Bamboo wine brewing leads to high abundance of Basidiomycetes rather than Ascomycetes of the soil fungal community in Phyllostachys edulis (moso bamboo) forest

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Research Article

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Abstract

Purpose Environmental conditions have an important impact on soil microbial communities. This study aimed to explore the effects of special management of bamboo wine brewing on the soil fungal community structure and dominant flora in *Phyllostachys edulis* (moso bamboo) forest.

Methods In this experiment, the geometric center of moso bamboo forest was taken as the core, and three kinds of soils including the core area of bamboo forest (J1), non-bamboo forest area (J2) and marginal area of bamboo forest (J3) were selected as the research objects.The community structure and diversity of soil fungi and FunGuild function prediction were analyzed by high-throughput sequencing on IonS5^{TM}XL sequencing platform using ITS sequencing technology.

Results The results showed that: (1) in contrast to other bamboo forests and woodlands, the abundance of Ascomycetes in bamboo forest soil was higher than that of Basidiomycetes under bamboo wine making environment. (2) In the core area of bamboo forest J1, the soil fungal community complexity and species diversity were higher because of this special management. As samples gradually approached the core area of bamboo forest J1 from the non-bamboo forest J2, the dominant species of Basidiomycetes transitioned from *Saitozyma podzolica* to *Burgoa anomala*. Meanwhile, the dominant species of the Ascomycota shifted from *Gliocladiopsis forsbergii* to *Fusarium oxysporum* and *Cyberlindnera saturnus*. (3) The special bamboo forest management of bamboo wine brewing could reduce the abundance of plant pathogens and animal pathogens in bamboo forest soil, and *Saitozyma* and *Penicillium*, which had antibacterial effects, were important. As a result, the abundance of Fungal parasite-Undefinded Saprotroph (26.86%), Ectomycorrhizal (10.46%) and Ericoid Mycorrhizal (6.66%) increased significantly.

Conclusions Our study indicated that the special management of bamboo wine brewing could change soil fungal community structure and diversity by increasing Basidiomycete abundance and inhibiting soil pathogens in moso bamboo forest.

Introduction

The growth of plants depends entirely on indigenous microbial diversity (Fuke et al. 2021). As an important part of soil microorganisms, soil fungi play a unique and important role in maintaining plant productivity (Yang et al. 2022) and the balance of woodland ecosystems (Zhang et al. 2004), including Ascomycetes, Basidiomycetes, Zygomycetes, Chytridiomycetes and Glomeromycota, etc., among which *Cenococcum*, *Lactarius* and *Russula* are the dominant genus (Chen et al. 2019;Jiang et al. 2021) From the 5 top most frequent words extracted in the web of science by CiteSpace software on soil fungi and bamboo published in recent ten years, "*Arbuscular mycorrhizal* fungi" "soil microbial community" "diversity" "biomass" "nutrient use efficiency" were the most frequent, and related research mainly came from subtropical China (Chen et al. 2019; Gai et al. 2021; Xing et al. 2021). Although bamboo forest vegetation is relatively simple, there are more than 1100 fungi species that have been reported or described in the world, consisting of 630 species of Ascomycetes, 150 species of Basidiomycetes which

account for about 13% of the reported fungi in bamboo forests (Zhang 2009) and about 330 species of mitotic fungi (Dai et al. 2017), which accounting for about 13% of reported fungi in bamboo forests(Hyde et al. 2002). Ascomycota are thought to have an ability to tolerate maximum stress than the Basidiomycota(Zhang et al. 2020). Studies on *Phyllostachys iridescens* and *Phyllostachys glauca* forest also showed that Ascomycota accounted for the highest abundance (75.17%, 69.46%, respectively), followed by Basidiomycota (9.56%, 8.25%, respectively) (Fu et al. 2022). The *Phyllostachys edulis* (moso bamboo) is a common species of giant bamboo in China (Wenhui et al. 2019), and the abundance of Ascomycetes (53.1%-78.6%) was also higher than that of Basidiomycetes (14.8%-34.4%)(Ye 2020). However, the soil fungi in *Phyllostachys praecox* mainly included *Trichoderma* sp., *Trichoderma asperellum* and *Trichoderma hamatum*, *Hypocrea cordyceps*, etc., which belong to Deuteromycotina, Ascomycotina and Zygomycotina at the phylum level (Chen et al. 2016).

Bamboo wine is usually made of bamboo juice in China (Sangija 2020), but in our study it was obtained by infusing the base wine into the living bamboo cavity for fermentation. In the process of base wine injection and live bamboo wine extraction, the wine is easily mixed into the soil, which leads to the change of soil microbial community. Nevertheless, the effect of this special bamboo forest management on soil fungal community structure and diversity is still unclear. This paper aims to study the community structure and diversity of soil fungi in *Phyllostachys edulis* (moso bamboo) forest under wine making environment using high-throughput sequencing of the Ion S5^{TM}XL sequencing platform, explore the specific microbial flora, enrich the soil fungi resources of bamboo forest, and provide a theoretical basis for the development and sustainable development of bamboo forest resources.

Materials And Methods

Study Area and Site Description

The experimental site was located in the Bamboo Wine Brewing Ecological Garden, which was closed to the Southern Sichuan Bamboo Sea in Changning County of Sichuan Province in China.The bamboo wine brewing garden was founded in 1984. The experimental site is a mid-subtropical humid monsoon climate, warm and humid, with rain and heat in the same season. The local average annual temperature is 18.3°C, and an average annual precipitation is 1141.7 mm. The annual sunshine hours are 987.6 hours, and the annual frost-free period is 357 days. The climate and environment are suitable for bamboo growth. The park covers an area of 150 acres and more than 20,000 bamboo trees, which species are mainly *P. edulis* (accounting for more than 90%). The traditional brewing technique of bamboo wine in the ecological garden is an intangible cultural heritage of Changning County, with an annual output of more than 6,000 tons of high-quality bamboo wine.

Experimental design

The current experiment took the geometric center of the bamboo forest in the Bamboo Wine Brewing Ecological Garden as the core, selected points where the habitat factors were basically the same, and

used the five-point sampling method to collect the 0–15 cm topsoil which was 50–60 cm away from the bamboo root in the bamboo forest core area (J1) and edge area (J3). The soil in the adjacent non-bamboo forest area (J2) was used as a comparison. The soil samples were put into sterile sampling bags, and store at -85°C for high-throughput sequencing.

Determination of soil physical and chemical properties

Soil pH value (H_2O) was determined by pH acidity meter (PXS-450, Shanghai), with a soil to water ratio of 2.5:1. The soil moisture content was measured by drying method. Soil total organic carbon (TOC) and total nitrogen (TN) were determined by TOC total organic carbon analyzer (Elementar vario, Germany). The content of total phosphorus (TP) in soil was determined by sulfuric acid elimination - molybdenum-antimony method. The content of soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were determined by spectrophotometric method (Clever Chem 200, Germany). Soil available phosphorus was determined by Bray-1 reagent (0.03M NH_4F-0.025M HCl) molybdenum blue colorimetric method (soil and water ratio 1:4) on Clever Chem 200, Germany.

High-throughput sequencing

Total microbial DNA in soil samples were extracted with the FastDNA® Spin Kit for Soil kit. DNA integrity and concentration were detected by 1% agarose gel electrophoresis, Nanodrop and Qbit (od260/280 = 1.8). Using diluted genomic DNA as template, its sequencing primers were ITS5-1737F and ITS2-2043R, Phusion® High-Fidelity Polymerase Chain Reaction (PCR) Master Mix with GC Buffer and high efficiency high fidelity enzyme for PCR. The reaction system was 50μL, including 0.4 μM positive and negative primers respectively, 200 μM of four dNTP, 1.5 mM $MgCl_2$, 1U Taq DNA polymerase (Kapa biosystems, Boston, US). The reaction condition was pre-denaturation at 95 ℃ for 6 min, a total of 22 cycles, and 95℃ for 1min, 52 ℃ for 1 min, 72 ℃ for 1 min, 72 ℃ for 7 min. PCR products were detected by electrophoresis with 1.5% agarose gel and the target bands were recovered with QIAGEN MinElute® gel extraction kit (Cat.no. 28604, USA). The library was constructed using the Ion Plus Fragment Library Kit 48 rxns of thermofisher company. After the constructed library passed Qubit quantitative and library detection, it was sequenced and analyzed on the Ion S5^{TM}XL sequencing platform of thermofisher company of China Food Fermentation Industry Research Institute.

Data analysis

Excel 2020 and SPSS 26.0 were used for statistical analysis.

Results

Soil physical and chemical properties

Soil pH value in the sampling area was between 3.57 and 5.01, which had a significant difference between bamboo forest area and the non-bamboo forest area (Table 1). As the samples from the non-bamboo forest area (J2) and marginal area of bamboo forest (J3) gradually approached to the core area

of bamboo forest (J1), soil water content, total organic carbon (TOC) and NO_3^--N gradually decreased, and the difference between J2 and J1 was significant.

Table 1
Determination of soil physical and chemical properties in the study area

sample	pH	water content(%)	SOM (g/kg)	TN (g/kg)	TP (g/kg)	NO_3^--N(mg/kg)	NH_4^+-N(mg/kg)	P_{avi} (mg/kg)
J1	4.06 ± 0.17b	40.91 ± 1.98b	163.95 ± 8.15b	3.56 ± 0.14b	1.10 ± 0.05c	0.62 ± 0.03b	3.03 ± 0.13a	4.17 ± 0.20c
J2	4.76 ± 0.25a	47.78 ± 2.35a	183.45 ± 9.07a	6.82 ± 0.29a	9.90 ± 0.50b	0.84 ± 0.04a	2.12 ± 0.09b	5.16 ± 0.23b
J3	3.73 ± 0.16b	42.22 ± 2.01b	165.59 ± 8.26b	1.94 ± 0.07c	15.93 ± 0.71a	0.80 ± 0.04a	1.28 ± 0.06c	6.36 ± 0.33a

*Note: different lowercase letters in the same column indicated significant differences at the level of 0.05.

Fungal sequence validity and Venne diagram

In the study area, 252036 effective sequences were obtained. The average length of the sequences used for subsequent analysis was 225 ~ 233nt (Fig. 1*(a)*). The quality of sequencing data was mainly distributed above Q20 (Fig. 1*(b)*), which could ensure the normal progress of subsequent analysis.

Valid sequences were clustered according to 97% similarity (Fig. 2). Through clustering, a total of 718 fungal OTUs were obtained from the study area. The number of fungal OTUs in the core area of bamboo forest (J1), non-bamboo forest area (J2) and marginal area of bamboo forest (J3) were 463, 377 and 431, and the number of unique fungal OTUs was 144, 94 and 112, respectively. The number of co-ownership OTUs was 185 among the three plots. On the whole, the ratios of OTUs number and the unique OTUs number to total OTUs number were shown as lowest in J2 (29.66%, 7.40%, respectively), increased in J3 (33.91%, 8.81%), and highest in J1 (36.43%, 11.33%). The results indicated that both fungal number and endemic species increased when the sample gradually transitioned from non-bamboo forest area to the core area of bamboo forest.

Soil microbial community diversity

The Alpha diversity indices of soil fungal communities under the 97% consistency threshold were shown in Table 2. The Goods coverage indices of the three samples were closed to or equaled to 1, which indicated that the sequencing depth was reasonable and the species coverage was high. The number of observed fungal species increased from the non-bamboo forest area (J2) to the core area of bamboo forest (J1), and the lineage diversity index (PD whole tree) also showed similar results. It suggested that

the fungal community in J1 was more complex and had greater species diversity, and its fungal community diversity and uniformity were higher than those of the marginal area of bamboo forest (J3), however its species richness and the number of rare species were lower than those of J3.

Table 2
Analysis of sample species diversity

sample	Goods coverage	Observed species	PD Whole Tree	Shannon	Simpson	Chao1	ACE
J1	0.999	463	131.71	5.85	0.95	487.26	487.12
J2	1.000	377	93.55	5.06	0.92	387.48	387.40
J3	1.000	431	107.88	5.87	0.96	440.29	441.87

In order to reveal the differences in community composition among samples, the Beta diversity analysis was carried out (Fig. 3). The difference coefficient between J1 and J2 was the largest (1.242), followed by J1 and J3 (0.957), and then J2 and J3 (0.768). It indicated that compared with J2 and J3, J1 had a big difference in the fungal community structure, which was consistent with the cluster analysis results, indicating that the bamboo forest ecosystem had a unique fungal community structure.

Differences of soil fungal community structure and composition

The superiority communities of soil fungi in the study area were Ascomycota (relative abundance 61.21%) and Basidiomycota (37.56%) at the phylum level, and the total proportion was as high as 98.77%. The abundance of Kickxellomycota and Gloneromycota accounted for 0.8% and 0.43% respectively.

Based on the Weighted Unifrac distance matrix, two samples were compared to study the differences in fungal community composition between samples (Fig. 4). The non-bamboo forest area (J2) and the marginal area of bamboo forest (J3) were similar in soil fungal community structure, and they were grouped into one class. The difference between them and the core area of bamboo forest (J1) was great, which indicated that there was a relatively independent and unique community of soil fungi in J1. Ascomycetes were mainly distributed in J2, followed by J3, with the lowest abundance in J1. Basidiomycetes were mainly scattered in J1, that is, as the sample gradually approached the core area of bamboo forest, the dominant fungi of soil fungal community gradually changes from Ascomycetes to Basidiomycetes at the phylum level.

At the genus level (Fig. 5), the above transformation was shown by *Gliocladiopsis Ilyonectria Cylindrocarpon Fusarium* of Ascomycetes in non-bamboo forest area (J2), transit to *Fusarium Nectria Plectosphaerella Pseudeurotium Cylindrocarpon* of Ascomycetes and *Saitozyma* of Basidiomycetes in the marginal area of bamboo forest (J3), and then *Saitozyma*, *Laccaria*, *Burgoa*, *Auricularia*, *Crustoderma*

of Basidiomycetes and *Fusarium*, *Cyberlindnera*, *Penicillium, Oidiodendron* of Ascomycetes in the core area of bamboo forest (J1). In general, the fungal communities in the soil samples mostly had a common origin, and there was a certain evolutionary correlation between them at the genus level.

At the species level, the abundance of individual fungal populations had undergone significant changes, such as the unique dominant species *Gliocladiopsis forsbergii* (relative abundance 26.34%) and the dominant species *Aspergillus niger* (1.66%) of Ascomycota in the non-bamboo forest area (J2), transitioned to the unique dominant species *Nectria diminuta* (6.29%), *Monographella nivalis* (2.12%) and dominant species *Thelonectria rubi* (2.49%), *Gibellulopsis nigrescens* (1.23%) of Ascomycota in the marginal area of bamboo forest (J3), and then transformed into the dominant species *Saitozyma podzolica* (33.02%), *Burgoa anomala* (unique,3.28%) of Basidiomycota and *Fusarium oxysporum* (12.15%), *Cyberlindnera saturnus*(2.89%), *Gibberella acuminata* (1.23%) of Ascomycota in the core area of bamboo forest (J1) .

FunGuild function prediction

In order to further analyze the ecological function of fungal species in moso bamboo brewing forest, the function prediction of FUNGuild was carried out. According to the results demonstrated in Fig. 6, soil fungi were mainly saprophytic, symbiotic and pathological nutrition in study plots. Soil fungal functional flora in non-bamboo forest area (J2) were dominated by undefined saprophytes and unassigned fungi, followed by plant pathogen, plant pathogen-soil saprotroph-wood saprotroph, etc. Soil fungal functional flora in J2 were dominated by undefined saprophytes and unassigned fungi, followed by plant pathogen, plant pathogen-soil saprotroph-wood saprotroph, etc. As the sample gradually approached to the core area of bamboo forest, the marginal area of moso bamboo forest (J3) was dominated by unassigned fungi, and there were certain distributions of plant pathogen, undefined saprotroph, animal pathogen-endophyte-fungal parasite-lichen parasite-plant pathogen-wood saprotroph, endophyte-plant pathogen, etc. The core area of moso bamboo forest (J1) was dominated by fungal parasite-undefined saprotroph and unassigned fungi, and undefined saprotroph, ectomycorrhizal and ericoid mycorrhizal were distributed to a certain extent.

Discussion

Effects of bamboo wine brewing on soil fungal community structure

Consistent with other woodlands (Chen et al. 2019; Jiang et al. 2021), Ascomycetes and Basidiomycetes were also the dominant fungi in the core area of moso bamboo forest (J1) under bamboo wine brewing environment. However, their abundances were different from that of other woodlands. A study from Hangzhou, Zhejiang Province (Ye 2020) found that soil Ascomycetes abundance in the *P. edulis* forest was between 53.1% and 78.6%, followed by Basidiomycetes (14.8–34.4%), and there was a certain distribution of Zygomycota (1.2–5.4%) and Glomeromycota (1.4–8.7%). Another study on *P. edulis, P.*

iridescens and *P. glauca* from Chenshan in Shanghai (Fu et al. 2022) showed that the average abundance of Ascomycetes and Basidiomycetes were 77.4% and 8.8%, respectively, while Mortierellomycota (4.4%) and Glomeromycota (1.5%) were distributed to a certain extent. In this study, the abundance of Basidiomycetes (42.3%) in J1 was higher than that of Ascomycetes (35.2%) and the Mortierellomycota (5.23%) was also distributed in J1, which was consistent with *Phyllostachys vivax* f. *aureocaulis* forest (Li et al. 2020). Compared with the non-bamboo forest area (J2) and the marginal area of bamboo forest (J3), the fungal community structure in the core area of bamboo forest (J1) was significantly different. The alpha diversity index also showed that the unique management of bamboo wine brewing made the soil fungal community more complex and the species diversity greater in J1.

Effects of special management on dominant flora of soil fungi

Previous studies found that yeast and Ascomycetes are dominant fungi in the middle and late stage of Baijiu fermentation (Han et al. 2021a; Han et al. 2021b), and their complex metabolism had a significant impact on liquor fermentation (Ma et al. 2020). Soil yeast can protect plants from pathogens and promote the growth of plant roots, which has a significant ecological role in the community. Yeast *Saitozyma podzolica*, which can be used to extract oil, is a dominant species of Basidiomycetes in many regions (Moreira 2018; Gorte et al. 2020). Our study found that *Saitozyma podzolica* existed in moso bamboo wine brewing forest soil, and it was the main species in J1 (abundance of 33.02%). Moreover, *Cyberlindnera* and *Penicillium* of Ascomycetes were also dominant species in J1, which might be related to the unique environment of bamboo wine brewing, or could effectively inhibit the distribution of plant pathogens and animal pathogens in bamboo soil.

Fungal pathogens can directly affect plants, such as reducing their growth and productivity, or even killing them (Hodge 2013).Previous studies considered that adult plants attracted host-specific enemies to enhance the performance of heterospecific relative to conspecific seedlings in their own surroundings (Daniel 1970). Mycorrhizal fungi form symbiotic associations with plant roots, contributing to host plant nutrient uptake (Yang et al. 2022). The results of FunGuild function prediction also indicated that the special management of bamboo wine brewing reduced the abundance of soil animal pathogens and plant pathogens, and increased the abundance of Fungal Parasite-Undefinded Saprotroph (26.86%), Ectomycorrhizal (10.46%) and Ericoid Mycorrhizal and other communities in J1. For example, the *Laccaria* which was an important Ectomycorrhizal fungus since it was discovered by Berkeleyin 1883 (Yin 2012;Mu 2015) was also widely distributed in J1.

In addition, the *Bulgoa anomala*, which was first reported in the Kitora Tumulus, Japan in 2010, and belonged to Cantharelloid branch together with isomorphic *Sistotrema* (Kiyuna et al. 2015), was a unique species with its abundance accounting for 3.28% in J1. That is, the special management of bamboo wine brewing made the forest soil fungal community unique.

Conclusions

As the samples gradually approached the core area of moso bamboo forest (J1) from the non-bamboo forest area (J2), *Fusarium oxysporum* and *Cyberlindner saturnus* turnded into the main species of Ascomycetes while *Saitozyma podzolica* and *Burgoa anomala* became the dominant species of Basidiomycetes in bamboo brewing environment. The yeast *Saitozyma podzolica* was found to exist in the moso bamboo forest under this unique environment, and *Burgoa anomala* was the unique dominant species in this special environment. In addition, the bamboo wine brewing could reduce plant pathogens and animal pathogens, increase Fungal Parasite and Mycorrhizal in moso bamboo soil, and benefit to the healthy growth of bamboo.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors agreed to publish this research (including any individual details, images or videos)

Availability of data and materials

The authors declare that [the/all other] data supporting the findings of this study are available within the article [and its **supplementary information** files].

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper "**Bamboo wine brewing leads to high abundance of Basidiomycetes rather than Ascomycetes of the soil fungal community in Phyllostachys edulis (moso bamboo) forest**".

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Authors' contributions

All authors contributed to the study conception and design. The first draft of the manuscript and the drawing of the diagram were completed by **Yan Peng** and all authors commented on previous versions of the manuscript. Manuscript writing guidance, the first draft revision was completed by the **Wei Jiang ,Cheng Yang** and **Jian Zhu**. Experimental operation, data collection and sample collection were performed by **Wenkai Deng, Jianru Su, Jie Wei** and **Tingting Li.**

All authors read and approved the final manuscript.

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Figures

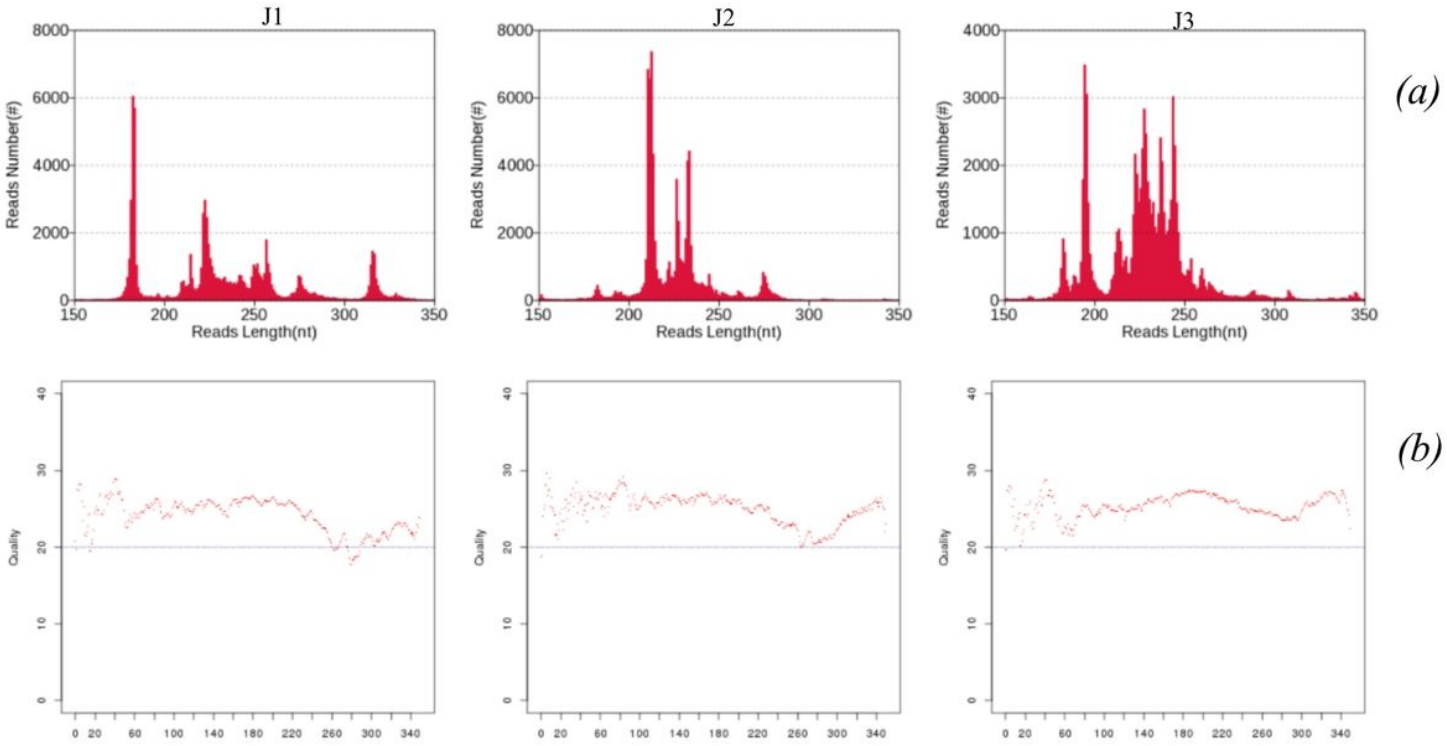

Figure 1

Length *(a)* and quality *(b)* distribution of sample sequencing sequences

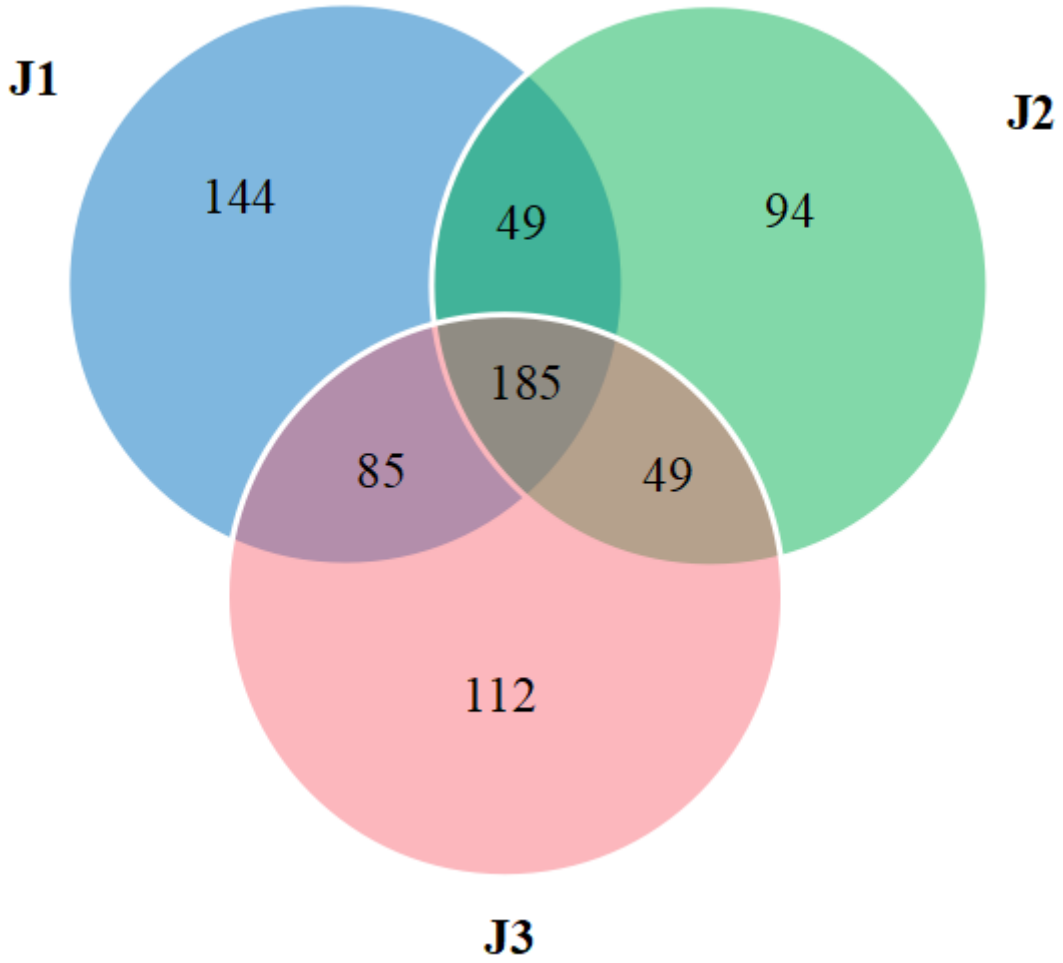

Figure 2

Venn diagram based on OTUs

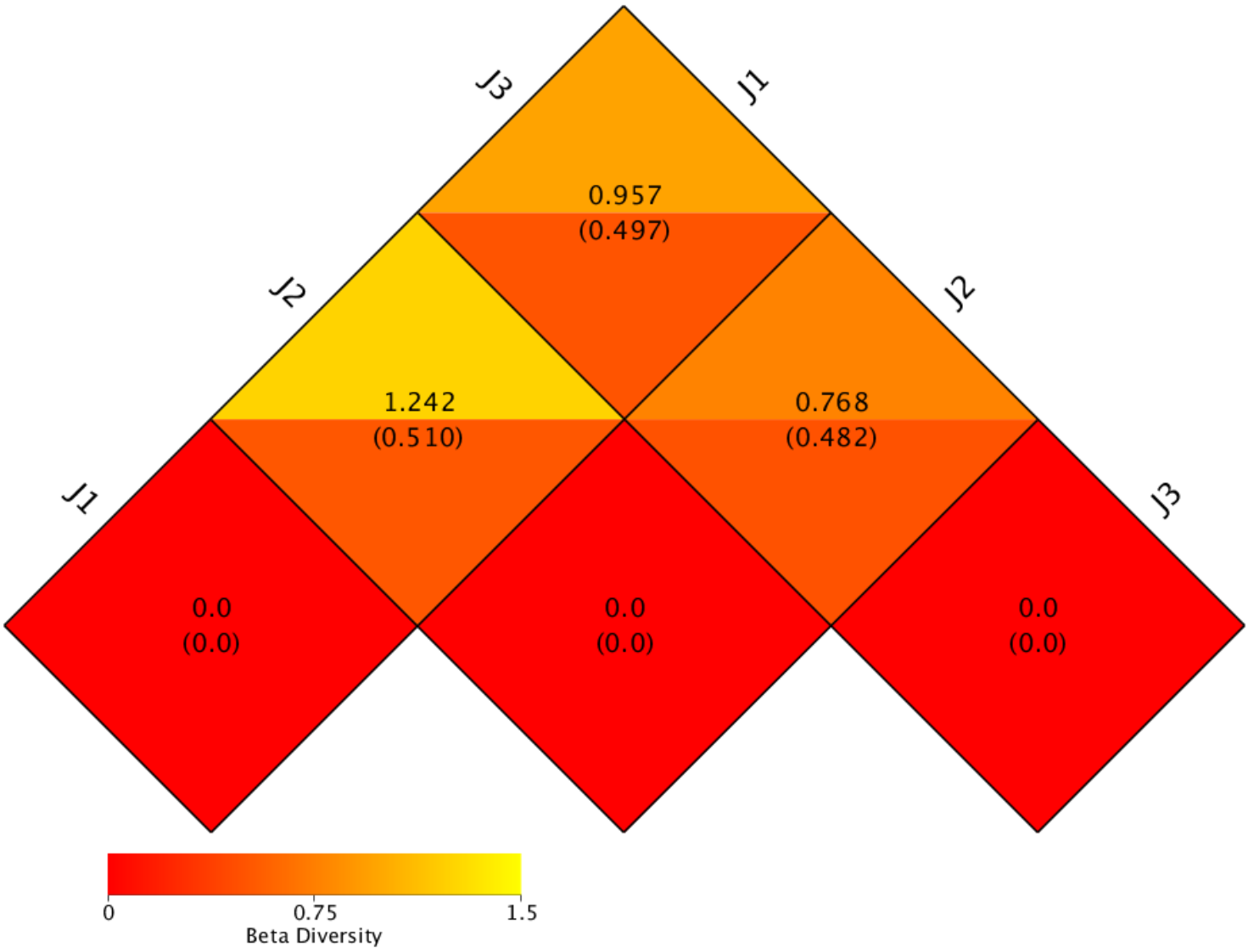

Figure 3

Heat map of beta diversity index

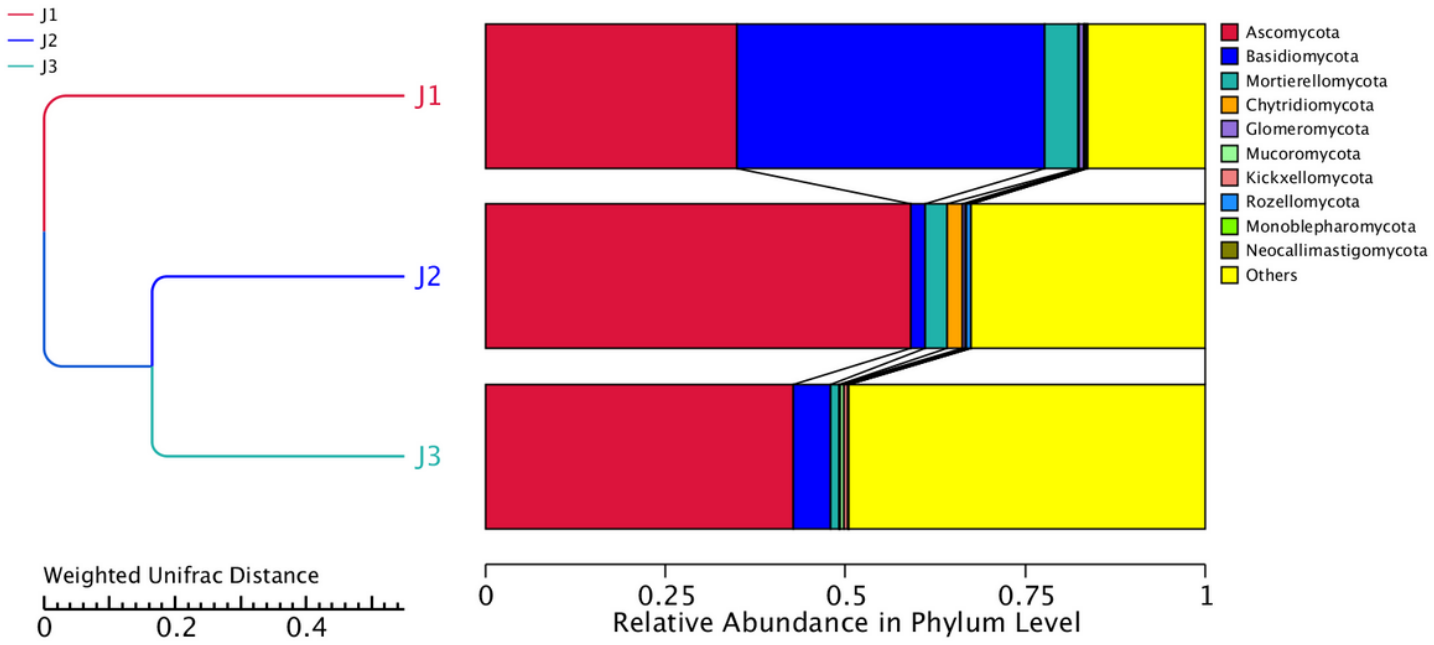

Figure 4

Cluster analysis at the phylum level

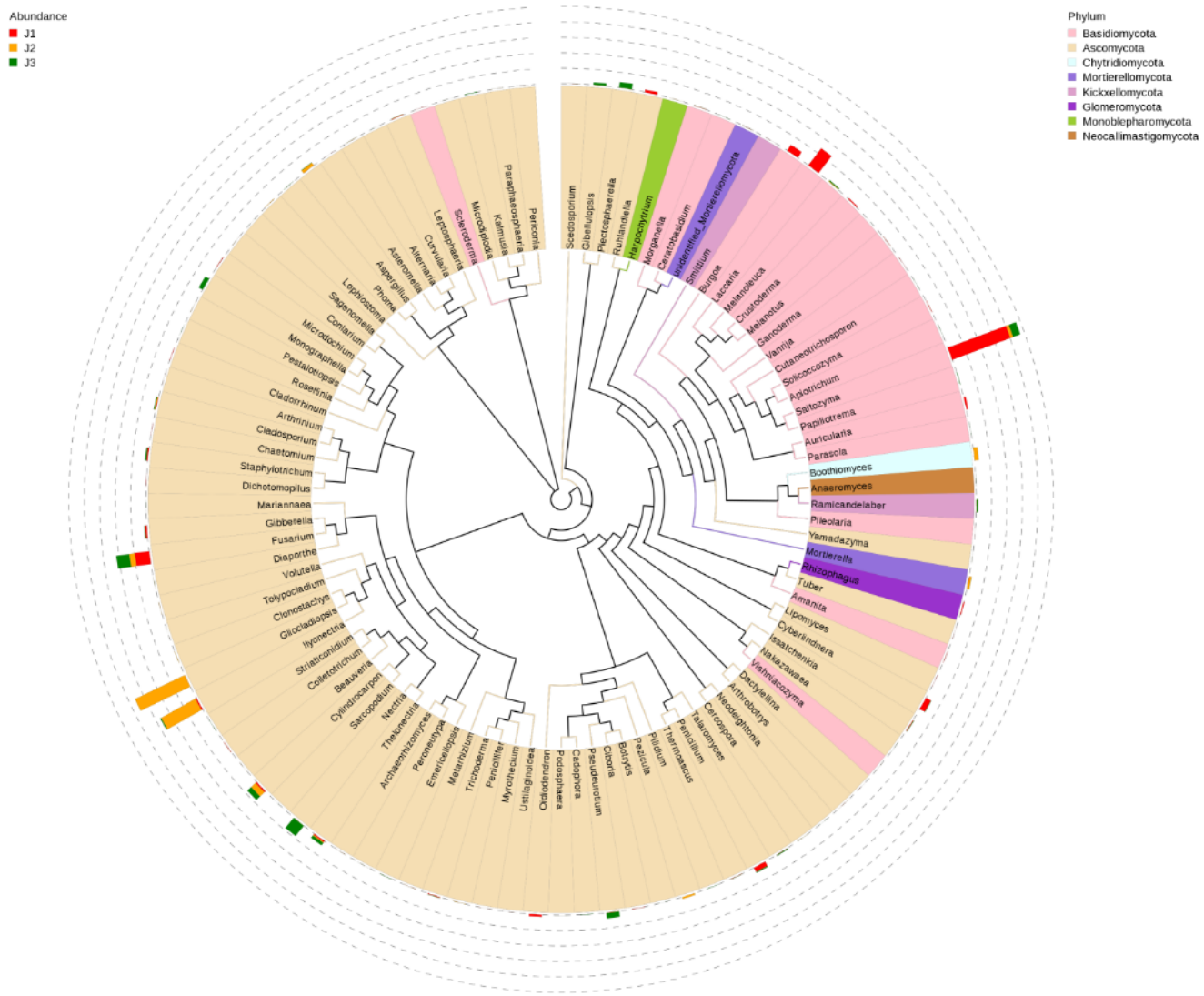

Figure 5

Evolutionary tree analysis of Top100 species at genus level

*Note: The color of a branch represents its corresponding phylum, and each color represents one phylum.

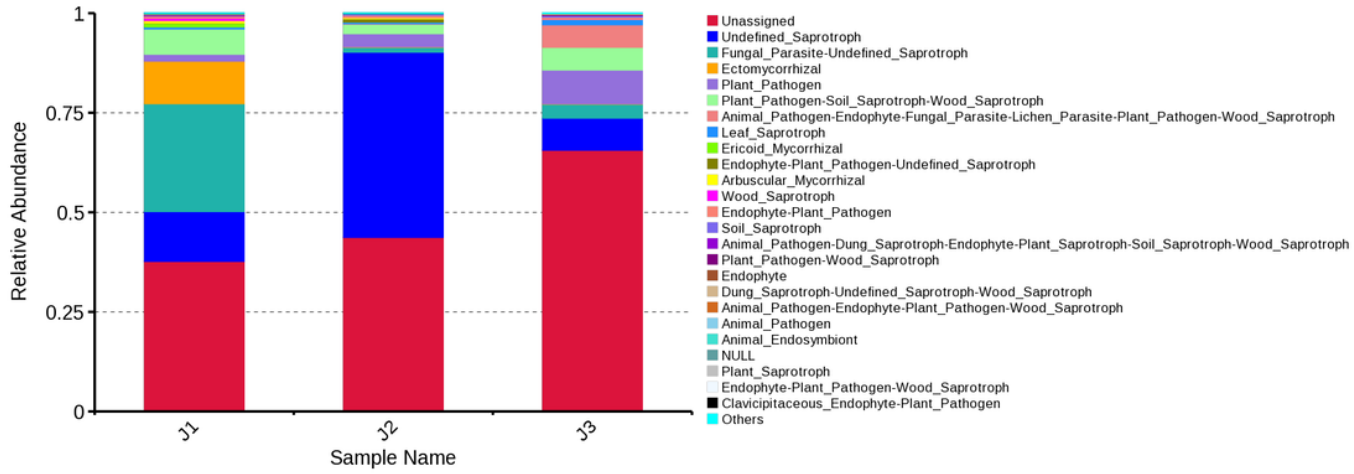

Figure 6

Relative abundance histogram of FunGuild function prediction

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 5SupplementaryMaterial.docx