Polish Journal of Microbiology
2021, Vol. 70, No 3, 373–385
https://doi.org/10.33073/pjm-2021-035

ORIGINAL PAPER

Bacterial Community Analysis and Potential Functions of Core Taxa in Different Parts of the Fungus *Cantharellus cibarius*

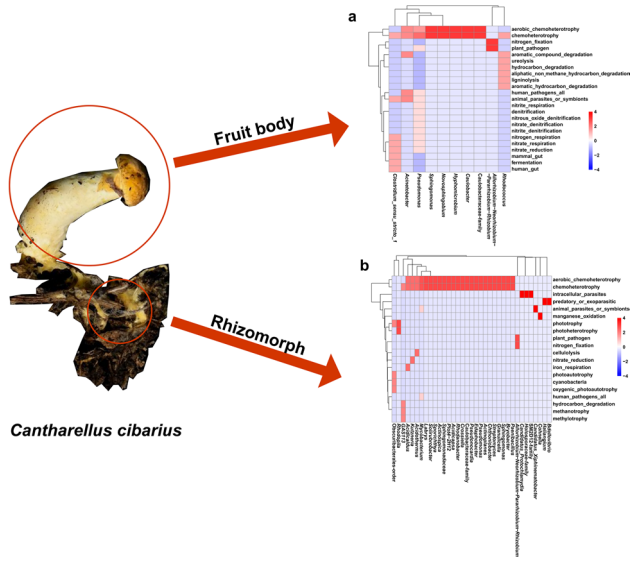
WEI GE[1], ZHI-YUAN ZHANG[1], CHUN-BO DONG[1], YAN-FENG HAN[1]*, SUNIL K. DESHMUKH[2] and ZONG-QI LIANG[1]

[1] Institute of Fungus Resources, Department of Ecology, College of Life Sciences, Guizhou University, Guiyang, China
[2] TERI-Deakin Nano Biotechnology Centre, The Energy and Resources Institute, New Delhi, India

Submitted 17 April 2021, revised 25 July 2021, accepted 25 July 2021

Abstract

Cantharellus cibarius is a widely distributed, popular, edible fungus with high nutritional and economic value. However, significant challenges persist in the microbial ecology and artificial cultivation of *C. cibarius*. Based on the 16S rRNA sequencing data, this study analyzed bacterial community structures and diversity of fruit bodies and rhizomorph parts of *C. cibarius* and mycosphere samples (collected in the Wudang District, Guiyang, Guizhou Province, China). It explored the composition and function of the core bacterial taxa. The analyzed results showed that the rhizomorph bacterial community structure was similar to mycosphere, but differed from the fruit bodies. Members of the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* complex had the highest abundance in the fruit bodies. However, they were either absent or low in abundance in the rhizomorphs and mycosphere. At the same time, members of the *Burkholderia-Caballeronia-Paraburkholderia* complex were abundant in the fruit bodies and rhizomorphs parts of *C. cibarius*, as well as mycosphere. Through functional annotation of core bacterial taxa, we found that there was an apparent trend of potential functional differentiation of related bacterial communities in the fruit body and rhizomorph: potential functional groups of core bacterial taxa in the fruit bodies centered on nitrogen fixation, nitrogen metabolism, and degradation of aromatic compounds, while those in rhizomorphs focused on aerobic chemoheterotrophy, chemoheterotrophy, defense against soil pathogens, decomposition of complex organic compounds, and uptake of insoluble inorganic compounds. The analysis of functional groups of bacteria with different structures is of great significance to understand that bacteria promote the growth and development of *C. cibarius*.



K e y w o r d s: *Cantharellus cibarius*, bacteria, core bacterial taxa, potential function

Introduction

Ectomycorrhizal fungi (EMF) are important participants in the nutrient cycling of forest ecosystems and play significant roles in enhancing water and nutrient absorption of plants. They promote the biogeochemical cycles of the chemical elements in the ecosystem (Calvaruso et al. 2007; Chen et al. 2009). EMF can be symbiotic with many plants, produce fruit bodies and rhizomorphs, and establish potential symbiotic relationships with the microbes in peripheral soils designated as "the mycosphere" (van Elsas and Boersma 2011; Haq et al. 2014; Liang et al. 2020). In this nested symbiosis model, bacteria on the surface of fungal

* Corresponding author: Y.-F. Han, Institute of Fungus Resources, Department of Ecology, College of Life Sciences, Guizhou University, Guiyang, China; **e-mail: swallow1128@126.com**

hyphae can interact with host fungi and colonize mycelium and fruit body tissues, thus forming unique forms of symbiosis or metabolic complementarity. Fungi use endobacteria, which live in the fungal vegetative hyphae or reproductive structures, to make minerals weathered and obtain nutrients such as nitrogen and phosphorus from the soil to improve their adaptability and biomass (Barbieri et al. 2010; Fontaine et al. 2016; Salvioli et al. 2016; Pent et al. 2017). Simultaneously, the mycelium and fruit bodies of fungi can also provide different energy sources and habitats for bacteria to promote their growth (Schulz-Bohm et al. 2017). Mutualistic and antagonistic relationships between bacteria and fungi are prominent when adapting to environmental changes. Recognition of the role of the EMF and their related microbial community in the development of primordium and fruit bodies has resulted in the ecology of microbiota associated with mycorrhizal fungi, which has become a hot topic of research (Marupakula et al. 2015; Deveau et al. 2016; Pent et al. 2017).

Cantharellus cibarius Fr. is one of the six well-known edible fungi globally and is extremely valuable for both medicine and food. This fungus is ectomycorrhizal with economically valuable trees and plays a significant ecological role (Zhang et al. 2010). The unique evolutionary history and substantial economic value of *C. cibarius* have attracted considerable researchers to focus on its ecology, physiology, and phylogeny (Dunham et al. 2003; Kumari et al. 2013). In the last century, Straatsma et al. (1986a, 1986b) studied the role of carbon dioxide and carboxylated metabolic intermediates in the vegetative growth stage of *C. cibarius*. They found that hyphal fragments of *C. cibarius* grew strongly in a nutrient solution supplemented with malic acid, thymine, and Tween 80. The mixture of these three substances replaced CO_2 or a living root as a growth factor. It is related to the fact that these factors promote hyphae growth by immobilization of CO_2 into Krebs cycle intermediates and biosynthesis pathways of pyrimidines and fatty acids. Subsequently, Danell et al. (1993, 1994, 1997) discussed the influence of different factors on the fruit body differentiation of *C. cibarius*. They tried to explore the formation of fruit bodies and relate it with the effects of bacteria (*Pseudomonas fluorescens*) on mycorrhizal formation and mycorrhizal synthesis *in vitro*, although no obvious inducing factors were found. However, it also provides some valuable information for the artificial cultivation of *C. cibarius*, for example, the change of pH value controls the growth of mycelium and *P. fluorescens*, and enough hyphal biomass may form fruit bodies.

In recent years, rapid developments in high-throughput sequencing technology have facilitated analysis of the dynamics and diversity of bacteria related to *C. cibarius*. For example, Pent et al. (2017) compared and analyzed the diversity and structural composition of some EMF, including *C. cibarius*, by combining high-throughput sequencing with the assessment of the physical and chemical properties of the mycosphere. These authors suggested that the soil pH and host identity were the predominant factors affecting bacterial community composition. Pent et al. (2020) continued to merge high-throughput sequencing and chemical composition determination to compare and analyze the chemical content and bacterial community composition of the fruit bodies of EMF, including *C. cibarius*, and revealed that the differences in the chemical composition of fruit bodies also markedly impacted bacterial community composition. Recently, Gohar et al. (2020) analyzed bacterial community potential function variation at different development stages (young, middle-aged, and old) and in internal (cap, stipe lower internal, stipe middle internal) and external (gills, cap surface, stipe lower external, and stipe middle external) compartments of fruit bodies of *C. cibarius*, and compared the bacteria with that of other ectomycorrhizae. Their results demonstrated that bacterial community structure differed between internal and external parts of the fruit body but not between inner tissues. The structure of the bacterial communities showed significant variation across fruit body developmental stages. In addition, some functional groups, such as nitrogen fixation, persisted in fruit bodies during the maturation but were replaced by putative parasites/pathogens afterward.

However, despite these detailed studies, microbiota's occurrence and interaction(s) with *C. cibarius* are still poorly understood. Basic information related to microbial communities and their functions in distinct ecological niches needs to be supplemented. The rapid development of bioinformatics and molecular ecology has expediated core microbiome research. The core microbiome, which is a critical component of the primary function of holobionts, is preserved, enriched, and inherited through natural selection during evolution and plays an essential ecological role in different environmental samples (Lemanceau et al. 2017). Core microbiome study has been employed in studying various samples, including plants (Dong et al. 2020), insects (Segata et al. 2016), soil (Mendes et al. 2013), and water (Ji et al. 2015), but it is scarce in the research field of *C. cibarius* and other EMF.

The current study used the high-throughput sequencing technology to reveal the bacterial community structure composition and dynamic changes in the fruit bodies and rhizomorphs parts of *C. cibarius* and mycosphere. It also compared their liquidity and correlation and further explored the potential function of core bacterial taxa of the fruit bodies and rhizomorphs. We hypothesized that (i) the structure of the rhizomorphs' bacterial communities was similar to that of

the mycosphere, but different from that of the fruit bodies; (ii) there are differences not only in composition but also in potential function between the core bacterial taxa of fruit bodies and rhizomorphs.

Experimental

Materials and Methods

Sample collection and processing. Five middle-aged *C. cibarius* were collected from Mount Yangchang, Yangchang Town (26°49′52″N, 106°87′56″E) in the Wudang District, Guiyang, Guizhou Province, China, in August 2019. The spacing of each sample was greater than 1 m to ensure that the samples collected were from a different host. With the fruit body as the center of the circle, the soil (10 cm in diameter and 5 cm in depth) was shoveled out with a sterile shovel to protect the samples of fruit body, the rhizomorph at the base of the fruit body, and the mycosphere. The samples were collected and rapidly transported to the laboratory in an ultra-clean box at 4°C for processing (Warmink and van Elsas 2008; Oh et al. 2016). While wearing sterile gloves, the connection between the cap and stipe of the fruit body was separated with a sterile scalpel. The cap was divided into two halves by the hands, and ~ 2 g internal tissues (unexposed and untouched part of the sterile scalpel) were picked out with sterile inoculation needles for each fruit body. Rhizomorph was the thread-like or cordlike structure in fungi made up of parallel hyphae, branched tubular filaments (Townsend 1954). Mycosphere was obtained by finding the rhizomorph, holding the stipe, slowly moving out the rhizomorph attached soil, and gently shaking the soil attached to the surface of the rhizomorphs. Then residual soil on the rhizomorphs was removed with a sterile inoculation needle (Warmink and van Elsas 2008). 2 g of mycosphere soil corresponding to each fruit body sample was collected. Rhizomorphs with the soil removed were sterilized under UV light for 5 min after disinfection with 75% alcohol for 30 seconds, and 2 g was harvested for each sample. All samples were processed and placed in a sterile centrifuge tube for the subsequent DNA extraction.

DNA extraction and PCR amplification. Total genomic DNA was extracted from fruit bodies and rhizomorphs of *C. cibarius*, as well as mycosphere soil samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA), according to the manufacturer's protocols. DNA concentration and purity were determined with a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, USA). Variable V3-V4 regions of the 16S rRNA gene were amplified using the bacterial primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') in the GeneAmp 9700 PCR system (ABI, USA). The PCR program comprised an initial denaturation step at 95°C for 3 min, 27 cycles of 30 s at 95°C, 30 s for annealing at 55°C, and 45 s elongation at 72°C, followed by a final extension at 72°C for 10 min and then storing at 4°C. PCR reactions were performed in triplicate in a 20 μl mixture containing 4 μl of 5 × TransStart FastPfu Buffer, 2 μl of 2.5 mM dNTPs, 0.8 μl of each primer (5 μM), 0.4 μl of TransStart FastPfu Polymerase, and 10 ng of template DNA. There were three replicates per sample (Dong et al. 2020).

Illumina Miseq sequencing. PCR products were extracted from a 2% agarose gel, further purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using a Quanti™ Fluorometer (Promega, USA), according to the manufacturer's protocol. A NEXTFLEX®Rapid DNA-Seq Kit was used to build the library via the following steps: (1) joint link; (2) screening with magnetic beads and removal of joint self-continuous segments; (3) enrichment of library templates via PCR amplification; (4) recovery of PCR products from magnetic beads to obtain the final library. Purified amplicons were sequenced by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) on an Illumina MiSeq PE300 platform (Illumina, San Diego, USA), according to the standard protocols. Sequences obtained from all samples were submitted to the Sequence Read Archive (SRA) and are available under BioProject PRJNA670583 (BioSample accession numbers SAMN16515864).

Statistics and analysis of sequencing data. Raw data files were quality-filtered by fastp (https://github.com/OpenGene/fastp, version 0.20.0) (Chen et al. 2018) and merged by FLASH (http://www.cbcb.umd.edu/software/flash, version 1.2.7) with the following criteria (Magoc et al. 2011): (1) reads were truncated at any site receiving an average quality score < 20 over a 50-bp sliding window; (2) according to the overlap relation between PE reads, pairs of reads were merged into a sequence with a minimum overlap length of 10 bp; (3) the maximum mismatch ratio allowed in the overlap region of the merged sequence was 0.2, and the non-conforming sequence was screened; (4) according to barcodes and primers at the beginning and end of the sequence, the samples were distinguished, and the sequence direction was adjusted. The allowable mismatch number of barcodes was 0, and the maximum primer mismatch number was 2.

Sequences with ≥ 97% similarity were assigned to the same operational taxonomic units (OTUs), and the chimeras were filtered using UPARSE (http://drive5.com/uparse/version 7.1) (Stackebrandt and Goebel 1994; Edgar 2013). OTUs were classified and annotated by the RDP classifier (http://rdp.cme.msu.edu,

version 2.2), and compared with the SILVA 16S rRNA database (v138), setting the comparison threshold at 70% (Wang et al. 2007).

Alpha-diversity analyses, including community diversity parameters (Shannon), community richness (ACE), and a sequencing depth index (Shannon), were calculated using the mothur software(Schloss et al. 2011). Beta-diversity measurements, including microbiota trees, were calculated as previously described (Jiang et al. 2013), and principal coordinate analyses (PCoA) based on OTU compositions were determined. Bacterial taxonomic distributions of sample communities were visualized using R package software. A Venn diagram was implemented using the R package to show unique and shared OTUs. The stats package in R (3.5.0) was used for the clustering calculations and data normalization in the heatmap, and the heatmap package in R was used to generate the heatmap. Differences between populations were analyzed using a Kruskal-Wallis *H* test, and $p \leq 0.05$ was considered statistically significant.

Correlation analysis of bacterial core taxa and FAPROTAX function prediction. MetaCoMET (http://probes.pw.usda.gov/MetaCoMET) was used to define the core microbiome and obtain the visual results (Wang et al. 2016). The raw bacterial dataset of *C. cibarius* was adjusted to a tab-delimited text file, which contained the OTU classification information and relative abundance values for each sample. Qiime 2.0 was used to convert the text format into the biom format data file matching MetaCoMET. According to the analysis method defined by the membership, the analysis parameters were set on the MetaCoMET platform and submitted to the network platform to obtain the results (Dong et al. 2019).

FAPROTAX is a database based on the current manual collection of cultivable bacteria, and contains more than 7,600 functional annotations collected from many prokaryotic microbiomes (Louca et al. 2016). In this study, FAPROTAX (http://www.ehbio.com/ImageGP/index.php/Home/Index/FAPROTAX.html) was used to predict the function of the core bacterial taxa that existed in fruit bodies and rhizomorphs, respectively, and the differences between the functional groups were compared and analyzed.

Results

Bacterial alpha-diversity of fruit bodies, rhizomorphs, and mycosphere. Among the 15 samples obtained from the fruit bodies, rhizomorphs, and mycosphere, a total of 860,689 sequences were detected by 16S rRNA gene amplicon sequencing; the sequence range for each sample was 35,483–74,379, with an average length of 408.85–414.07 bp. Rarefaction curves of the Shannon index indicated that the sequencing data depth in this experiment could comprehensively reflect the microbial information because the number of sample sequences increased and the curve gradually flattened out (Fig. S1) (Mao et al. 2015).

After strict quality filtering, resulting sequences were gathered into OTUs with a similarity ≥97%. A total of 3,628 OTUs were detected, belonging to 43 phyla, 98 classes, 243 orders, 427 families, 751 genera, and 1,396 species, and the alpha diversity indexes are shown in Fig. 1. Shannon index values were positively correlated with diversity (Dong et al. 2020). Shannon (Fig. 1a) indexes showed that the bacterial

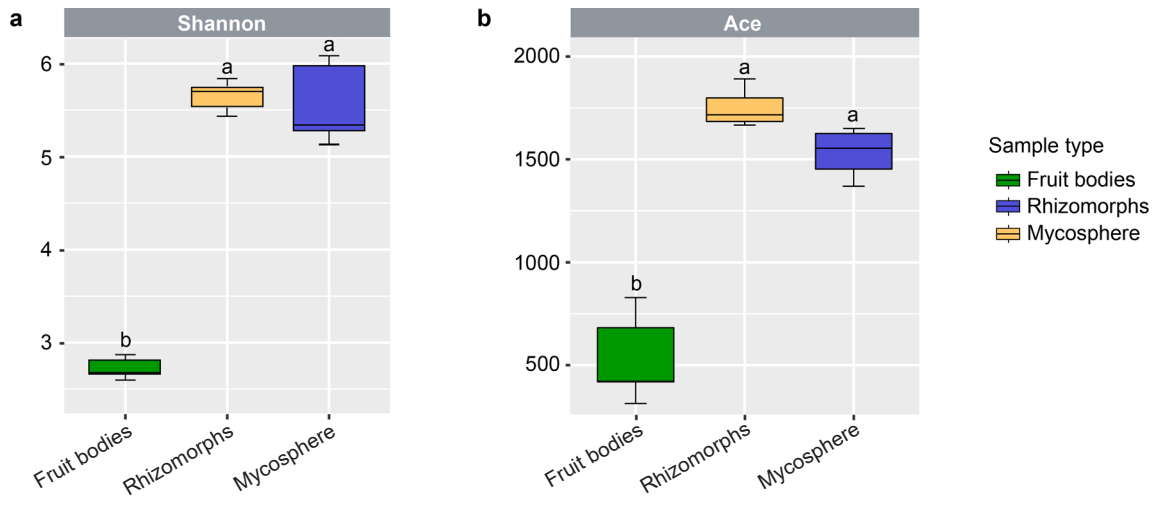


Fig. 1. Alpha-diversity comparison among the fruit bodies, rhizomorphs of *Cantharellus cibarius*, and mycosphere samples based on a) the Shannon and b) Ace indexes using the 16S rRNA gene amplicons sequencing data. Samples with the same letter do not differ significantly by Tukey's test at $p > 0.05$; samples with different letters are significantly different by Tukey's test at $p < 0.05$. The center value of each sample represents the median for the different indexes.

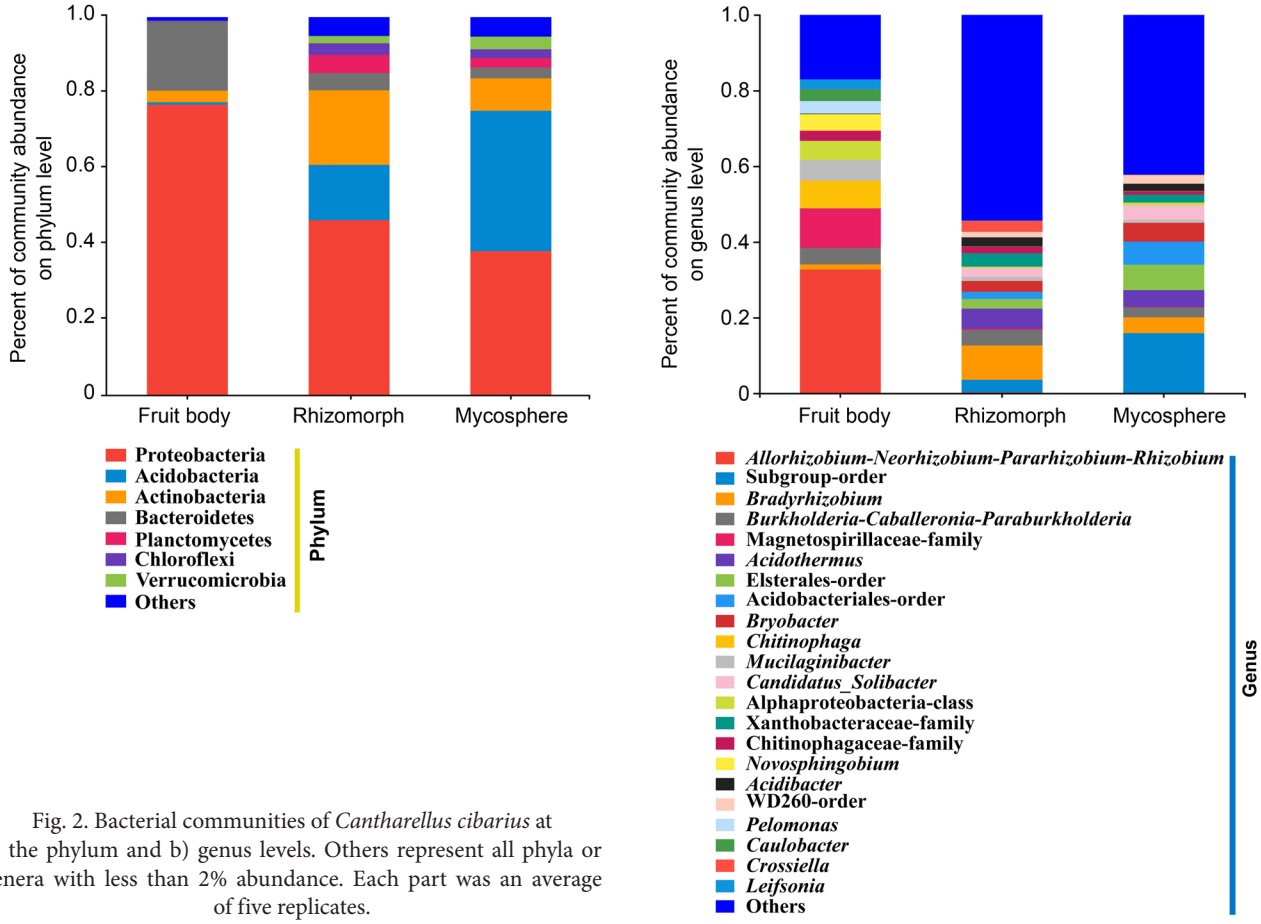


Fig. 2. Bacterial communities of *Cantharellus cibarius* at a) the phylum and b) genus levels. Others represent all phyla or genera with less than 2% abundance. Each part was an average of five replicates.

diversity of both rhizomorphs and mycosphere samples was significantly greater than that of fruit bodies ($p < 0.05$), but the diversity of rhizomorphs was similar to that of mycosphere samples ($p < 0.05$). The Ace (Fig. 1b) indexes both indicated that the bacterial richness of rhizomorphs and mycosphere samples were significantly higher than that of fruit bodies ($p < 0.05$). In contrast, the richness in rhizomorphs and mycosphere samples was similar ($p < 0.05$).

Bacterial taxonomic composition of fruit bodies, rhizomorphs, and mycosphere. Forty-three prokaryotic phyla were identified from the 16S rRNA gene amplicon sequences (Fig. 2a). Proteobacteria was the dominant phylum with the highest abundance in all three sampled parts of *C. cibarius*, and the proportion of Proteobacteria in the fruit bodies was as high as 76.89%. In addition, the abundance of Bacteroidetes in the fruit bodies was higher than in rhizomorphs and mycosphere soil. At the same time, Acidobacteria and Actinobacteria were predominantly concentrated in the rhizomorphs and the mycosphere.

At the genus level, the detected OTUs were distributed among 751 different bacterial genera in total (Fig. 2b). The top five abundant genera in each of the three sampled parts of *C. cibarius* are listed in Table I. The dominant genera of rhizomorph and mycosphere were similar but different from those of fruit body. It means that the dominant genera of bacteria are significantly different among the fruit body, the rhizomorph, and the mycosphere, and the relative abundance of the same taxa is also different between the rhizomorph and the mycosphere.

Differences were observed in the relative abundance of microbiota at the phylum level among the three populations ($p < 0.05$) (Fig. 3a). The relative abundance of Proteobacteria distributed in fruit bodies was quite high, but the relative abundance of Acidobacteria, Actinobacteria, and Planctomycetes were rarely distributed in the fruit bodies. Simultaneously, there were also differences in the relative abundance of microbiota at the genus level (Fig. 3b). The relative abundance of *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* complex and the bacteria of Magnetospirillaceae-family in the fruit body increased significantly ($0.001 < p \leq 0.01$). However, there was no significant difference in the member of the *Burkholderia-Caballeronia-Paraburkholderia* complex, *Chitinophaga*, and bacteria of Chitinophagaceae-family ($p > 0.05$) among the three parts.

Bacterial community structures of fruit bodies, rhizomorphs, and mycosphere. The principal coordinate analysis (PCoA) was used to explore the community structures of the microbiota in the three

Table I
Top five abundant genera in the fruit body, rhizomorph, and mycosphere of *Cantharellus cibarius*.

Sample parts	Genus	Relative abundance (%)
Fruit body	*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*	32.68
	Magnetospirillaceae-family	10.44
	Chitinophaga	7.31
	Mucilaginibacter	5.49
	Bradyrhizobium	1.37
Rhizomorph	*Bradyrhizobium*	9.06
	Acidothermus	5.32
	Subgroup-order	3.33
	Elsterales-order	2.57
	Acidobacteriales-order	1.88
Mycosphere	norank_o__Subgroup	15.78
	norank_o__Elsterales	6.74
	norank_o__Acidobacteriales	6.11
	Acidothermus	4.43
	Bradyrhizobium	4.19

parts sampled (Fig. 4b). The result showed that the bacterial communities from the rhizomorphs and mycosphere samples clustered tightly and were separated from the fruit bodies along principal coordinate axis 1 (PC1), which explained the large variation (62.01%). It indicates that the samples of rhizomorph and myco-

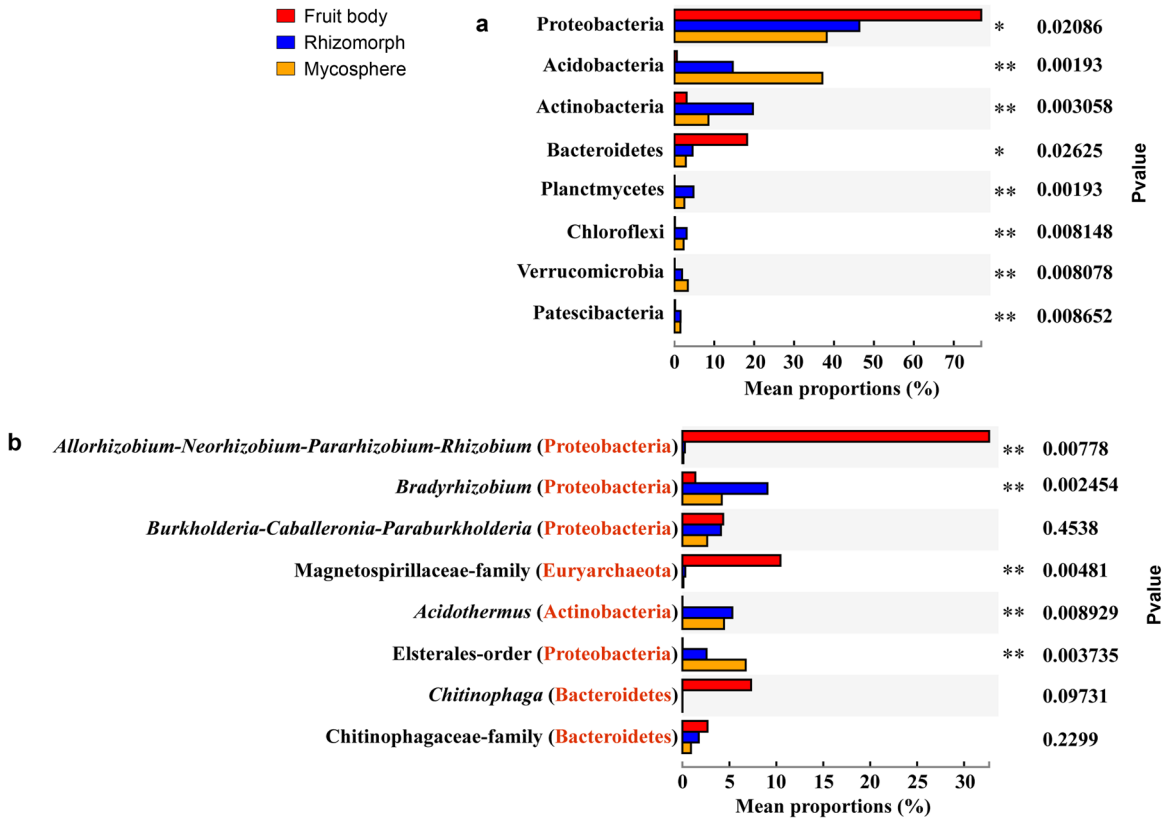


Fig. 3. Statistical comparison of the relative abundance of microbiota among the three sampled parts of *Cantharellus cibarius*. Comparison of a) dominant phyla and b) dominant genera in the fruit bodies, rhizomorphs, and mycosphere samples. The y-axis represents names of taxa at the dominant phyla or genera level; the x-axis represents average relative abundance; colored columns represent different sampled parts of *C. cibarius*. Values on the far right are the *p* values, $*0.01 < p \leq 0.05$, $**0.001 < p \leq 0.01$, $***p \leq 0.001$.

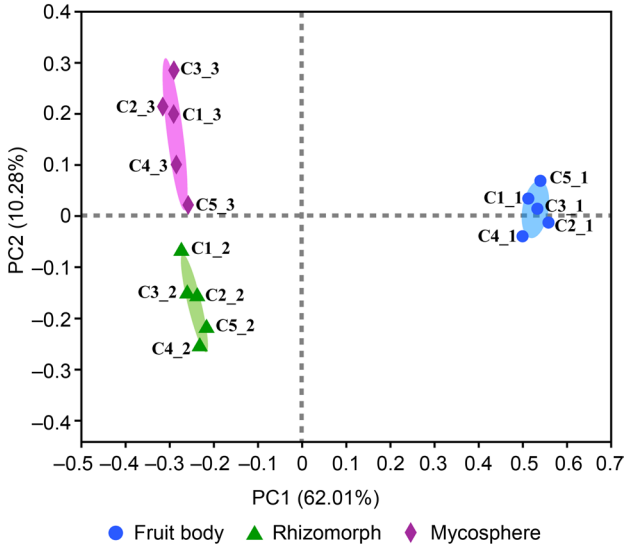


Fig. 4. Principal coordinate analysis (PCoA) of microbial communities in three sampled parts of *Cantharellus cibarius*. Circles, triangles, and diamonds represent the fruit bodies, rhizomorphs, and mycosphere, respectively. Distances between symbols on the ordination plot reflect relative dissimilarities in community structures.

sphere were similar but differed from the samples of the fruit bodies.

The shared and unique bacterial genera of fruit bodies, rhizomorphs, and mycosphere. The bar chart analysis revealed that there were 523, 527, and 470 bacterial genera in the fruit bodies, rhizomorphs, and mycosphere, respectively (Fig. 5). It indicates that the distribution of bacterial taxa at the genus level is relatively uniform. The Venn diagram analysis showed 264 bacterial genera were common to all three sample parts of *C. cibarius*. Fruit bodies and rhizomorphs shared fifty-three genera, 12 genera were common to both fruit bodies and mycosphere samples, and the rhizomorphs and mycosphere shared 176 genera. The results showed that the shared genera of rhizomorphs and mycosphere were similar but different from the fruit body.

Analysis of the unique genera of each site showed that there were 194, 34, and 18 unique genera in the fruit bodies, rhizomorphs, and mycosphere, respectively. This result indicated that differences in the fruit bodies were also reflected in the unique species composition compared with the rhizomorphs and the mycosphere.

Functional groups of the core bacterial taxa of fruit bodies and rhizomorphs. Based on the similarities in bacterial community composition between rhizomorphs and mycosphere, the fruit bodies and rhizomorphs were selected to identify members of the core bacterial taxa and predict potential functions. Visual comparison and analysis of the heatmaps showing the core bacterial taxa and potential functional group indicated that the potential functions of the core bacterial taxa from the fruit bodies were distinct from those of rhizomorphs. In addition to the unidentified taxa, the core bacterial taxa of fruit bodies comprised ten genera, including *Pseudomonas*, *Novosphingobium*, *Rhodococcus*, *Caulobacter*, *Hyphomicrobium*, and members of the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* complex, which belonged to 23 functional groups (Fig. 6a). The majority of the OTUs assigned to nitrogen-fixing bacteria belonged to the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* complex. At the same time, *Pseudomonas* participated in the nitrogen cycle (e.g., nitrate denitrification, nitrite denitrification, and nitrogen respiration). *Rhodococcus* had the potential function of aromatic compound degradation, hydrocarbon degradation, and aromatic hydrocarbon degradation. The results showed that most of the potential functions of the core bacterial taxa members in the fruit bodies were related to the nitrogen cycle, and degradation of aromatic compounds.

The core bacterial taxa of rhizomorphs included 35 genera (e.g., *Bryobacter*, *Mycobacterium,* and *Acidothermus*) belonging to 20 functional groups, which included aerobic chemoheterotrophy, chemoheterotrophy, intracellular parasites, predatory or exoparasitic, animal parasites or symbionts, and manganese oxidation (Fig. 6b). The result showed that the potential functional groups of the core bacterial taxa of the rhizomorphs were predominantly linked to nutrient mode and mineral decomposition.

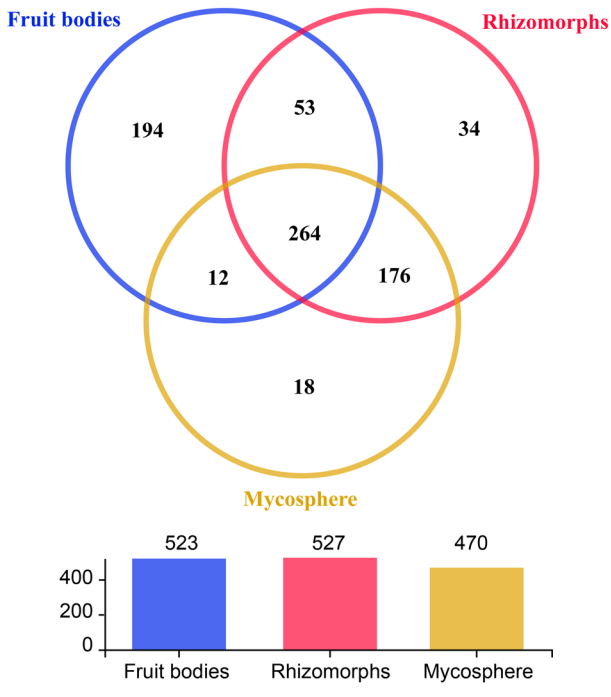


Fig. 5. Shared and unique genera in fruit bodies, rhizomorphs, and mycosphere of *Cantharellus cibarius*. The bar chart shows the total number of genera (shared and unique) in each sample.

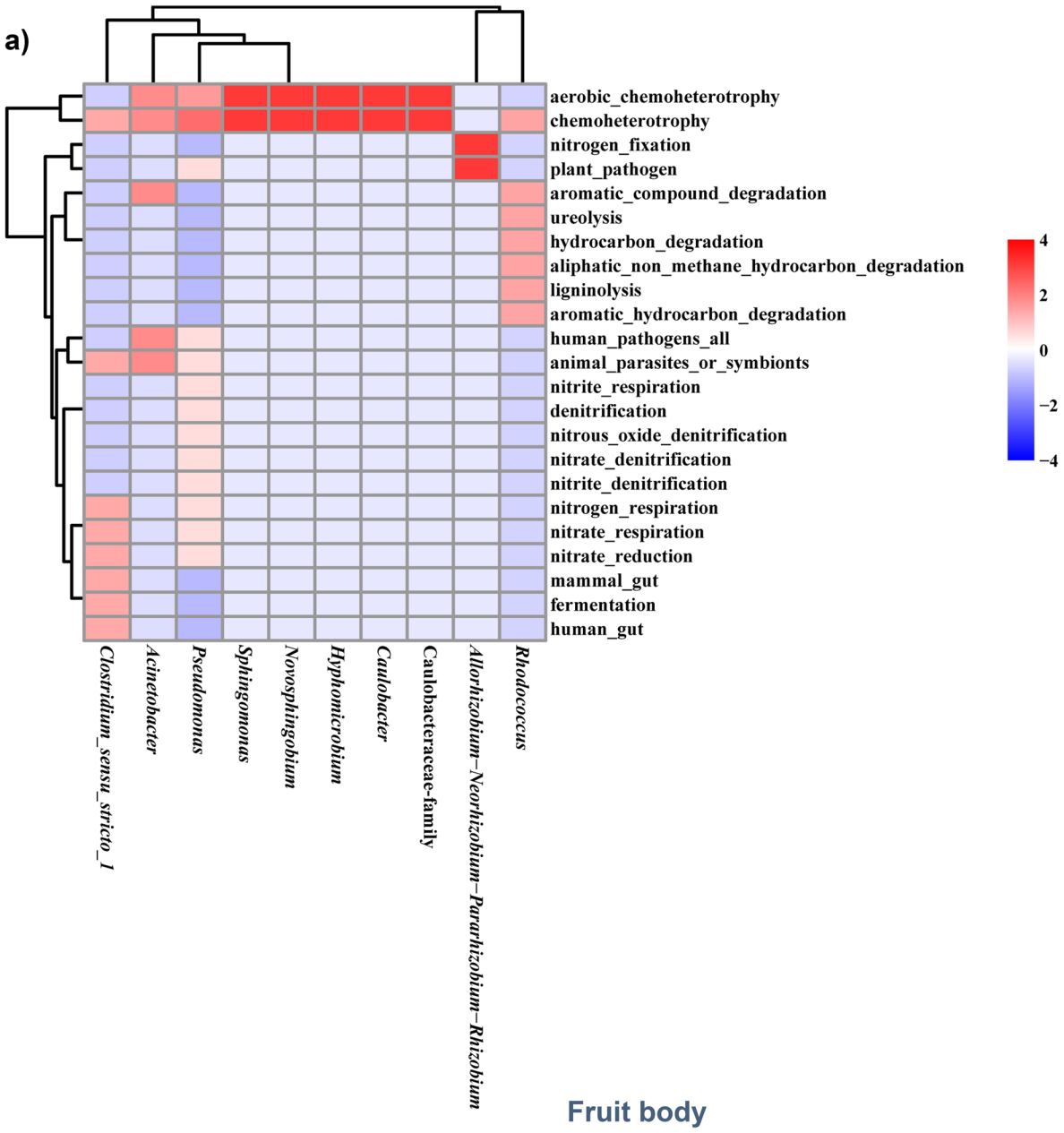


Fig. 6 (a) Heatmaps representing the differences in core bacterial group members and potential functions between a) fruit bodies and b) rhizomorphs of *Cantharellus cibarius*. The abscissa represents the members of the core bacterial group in each sampled part of *C. cibarius*, and the ordinate represents the potential functional types.

Discussion

This study revealed abundant bacterial taxa in both the fruit bodies and the rhizomorphs of *C. cibarius*. These taxa included 43 phyla, 98 classes, 243 orders, 427 families, and 751 genera. The comparison of bacterial community composition between fruit body and rhizomorph parts reflected apparent differences in the distribution of bacteria in the longitudinal structure of *C. cibarius*, which may be related to the selectivity of bacteria (Warmink et al. 2009). In addition, there are differences in bacterial community structure between fruit bodies and mycosphere, which is consistent with the results of Pent et al. (2017). Gohar et al. (2020) reported that the structure of bacterial communities in different internal parts of the stipe and cap were not significantly different. However, we chose the rhizomorphs in the lower part of the stalk and found significant differences between the rhizormorphs and fruit bodies. These differences may be caused by the close contact between the rhizomorphs and mycosphere. It further supported that the bacterial community in the lower part of the fruit body was closely related to the bacterial pool in the mycosphere (Gohar et al. 2020).

At the genus level, numerous members of the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*

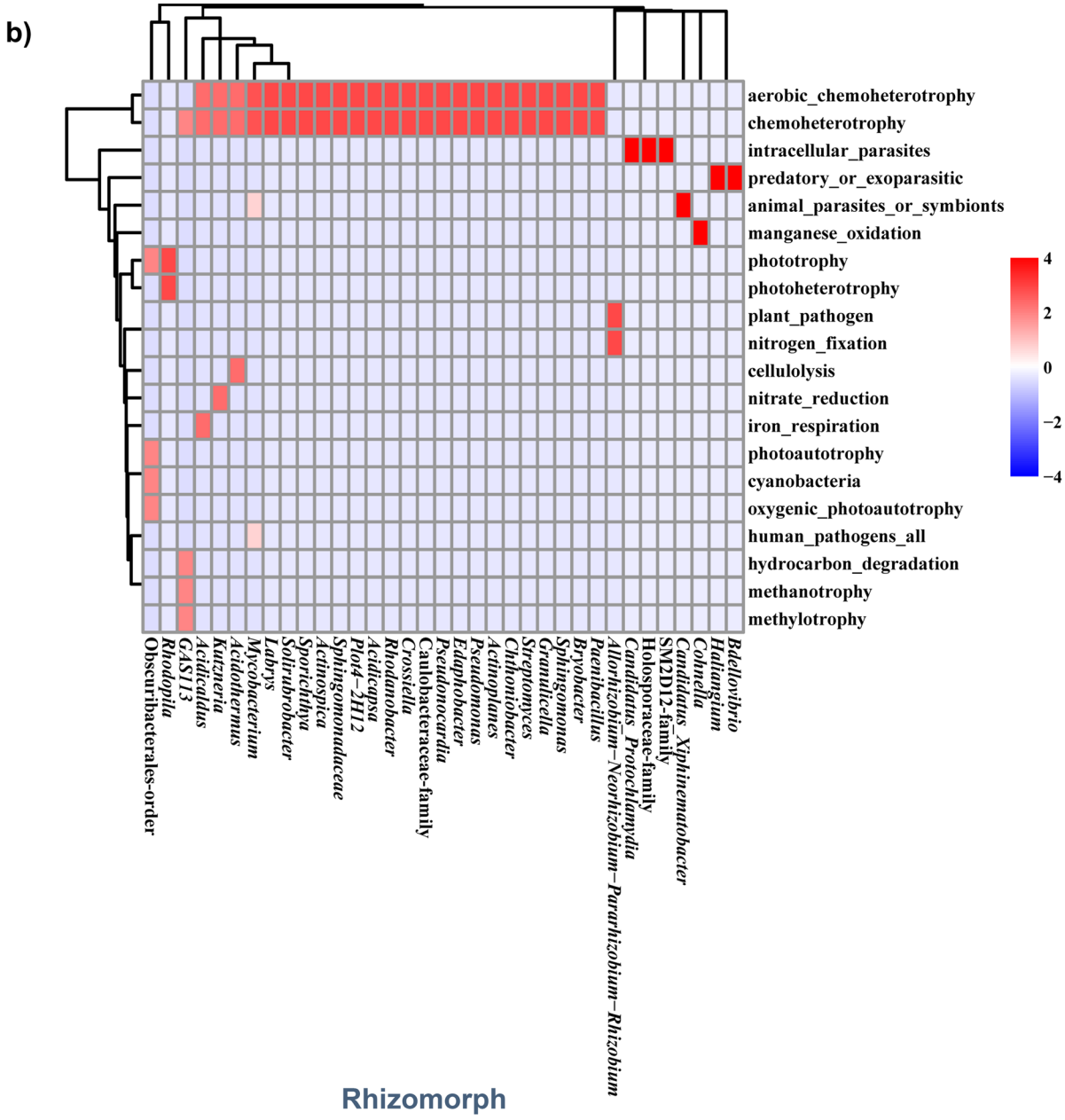


Fig. 6 (b) Heatmaps representing the differences in core bacterial group members and potential functions between a) fruit bodies and b) rhizomorphs of *Cantharellus cibarius*. The abscissa represents the members of the core bacterial group in each sampled part of *C. cibarius*, and the ordinate represents the potential functional types.

complex were distributed in the fruit bodies of *C. cibarius*, which was consistent with the observations of Pent et al. (2017, 2020), Rinta-Kanto et al. (2018), and Gohar et al. (2020). Together with other symbiotic bacteria, these genera may play a key role in maintaining the health of the fruit body (Gohar et al. 2020). Recently, Barbieri et al. (2005, 2010) have detected the bacterial genus *Bradyrhizobium* in truffles such as *Tuber borchii* and *Tuber magnatum*, and proved that this bacterial taxon was related to the nitrogen-fixation function in the fruit body. However, identifying the core bacterial taxa in the fruit body and rhizomorph samples in the current study revealed that members of the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* complex were still components of the core taxa of both these parts of *C. cibarius*. Through FAPROTAX function prediction, most of the OTUs with potential nitrogen-fixation function belonged to members of the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* complex. Although *C. cibarius* has a limited ability to absorb nitrogen sources (Rangel-Castro et al. 2002), there is a lack of evidence that the enrichment of members of the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* complex in the fruit body is directly related to the nitrogen fixation in *C. cibarius*. In addition, the functional groups of the core bacterial taxa in the fruit

body indicated that multiple bacteria had potential roles in nitrogen metabolism. Therefore, we speculated that the acquisition of nitrogen by *C. cibarius* might require the assistance of various symbiotic bacteria, and the specific mechanism still needs to be further explored.

In addition to the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* complex, dominant genera with high abundance in the fruit body included bacteria Magnetospirillaceae-family, *Chitinophaga*, and *Mucilaginibacter*. *Chitinophaga* was enriched in the old fruit body, could degrade the fruit body, and released organic compounds for the growth of other bacteria (McKee et al. 2019; Gohar et al. 2020). This activity may be related to the recruitment of fungi as well as the selection of bacteria. The Venn diagram indicated a high number of unique genera in mycosphere, which matched the "Fungipiles" preference of the fruit body (Warmink et al. 2009). Studies have shown that the characteristics of the soil may determine the initial selection of bacteria, especially the mycosphere (Warmink et al. 2009), as well as the characteristics of the fruit body, such as the presence of different metabolites and compounds, host identity, pH, and other factors (Danell et al. 1993; Pent et al. 2017). The current study focused on understanding the bacterial community structure in different parts of *C. cibarius* and did not test the related physical and chemical properties; consequently, further research is required in this area.

The genus *Rhodococcus* has the potential to degrade aromatic compounds (including aromatic hydrocarbons) and hydrocarbons. Saidi et al. (2016) reported that six strains of bacteria capable of producing aromatic odors were obtained from the strains of *C. cibarius*. Aromas synthesized by bacteria are of great biological significance for EMF, allowing the fungi to recruit specific microorganisms, defend against pathogens, and attract the animals for perpetuation through propagules or spores (Splivallo et al. 2011; Kanchiswamy et al. 2015; Splivallo et al. 2015; Ge et al. 2021). A research survey reported that every year in the forest, pathogenic microorganisms, insects, and other infections damaged 40–80% of mushrooms. In comparison, the proportion of damage to the fruit body of *C. cibarius* was less than 1% (Hackman and Meinander 1979). Interestingly, the fruit body of *C. cibarius* can persist for up to a month. In contrast, most of the other mushrooms usually start decaying within a few weeks or even days after production of the fruit body. Thus, it is tempting to speculate that the bacteria with the potential to degrade aromatic hydrocarbons may be related to the unique aroma of the *C. cibarius* and may play important roles in recruiting specific microorganisms, safeguarding the external health of the fruit body, and reproduction.

Many Acidobacteria and *Burkholderia*, which occurred mainly in rhizomorphs, are commonly found in forest soil. They were reported as significant players in mineral weathering (phosphate-solubilizing bacteria), the secretion of the involved in organic matter decomposition (ligninase and cellulase), and elemental cycling (Lepleux et al. 2012; Sun et al. 2014; Johnston et al. 2016). Some studies have shown that bacterial colonization of fungi lacking the ability to self-solubilize phosphorus could help the fungi effectively absorb phosphorus (Warmink et al. 2011; Nazir et al. 2012; Fontaine et al. 2016). The current study also found that members of the *Burkholderia-Caballeronia-Paraburkholderia* complex were distributed in fruit bodies, rhizomorphs, and mycosphere. The differences between these samples were not significant ($p > 0.05$). The discovery of these shared and unique genera among the sampled parts of *C. cibarius* is interesting. However, a significant limitation of the study is that the physicochemical properties of the fruit body, rhizomorph, mycosphere, and surrounding soil were not evaluated. Further studies should explore whether the colonization strategies of specific genera are associated with the growth and development of the *C. cibarius*.

Gohar et al. (2020) reported the changes of potential functional groups of bacterial communities at different developmental periods in the fruit body. For instance, the relative abundance of aerobic chemoheterotrophy and intracellular indices increased significantly in older fruit bodies, while the potential nitrogen-fixing function decreased significantly in the same samples. In contrast, we have compared the potential functions of bacterial communities in different structural parts of *C. cibarius* at the same developmental (middle-aged) period. We have found that the relative abundance of aerobic chemoheterotrophy and intracellular indices in the rhizomorphs was significantly higher than those in the fruit bodies. The potential nitrogen-fixing function was significantly lower in rhizomorphs than in the fruit bodies. Therefore, it can be speculated that the abundance and potential specific function(s) of the bacteria may differ not only in the different developmental periods of the same structure of *C. cibarius*, but also in the different structural parts at the same developmental period. In addition, the relative abundance of potential predatory or exoparasitic, animal parasites or symbionts were significantly increased in rhizomorph samples. However, only a few of these were found in fruit bodies. These phenomena indicate that the rhizomorphs may be more concentrated in protecting of the ideal habitat for the development of the fruit bodies and their symbiotic partners. This supports the symbiotic bacteria and fruit bodies to enhance the absorption and utilization of the mineral nutrients and complex organic compounds. The functions of fungus-associated bacteria should be further explored in future studies.

Conclusions

This study revealed differences in bacterial community composition, structures, and diversity in different parts of *C. cibarius*, and identified the members and potential functions of core bacterial taxa of fruit bodies and rhizomorphs from the perspective of the microbiome. There was an obvious trend of potential functional differentiation of related bacterial communities in the fruit body and rhizomorph: (i) the functional groups of the core bacterial taxa in the fruit bodies were more concentrated in nitrogen nutrition and aromatic compounds degradation to support their growth and development; (ii) bacterial taxa of the rhizomorphs preferred to decompose complex organic matter and absorb mineral nutrients, and this might prevent the infestation of pests and diseases in the soil environment to ensure the differentiation and growth of fruit bodies. The rhizomorphs were more like a "barrier" and "energy supply bank" for the healthy growth and development of fruit bodies. Therefore, it is necessary to understand EMF-associated microbiota's structure, dynamics, and function in rhizomorphs. Clarification is needed on the constituents of the *C. cibarius* holobiont and their effects on adaptability and morphology. This microbiota may facilitate understanding *C. cibarius* transition from the vegetative phase to the reproductive phase, thus maintaining normal growth and development. Although the prediction of the potential function(s) of the core bacterial taxa among the fruit body and rhizomorphs in this study may further highlight the specific role of bacteria in different parts of *C. cibarius*, the FAPROTAX functional prediction platform does have some limitations. Such drawbacks are that some bacteria do not belong to a single functional group. Many taxa still have numerous unexplored functions and data that have not been uploaded to the database (Louca et al. 2016; Sansupa et al. 2021), and the actual functions still need direct experimental proof. In the current study, further work is necessary to determine whether the extensive distribution of the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* complex in the fruit body is directly related to *C. cibarius* nitrogen fixation. Moreover, the reasons for the wide distribution of members of the *Burkholderia-Caballeronia-Paraburkholderia* complex in separate parts of *C. cibarius* need to be elucidated.

Acknowledgements

This work was financially supported by Key Areas of Research and Development Program of Guangdong Province (No. 2018B020205003), "Hundred" Talent Projects of Guizhou Province (Qian Ke He [2020] 6005), the Natural Science Foundation of China (No. 32060011) and Construction Program of Biology First-class Discipline in Guizhou (GNYL [2017] 009).

Author contributions

Yanfeng Han and Zongqi Liang conceived and designed the project, Wei Ge, Zhiyuan Zhang, and Chunbo Dong executed the experiments. Wei Ge wrote the paper with input from the co-authors, Zongqi Liang, Zhiyuan Zhang, and Chunbo Dong carried out the analyses. Sunil K. Deshmukh revised the English in the manuscript.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Barbieri E, Bertini L, Rossi I Ceccaroli P, Saltarelli R, Guidi C, Zambonelli A, Stocchi V. New evidence for bacterial diversity in the ascoma of the ectomycorrhizal fungus *Tuber borchii* Vittad. FEMS Microbiol Lett. 2005 Jun 1;247(1):23–35. https://doi.org/10.1016/j.femsle.2005.04.027

Barbieri E, Ceccaroli P, Saltarelli R, Guidi C, Potenza L, Basaglia M, Fontana F, Baldan E, Casella S, Ryahi O, et al. New evidence for nitrogen fixation within the Italian white truffle *Tuber magnatum*. Fungal Biol. 2010 Nov-Dec;114(11–12):936–942. https://doi.org/10.1016/j.funbio.2010.09.001

Calvaruso C, Turpault M, Leclerc E, Frey-Klett P. Impact of ectomycorrhizosphere on the functional diversity of soil bacterial and fungal communities from a forest stand in relation to nutrient mobilization processes. Microb Ecol. 2007 Oct;54(3):567–577. https://doi.org/10.1007/s00248-007-9260-z

Chen MM, Chen BD, Xu Y, Tian HY, Deng H. [Mycorrhizal fungi in bioremediation of petroleum-contaminated soil: A review] (in Chinese). Chin J Ecol. 2009 Jun 15;28(06):1171–1177. http://www.cje.net.cn/EN/abstract/abstract15380.shtml

Chen S, Zhou Y, Chen Y, Gu J. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018 Sep 1;34(17):i884–i890. https://doi.org/10.1093/bioinformatics/bty560

Danell E, Alström S, Ternström A. *Pseudomonas fluorescens* in association with fruit bodies of the ectomycorrhizal mushroom *Cantharellus cibarius*. Mycol Res. 1993 Sep 1;97(9):1148–1152. https://doi.org/10.1016/S0953-7562(09)80519-4

Danell E, Camacho FJ. Successful cultivation of the golden chanterelle. Nature. 1997 Jan 1;385(6614):303. https://doi.org/10.1038/385303a0

Danell E. Formation and growth of the ectomycorrhiza of *Cantharellus cibarius*. Mycorrhiza. 1994 Dec 1;5(2):89–97. https://doi.org/10.1007/BF00202339

Deveau A, Antony-Babu S, Le Tacon F Robin C, Frey-Klett P, Uroz S. Temporal changes of bacterial communities in the *Tuber melanosporum* ectomycorrhizosphere during ascocap development. Mycorrhiza. 2016 Jul;26(5):389–399. https://doi.org/10.1007/s00572-015-0679-7

Dong CB, Yao T, Zhang ZY, Chen WH, Liang JD, Han YF, Huang JZ, Deshmukh SK, Liang ZQ. Structure and function of bacterial microbiota in *Eucommia ulmoides* bark. Curr Microbiol. 2020 Nov;77(11):3623–3632. https://doi.org/10.1007/s00284-020-02157-2

Dong CB, Zhang ZY, Han YF, Liang ZQ. [Research and application prospect of core microbiome] (in Chinese). Mycosystema. 2019 Dec 25;38(01):1–10. https://doi.org/10.13346/j.mycosystema.180214

Dunham S M, Odell T E, Molina R. Analysis of nrDNA sequences and microsatellite allele frequencies reveals a cryptic chanterelle

species *Cantharellus cascadensis* sp. nov. from the American Pacific Northwest. Mycol Res. 2003 Oct;107(10):1163–1177. https://doi.org/10.1017/s0953756203008475

Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013 Oct;10(10):996–998. https://doi.org/10.1038/nmeth.2604

Fontaine L, Thiffault N, Paré D, Fortin JA, Piché Y. Phosphate-solubilizing bacteria isolated from ectomycorrhizal mycelium of *Picea glauca* are highly efficient at fluorapatite weathering. Botany. 2016 Jun 8;94(12):1183–1193. https://doi.org/10.1139/cjb-2016-0089

Ge W, Zhang ZY, Dong CB, Shao QY, Liu YX, Han YF, Liang ZQ. [Diversity and functional analysis of the culturable microbes isolated from the fruitbodies of wild *Cantharellus cibarius*] (in Chinese). Mycosystema. 2021 Mar 30;40(05):1054–1073. https://doi.org/10.13346/j.mycosystema.210044

Gohar D, Pent M, Põldmaa K, Bahram M. Bacterial community dynamics across developmental stages of fungal fruiting bodies. FEMS Microbiol Ecol. 2020 Oct 1;96(10):fiaa175. https://doi.org/10.1093/femsec/fiaa175

Hackman W, Meinander M. Diptera feeding as larvae on macrofungi in Finland. Ann Zool Fennici. 1979;16(1):50–83.

Haq IUI, Zhang MZ, Yang P, van Elsas JD. The interactions of bacteria with fungi in soil: emerging concepts. Adv Appl Microbiol. 2014;89:185–215. https://doi.org/10.1016/B978-0-12-800259-9.00005-6

Ji P, Parks J, Edwards MA, Pruden A. Impact of water chemistry, pipe material and stagnation on the building plumbing microbiome. PLoS One. 2015 Oct 23;10(10):e0141087. https://doi.org/10.1371/journal.pone.0141087

Jiang XT, Peng X, Deng GH, Sheng HF, Wang Y, Zhou HW, Tam NFY. Illumina sequencing of 16S rRNA tag revealed spatial variations of bacterial communities in a mangrove wetland. Microb Ecol. 2013 Jul;66(1):96–104. https://doi.org/10.1007/s00248-013-0238-8

Johnston SR, Boddy L, Weightman AJ. Bacteria in decomposing wood and their interactions with wood-decay fungi. FEMS Microbiol Ecol. 2016 Nov;92(11):fiw179. https://doi.org/10.1093/femsec/fiw179

Kanchiswamy CN, Malnoy M, Maffei M. Bioprospecting bacterial and fungal volatiles for sustainable agriculture. Trends Plant Sci. 2015 Apr;20(4):206–211. https://doi.org/10.1016/j.tplants.2015.01.004

Kumari D, Reddy MS, Upadhyay RC. Diversity of cultivable bacteria associated with fruiting bodies of wild Himalayan *Cantharellus* spp. Ann Microbiol. 2013;63(3):845–853. https://doi.org/10.1007/s13213-012-0535-3

Lemanceau P, Blouin M, Muller D, Moënne-Loccoz Y. Let the core microbiota be functional. Trends Plant Sci. 2017 Jul; 22(7):583–595. https://doi.org/10.1016/j.tplants.2017.04.008

Lepleux C, Turpault M, Oger P, Frey-Klett P, Uroz S. Correlation of the abundance of betaproteobacteria on mineral surfaces with mineral weathering in forest soils. Appl Environ Microbiol. 2012 Oct;78(19):7114–7119. https://doi.org/10.1128/AEM.00996-12

Liang ZQ, Han YF, Liang JD, Chen WH, Zhang ZY, Dong CB, Shao QY, Ge W, Liu YX. [From intracellular symbiosis to multiscale symbiosis: review and prospect] (in Chinese). Mycosystema. 2020 Oct;39(12):2202–2217. https://doi.org/10.13346/j.mycosystema.200269

Louca S, Parfrey L W, Doebeli M. Decoupling function and taxonomy in the global ocean microbiome. Science. 2016 Sep 16; 353(6305):1272–1277. https://doi.org/10.1126/science.aaf4507

Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011 Nov 1;27(21):2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Mao WH, Wu SL, Zhang X. [Establish and application of the high throughput sequencing method for soil microbia 16SrDNA using Ion Torrent PGM] (in Chinese). Acta Agric Zhejiangensis. 2015 Dec;27(12):2165–2170. https://doi.org/10.3969/j.issn.1004-1524

Marupakula S, Mahmood S, Finlay RD. Analysis of single root tip microbiomes suggests that distinctive bacterial communities are selected by *Pinus sylvestris* roots colonized by different ectomycorrhizal fungi. Environ Microbiol. 2015 May;18(5):1470–1483. https://doi.org/10.1111/1462-2920.13102

McKee LS, Martínez-Abad A, Ruthes AC, Vilaplana F, Brumer H. Focused metabolism of β-Glucans by the soil *Bacteroidetes* species *Chitinophaga pinensis*. Appl Environ Microbiol. 2019 Jan 9;85(2): e02231-18. https://doi.org/10.1128/AEM.02231-18

Mendes R, Garbeva P, Raaijmakers JM. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev. 2013 Sep; 37(5): 634–663. https://doi.org/10.1111/1574-6976.12028

Nazir R, Zhang MZ, de Boer W, van Elsas JD. The capacity to comigrate with *Lyophyllum* sp. strain Karsten through different soils is spread among several phylogenetic groups within the genus *Burkholderia*. Soil Biol Biochem. 2012 Jul;50:221–233. https://doi.org/10.1016/j.soilbio.2012.03.015

Oh SY, Fong JJ, Park MS, Lim YW. Distinctive feature of microbial communities and bacterial functional profiles in *Tricholoma matsutake* dominant soil. PLoS One. 2016 Dec 15;11(12):e0168573. https://doi.org/10.1371/journal.pone.0168573

Pent M, Bahram M, Põldmaa K. Fruitbody chemistry underlies the structure of endofungal bacterial communities across fungal guilds and phylogenetic groups. ISME J. 2020 Aug;14(8):2131–2141. https://doi.org/10.1038/s41396-020-0674-7

Pent M, Põldmaa K, Bahram M. Bacterial communities in boreal forest mushrooms are shaped both by soil parameters and host identity. Front Microbiol. 2017 May 10;8:836. https://doi.org/10.3389/fmicb.2017.00836

Rinta-Kanto JM, Pehkonen K, Sinkko H, Tamminen MV, Timonen S. Archaea are prominent members of the prokaryotic communities colonizing common forest mushrooms. Can J Microbiol. 2018 Oct;64(10):716–726. https://doi.org/10.1139/cjm-2018-0035

Saidi N, Deshaware S, Romdhane IB, Nadim M, Laribi M, Ltifi A, Kremer RJ, Shamekh S. Endogenous starter bacteria associated to chanterelle mycelia enhance aroma, color and growth of mycelia. IJEAS. 2016;9(3):58–65.

Salvioli A, Ghignone S, Novero M, Navazio L, Venice F, Bagnaresi P, Bonfante P. Symbiosis with an endobacterium increases the fitness of a mycorrhizal fungus, raising its bioenergetic potential. ISME J. 2016 Jan;10(1):130–144. https://doi.org/10.1038/ismej.2015.91

Sansupa C, Wahdan SFM, Hossen S, Disayathanoowat T, Wubet T, Purahong W. Can we use functional annotation of prokaryotic taxa (FAPROTAX) to assign the ecological functions of soil bacteria? Appl Sci. 2021 Jan;11(688):688. https://doi.org/10.3390/app11020688

Schloss PD, Gevers D, Westcott SL. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PLoS One. 2011;6(12):e27310. https://doi.org/10.1371/journal.pone.0027310

Schulz-Bohm K, Tyc O, de Boer W, Peereboom N, Debets F, Zaagman N, Janssens TKS, Garbeva P. Fungus-associated bacteriome in charge of their host behavior. Fungal Genet Biol. 2017 May; 102:38–48. https://doi.org/10.1016/j.fgb.2016.07.011

Segata N, Baldini F, Pompon J, Garrett WS, Truong DT, Dabiré RK, Diabaté A, Levashina EA, Catteruccia F. The reproductive tracts of two malaria vectors are populated by a core microbiome and by gender-and swarm-enriched microbial biomarkers. Sci Rep. 2016 Apr; 6:24207. https://doi.org/10.1038/srep24207

Splivallo R, Deveau A, Valdez N, Kirchhoff N, Frey-Klett P, Karlovsky P. Bacteria associated with truffle-fruiting bodies contribute to truffle aroma. Environ Microbiol. 2015 Aug;17(8):2647–2660. https://doi.org/10.1111/1462-2920.12521

Splivallo R, Ottonell S, Mello A, Karlovsky P. Truffle volatiles: from chemical ecology to aroma biosynthesis. New Phytol. 2011 Feb; 189(3):688–699. https://doi.org/10.1111/j.1469-8137.2010.03523.x

Stackebrandt E, Goebel BM. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Evol Microbiol. 1994; 44(4):846–849. https://doi.org/10.1099/00207713-44-4-846

Straatsma G, Bruinsma J. Carboxylated metabolic intermediates as nutritional factors in vegetative growth of the mycorrhizal mushroom *Cantharellus cibarius* Fr. J Plant Physiol. 1986a Oct;125(3–4): 377–381. https://doi.org/10.1016/S0176-1617(86)80161-4

Straatsma G, van Griensven LJLD. Growth requirements of mycelial cultures of the mycorrhizal mushroom *Cantharellus cibarius*. Trans Br Mycol Soc. 1986b Aug;87(1):135–141. https://doi.org/10.1016/S0007-1536(86)80013-4

Sun H, Terhonen E, Kasanen R, Asiegbu FO. Diversity and community structure of primary wood-inhabiting bacteria in boreal forest. Geomicrobiol J. 2014;31(4):315–324. https://doi.org/10.1080/01490451.2013.827763

Townsend BB. Morphology and development of fungal rhizomorphs. Trans Br Mycol Soc. 1954;37(3):222–233. https://doi.org/10.1016/S0007-1536(54)80004-0

van Elsas JD, Boersma FG. A review of molecular methods to study the microbiota of soil and the mycosphere. Eur J Soil Biol. 2011 Mar-Apr;47(2):77–87. https://doi.org/10.1016/j.ejsobi.2010.11.010

Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007 Aug;73(16):5261–5267. https://doi.org/10.1128/AEM.00062-07

Wang Y, Xu L, Gu YQ, Coleman-Derr D. MetaCoMET: a web platform for discovery and visualization of the core microbiome. Bioinformatics. 2016 Nov 15;32(22):3469–3470. https://doi.org/10.1093/bioinformatics/btw507

Warmink JA, Nazir R, Corten B, van Elsas JD. Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae. Soil Biol Biochem. 2011 Apr;43(4):760–765. https://doi.org/10.1016/j.soilbio.2010.12.009

Warmink JA, Nazir R, van Elsas JD. Universal and species-specific bacterial 'fungiphiles' in the mycospheres of different basidiomycetous fungi. Environ Microbiol. 2009 Feb;11(2):300–312. https://doi.org/10.1111/j.1462-2920.2008.01767.x

Warmink JA, van Elsas JD. Selection of bacterial populations in the mycosphere of *Laccaria proxima*: is type III secretion involved? ISME J. 2008 Aug;2(8):887–900. https://doi.org/10.1038/ismej.2008.41

Zhang CL, Yang FJ, Gui XM, Li XM, Bai CY. [Research status and prospect of the *Cantharellus cibarius*] (in Chinese). Trop Agric Sci Technol. 2010 Sep;33(03):35–39. https://doi.org/10.16005/j.cnki.tast.2010.03.007

Supplementary materials are available on the journal's website.