tables S7 and S8). In the fungal community, *Hebeloma* was more abundant in the Hh2 group than in the other groups, *Paraphoma* was more abundant in the Hh1 group. In the bacterial community, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Phenylobacterium*, *Massilia*, and *Burkholderia-Caballeronia-Paraburkholderia* were more abundant in the Hh2 group than in the other groups; *MND1* was more abundant in the CK group.

Species correlation analysis

Fungal correlation analysis at the genus level revealed that both *Hebeloma* and *Peziza* were negatively correlated with the majority of fungi, including *Penicillium* and *Trichoderma* (Fig. 7). Although the fungal correlation analysis did not show a significant positive correlation between *Hebeloma* and *Peziza*, a comparison of their abundance across the three groups indicated an increase in *Peziza* following the presence of *Hebeloma* (Fig. 5b).

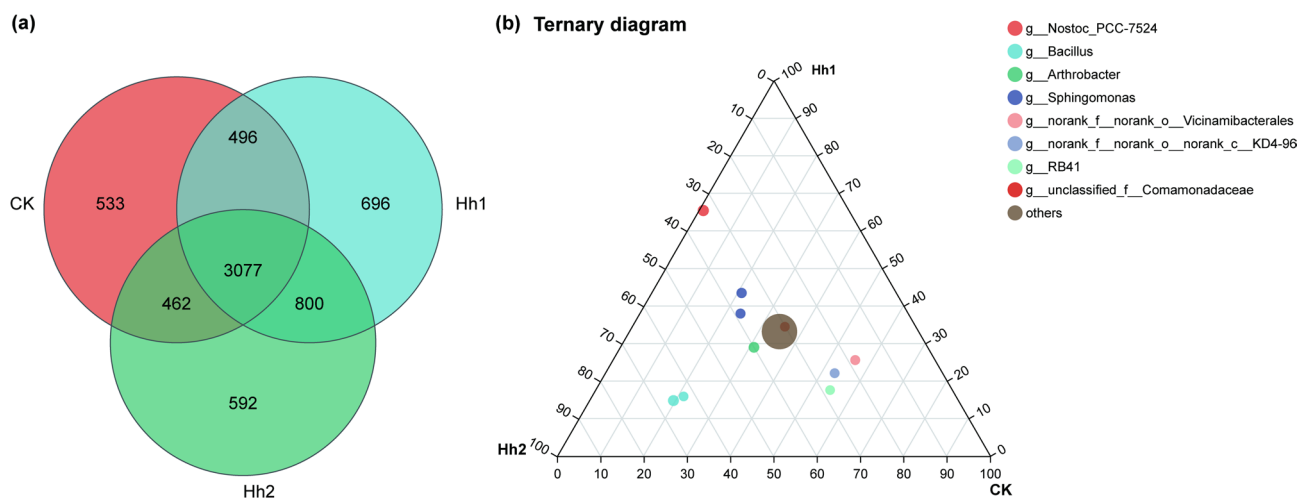


Fig. 4. Bacterial community composition among three groups. (a) Venn diagram of bacterial OTU number; (b) The ternary diagram of dominant bacterial genera on genus level.

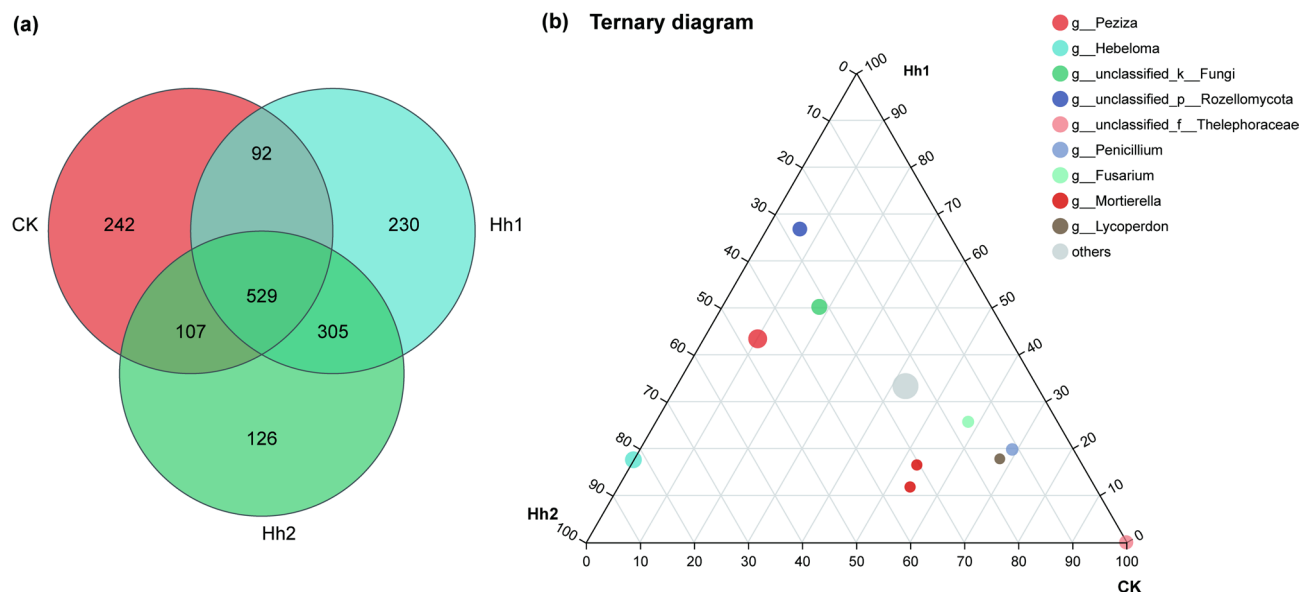


Fig. 5. Fungal community composition of the three groups. **(a)** Venn diagram of fungal OTU number; **(b)** The ternary diagram of dominant fungal genera.

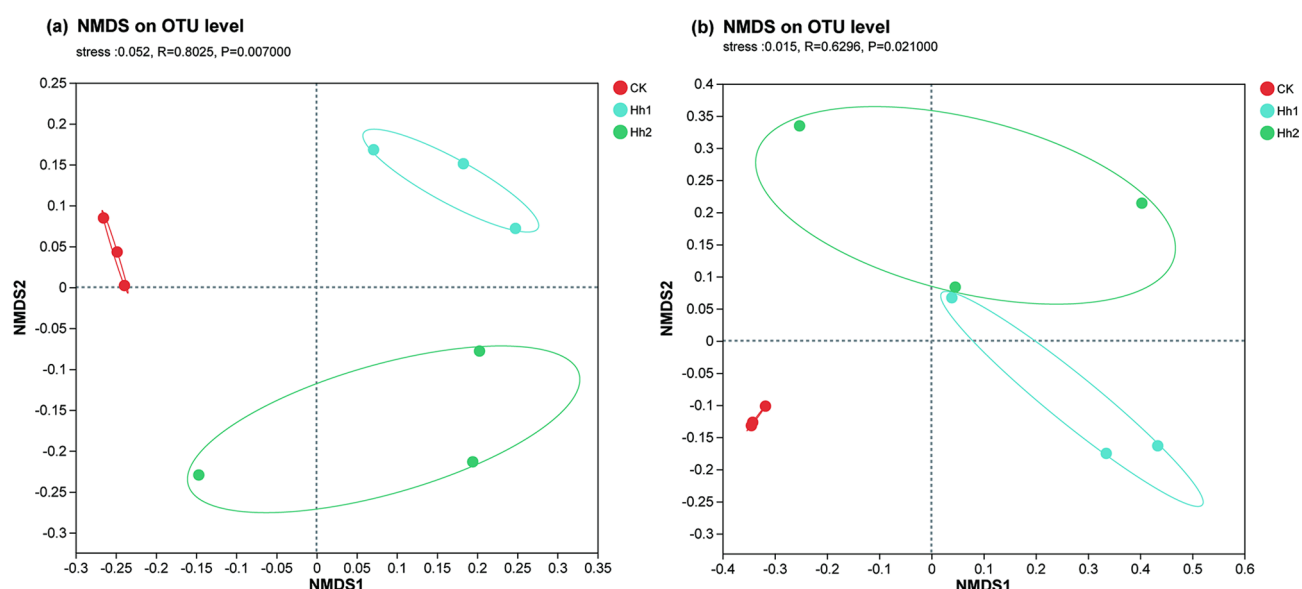


Fig. 6. Differences in microbial community structure among three groups. NMDS analysis of bacterial **(a)** and fungal **(b)** communities at OTU level.

Functional predictions

FUNGuild was used to predict the functional profiles of fungal communities (Fig. S3). The results showed that ectomycorrhizae, represented by *H. hiemale*, and endophytes were significantly more abundant in the Hh2 group than in the CK group. Conversely, plant pathogen decreased when *H. hiemale* abundance increased.

Functional predictions of bacterial communities were performed using PICRUSt2, BugBase, and FAPROTAX. Notably, PICRUSt2 functional predictions revealed an increase in the abundance of *H. hiemale* resulted in an increase in the enzyme-level abundance of Monosaccharide-transporting ATPase and Serine-type D-Ala-D-Ala carboxypeptidase (Fig. S4), as well as the glyoxylate cycle and superpathway of the glyoxylate bypass-TCA in the MetaCyc pathway (Fig. S5), with the highest abundance observed in the Hh2 group. BugBase analysis revealed that the abundance of Contains Mobile Elements traits was significantly higher in the Hh groups than in the CK group (Fig. S6). In contrast, Potentially Pathogenic bacteria were significantly lower in the Hh groups than in the CK group, and Anaerobic bacteria were significantly lower in the Hh2 group than in the CK group. FAPROTAX

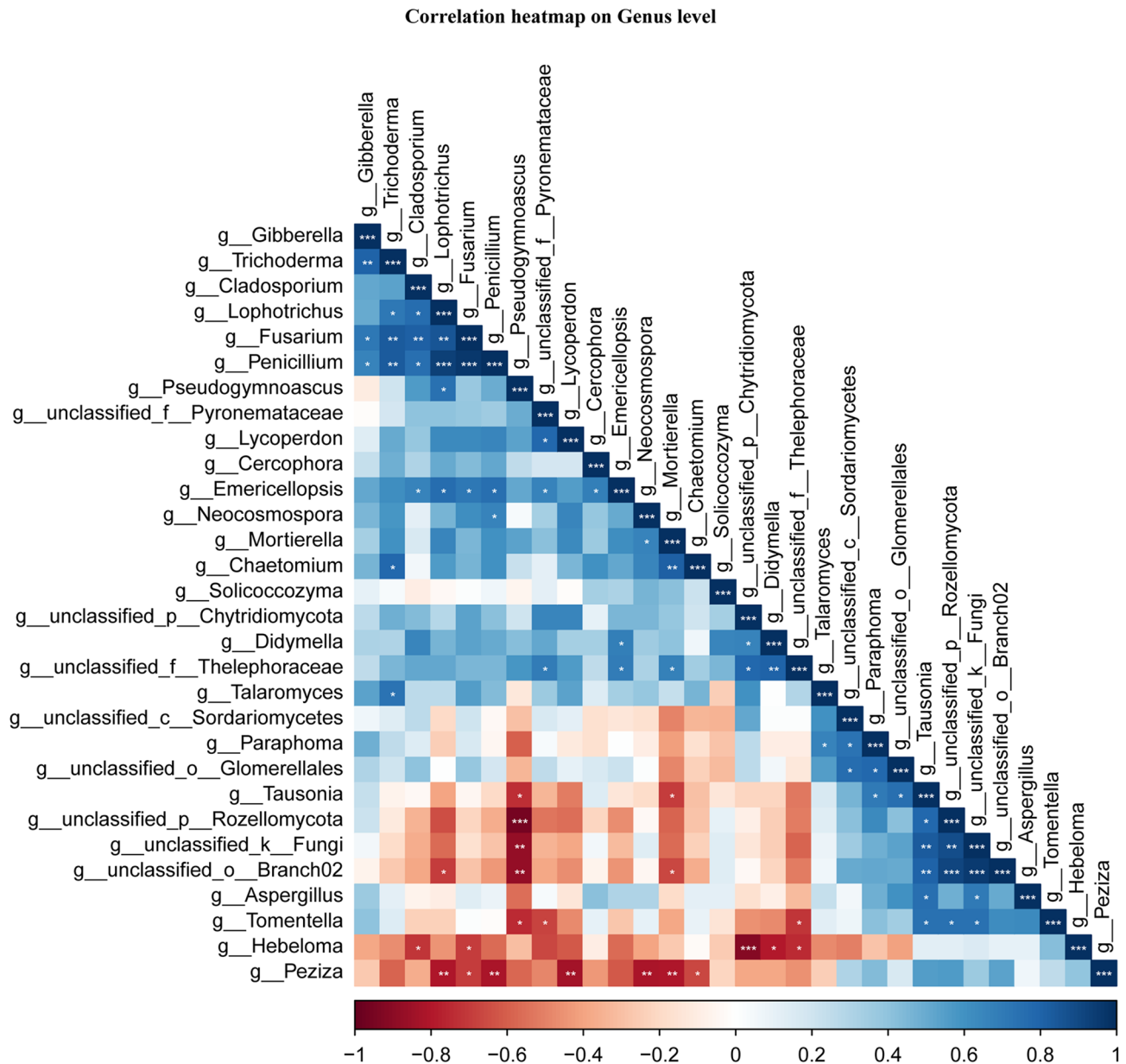


Fig. 7. Fungal correlation heatmap analysis at genus level. Asterisks denote statistical significance levels: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

functional predictions indicated that following *H. hiemale* inoculation, functions related to nitrogen fixation and photoautotrophy were enhanced, with a higher abundance in the Hh groups than in the Ck group (Fig. S7).

Discussion

Analysis of microbial diversity and community structure

Microbial diversity analyses revealed that inoculation with *H. hiemale* significantly affected both the diversity and evenness of the fungal and bacterial communities (Fig. 3). The bacterial Shannon and Chao indices decreased in the Hh1 and Hh2 groups compared with the CK group, with Hh2 showing the lowest values. In contrast, although the fungal Chao index increased with *H. hiemale* inoculation, the fungal Shannon index decreased, reaching its lowest value in the Hh2 group. NMDS analysis revealed distinct clustering patterns among the three groups (Fig. 6), suggesting that *H. hiemale* inoculation differentially influenced the structure of the bacterial and fungal communities. The bacterial community appeared to diverge more than the fungal community did after inoculation with *H. hiemale*. These results suggest that *H. hiemale* may favor certain microbes in bacterial and fungal communities, thereby reducing the overall bacterial diversity while reshaping fungal evenness. Similar observations of mycorrhizal fungi altering rhizosphere diversity have been reported in other studies^{52,53}. The reduced bacterial diversity suggests competitive exclusion of some microbes by those that are better adapted

to the ectomycorrhizal environment^{54,55}. A possible explanation for this is that ECM can release exudates or modify root exudation patterns that selectively promote or suppress certain microbes^{8,56}.

Analysis of microbial community composition

Analysis of microbial bacterial community composition

In the present study, we observed significant differences in the abundances of several bacterial genera among the three groups. These findings suggest that the presence of *H. hiemale* can significantly influence the composition of bacterial communities in the rhizosphere of host plants. Previous research has shown that ECM symbionts can selectively affect associated bacterial communities⁵⁵. Our results further support this finding, indicating that *H. hiemale* modulates the rhizosphere microbiome by promoting the growth of specific microbial communities.

Notably, the abundances of *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Massilia*, *Burkholderia-Caballeronia-Paraburkholderia*, *Luteibacter*, *Dyella*, *Chitinophaga* were significantly higher in the CK group than in the Hh2 group. *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* is known for its nitrogen-fixing capabilities, which play crucial roles in enhancing host plant growth by improving nitrogen availability^{57,58}. *Massilia* has been identified as possessing phosphorus-solubilization abilities⁵⁹ which can facilitate the absorption of available phosphorus by the host plant, thereby promoting host plant growth. Additionally, a species of the genus *Massilia* has been found to produce dimethyl disulfide, offering a potential alternative soil fumigant to replace methyl bromide⁶⁰, which could help mitigate the adverse effects of soil-borne diseases and nematodes. *Burkholderia-Caballeronia-Paraburkholderia* plays a significant role in degrading atrazine, a pesticide residue, showing a strong positive correlation with the rate of atrazine degradation⁶¹. *Luteibacter*, which is associated with cellulose degradation, contributes to enhancement of soil fertility⁶², and has also been shown to degrade methamidophos, another pesticide residue⁶³. *Dyella* participates in the selenium cycle by converting organic selenium into selenium-oxygen anions, which facilitate selenium uptake by the host plant^{64,65}. *Chitinophaga* has emerged as a potential key player in suppressing significant plant pathogens, such as *Ralstonia solanacearum*, contributing to the healthy growth of the host plant⁶⁶. Their diverse ecological roles, such as nitrogen fixation, and pollutant degradation, contribute to the maintenance of soil fertility and promotion of sustainable agricultural practices. Notably, *Luteibacter* was exclusively detected in the Hh groups and was absent in the CK group. Thus, *H. hiemale* may indirectly contribute to improved host plant health and growth by selectively shaping bacterial community composition.

Analysis of microbial fungal community composition

Inoculation with *H. hiemale* altered the fungal community composition in the rhizosphere of the host plant, significantly increasing the abundance of *unclassified_p_Mortierellomycota* and *Lecythophora*. A notable increase in their relative abundance was observed in the CK group compared to that in the Hh2 group. Interestingly, *Mortierellomycota* has been reported to exhibit a significant negative correlation with biogenic Cd levels⁶⁷ and may represent a key component of the rhizosphere microbiome that enhances the resistance of alfalfa to Cd⁶⁸. This suggests that the increased abundance of *unclassified_p_Mortierellomycota*, induced by *H. hiemale* inoculation, may contribute to the mitigation of Cd toxicity in *Q. mongolica*. Furthermore, the increased abundance of *Lecythophora* in response to *H. hiemale* inoculation may have environmental implications. *Lecythophora* exhibits significant resistance to Hg(II) and can volatilize Hg(II) to Hg(0), potentially improving mercury-contaminated environments^{69,70}. *Lecythophora* abundance increased with *H. hiemale* inoculation, which could potentially enhance this mercury-remediation process, contributing to the overall health of the ecosystem. In addition, the ternary diagram (Fig. 5) revealed that *Peziza* abundance was higher in the Hh groups than in the CK group. The fungal correlation heatmap analysis (Fig. 7) also indicated a positive correlation between *Peziza* and *Hebeloma*. This observation aligns with previous findings that *Peziza* frequently co-occurs with a specific ECM, suggesting potential facilitative interactions or similar nutrient requirements^{71,72}.

In contrast to its positive effects on *unclassified_p_Mortierellomycota* and *Lecythophora*, *H. hiemale* inoculation appeared to inhibit the abundance of *Fusarium*. As the concentration of *H. hiemale* increased, a corresponding decrease in the relative abundance of *Fusarium* was observed. This suggests that *H. hiemale* possesses certain compounds or properties that selectively suppress the growth of *Fusarium*. Given the pathogenic nature of some species within this genera^{73–75}, the inhibitory effect of *H. hiemale* may provide an additional layer of protection for the host plant, promoting its overall health and resilience. Inoculation with *H. hiemale* influenced the fungal community composition in the rhizosphere of the host plant, with notable increases in the abundance of *unclassified_p_Mortierellomycota* and *Lecythophora*, and a concomitant decrease in *Fusarium*. These changes in fungal community structure may have important implications for the host plant, including reduced Cd uptake, enhanced Hg remediation, and increased protection against potential pathogens.

Functional predictive analysis of microbial communities

Functional prediction analysis of fungal community

FUNGuild predicted that functional analysis of the fungal community would reveal significant changes after *H. hiemale* inoculation. The results demonstrated that ectomycorrhizal and endophytic fungi were significantly increased in the Hh2 group compared with the CK group. Previous studies have shown that the community structures of ectomycorrhizal and endophytic fungi can influence one another through interspecific competitive and facilitative interactions in plant roots^{76,77}. Therefore, the observed increase in endophytes suggests the potential for facilitative interactions between *H. hiemale* and endophytes.

Conversely, FUNGuild predicted that the plant pathogens would decrease with *H. hiemale* abundance increased. For example, within the fungal community, the presence of *H. hiemale* also leads to a significant decrease in the abundance of *Fusarium*, a genus of filamentous fungi that includes many plant pathogens^{73–75}.

H. hiemale may indirectly promote the growth and health of host plants by suppressing the growth of plant pathogens. The observed changes in fungal community composition and function following *H. hiemale* inoculation highlight the complex interactions among ECM, microbial community, and host plants. This suggests a potential mechanism by which the ECM can shape the microbiome to benefit the host plant.

Functional prediction analysis of bacterial community

In this study, we employed a multifaceted approach to investigate the effect of ectomycorrhiza on the functional profiles of bacterial communities. PICRUSt2 showed that the enzyme-level abundance of monosaccharide-transporting ATPase increased with the increasing abundance of *H. hiemale*, with the highest abundance observed in the Hh2 group. Furthermore, MetaCyc pathway analysis revealed an increase in the abundance of the superpathway of glyoxylate bypass-TCA and glyoxylate cycle. This phenomenon may be attributed to the competitive dynamics established between *H. hiemale* and the bacterial community. The competition for carbon sources between *H. hiemale* and the bacterial community likely drove the elevation of the enzyme-level abundance of monosaccharide-transporting ATPase within the bacterial community, as it is critical for accelerated carbon metabolism⁷⁸. Moreover, competition might have resulted in carbon source deficiency, consequently stimulating biomolecule synthesis by upregulating the superpathway of glyoxylate bypass-TCA and glyoxylate cycle, which are essential pathways for the synthesis of biomolecules under carbon-limiting conditions⁷⁹.

BugBase prediction analysis showed that the abundance of Containing Mobile Elements was higher in the Hh groups than in the CK group. Conversely, Anaerobic was significantly lower in the Hh2 group than in the CK group. Mobile elements, such as transposons and plasmids, facilitate horizontal gene transfer in bacteria, which can profoundly impact their fitness and evolution^{80,81}. The observed increase in Containing Mobile Elements may indicate an adaptive response of the bacterial community to the presence of *H. hiemale*. Mycorrhizal activity can influence the soil pore spaces available for microbial habitation, resulting in altered oxygen concentrations⁸². The observed decrease in anaerobes suggests that *H. hiemale* inoculation may modify oxygen availability in the rhizosphere, thereby potentially suppressing the growth of anaerobic bacteria. Predictions generated by the FAPROTAX function indicated elevated levels of nitrogen fixation with increasing *H. hiemale* inoculation. Nitrogen fixation activity may indicate enhanced nitrogen uptake by the host plant upon inoculation with *H. hiemale*, as this process is crucial for the conversion of atmospheric nitrogen into bioavailable forms for plant uptake^{83,84}.

Conclusion

In this study, diversity and composition of microbial communities in the rhizosphere of the host plant were compared after inoculation with *H. hiemale*. Compared with the CK group, *H. hiemale* inoculation altered the evenness of both fungal and bacterial communities and reduced the diversity of the bacterial community. Notable changes were observed in the bacterial community composition, with several genera exhibiting an enhanced capacity for nutrient cycling, pollutant degradation, and host plant protection. Shifts in the fungal community structure may have significant implications for the host plant, such as reduced Cd uptake, enhanced mercury remediation, and increased protection against potential pathogens. It provides valuable insights into the intricate interactions between ECM and microbial communities in the rhizosphere, underscoring the need to account for multi-species interactions to understand the ecological implications of plant–microbe relationships. Further research is necessary to elucidate the specific mechanisms underlying these effects and their potential impact on host plant fitness. Future studies should explore the potential applications of *H. hiemale* in sustainable agriculture and environmental management, which could lead to novel strategies for increasing crop productivity and mitigating environmental pollution.

Data availability

The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found below: National Center for Biotechnology Information (NCBI) BioProject (PRJ-NA1116842).

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Author contributions

HB-G: Writing – original draft, review & editing, Methodology, Formal analysis. JC-Z: Writing – original draft, Methodology, Software, Formal analysis. WY-L: Writing – original draft, Investigation, Formal analysis, Data curation. YD-B: Writing – original draft, Visualization, Conceptualization. LA-S: Writing – review & editing, Visualization, Conceptualization. XD-Y: Writing – review & editing, Supervision, Conceptualization.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to X.-D.Y.

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