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ORIGINAL ARTICLE

# Diversity and community structure of wood-inhabiting fungi found in Japanese wooden houses analyzed by the next-generation sequencing

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**Abstract** The diversity and community structures of wood-inhabiting fungi in 16 decayed wood samples from ten wooden houses in Japan were analyzed using a next-generation sequencing (NGS) to determine the fungi responsible for wood decay. DNA of fungi in decayed wood was extracted directly, the internal transcribed spacer (ITS) region in ribosomal DNA (rDNA) was amplified by polymerase chain reaction (PCR), and then, sequences of tagged ITS fragments were analyzed by NGS. Results of sequencing indicated that 68 species of ascomycetes, 37 species of basidiomycetes, and one fungus each from Mortierellales and Mucoromycetes were detected. The fungal community structures showed diversity and included various species of ascomycetes. A microscopic examination of cell wall structure in decayed wood samples suggested that some ascomycetes were soft-rot fungi. Heat map analysis indicated that the similarity in the structures of fungal communities was influenced to a greater extent by the wood species of samples than where they were used as a component.

**Keywords** Wood-rotting fungi · Fungal community · Diversity · Wooden house · Next-generation sequencing

## Introduction

Wood is a useful natural material that is commercially produced in forests and used for the construction of wooden houses. Wood is a durable natural material, but microbes such as wood-rotting fungi can attack its cell walls, causing serious deterioration of wood strength. Twenty-to-thirty species of wood-rotting fungi found in Japanese wooden houses have been reported [1, 2],

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however, yet undiscovered fungi are likely to exist. A 6-year long investigation in Germany reported that more than 70 species wood-rotting fungi were found in wooden building materials [3]. Therefore, a comprehensive nationwide investigation to determine the microbes that damage to the wooden houses in Japan is needed. Using the conventional methods, fungal species are identified morphologically and physiologically, which sometimes require strains to be completely isolated. These methods consume considerable time, efforts, and experience and the results can vary according to examiner's knowledge and experiences with fungal identification. In contrast, identification based on DNA sequences provides more accurate, objective, and reproducible results in a shorter time [4–7]. Furthermore, analyzing DNA extracted directly from decayed wood allows the detection of fungi that may be difficult to culture or appear in low numbers [8–10]. Next-generation sequencing (NGS) can obtain a large amount of information which quickly provides comprehensive microbial community analysis data [11–14]. In fungal species identification based on nucleotide sequences, internal transcribed spacer (ITS) genes, which are a noncoding part of ribosomal DNA (rDNA), are employed as marker sequences [15]. Using sequencing of ITS region allows a sufficient analysis of the diversities of nucleotide sequences and length enough to identify fungal species. By directly extracting DNA from decayed wood specimens, whole microbial communities that are present can be analyzed [9, 10, 16]. Wood decay is caused not by a single fungus but by groups of fungi, whose members change as decay progresses. Therefore, fungal communities should be comprehensively investigated holistically to better understand wood decay.

In the present study, fungal community structures in decayed wood samples collected from ten wooden houses in Japan were analyzed.

## Materials and methods

### Decayed wood samples

Sixteen-decayed wood samples were collected from houses in Tokyo, Kanagawa, Nara, Osaka, Hyogo, and Miyazaki prefectures in Japan between September 2011 and August 2012 (Table 1; Fig. 1). The collected samples were stored separately in instrument sterilization pouches and kept at room temperature. Samples taken from separate locations in the same house were treated separately. Wood species were anatomically identified to the genus level by observation of optical and electron microscopes.

### Estimation of decay type by microscopic observation

The morphology of cavities formed in cell walls was observed to estimate the type of decay [17, 18]. A piece of decayed wood was placed in a glass tube with 16.5% peracetic acid and was incubated at 80 °C for 1 h. After the old liquid was removed, fresh peracetic acid solution was put in the tube and heated again. This cycle was repeated several times to degrade lignin completely in the sample piece. The delignified samples were observed under a polarizing microscope (Olympus BX50F4) to determine the type of decay (Fig. 2).

### DNA extraction and polymerase chain reaction (PCR)

Ten-gram specimens taken from each wood sample were placed in a stainless steel chamber and then ground with by TissueLyser (Qiagen, Venlo, The Netherlands). DNA was extracted from 1 g of the powdered wood sample by the modified benzyl chloride method [19] using the ISO-PLANT II DNA extraction kit (Nippon Gene, Tokyo, Japan) following the manufacturer's instruction with some modifications. Crude DNA was purified using PowerSoil®DNA Isolation Kit (Mo bio, CA, USA). Whole-genome amplification was conducted using illustra GenomiPhi V2 Kit (GE Healthcare, WI, USA) when PCR amplicon was not obtained.

The ITS I region was amplified from extracted DNA by PCR with the universal primer pair ITS1 (5′-TCC GTA GGT GAA CTT GCG G-3′) and ITS2 (5′-GCT GCG TTC TTC ATC GAT GC-3′) [20] using Takara Mighty Amp DNA Polymerase Kit (TakaraBio, Shiga, Japan). Amplicon sizes were confirmed by agarose gel electrophoresis.

### NGS analysis

To prepare the sequence samples with attached tags for NGS analysis, the second PCR was conducted using primers with the adapter sequence (5′-CGT ATC GCC TCC CTC GCG CCA TCA G), tag sequence, and ITS1-primer as the forward primer and another adapter (5′-CTA TGC GCC TTG CCA GCC CGC TCA G) and ITS2-primer as the reverse primer [21]. The second amplicon was separated via gel electrophoresis in 2% agarose, and then, the bands were excised from the gel to purify the amplicon. The double-strand DNA in purified samples was quantified using the Quant-iT dsDNA BR assay kit (Invitrogen, Carlsbad, CA, USA). The sequence library was prepared and purified using Agencourt AMPure XP (Beckman Coulter, Fullerton, CA, USA) and quantified with an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). Pyrosequencing was performed on the GS FLX (Roche

**Table 1** Collecting site, component, wood species, and decay type of decay samples

| | Sample collection site | | | Wood genus (family) | Decay type** |
|---|---|---|---|---|---|
| | House no. | Prefecture* | Component | | |
| HIM1 | 1 | Kanagawa (35.56N, 139.52E) | Under floor member of hallway | *Abies* or *Tsuga* (Pinaceae) | S1, S2 |
| HIM2 | 1 | Kanagawa (35.56N, 139.52E) | Under floor member of bathroom | *Shorea* (Dipterocarpaceae) | S2 |
| HIM3 | 2 | Kanagawa (35.55N, 139.52E) | Floor member of open verandah | *Tsuga* (Pinaceae) | – |
| HIM4 | 2 | Kanagawa (35.55N, 139.52E) | Floor member of open verandah | *Tsuga* (Pinaceae) | B, S1 |
| HIM5 | 3 | Hyogo (34.75N, 135.41E) | Under floor sill of hallway | *Abies* (Pinaceae) | S2 |
| HIM6 | 4 | Osaka (34.68N, 135.44E) | Under floor member of Japanese-style room | *Abies* (Pinaceae) | B |
| HIM7 | 4 | Osaka (34.68N, 135.44E) | Under floor member of kitchen | *Abies* (Pinaceae) | S1 |
| HIM8 | 5 | Nara (34.56N, 135.72E) | Column of bathroom | *Tsuga* (Pinaceae) | S1 |
| HIM9 | 6 | Hyogo (34.74N, 135.32E) | Blindfold fence of bathroom | *Larix* or *Picea* (Pinaceae) | B |
| HIM10 | 6 | Hyogo (34.74N, 135.32E) | Blindfold fence of bathroom | *Xanthocyparis* (Cupressaceae) | S1 |
| HIM11 | 7 | Kanagawa (35.32N, 139.34E) | Brace of bathroom | *Abies* (Pinaceae) | ba |
| HIM12 | 7 | Kanagawa (35.32N, 139.34E) | Window sill of kitchen | *Shorea* (Dipterocarpaceae) | S2 |
| HIM13 | 7 | Kanagawa (35.32N, 139.34E) | Roof member of bathroom | *Cryptomeria* (Cupressaceae) | B |
| HIM14 | 8 | Kanagawa (35.37N, 139.39E) | Column of bathroom | *Tsuga* (Pinaceae) | S2 |
| HIM15 | 9 | Miyazaki (31.91N, 131.38E) | Window frame of kitchen | *Cryptomeria* (Cupressaceae) | W, S1 |
| HIM16 | 10 | Tokyo (35.78N, 139.71E) | Under floor member of kitchen | – | B, S1 |

*W* white rot, *B* brown rot, *S1* soft-rot type 1 (diamond-shaped cavity), *S2* soft-rot type 2 (cell wall erosion), *ba* bacterial attack

* Parentheses indicate approximate location

** Decay type estimated by microscopic observation

Diagnostics, Basel, Switzerland) according to the manufacturer's instructions.

### Sequence data analysis

Sequences were sorted according to tags and low quality, and chimeric sequences were removed using CLC Genomics Workbench version 6.0 (CLC Bio, Cambridge, MA, USA). After eliminating reads under 100 bp and deleting adapter and tag sequences, the remaining sequences were clustered into the operating taxonomic units (OTUs) using Usearch version 7 [22] with default parameters except for identity cutoff, which was set at '-id 0.95'. The BLASTN algorithm [23] was used to identify the sequences for representative OTUs using NCBI nt database.

Species richness and Shannon–Wiener index ($H'$) were calculated as indicators of diversity [24, 25]. Species richness was the number of species in a community. $H'$ was computed using the following equation:

$$H' = -\sum (p_i \log p_i)$$

where $p_i$ is the proportion of individuals for identified species. To compare the structures of fungal communities, heat maps were generated using heatmap.2 from the gplots package in R version 3.0.2 [26]. Clustering of communities was performed using the amap package with group-average method and Euclidean distance. Heat map data were converted to the base-two logarithms and displayed as a color rage with light orange indicating '0' and deep red as '12'.

## Results

### Microscopic observation of cell wall to assess the type of wood decay

The wood samples in this study were well decayed in appearance. After lignin was removed from the cell walls

**Fig. 1** Photographs of decayed wood samples. *Scale bars* show 2 cm

using peracetic acid, a longitudinal surface was observed to assess the type of wood decay (i.e., white rot, brown rot, soft-rot type 1, and soft-rot type 2) [27–30]. White rot was identified by bore holes formed on cell walls. Brown rot was identified by cell wall thinning and/or crevices along the direction of microfibrils. Soft-rot type 1 (S1) was identified by cavities with conical ends inside the walls and Soft-rot type 2 (S2) was identified by irregular erosion on surface of inner cell wall (Table 1; Fig. 2). Only sample HIM15 was observed to possess white rot through microscopic investigation. Bacteria were observed on the surface of cell walls in HIM11.

## Analysis of fungal community structures

NGS reads longer than 100 bp were sorted for each sample according to tag sequences. More than 2000 reads per sample were included using CLC Genomics Workbench, as described above (Table 2). Representative sequences of OTUs selected from clustering were 'identified' species. The ratios of basidiomycetes to ascomycetes varied considerably among in the samples (Fig. 3a). Almost all fungal communities were composed of both basidiomycetes and

ascomycetes, except for HIM12, which was composed only of ascomycetes. Sorting by ascomycete contents, the communities in which ascomycetes and basidiomycetes existed together had greater species richness and diversity (Fig. 3a, c). Therefore, the more diversified communities included both ascomycetes and basidiomycetes with higher species richness.

## Ascomycetes and basidiomycetes in community structures

In this study, fungal species detected by NGS contained 68 species of ascomycetes, 37 species of basidiomycetes, and one species each in the orders Mortierellales and Mucoromycetes. Fifteen species of white rot and three species of brown-rot fungi were detected. Ascomycete and basidiomycete community structures in the samples are illustrated in Fig. 4a, b, respectively, while the relative abundance of ascomycetes and basidiomycetes is shown in Fig. 3a.

Among the basidiomycetes, Corticiaceae and Coriolaceae were the most frequently detected families.
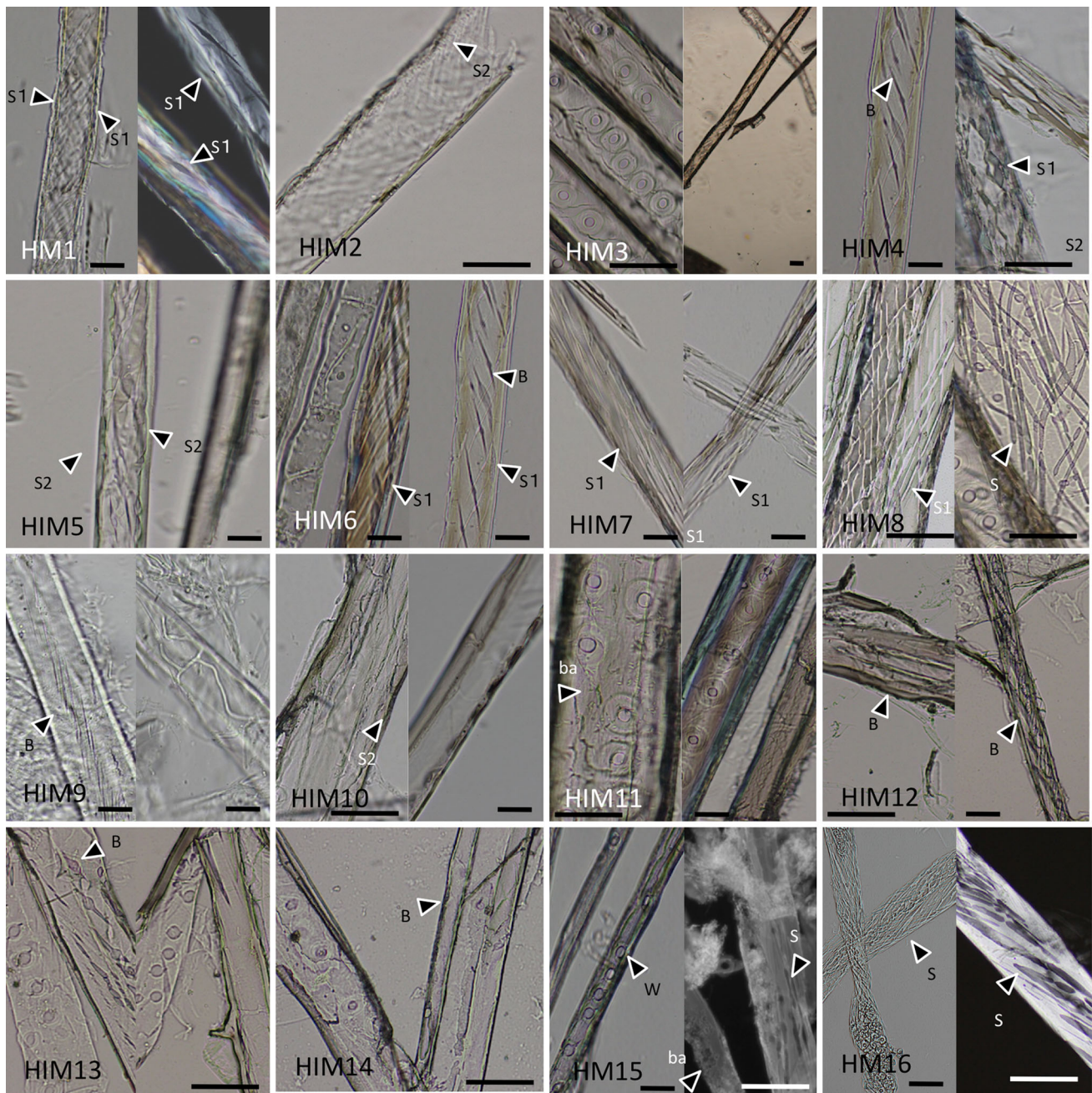
**Fig. 2** Microscopic images of cell walls delignified with peracetic acid. *Bars* show 20 μm. *S* crevice along the angle cellulose microfibril by soft-rot fungi, *S1* cavity with conical ends inside the wall created by soft-rot fungi type 1, *S2* irregular erosion on surface of inner cell wall by soft-rot fungi type 2, *B* cell wall thinning and/or crevices along direction of microfibril by brown-rot fungi, *W* bore hole formed by white-rot fungi, and *ba* bacteria cells

Corticiaceae contained *Hyphodontia* sp., which dominated fungus in HIM4 and HIM8. *Coriolaceae* spp. were prevalent among basidiomycetes in communities with >40% ascomycetes, except for HIM12. Coriolaceae included *Trametes hirsuta, Trametes versicolor,* and *Tyromyces chioneus*. The brown-rot fungus *Antrodia sinuosa*, which belongs to Fomitopsidaceae, was dominant species in the HIM9 and HIM16 samples.

Ascomycetes detected in this study were more varied than basidiomycetes (Fig. 3). The families Aspergillaceae, Leotiomycetes, and genus *Scytalidium* were detected frequently. *Scytalidium lignicola* was the most frequently detected ascomycete species in this study, which was classified as soft-rot fungus type 2 [29, 30]. Fungal species that comprised more than 5% of fungal community in each sample are listed in Table 2. The white-rot fungi

**Table 2** Dominant species (more than 5%) and number of NGS reads of each sample. Number and letter in parenthesis indicate occupancy (%) and decay type

| | Dominated wood rot basidiomycetes | Dominated ascomycetes | Number of reads* |
|---|---|---|---|
| HIM1 | – | *Geomyces* sp. (83.3), *Chrysosporium* sp. (8.6, S1+2) | 25284 |
| HIM2 | – | *Chrysosporium* sp. (96.9, S1+2), | 17802 |
| HIM3 | *Hyphodontia* sp. (97.1, W) | – | 12160 |
| HIM4 | *Hyphodontia* sp. (72.3, W) | – | 3238 |
| HIM5 | *Trametes versicolor* (7.1, W), *Trametes hirsuta* (12.1, W) | *Phialophora hyalina* (39.8, S1+2), *Scytalidium lignicola* (34.7, S2) | 3282 |
| HIM6 | *Polyporus brumalis* (18.1, W), *Trametes hirsuta* (5.5, W) | *Aspergillus versicolor* (5.7, S), *Aspergillus chlamydosporus* (39.0), *Phialosimplex chlamydosporus* (12.2) | 2064 |
| HIM7 | *Trametes hirsuta* (42.6, W), *Hyphodontia* sp. (11.7, W) | *Phialophora* sp. (34.6, S) | 1934 |
| HIM8 | *Hyphodontia* sp. (99.3, W) | – | 5569 |
| HIM9 | *Antrodia sinuosa* (99.9, B) | – | 2434 |
| HIM10 | *Sterigmatosporidium polymorphum* (48.9, B) | *Penicillium lignorum* (10.8), *Phialocephala dimorphospora* (10.1, S1) | 6981 |
| HIM11 | *Cabalodontia* aff. *subcretacea* (41.1), *Ganoderma* sp. (12.4, W) | *Dothideomycetes* sp. (29.1), *Penicillium citreonigrum* (11.4) | 2205 |
| HIM12 | – | *Penicillium citreonigrum* (77.8), *Scytalidium lignicola* (22.1, S2) | 7489 |
| HIM13 | – | *Exophiala xenobiotica* (8.8) | 2167 |
| HIM14 | – | *Penicillium rubidurum* (99.6) | 2500 |
| HIM15 | *Peniophorella praetermissa* (36.0, W), *Trametes hirsuta* (32.6, W), *Tyromyces chioneus* (15.4, W), *Hyphodontia* sp. (7.6, W) | *Penicillium pimiteouiense* (5.4) | 2318 |
| HIM16 | *Antrodia sinuosa* (20.4, B), *Hyphodontia* sp. (6.6, W) | *Scytalidium lignicola* (69.2, S2) | 3410 |

*S1* soft-rot type 1, *S2* soft-rot type 2, *S* soft rot, *W* white rot, *B* brown rot

* After trimming and filtering

*Hyphodontia* sp. and *Trametes hirsuta* and the brown-rot fungus *Antrodia sinuosa* were dominant among basidiomycetes. *Chrysosporium* sp., *Phialophora hyaline, Scytalidium lignicola, Aspergillus versicolor,* and *Phialophora* sp. were dominant among ascomycetes that were classified as soft-rot fungi.

## Comparing the structures of fungal communities

Clustering and heat map analysis visualizing the OTUs present in each sample were conducted to examine the effect of the sample origin (collection place) and wood species on the fungal communities (Fig. 5). The fungal communities of four samples, namely, HIM1, HIM2, HIM3, and HIM4 (Kanagawa 1 and 2), were not similar, even though the sampling sites were close. Furthermore,

the fungal communities on samples HIM6 and HIM7 (Osaka 4) and HIM9 and HIM10 (Hyogo 6) were not similar, despite the samples being collected from the same houses. Thus, the heat map showed that no special relationship existed between the fungal community structures and sampling origin. However, there was a relationship between the wood species of samples and the composition of fungal communities. Community structures on *Abies* sp. samples were similar as were those on *Tsuga* sp. suggesting that wood species was related to fungal community structure. Finally, the fungal species that were most widely detected among the samples in this study were white-rot fungi of *Trametes hirsuta, Hyphodontia* sp. and soft-rot fungi *Penicillium* spp., *Scytalidium* spp. These results suggested that these fungi are broadly found in wooden house decay.

**Fig. 3** Diversity of the fungal communities. Proportion of ascomycetes, basidiomycetes, and other fungi (**a**), Shannon–Wiener index *H'* (**b**), and species richness (**c**)
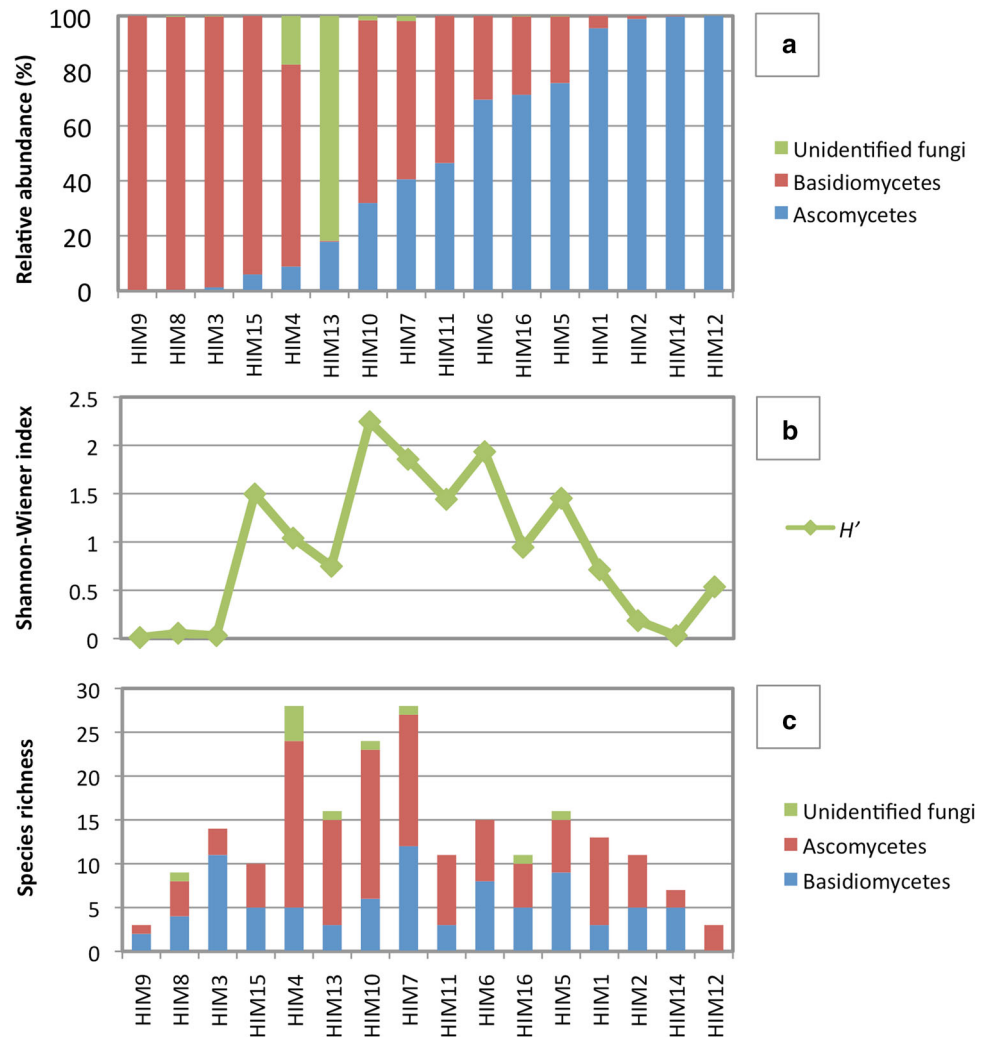


## Discussion

All of the woods sampled in this study were softwoods except for HIM2 and HIM12, which were hardwood and HIM16 (species unknown). The wooden houses primarily built with conifer woods were thought to be attacked by brown-rot fungi [31]. The results of this study indicate that various fungi types and species, including ascomycetes, were involved in decaying the sampled wooden houses. The white-rot fungi such as *Trametes hirsuta, Trametes versicolor,* and *Tyromyces chioneus* were detected in the communities in which ascomycetes and basidiomycetes were coexisted. HIM 9 and HIM10 were taken from the bathroom of the same house, and brown-rot fungi *Antrodia sinuosa* and *Sterigmatosporidium polymorphum* were predominant, respectively. These results suggest that both white-rot and brown-rot fungi are responsible for damage to wooden houses. Both white-rot and brown-rot fungi (*Antrodia sinuosa* and *Hyphodontia* sp.) were detected in the fungal community in sample

HIM16. The results of other analyses suggested that observations of wood surfaces were not sufficient to determine wood decay type. The wood species of hardwood samples such as HIM2 and HIM12 were identified as *Shorea* sp. and ascomycete species were dominant in these samples, basidiomycete abundance making up a minority at less than 5%. Many species of ascomycetes were detected in this study. One reason for this prevalence may be that the wood decay in the houses sampled occurred in wet area (e.g., bathrooms and kitchens), which promoted the growth of soft-rot fungi.

The influence of the samples' origin on their fungal community structures was negligible. However, wood species appeared to have an effect on the composition of fungal communities. It has been reported that wood species has a considerable influence on the species-level composition of fungal communities in decaying fallen logs in forests [32, 33]. No characteristics due to the samples' origin regarding the composition of fungal communities were found in this study, because variation of the

**Fig. 4** Microbial structures at the family level of ascomycetes (**a**) and basidiomycetes (**b**)
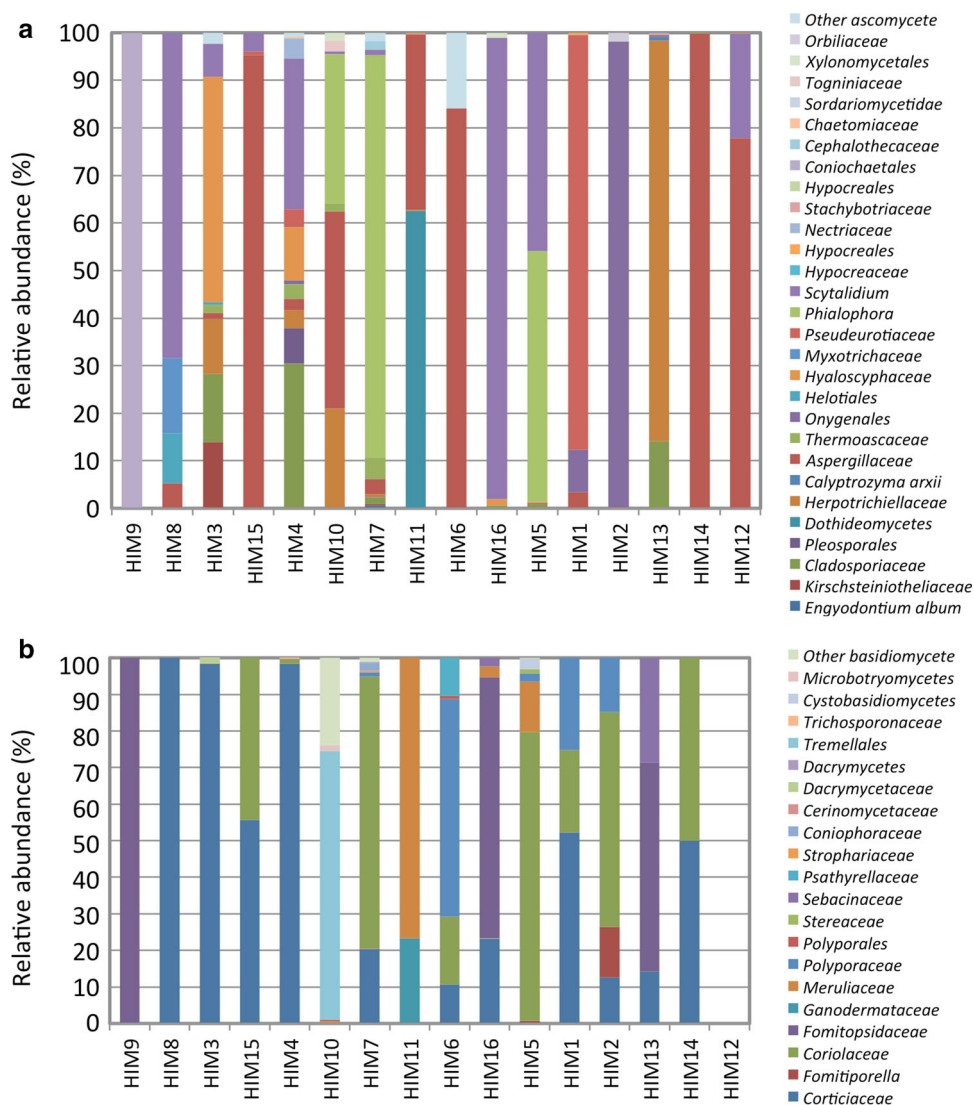


environmental conditions in houses was smaller than natural environment. Thus, the diversity of fungal species is thought to be smaller. In the fungal communities formed on *Abies* spp. and *Tsuga* spp. wood in this study, *Trametes hirsuta* and/or *Hyphodontia* sp. were dominant; on the samples, the species and abundance of ascomycetes are also varied.

Ascomycetes detected in this study included soft-rot fungi, which was supported by microscopic observation. In natural environments, ascomycetes that depend on soluble sugar invade in the early stages of wood decay [34]. Later, they are replaced by other ascomycete group with basidiomycetes [35, 36]. In the later stage, soft-rot fungi are thought to become dominant as the moisture content of the wood increases [37, 38]. Sorting the fungal communities by

the ascomycete abundance, communities with low ascomycete abundance had low diversity and high proportions of basidiomycetes; communities with low basidiomycete abundance had low diversity and high proportions of ascomycetes (Fig. 3). The compositions of fungal communities with high proportions of ascomycetes, such as HIM2 and HIM12, were not similar to one another: one compromised *Penicillium* spp. and *Scytalidium* spp. almost exclusively, while the other comprised a variety of various ascomycetes. This suggests that the process of fungal succession in decayed wood within houses may differ from succession in natural environments. One of the reasons for this may be diversification that occurs during the middle stage of decay [38, 39]. Another reason is that the samples in this study were the house components at water gathering
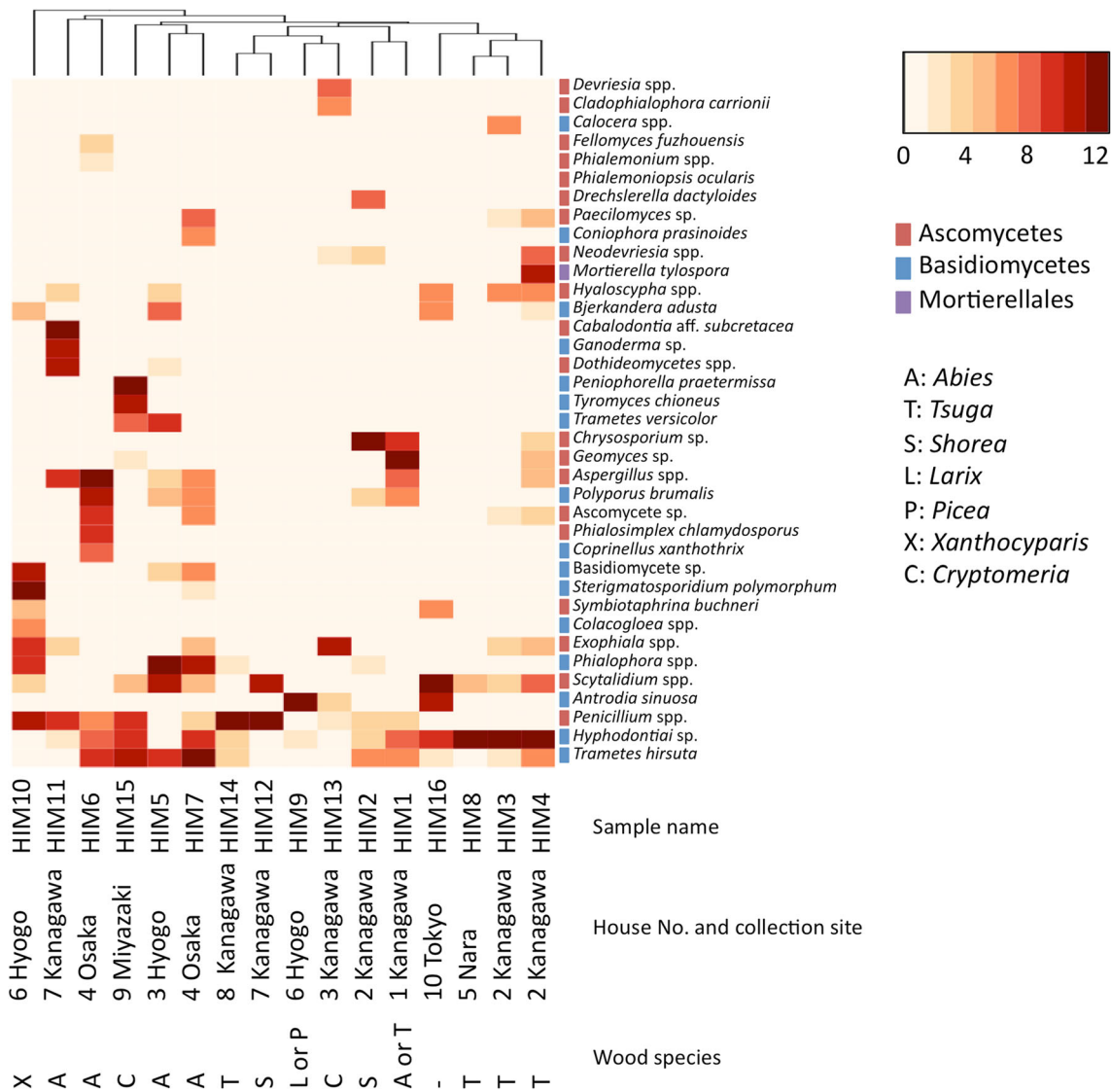
**Fig. 5** Cluster and heat map analysis of the fungal communities formed on the decayed wood samples from wooden houses

place. In this study, cooperative relationship was not revealed among fungal groups. Investigating the composition of fungal communities throughout the stages of decay is necessary to better understand the process of wood decay.

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