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Analysis of microbial diversity and its degradation function in wooden piles at Shahe ancient bridge site in Xi'an and protection measures

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Abstract

Microorganisms possess the capability to impact virtually any form of cultural heritage, leading to contamination, degradation, and even combined degradation, where diverse microorganism communities facilitate the decay of wooden materials. This study aims to analyze the microbial diversity of the wooden structure bridge at the Shahe Ancient bridge site in Xi'an. The objective is to investigate the correlation between microbial species and the state of wood degradation, as well as explore the relationship between wood microbial species and the potential influence of the wood preservation environment on its long-term preservation. Simultaneously, the conventional laboratory separation technique was employed to isolate the microorganisms that were contaminated by experimental ancient wood. This was done to assess the inhibitory effect of two fungicides on the dominant microorganisms found in ancient wood, identify the most effective inhibitor, and subsequently establish an experimental foundation for safeguarding ancient bridge wood structures. The primary objective of this research was to establish microbial control criteria for the enduring conservation of historical sites, while also equipping conservators with scientifically-guided data to inform future research and restoration endeavors.

Keywords Wood-degrading microorganism, High-throughput sequencing, Microbial diversity, Functional prediction

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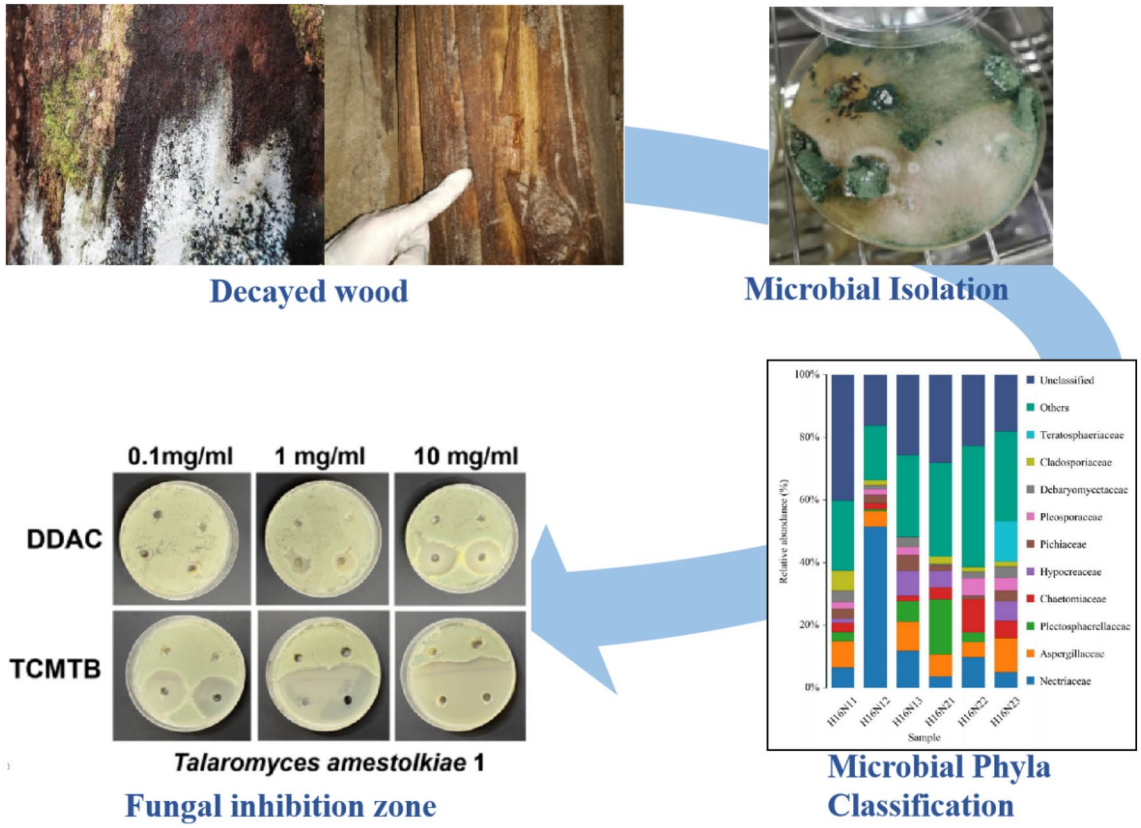
Graphical Abstract



Introduction

Wood is a natural biomass material composed of multicomponent cell walls, which is composed of a compound intercellular layer, primary wall and secondary wall. When wood is preserved in ancient buildings or in saturated soil environments for hundreds or even thousands of years, the main chemical components of its cell wall will not be rapidly degraded. However, over a longer period of time, microbial or acid–base hydrolysis will continuously promote this process, thus, resulting in changes in the cell structure of the main components of cell walls [1]. The cell walls of ancient wooden structures are primarily degraded by fungi that cause white rot, brown rot and soft rot. This can happen when the fungi have been in the natural environment for a long time [2, 3]. Wooden cultural relics that are buried underground are often destroyed by physical deformation, the crystallization of salt particles and microbial degradation owing to contact with the soil environment and sometimes the joint action of groundwater. Owing to the relatively closed burial environment, the decomposition of wooden cultural relics is in a state of dynamic balance. Corrosion by anaerobic microorganism occupies a dominant position, and the wooden cultural relics decompose slowly and incompletely. However, after wooden cultural relics are unearthed, they are exposed to the air for a long time, and aerobic microorganisms gradually revive. Aerobic microbial not only quickly metabolize and strongly corrode, but they also interact synergistically effect with anaerobic microorganism, causing a series of microbial decay problems and rapidly destroying cultural relics [4].

Historical sites and cultural relics widely distributed all over the world are valuable assets shared by all mankind, but the existence of microorganisms poses a serious threat to their protection [5, 6]. The corrosion and destruction of cultural relics is a completely spontaneous and long-term process, and the purpose of protecting cultural relics is to reduce the speed of their destruction and apply technology to resist natural destruction. Clades across the tree of life, from Archaea to animals, inhabit deadwood and in some cases also decay the wood. Wood-inhabiting fungi and bacteria are the key global agents of

wood degradation [7, 8]. Among fungi, saprotrophic taxa produce an array of extracellular enzymes to degrade and consume wood constituents, including lignin, hemicellulose and cellulose. These saprotrophic taxa are primarily but not exclusively members of the *Basidiomycota*. Fungi that lack these abilities can survive in deadwood by scavenging simple sugars generated by the saprotrophs, degrading or parasitizing saprotrophs or as symbionts of neighboring plants [9]. The influences of environmental and biotic interactions on the decay of wood may change over time. Because deadwood is dynamic and changes in the structure and chemical composition as it decays, we hypothesize that the relative importance of environment and biotic interactions on microbial communities will change as decay proceeds, leading to shifts in microbial composition and function [10, 11].

In recent years, the methods and techniques related to molecular biology have played an important role in revealing the mechanisms of microbial degradation in cultural heritage, and related techniques have dramatically improved the ability to identify, characterize, and describe microbial diversity [12]. In the past decade, many nucleic acid-based technologies have been widely used for microbial community analysis, and the ability to detect and analyze DNA and RNA have facilitated substantial improvements in the study of biological degradation of cultural heritage structures [13]. The microbial response of metabolites identified by their environmental analysis can facilitate microbial function research, macrogenomics and the rapid development of high-throughput sequencing [14, 15]. Purahong et al. used next-generation sequencing technologies to evaluated conservation strategies for wood-inhabiting fungi, examined which tree species combinations promoted the highest richness of wood-inhabiting fungi [16]. Studies also used high-throughput sequencing techiniques to quantify the contribution of soil as a source and medium for the dispersal of wood-inhabiting fungi to deadwood [17]. Piñar et al. studied Leonardo autoritratto (self-portrait) via Oxford Nanopore sequencing technology, the drawings' microbial bio-archive showed a relatively high contamination with human DNA and a surprising dominance of bacteria over fungi, indicating the importance of environmental and storage conditions on the specific microbiota [18, 19]. Marvasi et al. reviewed on recent applications of omics technologies to advance our understanding of microbial biodeterioration of cultural heritage materials such as stone, cellulose-based substrates, parchment, and provide an extensive perspective section suggesting future approaches and concrete tools for conservators in diagnosis and treatment of biodeterioration [20]. Lee et al. research finding the relative influence of wouud construction versus spatial locations differed between fungi and bacteria, suggesting that life history characteristics of clades structure diversity differently across space and time in decomposing wood [21]. Thus, it is now easier to conduct these studies in a variety of environments. These methods can not only characterize microbial diversity, but they can also provide a better understanding of the function of microbial communities in the natural environment, activities, characteristics and dynamic situations.

This study utilized the Shahe ancient wooden bridge site in Xi'an, China as the research object. The area where the Shahe ancient bridge site is located belongs to a warm temperate semi-humid continental monsoon climate area with distinct cold, warm, dry and wet seasons. The area belongs to the Weihe River system of the Yellow River Basin and is located within the hydrogeological area that encompasses the river terrace. The Weihe River is the largest surface water system in the area. It flows from west to the east along the southern edge of the municipal area. The ruin was damaged by natural factors after excavation, and the temporary protective shed conditions were limited; therefore, the overall preservation condition of the site was poor. The main diseases of the exposed wooden bridge piles on the ground include decay, cracks, incomplete areas, microbial damages, crooked flashes, fractures, discolouration, pollution, man-made damages (fire), and salt-alkali corroded areas. Through bioinformatics technology analysis of wood microbial diversity, accurately analyze the disease types of ancient wood structure sites, while combining with traditional analysis direction, the relationship between the causes of damage to wooden bridges and the environment was discussed, which provides feasible protection countermeasures for the long-term preservation of wooden bridges.

Methods and materials

Materials

Potato Dextrose Agar (PDA) medium was purchased from Aobox biotechnology (Beijing, China) used mould isolation and inhibition experiment. (2-benzothiazolylthio) methyl thiocyanate (TCMTB) and didecyl dimethyl ammonium chloride (DDAC) were purchased from Yuanye Bio-Technology Co.,Ltd (Shanghai, China).

Study site

The site of Shahe Ancient Bridge is located in Shahe River (Fig. 1A), Qindu District, Xi'an, Shaanxi Province, China (Latitude 34°15 ′27.24 ″N, longitude 108°40′ 57.80″ E, altitude 388 m) (Fig. 1B). Excavated in 1989, it was in the Qin-Han period. In 1992, it was declared as one of the key Cultural Relics Protection Unit of Shaanxi Province. It was declared as the seventh batch of national

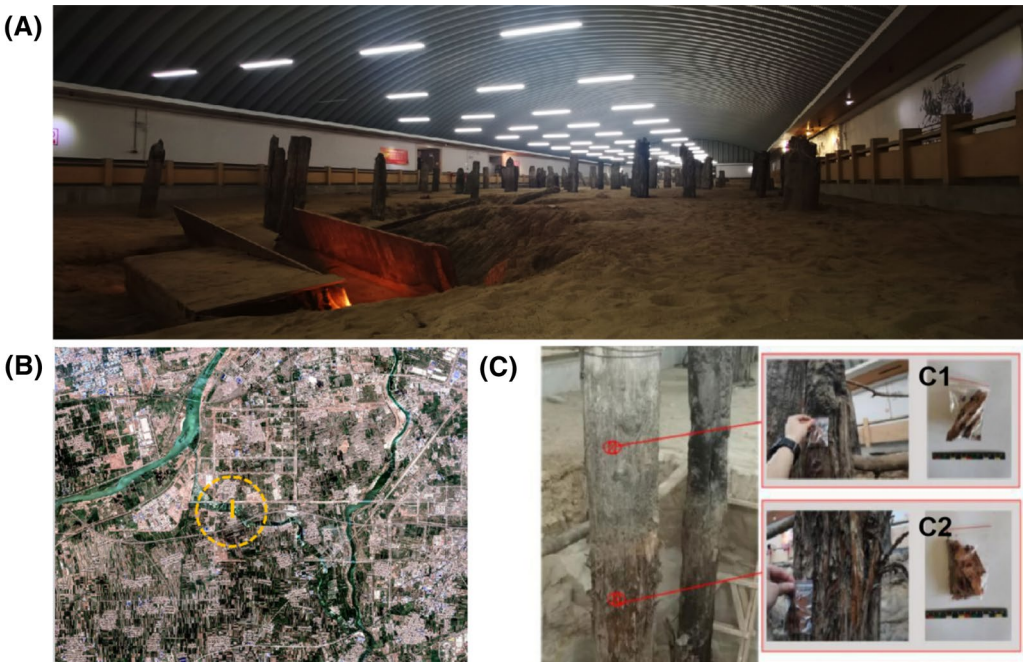


Fig. 1 Shahe ancient bridge site distribution and sampling sites. **A** Site photos; **B** Site river distribution; **C** Location distribution of sampling points

Key Cultural relics protection unit by The State Council in May 2013. The Shahe ancient bridge is a wooden bridge. Currently, only piles are left, and they are comprised of logs. Examination by experts have shown that the piles are composed of *phoebe zhennan*, *fir*, *camphorwood*, *cypress* and *pine with phoebe zhennan* as the main wood. Research on the characteristics of microbial wooden relics along with the analysis of influence of the timber microbial activity on wooden relics can predict its function and combine the morphological observations of decay with an analysis to effectively test the types of fungi that degrade ancient timber structures. Such targeted wood efforts should serve to help protect ancient ruins, so that they will continue to exist for a long time to provide certain theoretical bases and experimental foundations.

In the experiment, three samples were taken from the air contact (C1) and soil contact (C2) parts of excavated bridge piles of Shahe Bridge (Fig. 1C), respectively designated A1, A2, A3 for the air contact and B1, B2 and B3 for the soil contact. The samples were stored at − 20 ℃ for future study. An analysis of the diversity of wood microbial species was conducted in the Beijing Baimaike Biotechnology Co., Ltd. (Beijing, China) using an Illumina HiSeq sequencing platform (San Diego, CA, USA).

Bioinformatics analysis

Operational taxonomic units (OTUs) are the same marks artificially assigned to a taxon (strain, species, genus, and grouping), so that the relationship between organisms can be easily analyzed in phylogenetic research or population genetics research. According to different similarity levels, all the sequences are divided into OTUs [22]. Each OTU corresponds to a representative sequence. The number of OTUs of each sample was obtained at a similarity level of 97%. A Venn diagram could be used to show the number of common and unique OTUs between samples (number 2 to 5), which intuitively showed the coincidence of OTUs between samples. By combining the species represented by OTUs, common microorganisms in different environments can be identified. By comparing the representative sequences of the OTU with the microbial reference database, the species classification information that corresponds to each OTU can be obtained, and the community composition of each sample can then be counted at each level (phylum, class, order, family, genus and species). QIIME software was used to generate species abundance tables at different taxonomic levels, and the R language tool was used to draw community structure maps at different taxonomic levels. A heat map is a graphical display of the number of values in the data matrix and the similarity of the species or sample abundance similarity. The clustering of species at high and low abundance is used to reflect the similarities and differences of multiple sample communities using a color gradient and similarity. Based on the species composition and relative growth of each sample, the species of each taxonomic level was extracted, and the R

language tool was used to analyze the heat map cluster in the phylum, class, order, family, genus and classification level. Using QIIME software, the most abundant sequence of OTU in the taxonomic level was selected as the sequence of the representation, the multiple sequence comparison and the construction of the system evolution tree, and then the graphics of the python language tool. Based on the taxonomic database of existing microbial species provided by the NCBI, the evolutionary relationship and abundance difference of all the microorganisms in the sample are fully understood from the whole classification system using MEGAN software to return the species abundance information to the database. The specific classification analysis of single samples, and the analysis of single samples can be used to understand the distribution of sequences in individual samples at each taxonomic level. The variety of analyses can compare the differences in the abundances of sequences in different taxonomic branches [23].

Fungal isolation and identification

A small amount of 0.85% NaCl was applied to sterile cotton swabs, which were gently smeared on the wooden bridge to obtain samples. The cotton swabs were placed on PDA media for 3–5 days at 28 ℃, and a single colony with good growth was selected and inoculated onto a PDA plate. Based on the characteristics of the colony and microscopic examination results, the corresponding single colony was inoculated 3–4 times until a pure fungal colony was obtained.

Microscopical examination and strain identification

The fungal isolates were then grown on PDA medium for seven days at 28 ℃, after which the mycelia were harvested, and their total genomic DNA was extracted using the DNeasy Plant Mini-Kit (Qiagen, Valencia, CA, USA). The genomic DNA was amplified using primer, pair ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) targeting the ITS region of the rDNA. The PCR reactions were performed in a total volume of 25 µl, containing 2.5 µl of 10 × PCR buffer, 1 µl of deoxyribonucleotide triphosphate (dNTP; 2.5 mM), 0.5 µl of each primer (10 µM), 2.5 µl of 10 × buffer (with Mg^{2+}), 17.5 µl of sterile deionized water, and 0.5 µl of DNA template. The PCR conditions were as follows: 4 min at 94 ℃; denaturation for 45 s at 94 ℃; annealing for 1 min at 54 ℃; extension for 1 min at 72 ℃ (30 cycles); and a final extension for 10 min at 72 ℃. Amplicons were separated on 1% (w/v) agarose gel stained with ethidium bromide, and bands were visualized under a UV transilluminator. Next, the amplicons were purified using the EzWay PCR Clean-up kit (Sangon Biotech, China). The purified PCR products were sequenced using ITS primers at Sangon Biotech Co., Ltd (Shanghai, China).

Anti-fungal experiment

The isolated strains were grown on PDA in slant tubes placed in an incubator at 25 ℃. The fungal spores were isolated using 0.05% Tween 20 and filtered through gauze. The resulting spore suspension was diluted with phosphate-buffered saline (PBS) to adjust the number of fungi. The fungus was evenly coated on the medium. 0.85% NaCl was added to an Oxford cup control group, and TCMTB and DDAC were added to the treatment group, respectively. The growth of fungi was observed for 24 h.

Results

Sample collection

Sequence splicing quality control and cluster analysis of OTUs

Using 18S rDNA and ITS high-throughput sequencing, the average number of microbial original sequences on the Shahe ancient bridge was 453,060. A Venn diagram was used to show the number of common and unique OTU between samples. Based on the drawing of the two groups of samples, the OTU Venn of each sample is shown in Fig. 2. The average effective sequences that corresponded to A1, A2 and A3 were 73,331, 78,138 and 75,964, respectively, while the average effective sequences that corresponded to B1, B2 and B3 were 76,222, 77,790 and 70,490, respectively (Fig. 2A). A cluster analysis based on a 97% similarity standard showed that the OTUs of group A1, A2 and A3 were 176, 99 and 141, respectively (Fig. 2B), while the OTUs of group B1, B2 and B3 were 176, 99 and 141, respectively (Fig. 2C). The number of OTUs unique to group A and B was 123 and 142, respectively (Fig. 2D).

Gene composition of the microbial mats

The eight most prevalent microbial phyla in the Shahe ancient bridge samples were Ascomycota, Basidiomycota, Mortierellomycota, Chitridiomycota and Mucoromycota, Aphelidiomycota, Rozellomycota and Glomeromycota. This result was published in a previous journal of Heritage Science (2021, 9:99)[24]. In this study, we analyzed the whole microbial population of the Shahe ancient bridge site. As shown in Fig. 3, the five most prevalent microbial classes in the Shahe ancient bridge samples were Sordariomycetes, Eurotiomycetes, Dothideomycetes, Agaricomycetes, and Saccharomycetes. The five most prevalent microbial orders in the Shahe ancient bridge samples were Hypocreales, Eurotiales, Sordariales, Saccharomycetales, and Glomerellales. The five most prevalent microbial families in the Shahe ancient bridge samples were Nectriaceae, Aspergillaceae,

(A)

Sample	PE reads	Raw Tags	Clean Tags	Effective Tags	AvgLen(bp)	Effective(%)	OTU (number)
A1	80021	73987	73982	73331	230	91.64	176
A2	79860	78179	78177	78138	235	97.84	99
A3	80081	76000	75997	75964	240	94.86	141
B1	79897	76250	76248	76222	246	95.4	116
B2	79942	78120	77790	77790	245	97.31	228
B3	80123	70524	70492	70492	245	87.98	125

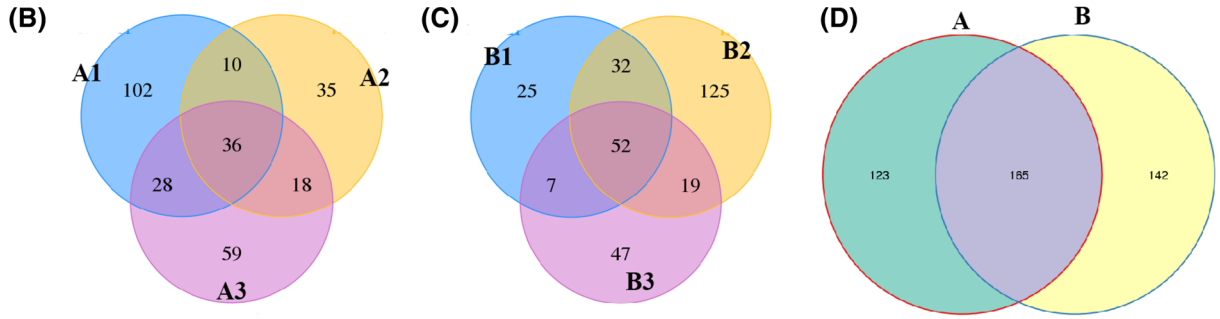


Fig. 2 OTUs Venn diagram of microbial cluster at two sampling sites. **A** High throughput sequencing basic information table; Venn diagram of sample point A (**B**) and sample point B (**C**); **D** Sample AB has a Venn diagram

Plectosphaerellaceae, Chaetomiaceae, and Hypocreaceae. The five most prevalent microbial family in the Shahe ancient bridge samples were Nectriaceae, Aspergillaceae, Plectosphaerellaceae, Chaetomiaceae, Hypocreaceae. The five most prevalent microbial genera in the Shahe ancient bridge samples were Xeromyces, Trichoderma, Verticillium, Pichia, and Eupenidiella. The most prevalent microbial species in the Shahe ancient bridge samples were *Xeromyces bisporus*, *Pichia kluyveri*, *Verticillium leptobactrum*, *Trichoderma virens*, *Eupenidiella cenezuelensis*, *Epichloe sibirici*, *Sterigmatomyces halophilus*, *Aspergillus cibarius*, *Thermonyces lanuginosus*, and *Sarocladium implicatum*.

Next, we identified the differences in fungi at the genus (Fig. 4A) and species (Figs. 4B) levels in different regions. A chart was drawn simultaneously for the microorganisms with a high number of species that were enriched at the sampling sites (Table 1). As shown in Fig. 4A, Tricholoma and Fusarium appeared in group A1. Chaetosphaeria appeared in group B2, and Trichoderma and Chaetosphaeronrma appeared in group A3. Soft rot bacteria grow in wet wood, partially decomposing lignin and cellulose, causing the surface of wood to soften and rot. Trichoderma, Fusarium and Chaetosphaeria belong to the taxa of soft rot bacteria.

Table 1 shows a variety of highly enriched microorganisms. Fusarium, Trametes, and Pholiota are all types of common white rot fungi, which indicate that the main degraders of the wooden piles sampled were white rot fungi.

QIIME software was used to select the sequences of OTU with the highest abundance at the taxonomic level as representative sequences, conduct multiple sequence alignments and build a phylogenetic tree. Graphs were then drawn using Python language tools. Each branch in an evolutionary tree represents a species, and the length of the branch is the evolutionary distance between two species, i.e., the degree of difference between the species. According to the phylogenetic tree, the strains collected were primarily distributed in Ascomycota, Basidiomycota, Mortierellomycota and Chytidiomycota (Fig. 5A). The multi-sample taxonomic tree compares the differences in abundance of sequences of different groups or samples on a branch. As shown in Fig. 5B, the phylogenetic tree indicates that Basidiomycota occupies the main component, and both the white rot and brown rot fungi that decompose wood are derived from the Basidiomycota.

FUNGuild (Fungi Functional Guild) (Fig. 6) is a tool that can be used to taxonomically resolve fungal OTUs by ecological societies by classifying large sequences into ecologically significant categories in a simple and consistent way. Pathotrophs, symbiotrophs and saprotrophs were divided into three groups according to their

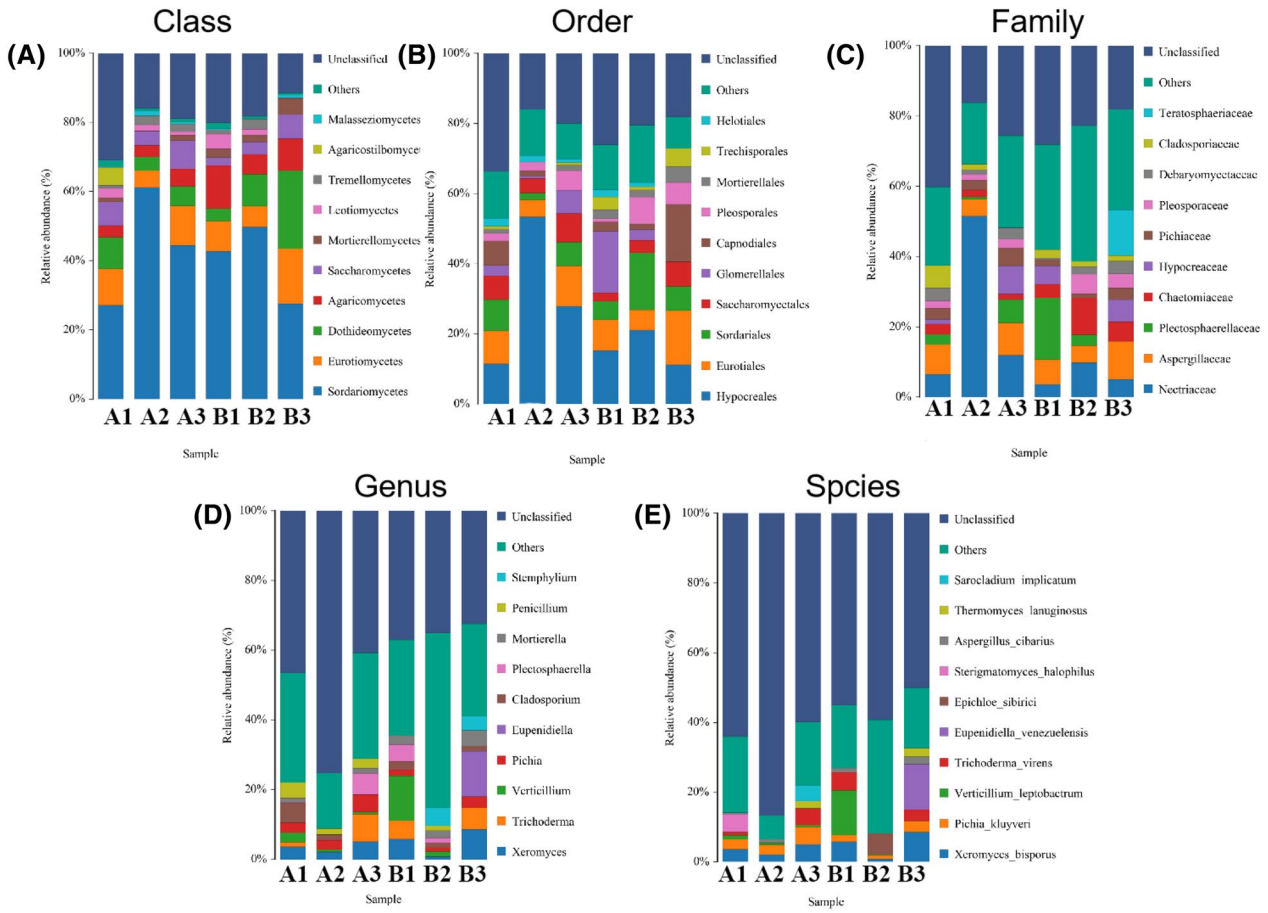


Fig. 3 Relative abundance (%) plot of (**A**) Class, **B** Order, **C** Family, **D** Genus, and **E** Species

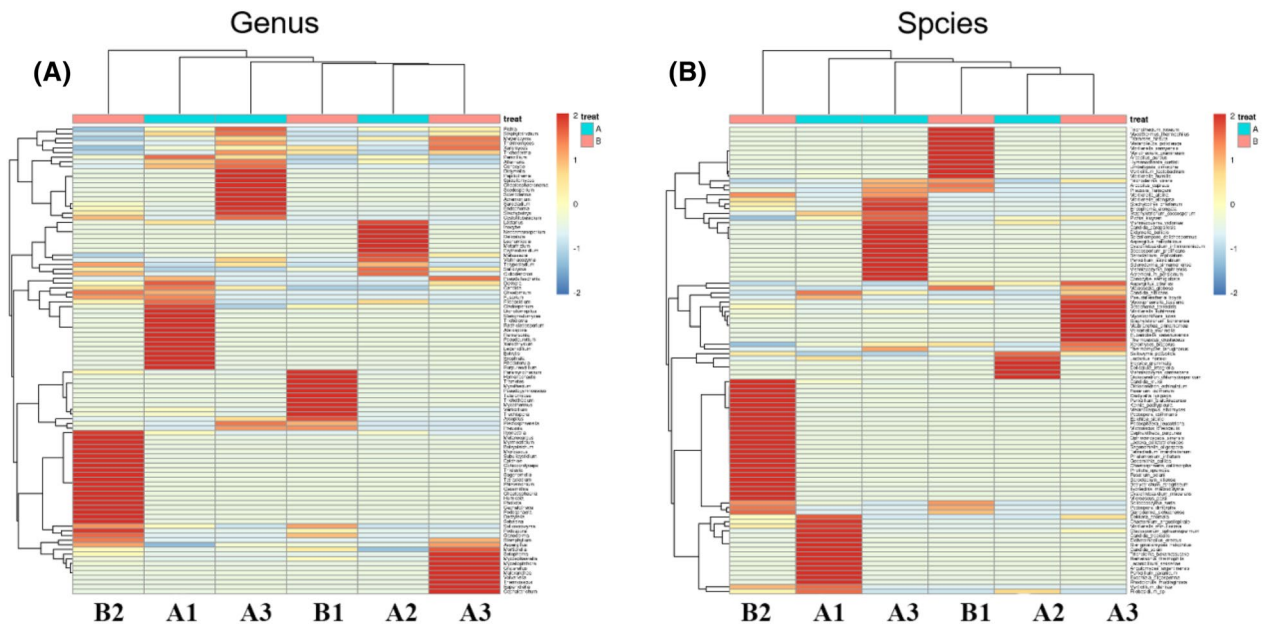


Fig. 4 Microbial differences at the genus/species level. **A** Genus level. **B** Species level

Table 1 Species abundance clustering of sampling site

A1	A2	A3	B1	B2		B3
Chaetomium angustispirale	Lactarius horakii	Stachybotrys chartarum	Trichothecium roseum	Candida kruisli	Phialemonium inflatum	Mycosphaeralla tassiana
Mortierella minutissima	Inocybe grammata	Endophoma elongate	Mycothermus thermophilus	Oidiodendron echinulatum	Geosmithia pallida	Setophoma terrestris
Cladosporium sphaerospermum	Delicatula integrella	Vishniacozyma victoriae	Trametes hirsuta	Fusarium culmorun	Chaetosphaeria callimorpha	Mortierella kuhlmanii
Candida tropicalis	Vishniacozyma carnescens	Candida parapsilosis	Melanoleuca polioleuca	Dactylella lysipaga	Pholiota spumosa	Myceliophthora lutea
Dichotompilus erectus	Oidiodendron chlamydosporicum	Didymella bellidis	Mortierella sarnyensis	Penicillium bialowiezense	Fusarium solani	Staphylotrichum boninense
Sterigmatomyces halophilus		Spizellomyces dolichospermus	Myurothecium gramineum	Kernia pachypleura	Sarocladium kiliense	Malbranchea cinnamomea
Candida solani		Aspergillus halophilicus	Arcopilus aureus	Melanocarpus albomyces	Botryotrichum atrogriseum	Volvariella murinella
Tercholoma bakamatsutake		Cystofilobasidium infirmominiatum	Hymenochaeta curtisii	Podospora communis	Iiuonectria macrodidyma	Eupenidiella venezuelensis
Remersonia thermophila		Scedosporium prolificans	Umbelopsis dimorpha	Epichloe sibirici	Cystodilobasidium macerans	Thermoascus crustaceus
Lecanicillium saksenae		Sarocladium implicatum	Verticillium leptobactrum	Podosphaera leucotricha	Microascus paisii	
Angulomyces argentinensis		Penicillium astrolabium	Mortierella humilis	Microascus brevicaulis	Lectera colletotrichoides	
Penicillium javanicum		Scleroderma sinnamariense		Cephalotheca purpurea	Sagenomella oligospora	
Exophiala oligosperma		Vishniacozyma tephrensis		Ophiocordyceps sinensis	Tetracladium marchalianum	
Rhodotorula mucilaginosa		Acremonium persicinum				
		Conocybe semiglobata				

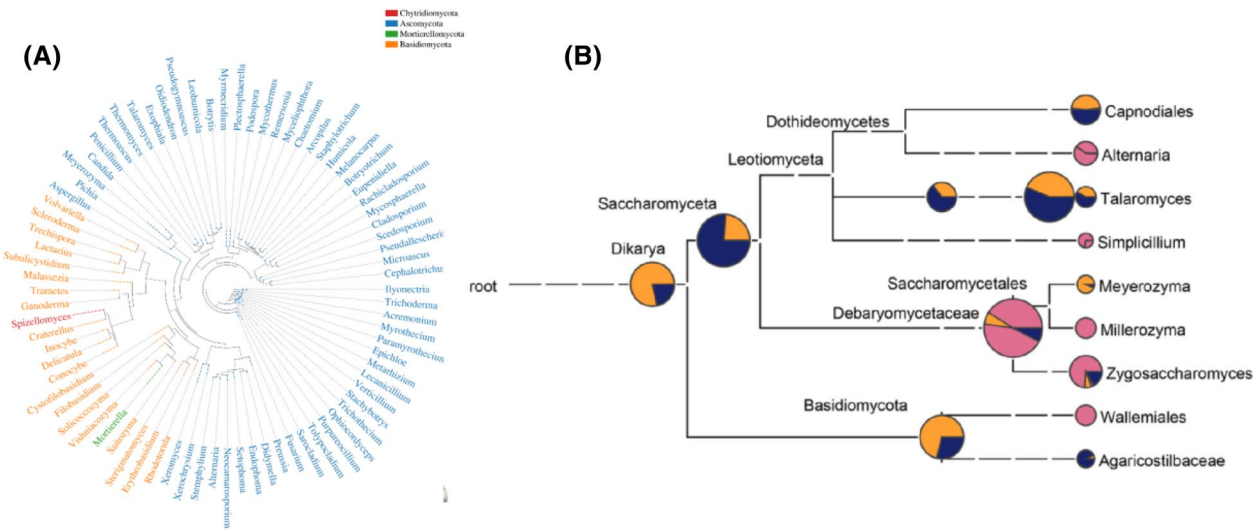


Fig. 5 **A** Phylogenetic tree of species, **B** Example diagram of MEGAN taxonomic tree

nutritional patterns. The three categories were divided further into 12 guilds. Pathotrophic microbes are primarily distributed in the Dothideomycetes and Agaricomycetes. Symbiotrophic microbes were primarily distributed in the Agaricomycetes, Dothideomycetes, Glomeromycetes and Leotiomycetes. Saprotrophic microorganisms primarily included the Agaricomycetes, Saccharromycetes, Sordariomycetes and Tremellomycetes. Based on

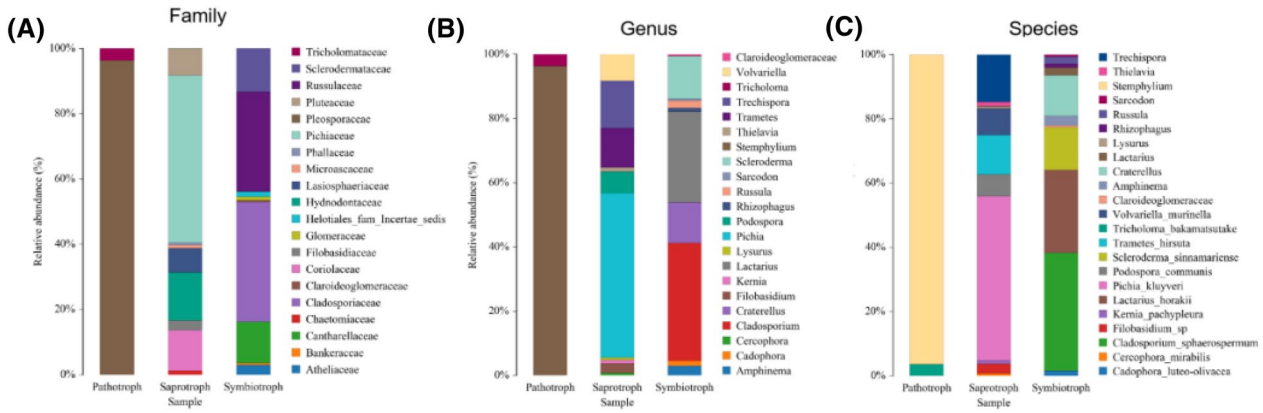


Fig. 6 Predictive analysis of functional genes

a species distribution, pathotrophic microbes were the most numerous, followed by symbiotrophic microbes, and saprotrophic microbes were the least numerous. These results are consistent with the patterns observed in wood decay.

Correlation analysis of core microbiota and metabolic pathways

A species correlation network graph was drawn based on Python. The top 50 genera with the highest correlation are shown in Fig. 7. The circle represents species, and the size of the circle represents the average abundance of species. The lines represent correlation between two species. The thickness of the line is consistent with the strength of the correlation, and the stronger the correlation, the thicker the line. Orange represents a positive correlation, and green represents a negative correlation. As shown in Fig. 8, No.1 is Xeromyces, No.10 is Aspergillus, No.13 is Penicillium, N0. 18 is Mortierella, No. 32 is Podospora, No. 36 is Stemphylium, No. 45 is Plectosphaerella and No. 48 is Candida were the most enriched species, among which, the positive correlation between species showed more relationships. Only five related species showed negative correlations. Pseudogymnoascus

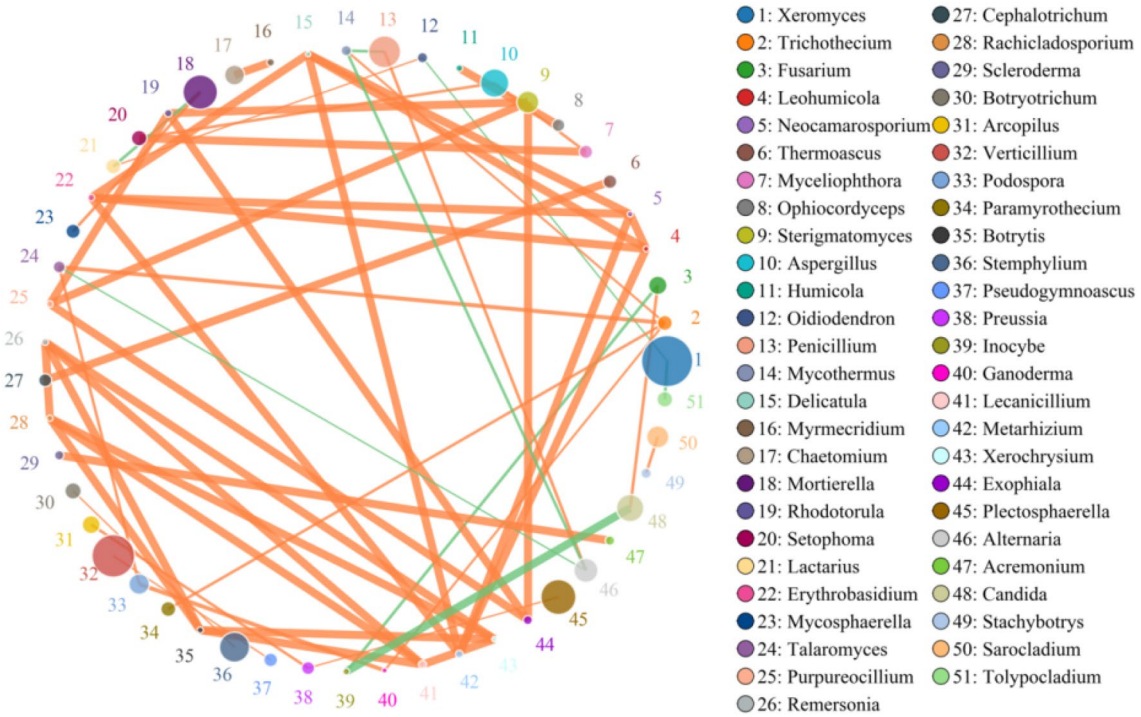


Fig. 7 Network diagram of species at genus level

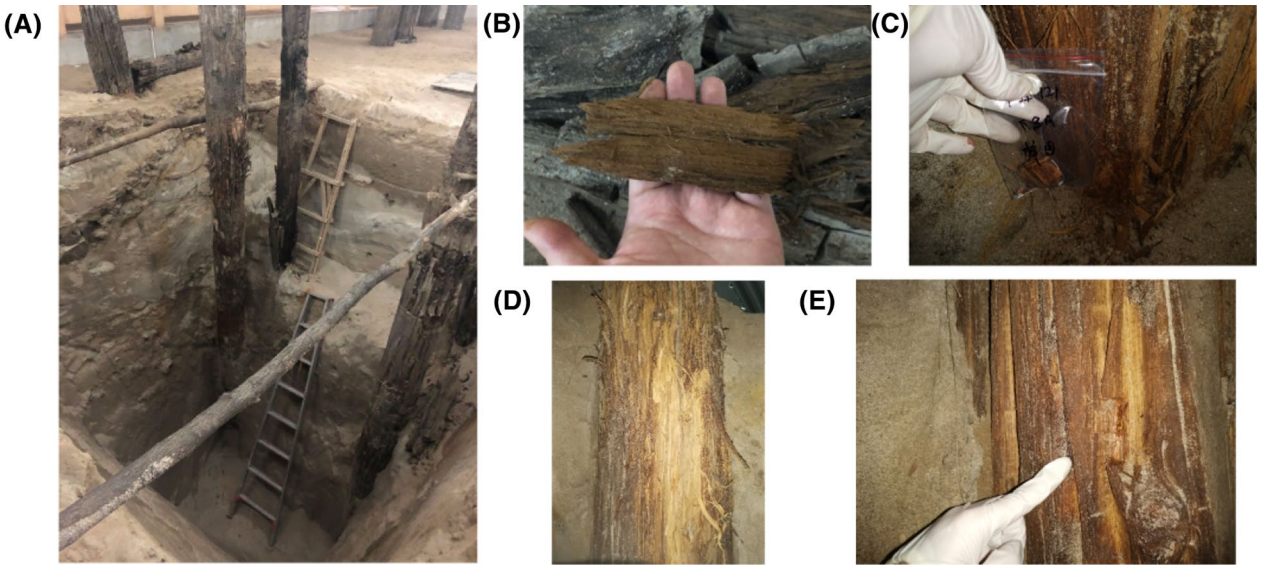


Fig. 8 Observation of microbial diseases. **A** Sampling site; **B**, **C**, **D**, **E** White rot of wood

No. 37 was a basidiomycota strain, Podospora No. 32 was a hemiptomycota strain, Podospora was positively correlated with Pseudogymnoascus strain. Both of the above two strains were common wood white blight fungi, which was consistent with the above wood disease results. Shows wood as white rot disease type. Inocybe No. 39 belongs to basidiomycota, and Fusarium No. 3 is a hemipygium, a disease of white rot fungi common to wood, and No. 39 is negatively correlated with No. 3. No. 39 was negatively correlated with No. 48, and No. 48 was Candida, suggesting that Inocybe of basidiomycetes was negatively correlated with Fusarium of basidiomycetes and Candida.

Status of wood preservation and microbial species

The results indicate that there are significant differences in the richness and diversity of pile samples in different regions. The dominant flora were classified as Ascomycota, Basidiomycota, Mortierellomycota, Chitridiomycota and Mucoromycota, Aphelidiomycota. Glomeromycota and Rozellomycota. Among them, Basidiomycota can form white rot, which is consistent with the observed pile rot [25, 26]. Fusarium, Trametes and Pholoitoa are all the strains that produce white rot in the middle of the more enriched species. The wood decay of Shahe Bridge site is shown in Fig. 8A. In the morphological observation of wood, as shown in Fig. 8B and 8E, the color of wood became dark and slightly brown, and its surface softened and streaked (Fig. 8C). In the later stage of degradation by white rot fungi, the wood would dry and shrink (Fig. 8D). This suggests that the detection of microbial diversity was consistent with the identification of the wood microorganisms.

Fungicidal effect of wood reinforcement agent

DDAC is the most commonly used preservative treatment for wood due to its colorless appearance, high permeability, low odor, and zero metal content [22]. TCMTB is used as fungicide in the paper, tannery, paint, and coatings industries [27]. Lecanicillium aphanocladii, 2 kinds of Talaromyces amestolkiae, Penicillium citreonigrum, Cladosporium cladosporioides and Aspergillus versicolor. These dominant microorganisms were consistent with those analyzed in the microbial diversity shown in Fig. 3. The results suggest that microbial diversity could provide an accurate research idea for wood disease prediction. The fungicidal effects of 0.1 mg/mL, 1 mg/mL, 10 mg/mL DDAC and TCMTB on six dominant strains isolated from Shahe Ancient Bridge piles were compared through the fungicidal circle. As shown in Fig. 9, 10 mg/mL DDAC and TCMTB had good inhibition effect on all six strains, and TCMTB had better effect than DDAC. The inhibitory effect of 1 mg/mL DDAC on fungi was not as good as that of 1 mg/mL TCMTB. 0.1 mg/mL DDAC has no obvious inhibition effect on mold, while 0.1 mg/mL TCMTB has a better inhibition effect on mold. Compared with DDAC, TCMTB at the same concentration has better fungicidal effect. In the follow-up wood reinforcement and protection experiment, TCMTB will be used for wood mould prevention reinforcement of Shahe Ancient Bridge.

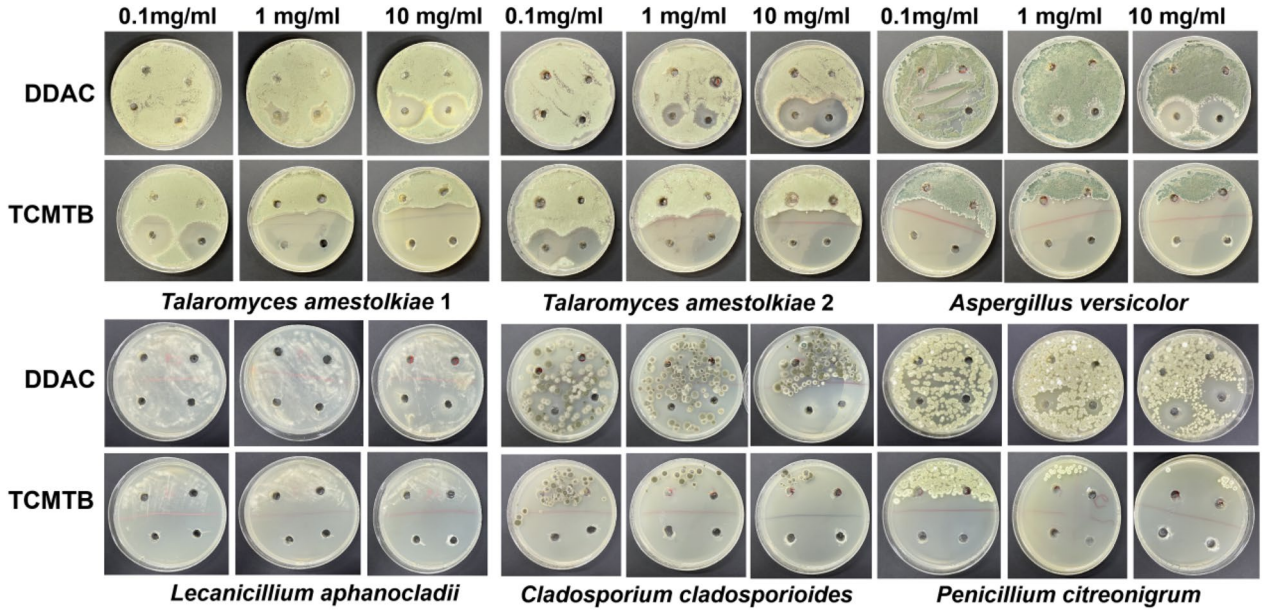


Fig. 9 Mould proof experiment of wood mould proof agents

Conclusion

In this study, high-throughput sequencing technology was used to analyze the changes in the diversity of surface microbial community and the distribution of dominant microorganism in different areas of wooden piles in the Shahe ancient bridge site. The results show that there are significant differences in the richness and diversity of pile samples in different regions. The dominant floras were classified as Ascomycota, Basidiomycota, Mortierellomycota, Chitridiomycota and Mucoromycota, Aphelidiomycota, Glomeromycota and Rozellomycota. Among them, Basidiomycota can form white rot, which is consistent with the observed pile rot. Fusarium, Trametes and Pholoitoa are all strains that produce white rot in the middle of the more enriched species. This suggests that the detection of microbial diversity was consistent with the results from the identification of the microorganisms found in the wood. Since the main decomposers of this wooden pile were white rot fungi, more effective measures will be taken to protect this wooden pile. TCMTB has better antifungi effect compared with DDAC at the same concentration. Therefore, TCMTB will be selected for the wood antifungal of Shahe Ancient Bridge. We will also use the same method to analyze the remaining pile microorganisms to find more accurate means of protection. The corroborative evidence derived from these analyses provides important scientific data that can be contributed to conservators to scientifically guide future research and restoration processes.

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Author contributions
The manuscript was prepared through contributions of all authors. Jing Cao planned the study together with Bingjie Mai, conducted all data analysis, and wrote most of the manuscript. HC, XL, DL, JW, and JL contributed to the collection of samples in the article. All authors have given approval to the final version of the manuscript.

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Availability of data and materials
All data generated and/or analyzed during this study are included in this published article. The datasets generated during and/or analysed during the current study are available in the NCBI. Sequence Read Archive (SRA) Submission: SUB10670341.

Declarations

Ethics approval and consent to participate
This article does not contain any data based on experiments in humans or animals.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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