

Analysis of fungal diversity of the rotten wooden pillars of a historic building

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Abstract

High-throughput sequencing technology was used to analyze the fungal community structure and its association with the cause of decay on the wooden pillars of an ancient archway in Beijing. The dominant fungi on the rotten pillars belonged to Ascomycetes regardless of the sampling position. Compared with the fungal community composition of discolored wood previously studied, the proportion of Basidiomycetes in rotten wood pillars increased at the highest value of 37.9%. High-throughput sequencing showed that the main fungi in the first pillar were Ascomycetes (*Phoma* , *Lecythophora* , and *Scenedosporium*) and Basidiomycetes (*Sporidiobolales*). Ascomycetes *Lecythophora* and Basidiomycetes *Cryptococcus* and *Postia* were the main fungi in pillar 2. *Phoma* , *Trichoderma*, and *Entoloma* were isolated from pillar 1, whereas *Alternaria* and *Phaeosphaeriaceae* were obtained from pillar 2 using culture isolation. Traditional isolation failed to obtain all dominant fungi. The importance of high-throughput sequencing technology in ancient wooden structure building biodeterioration analysis was further explained. At the three sampling sites, the contact-ground fungal community composition was similar to that of in-ground wood, whereas above-ground fungal community composition was significantly different from the other two sites. The high moisture content of the wood caused decay. The bottom of the pillar was immersed in groundwater, whereas that cement coating prevents the evaporation of water, cause the wood moisture content to be high. By comparing the fungal diversities of decaying wood and discolored and dry, decayed wood, the relative content of Basidiomycetes may be used as an indicator of wood decay state.

Introduction

Historical buildings, as landmarks of culture and diversity, could be conserved, reused, and be expected to deliver a good shape for future generations [1]. As Chinese ancient buildings are characterized by wooden structures, wood biodeterioration and protection are important concerns. Proposed effective maintenance projects for ancient wooden structures require information on wood deterioration degree. In the past, visual inspection and tapping methods were used, and they were later replaced several non-destructive or semi-destructive evaluation methods, such as the Pilodyn Wood Tester, Ultrasonics and stress wave technology, [2–5]. However, none of these tests revealed the cause of biodeterioration.

The most serious biological deterioration of wood is caused by fungi. Fungi not only cause serious decay of timber but can also spread through buildings from one timber location to another across non-nutritional surfaces, thus seriously affecting historical buildings [6]. Fungi have both direct and indirect effects on the health of building materials, structures, and occupants; thus, “building mycology” is defined as the branch of mycology dealing with the study of fungi in and around built environments [7, 8].

Traditional methods for identifying wood fungi include isolation of fungi from degrading building timbers and identification of fungal species based on culture characteristics. Several molecular methods based on DNA analyses have been used to provide efficient, sensitive, and rapid diagnostic tools for the detection and identification of wood decay fungi without requiring a prior fungal isolation step [9–12].

Wood decay is caused by various fungi. Microbial diversity has been traditionally assessed by cultivation techniques. The biodiversity of forest fungi can serve as an indicator of forest health [10]. The relationship among microbial diversity, environmental factors, and wood determines the degradation rate of timbers [13, 14]. Kahl et al. observed that the activity of wood-degrading enzymes laccase and endocellulase and fungal diversity are the most important predictors for wood decay when applying a multi-model inference approach [15]. Der Wal et al. and Pastorelli et al. reported that two basic parameters, namely, microbial community structure and diversity, are good indicators to predict the rate of wood mass loss and determine the rate of wood decay [16–18].

The two essential parameters of fungal diversity, namely, species richness (the number of species within a community) and species evenness (the sizes of species populations within a community), can be detected using amplicon high-throughput sequencing, which is the best method for in-depth analysis of fungal community composition [19, 20]. Amplicon high-throughput sequencing, also known as next-generation sequencing, provides the best understanding of microbial community diversity on any substrate, including building timbers [21–23]. Another advantage of amplicon high-throughput sequencing method is that testing can be conducted with as low as 0.1 g wood sample, minimizing the damage to in-service building timbers [22].

Experiencing more than 200 years of wind and rain, a historical memorial archway in Beijing has decayed to different degrees. With a history of almost 40 years, the two large round wood pillars buried in the ground were eventually replaced. Serious decay occurred in the pillars. Thus, the degree of decay and the assessment of decayed organisms should be carried out. In this study, a small amount of sawdust was sampled from the contact-ground wood (HC) pillar, 100 cm in-ground wood (HUP), and 100 cm above-ground wood (HD), and microbial DNA was directly extracted from the sawdust. High-throughput sequencing was used to analyze the fungal structure composition and the cause of decay on the wooden pillars to provide basis for the analysis of wood decay degree and disease development in historical buildings.

Materials And Methods

On-site inspection and sampling

The decay on sample location was observed, and the moisture content of the rotten pillar was measured using JINGTAI wood moisture meter SD-C50. Routine desk survey was performed. A more than 200-year-old decayed old wood beam of the ancient building was selected as the control (G6) (Fig. 1). Small pieces of wood (0.1 g) were sampled by knife aseptically.

Fungi isolation

The small wood chips detached from each of the five samples were surface-sterilized and then placed onto 2% agar plates. The plates were incubated at constant temperature and humidity incubator at 28 ± 10 °C. Fungal isolation and purification were conducted as described by Fang [24].

Fungal DNA extraction, polymerase chain reaction (PCR), amplicon quantification, MiSeq high-throughput sequencing

Sample processing, microbial genomic DNA extraction from wood, PCR protocol, and MiSeq high-throughput sequencing testing were conducted in accordance with the work of Ma et al [22]. The fungal internal transcribed spacer (ITS) region primer pairs were ITS1-F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') /ITS2 (5'-GCT GCG TTC TTC ATC GAT GC-3') [25,26].

PCR, amplicon quantification, and MiSeq high-throughput sequencing test were performed thrice.

Bioinformatics analysis

Trimmomatic, Pear (v1.10.5) and Flash (v1.2.10) software were used for bioinformatics analysis. The sequences were clustered into operational taxonomic units (OTUs). Clustering was conducted by Uchime (v4.1) based on 97% pairwise identity. Qiime was used as open-reference OTU picking strategy (<http://www.qiime.org>).

Results And Discussion

On-site inspection and sampling

The ancient memorial archway in Beijing was established more than 200 years ago. The two large, round wooden pillars buried in the ground were numbered No. 1 and No. 2. They were nearly 40 years old and seriously decayed and replaced in a later stage (Fig. 1). The wood species of No. 1 and No. 2 pillar were *Pseudotsuga menziesii* and *Picea* sp., respectively, according to maintenance records. The in-ground part was covered in cement, and the pillar base was submerged in underground water. The in-ground wood was seriously decayed. The sampling was conducted on in-ground, decaying wood (HUP); contact-ground, decaying wood (HC); and above-ground wood without progressing decay (HD). Samples from No. 1 pillar were coded as HC1, HUP, and HD1, and those from No. 2 pillar were HC2 and HD2. The buried part of No. 2 pillar had been cut off during the second maintenance, and the in-ground foundation was connected to the cement pillar. Thus, only the contact-ground and above-ground samples were available. The moisture content was measured at the corresponding sampling location. At least three points were measured in each location, and the average was obtained. All data are shown in Table 1.

Table 1 shows that the moisture content at the bottom of the wood pillar was extremely high (average moisture content: 56.3%). The contact-ground wood moisture content was also notably high and exceeded 20%, but that above-ground was less than 14%. The average moisture content of the control ancient wood (dry and rotten wood) was notably low at 8.9%. Mold infections start with 14% wood moisture content, whereas wood decaying fungi need more than 20% to initiate decay [27]. The moisture content in contact-ground and in-ground wood is suitable for the growth of decaying fungi and occurrence of wood decay.

What caused the high moisture content of the in-ground and above-ground wooden pillars? During on-site observation, the bottom of the wood pillar was soaked in ground water, whereas the in-ground pillar was encased in cement. The water at the bottom of pillar originated partly from underground water or accumulated rain, whereas the cement coating prevented water evaporation.

Fungal isolation

By traditional tissue isolation and purification, one species of mold *Phoma* sp. was isolated from HC1, *Trichoderma atroviride* from HUP, and *Entoloma* from HD1. Meanwhile, two species of Ascomycetes *Alternaria* and *Phaeosphaeriaceae* were isolated from HC2 of No. 2 pillar. However, no fungus was isolated from HD2 (Fig.2). *Entoloma* is a decaying fungus, whereas the rest are molds.

Microbial diversity estimation on five samples

α -Index diversity analysis

Table 2 sums up the richness and diversity estimation of fungal ITS sequencing libraries from the MiSeq sequencing test. The total numbers of fungal ITS reads obtained with clean tags from sample HC1, HUP, and HD1 were 39962, 31442, and 30700, respectively, which were clustered into 115, 122, and 95 OTUs, with almost 0.97 similarity in nucleotide identity. The total numbers of fungal ITS from samples HC2 and HD2 reached 28637 and 41029, which were clustered into 134 and 182 OTUs, respectively.

Good's coverage of six samples were close to 100%, with the mean obtained from the full sequence. The community diversity indicator Shannon index was 1.9–3.9, indicating the relatively simple fungal community composition of the sample. The abundance index Chao1 was 103.02–294.51, which was used to evaluate the community richness, and the value of phylogenetic diversity (PD_{whole tree}) was 21.89–69.39 (Table 2).

Fungal composition analysis

The community structure and relative abundance of the five samples in different taxonomic levels (phylum, class, order, family, and genus) are shown in a colored column chart (Fig. 3). Fig. 3 and Table 3 show that the dominant fungi were Ascomycota, followed by Basidiomycetes. Basidiomycetes accounted for the largest proportion (37.86%) in HC2. The second highest proportion was observed in HD2 at 27.02%. Similar to the control G, the proportion of Ascomycota in HD1 reached more than 90%, whereas that of Basidiomycetes approximated 5%. Compared with the microbial diversity structure of the discolored wood, the overall proportion of Basidiomycetes in rotten wood pillars increased but was less than 10% in the discolored wood [22].

Basidiomycetes are the main degradation fungi of wood decay. The proportion of Basidiomycetes is a possible indicator to predict whether ancient wood decay progresses. Further research is needed to prove this assumption.

Figure 4 shows the microbial structure composition of samples from three different sampling locations at the phylum and genus levels. The microbial structure composition of contact-ground wood (C) is similar to that of in-ground wood (UP), whereas that of above-ground wood (D) differs from the other two positions. The proportions of Ascomycota in the contact-ground and in-ground woods were about 74%, whereas the proportion of Basidiomycota reached 25.08% in contact-ground wood and 24.03% in in-ground wood. At the genus level, Lecythophora, Phoma and Cryptococcus were the main fungi, and their proportions slightly differed at 25.25%, 34.15%, and 10.77%, respectively, in the contact-ground wood, and 50.8%, 9.16%, and 3.32% in the above-ground wood.

Fig. 5 shows the fungal structure composition of two pillars at the phylum and genus levels. The wood species of No. 1 pillar was *Pseudotsuga menziesii* (Mirbel) Franco, and that of No. 2 pillar was *Picea* sp (Table 1). Both were coniferous wood. No. 1 pillar comprised 83.9% Ascomycota and 15.23% Basidiomycota. For No. 2 pillar, Ascomycota accounted for 67.4%, and Basidiomycota amounted to 31.88%. Among the fungal species that can be identified, Lecythophora was dominant at the genus level. The proportions reached 24.44% and 12% in pillars 1 and 2, respectively. *Scedosporium* accounted for the second highest content in pillar 1 (21.26%). The third was *Cryptococcus* (3.18%), which accounted for 7.46% in No. 2. *Postia* (6.21%) was also observed at a high proportion in No. 2 pillar.

Principal component analysis (PCA) and cluster tree

The relationship among the samples could be analyzed by PCA (Fig. 6) and cluster tree analysis (Fig. 7) based on the composition of each sample OTU. The closer the position in the PCA figure, a more clustering relationship occurs, and the more similar composition of samples. Fig. 7 shows that PC1 factor reached 53.78%, and PC2 totaled 28.82%. Three sequencing tests have shown excellent identity (Fig. 7). The cluster tree showed the close relationship between HC1 and HC2, whereas HC (HC1+HC2) and HUP exhibited a closer relationship. HD (HD1+HD2) was distantly located from HC and HUP, consistent with fungal composition analysis (Fig. 4).

Venn diagram and core microbiome analysis

Venn diagrams showed the common and exclusive fungal OTUs of the different wood samples (Fig. 8). A total of 23 OTUs were observed in all wood samples. Meanwhile, 75 OTUs were detected in three different locations, and 168 OTUs were identified in different pillars.

Core microbiome analysis of five samples showed 23 OTUs (Table 4). Two OTUs were identified at the phylum level, in which OUT_4 belonged to Ascomycota and OUT_8 to Basidiomycota. OTU_70 was identified under order Pleosporales. Five OTUs were identified at the family level. Fifteen OTUs were identified at the genus and species levels. The OTUs comprised *Lecythophora* sp. W3a2, *Aspergillus cibarius*, *A. subversicolor*, *A. caesiellus*, *Rhodotorula mucilaginosa*, *Aureobasidium pullulans*, *Cryptococcus albidus*, *Epicoccum* sp. NFW7, *Cladosporium*, *Alternaria*, *Fusarium*, *Cladophialophora immunda*, *Podospora ellisiana*, *Ilyonectria macrodidyma*, and *Kernia pachypleura*. Two species belonged to Basidiomycota (*R. mucilaginosa* and *C. albidus*), and the other thirteen OTUs all belonged to

Ascomycota. *Aspergillus* spp., *Fusarium* sp., *Aureobasidium pullulans*, *Trichoderma* sp., *Alternaria* sp., and *Cladosporium* sp. were the common mold fungi reported previously [22, 28-29].

Dominant fungal species analyzed by MiSeq sequencing and isolation

Table 3 shows the dominant fungal species analyzed with MiSeq sequencing and isolation. The dominant fungi in five wood samples were Ascomycota and Basidiomycota at the phylum level. For HC1, the fungal composition was 84.40% Ascomycota and 15.92% Basidiomycota. At the genus level, the fungi composition of HC1 was 52.21% *Phoma*, 22.46% *Lecythophora*, and 5.51% of *Cryptococcus*. One fungus (*Phoma*) was isolated by traditional method. Thus, *Phoma* is the dominant genus in HC1.

In sample HC2, the proportions of Ascomycota and Basidiomycota were 60.26% and 37.86%, respectively. At the genus level, HC2 was inhibited by *Lecythophora* (29.15%), *Cryptococcus* (18.11%), *Dacryopinax* (11.79%), *Phoma* (8.96%), and *Sporidiobolaceae* (7.72%). Two fungi (*Alternaria* and *Phaeosphaeriaceae*) were isolated from HC2. MiSeq sequencing revealed *Alternaria* accounted for 2.13% of the fungal proportion, and *Phaeosphaeriaceae* belonged to an unidentified fungi. The traditional isolation failed to determine the dominant fungus, indicating the need for high-throughput sequencing. However, several OTUs were insufficient to identify certain genus or species. Thus, the development of molecular identification of fungi is needed.

The fungal composition of HUP comprised 74.07% Ascomycota and 24.23% Basidiomycota. The fungal structure at the genus level was composed of 50.8% *Lecythophora*, 20.66% *Sporidiobolales*, 9.16% *Phoma*, 6.97% *Cladophialophora*, and 3.32% *Cryptococcus*. *Trichoderma* accounted for 0.006%, which had been obtained by isolation method. The dominant fungi (50.8% *Lecythophora*) was not obtained.

A high proportion (91.96%) of Ascomycota was observed in HD1. At the genus level, three genus were dominant: *Scedosporium* (70.71%), *Sporothrix* (10.53%), and *Entoloma* (4.5%). The Basidiomycota fungus *Entoloma* was obtained by isolation method. The strains obtained by isolation method were not always dominant.

In HD2, the proportion of Ascomycota and Basidiomycota reached 72.39% and 27.02%, respectively. Meanwhile, MiSeq sequencing identified 10.54% *Postia* (Basidiomycota), whereas 80.44% OTUs were unidentified at the genus level. Not fungus was obtained by isolation method.

Not only is fungal infection of timber an unsightly and potentially hazardous to human health, it can also adversely affect the structural integrity of timbers and disrupt the use of buildings [6]. The tests revealed numerous mold species at certain proportions in rotten pillars. Degradation substances by wood decay fungi provides most of the nutrients for the growth of saprophyte molds. Mold spores can cause a great risk to the human body. Therefore, timely repair of wood decay of ancient buildings is also important for tourist health.

On the other hand, the moisture content at the bottom of the wood pillar is extremely high; thus, the role of bacteria in decay formation should not be ignored based on the progressive infection mechanism of

microorganisms on wood [30]. The bacterial diversity of rotten pillars should be further analyzed.

Conclusions

This study detected fungal diversity on rotten wooden pillars of an ancient building archway in Beijing by amplicon high-throughput sequencing and analyzed the cause of decay. Comparison of microbial composition of discolored and dry rotten woods showed the increased proportion of Basidiomycetes with the highest value of up to 37.9%, although Ascomycetes remained the dominant phylum.

Fungal composition analysis showed the first pillar mainly contained Ascomycetes (Phoma, Lecythophora, and Scedosporium) and Basidiomycetes Sporidiobolales. Ascomycetes Lecythophora and Basidiomycetes Cryptococcus and Postia were the main fungi in pillar 2. Phoma, Trichoderma and Entoloma were isolated from pillar 1, and Alternaria and Phaeosphaeriaceae were obtained from pillar 2 using culture isolation method. Traditional isolation failed to determine the dominant fungi. The importance high-throughput sequencing technology in biodeterioration analysis of ancient wooden structural buildings was further explained.

Wood decay is caused by high moisture content. The bottom of the pillar was immersed in groundwater, and the cement coating prevented water evaporation. Comparison of fungal diversities of decaying wood and discolored and dry, decayed wood showed that the relative content of Basidiomycetes may be used as an indicator of wood decay state.

Declarations

Authors' contributions

Three authors contributed to the entire paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

No ethical issues.

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Tables

Table 1: Data of rotten pillars

Material	Wood species	Sampling	Decay state	Wood moisture content (%)
NO.1 pillar	<i>Pseudotsuga</i>	HC1	decaying	26.6%
	<i>menziesii</i>	HUP	decaying	56.3%
		HD1	not started	13.2
NO.2 pillar	<i>Picea sp.</i>	HC2	decaying	23.2%
		HD2	not started	10.4%
Beam	<i>Picea sp.</i>	G6	Rotten, dry	8.9%

Table 2: Richness and diversity estimation of fungal ITS sequencing libraries from the MiSeq.

Sample	Clean tags	OTUs	Chao 1	Good's coverage	Observed species	PD_whole tree	shannon
HC1	39962	115	120.43	0.999	104	20.87	2.5
HC2	28637	134	154.26	0.999	131	22.77	3.9
HUP	31442	122	146.44	0.999	115	22.55	2.6
HD1	30700	95	103.02	0.999	92	21.89	1.9
HD2	41029	182	193.59	0.999	167	37.51	3.3
G6	29171	258	294.51	0.995	225	69.39	2.0

OTU, operational taxonomic units; Chao1, community richness; Good's coverage, sequencing saturation; PD, phylogenetic diversity; Shannon, community diversity.

Table 3 Comparison of fungal species obtained by MiSeq sequencing and isolation.

Sample	MiSeq sequencing analysis		Isolation
HC1	<i>Ascomycota</i> 84.40%	<i>Phoma</i> 52.21%	<i>Phoma</i>
		<i>Lecythophora</i> 22.46%	
HUP	<i>Basidiomycota</i> 15.92%	<i>Cryptococcus</i> 5.51%	/
	<i>Ascomycota</i> 74.07%	<i>Lecythophora</i> 50.8%	<i>Trichoderma atroviride</i>
HD1	<i>Basidiomycota</i> 24.23%	<i>Phoma</i> 9.16%	
		<i>Cladophialophora</i> 6.97%	
	<i>Ascomycota</i> 91.96%	<i>Sporidiobolales</i> 20.66%	/
		<i>Cryptococcus</i> 3.32%	
HC2	<i>Ascomycota</i> 60.26%	<i>Scedosporium</i> 70.71%	
		<i>Sporothrix</i> 10.53%	
HD2	<i>Basidiomycota</i> 5.1%	<i>Entoloma</i> 4.5%	<i>Entoloma</i>
	<i>Ascomycota</i> 72.39%	unidentified 80.44%	
G6	<i>Ascomycota</i> 60.26%	<i>Lecythophora</i> 29.15%	<i>Alternaria</i>
		<i>Phoma</i> 8.96%	<i>Phaeosphaeriaceae</i>
HD2	<i>Basidiomycota</i> 37.86%	<i>Cryptococcus</i> 18.11%	/
		<i>Dacryopinax</i> 11.79%	
	<i>Ascomycota</i> 91.30%	<i>Sporidiobolaceae</i> 7.72%	
		unidentified 80.44%	
G6	<i>Basidiomycota</i> 27.02%	<i>Postia</i> 10.54%	/
	<i>Ascomycota</i> 91.30%	<i>Scedosporium</i> 77.78%	/
G6	<i>Basidiomycota</i> 5.97%	<i>Polyporaceae</i> 5.06%	/

Table 4: Core microbiome analysis of the samples of HC1/HC2/HUP/HD1/HD2.

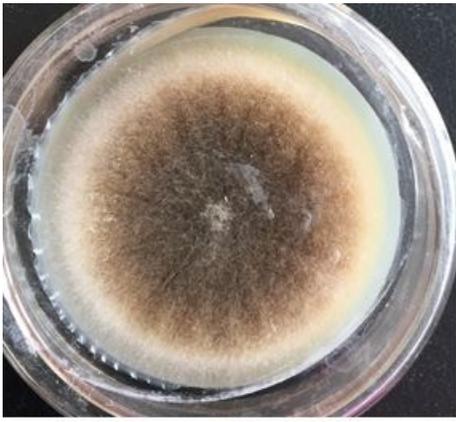
OTUs	level	taxonomy
OTU_3	family	<i>Incertae sedis</i>
OTU_5	family	<i>Incertae sedis</i>
OTU_1	species	<i>Lecythophora sp W3a2</i>
OTU_45	species	<i>Aspergillus cibarius</i>
OTU_32	species	<i>Rhodotorula mucilaginosa</i>
OTU_16	species	<i>Aureobasidium pullulans</i>
OTU_13	species	<i>Cryptococcus albidus</i>
OTU_49	family	<i>Nectriaceae</i>
OTU_28	family	<i>Incertae sedis</i>
OTU_4	phylum	<i>Ascomycota</i>
OTU_48	species	<i>Aspergillus subversicolor</i>
OTU_39	species	<i>Epicoccum sp NFW7</i>
OTU_66	genus	<i>Cladosporium</i>
OTU_22	genus	<i>Alternaria</i>
OTU_25	family	<i>Davidiellaceae</i>
OTU_70	order	<i>Pleosporales</i>
OTU_65	genus	<i>Fusarium</i>
OTU_12	species	<i>Cladophialophora immunda</i>
OTU_64	species	<i>Podospora ellisiana</i>
OTU_8	phylum	<i>Basidiomycota</i>
OTU_77	species	<i>Aspergillus caesiellus</i>
OTU_242	species	<i>Ilyonectria macrodidyma</i>
OTU_121	species	<i>Kernia pachypleura</i>

Figures

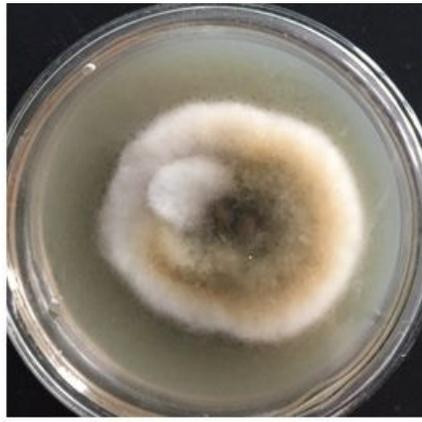


Figure 1

Sampling of two wood pillars and antient dry wood (G6).



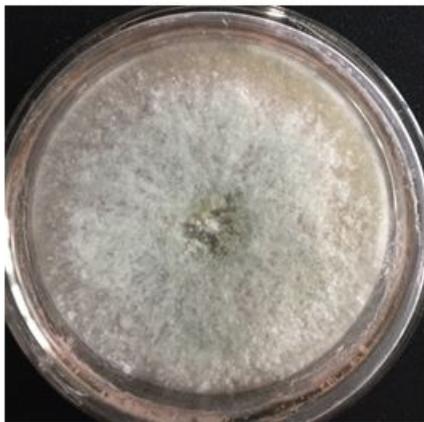
Phoma from HC1



Alternaria from HC2



Phaeosphaeriaceae from HC2



Trichoderma from HUP



Entoloma from HD1

Figure 2

Five different fungi isolated from rotten wood pillars. *Phoma* was isolated from sample HC1, *Alternaria* and *Phaeosphaeriaceae* from HC2, *Trichoderma* from HUP, and *Entoloma* from HD1.

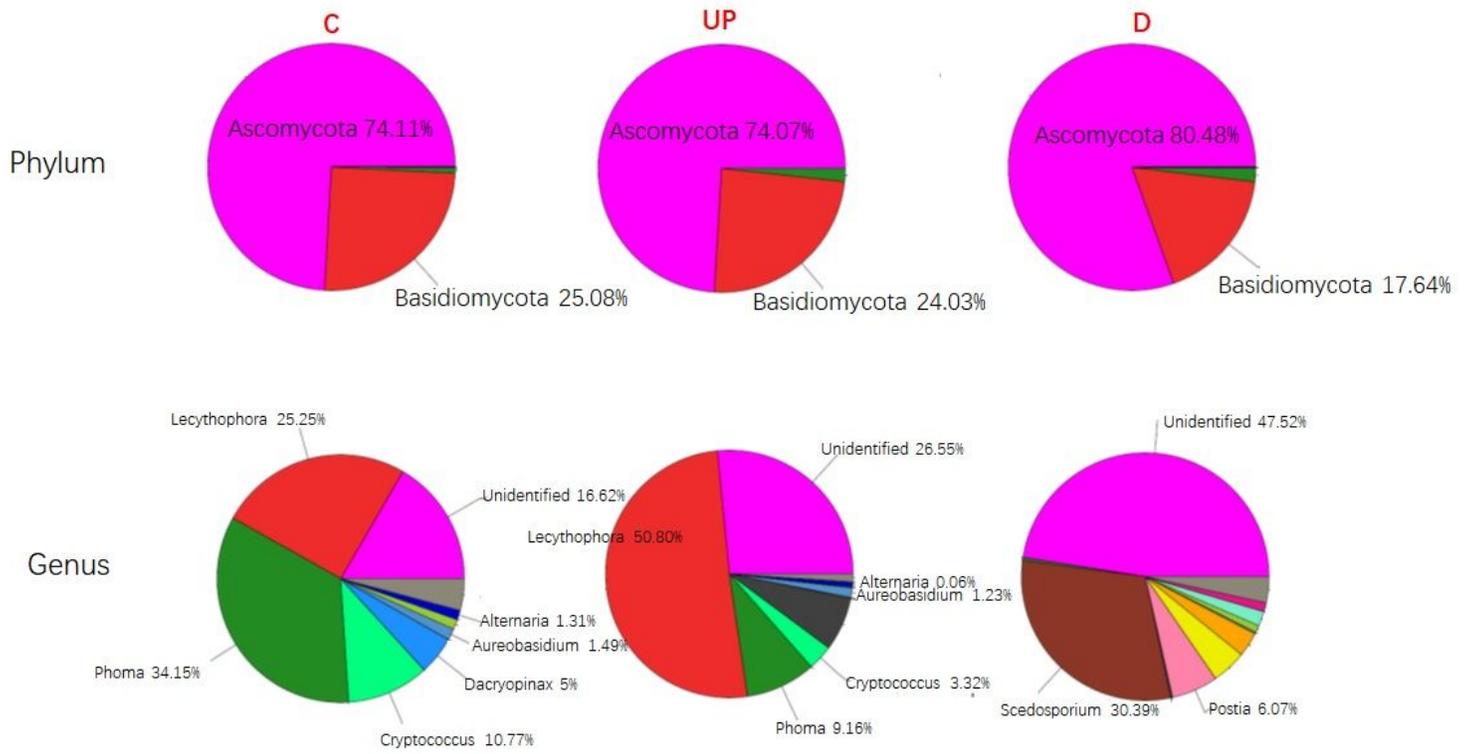


Figure 4

Fungal composition analysis at the phylum and genus levels on different positions.

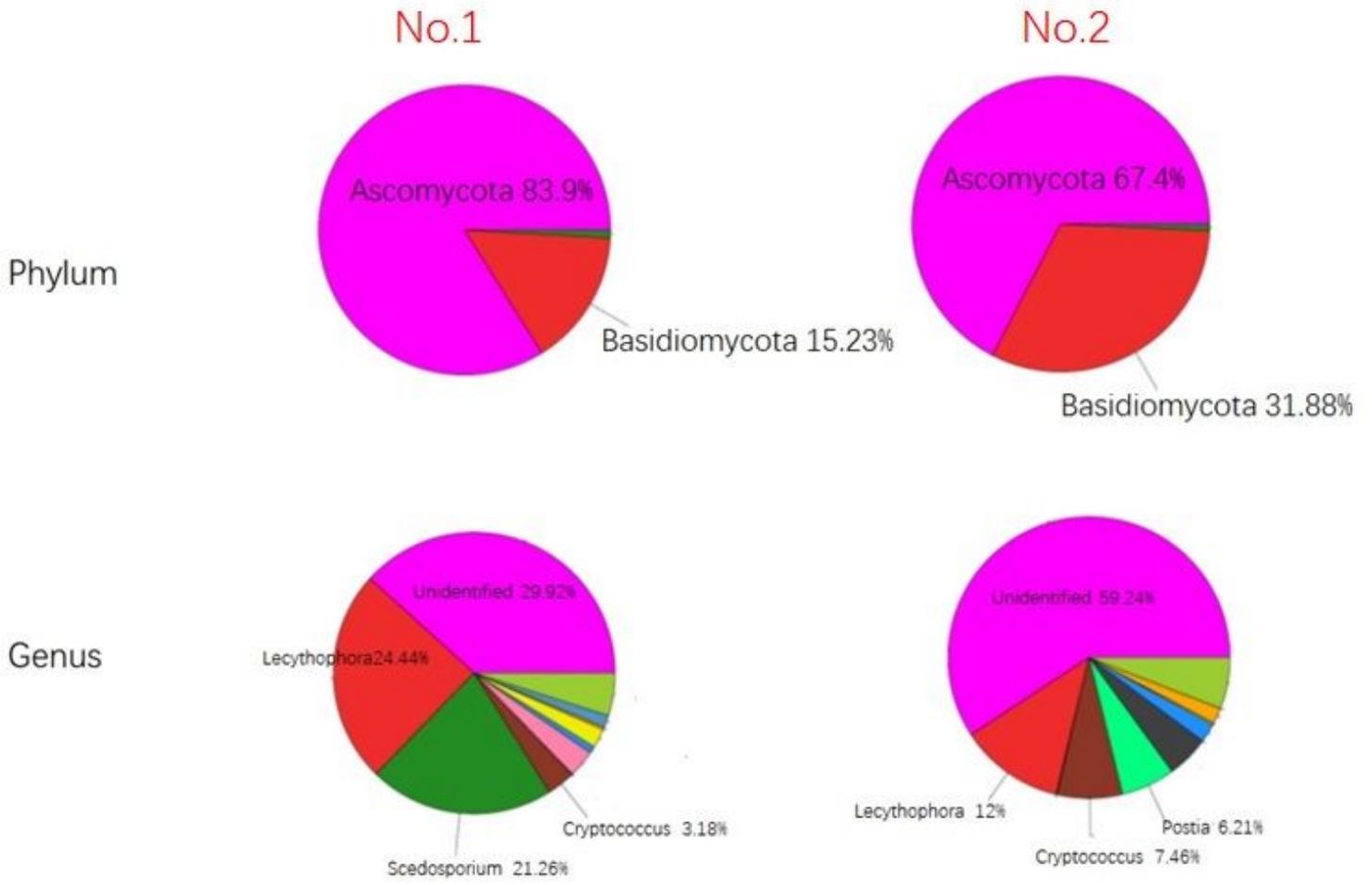


Figure 5

Fungal composition analysis at the phylum and genus levels of different pillars.

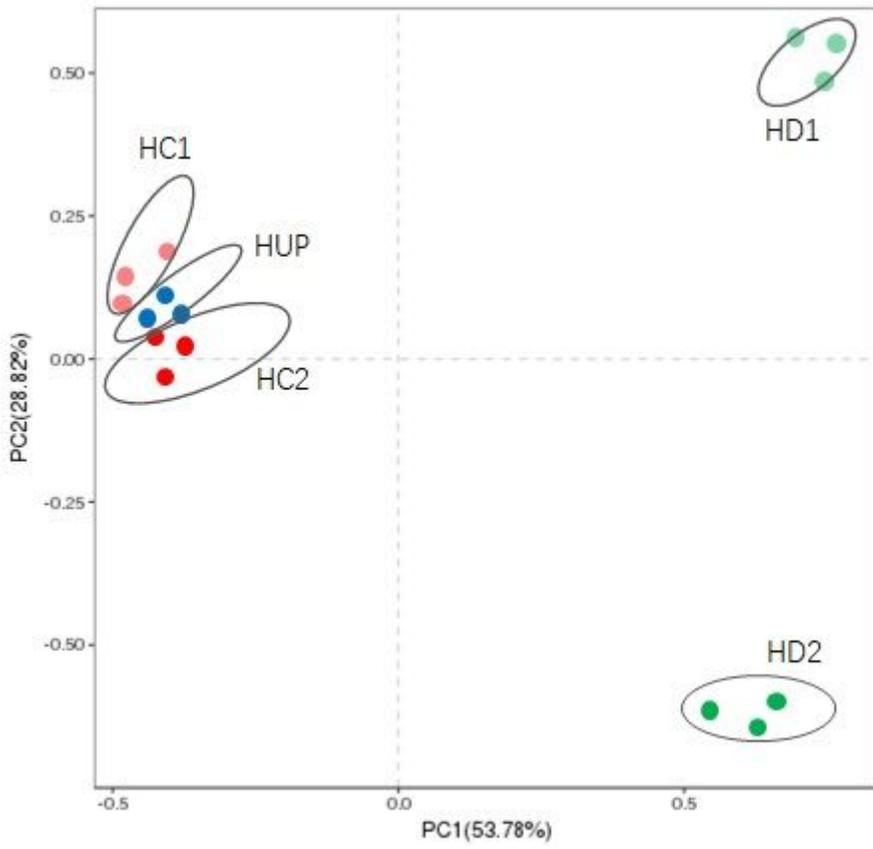


Figure 6

PCA based on the composition of each sample OTU.

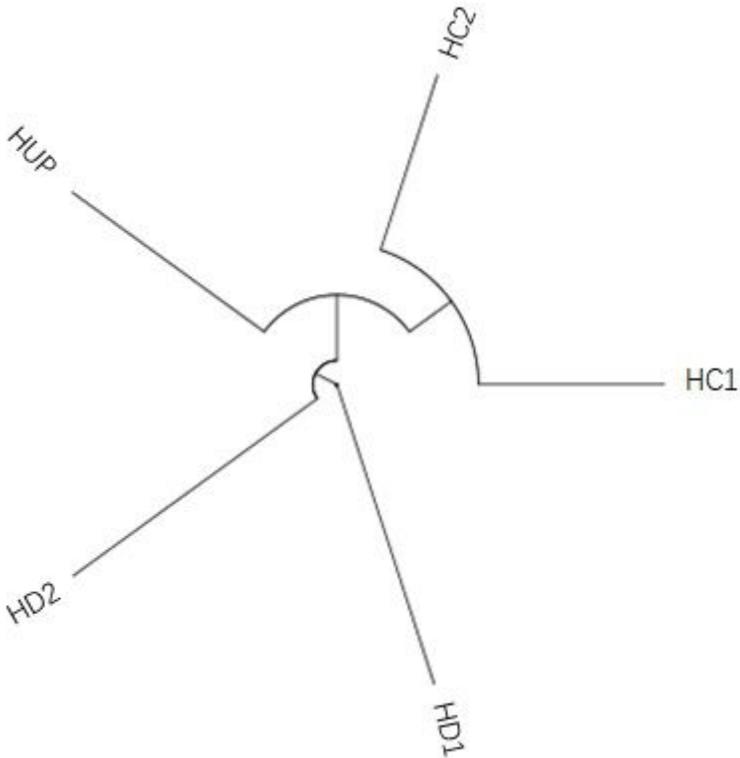


Figure 7

Cluster tree analysis based on the composition of each sample OTU.

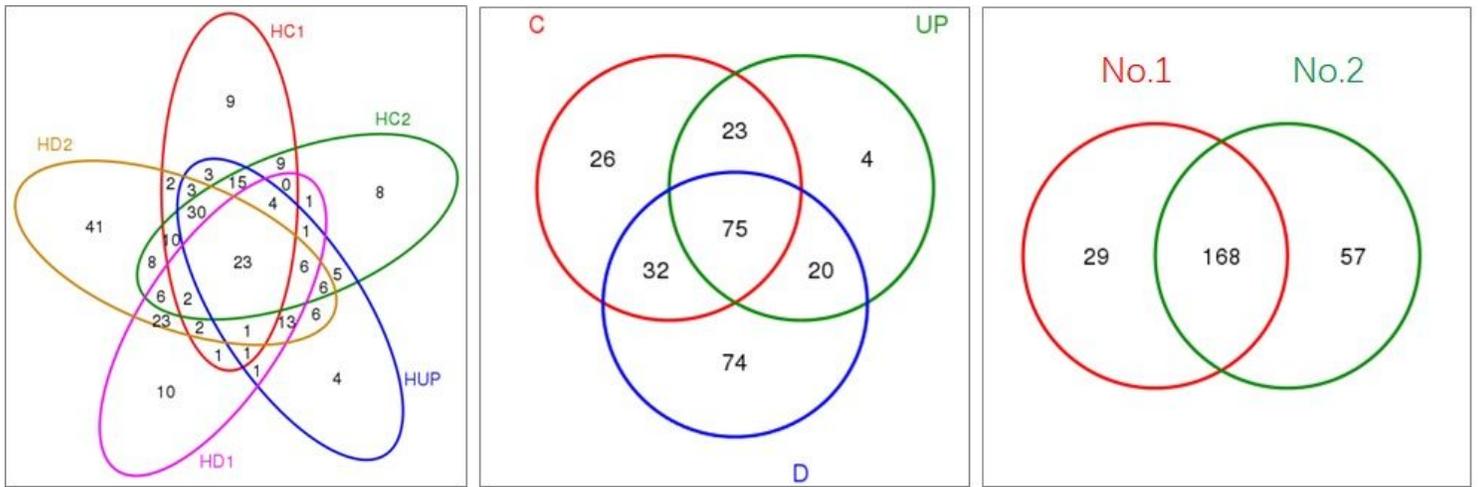


Figure 8

Venn diagrams of fungal OTUs of the wood samples.