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ORIGINAL ARTICLE

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Fungal succession and decomposition of *Pinus densiflora* snags

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Abstract Fungal decomposition of coarse woody debris has an essential role in the biodiversity and carbon and nutrient dynamics in forest ecosystems. However, our knowledge of the effects of fungal species and within-stem diversity on wood decay is limited. In this paper, I described the process of wood decay and fungal succession of pine (*Pinus densiflora*) snags using chronosequence method. The results showed that the decay process differed between sapwood and heartwood. Sapwood decayed faster than heartwood, despite their initial densities being the same. Sapwood decay occurred in two phases. The first phase involved a typical white-rot process wherein acid-unhydrolysable residue (lignin) and holocellulose decayed simultaneously. White-rot species, such as *Trichaptum abietinum* and *Phanerochaete sordida*, were the dominant fungi and were likely the functional decomposers in this phase. The second phase involved selective decomposition of holocellulose. The dominant fungi in this phase included soft-rot species, such as *Trichoderma* spp., that can decompose holocellulose in wood that was previously delignified by white-rot species. In contrast to sapwood, heartwood experienced less loss of wood density and no clear change in lignocellulose composition, even in later stages of decay. Dominant fungi in heartwood included the latent inhabitants of living pine trees, such as *Ascocoryne cylichnium*, which is known to reduce colonization and wood decay by strong decomposers. These results suggest that, in addition to decay-resistant chemicals present in heartwood, the differences in the fungal communities present in heartwood and sapwood were responsible for the differences in their decay rates.

Keywords Decay · Fungal community · Heartwood · Pine · Sapwood

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Introduction

Coarse woody debris (CWD), such as standing dead snags and fallen logs, have an crucial role in maintaining the biodiversity and carbon and nutrient cycles in temperate forest ecosystems (Harmon et al. 1986). Among wood-inhabiting organisms, fungi have a central role in wood decomposition (Rayner and Boddy 1988). Basidiomycota and a portion of the Ascomycota phylum include many strong decomposers of wood structural components, such as acid-unhydrolysable residue (AUR, Klason lignin) and holocellulose (cellulose + hemicellulose). There is a species-specific preference regarding the decay of wood structural components, which is traditionally categorized into decay types. In white-rot decay, AUR and holocellulose are decayed simultaneously or, in some cases, AUR is decayed preferentially, whereas in brown-rot decay, only holocellulose is decayed leaving AUR slightly modified (Eaton and Hale 1993). The majority of species in the Ascomycota phylum are weak decomposers of fresh wood, and some species are known as soft-rot fungi (Nilsson 1973). This is the third decay type with the ability to decompose wood holocellulose, particularly under high water-stress conditions (Eriksson et al. 1990), or wood previously delignified by AUR decomposers (Fukasawa et al. 2011). The changes in physicochemical properties due to the activities of those fungi, in turn, affect the wood decomposer communities (Bunnell and Houde 2010; Stokland et al. 2012) and the downstream decay processes (Koide et al. 2005). Demonstrating the dynamics of fungal communities on decaying woody debris is essential to understanding their roles in forest biodiversity and carbon and nutrient cycling.

Because trees construct rigid stems, a substantial percentage of dead stems start their decay process as standing snags (Wilhere 2003; Svoboda and Pouska 2008). An obvious feature of fungal communities in snags is the existence of a vertical gradient caused by both abiotic and biotic factors. An important abiotic factor affecting the fungal community composition is moisture. Levy (1982) recognized four zones with different moisture conditions along the vertical length of a wood pole. The varieties and activities of the fungi within each zone differed, and the difference between each zone progressively increased over time although initially the zones had a more or less uniform composition. Differences in fungal communities along a moisture gradient may be determined not only on the basis of the difference in the optimal moisture levels required for hyphal growth among fungal species but also on the basis of the hierarchy of fungal interspecific competition. Boddy et al. (1985) suggested that differences in wood moisture condition could reverse the outcome of fungal antagonistic interactions. Differences in colonization strategies among fungal species also affect vertical distribution along a snag (Coates and Rayner 1985). Fungi colonizing from airborne spores dominate the upper part of the snag, whereas fungi colonizing from the soil by cords or rhizomorphs dominate the lower part of the snag. Decay columns of latent inhabitants colonizing healthy wood tissues, such as cambium and sapwood, develop near the bark. Vertical position also affects other organisms, such as insects that are intimately associated with fungi. Insects are a biotic factor that can affect fungal communities. For example, species composition of bark beetles differs vertically in pine snags (Masuya et al. 2009; Masuya and Yamaoka 2012). Consequently, the occurrence of beetle-associated fungi, such as *Ophiostoma* and its anamorphic genus *Leptographium*, also shows height-dependent patterns.

In addition to vertical differences, horizontal differences in wood decay also exist within a snag. Decay-resistant heartwood is a feature of pine stems (Hillis 1987). It contains resins, polyphenols, and antifungal chemicals that reduce the hyphal growth of fungi. Consequently, fungal communities differ dramatically between sapwood and heartwood (Käärik 1967), and decay rates are significantly reduced in heartwood versus sapwood (Harju et al. 2003).

These reports suggest that the wood decay process and responsive fungal communities differ vertically and horizontally within the stems of standing dead trees. However, the majority of CWD decay studies were conducted for logs, but not for snags. Knowledge of within-stem heterogeneity during wood decay is important to estimate the carbon and nutrient turnover rates and to evaluate the importance of snags as a habitat for a variety of organisms (Stokland et al. 2012). In the present study, fungal communities of live and standing dead *P. densiflora*, one of the most dominant trees in rural forests in Japan, were identified with respect to height from the ground and wood position (sapwood versus heartwood) along the decay process using the chronosequence method. Fungal species associations with wood physicochemical properties (wood density, water content, and lignocellulose content) and their roles in *P. densiflora* snag decomposition are also discussed.

Materials and methods

Study area

This study was carried out in a *P. densiflora* plantation at Kawatabi Field Science Center of Tohoku University (38°46′N, 140°45′E, 547 m a.s.l.), Miyagi, Japan. The mean annual temperature recorded at the weather station (170 m a.s.l.) was 10.4 °C from 2001 to 2010 (Japan Meteorological Agency 2014). The mean monthly temperature ranged from − 1.0 °C in January to 22.6 °C in August. The mean annual precipitation was 1656.6 mm. The ground was covered by snow in the winter period from November to April (53 cm depth in max). *P. densiflora* dominates (basal area, 34.7 m^2 ha^{-1}) and is the only canopy tree in the study area, but succession to broad-leaved trees, such as *Carpinus laxiflora* and *Acer rufinerve*, is occurring. The average diameter at breast height of *P. densiflora* was 21.8 cm (n = 35) at the study site (Fukasawa 2016).

Fungal isolation and identification

During May to July 2012, five living trees and 20 standing dead snags of *P. densiflora* were cut down at the base (hereafter referred to as snags). The diameter at breast height of these snags ranged from 11 to 34 cm. I assigned each snag to five decay classes (DC) from 0 to IV (five snags per decay class) by employing the criterion of Fukasawa (2012) with a little modification: (0) alive wood; (I) wood hard, penetrable with a knife to only a few mm, bark and twigs (diameter < 1 cm) intact; (II) wood rather hard, penetrable with a knife to less than 1 cm, bark and twigs have begun to shed away, branches (diameter 1–4 cm) intact; (III) wood distinctly softened, penetrable with a knife to approximately 1–4 cm, bark and branches partially lost, original snag circumference intact; (IV) wood considerably decayed, penetrable with a knife to approximately 5–10 cm, bark lost in most places, original snag circumference has begun to disintegrate. Since the definition of DC V states that CWD already has lost its original shape (Fukasawa 2012), no snags were assigned to DC V. Although there must be within-stem variation in decay classes (Pyle and Brown 1999), I defined the decay class of the snags at breast height in this study.

From each snag, three disks of approximately 10-cm thickness each, were cut out at 0, 2, and 4 m height from the tree base using a chainsaw. The disks were stored at 4 °C in plastic bags, and processed within 48 h. From each disk, small wood chips (approximately 2 mm^3) were cut from the cross-sections at five fixed-positions

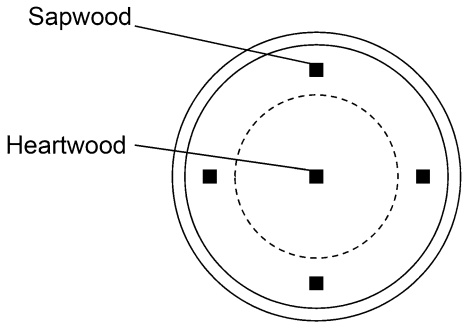

Fig. 1 Distribution of wood chip sampling points within a stem disk for fungal isolation. Black squares represents five fixed sampling points

corresponding to the locations of sapwood and heartwood (Fig. 1). In total, 375 wood chips were cut out.

All wood chips were removed aseptically from the disks and transferred to petri dishes containing malt extract agar medium (MA; 2% malt extract, 1.5% agar, w v^{-1}; Nacalai Tesque, Kyoto, Japan). The plates were incubated at 20 °C for 2 months. Once fungal colonies formed on the agar plates, each individual colony was transferred to a new agar plate to grow as a pure culture. Fungal identification was based on features and morphologies of the colonies, which were determined microscopically, particularly for anamorphic fungi. Identification of basidiomycetous isolates (defined by clamp-connections) and frequent sterile ascomycetous isolates (over 10% frequency in at least one decay class), were confirmed by DNA analysis. DNA was extracted from each isolated mycelium using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). The internal transcribed spacer (ITS) region of the basidiomycetous fungal rDNA was PCR-amplified using the primer pair ITS1F and ITS4 (Gardes and Bruns 1993). The PCR products were purified using Exo-Sap enzymes (SIGMA, St Louis, MO, USA). Sequencing was performed using the same primer pair. For acquired sequences over 400 bp in length, BLAST searches were performed against the public sequence database at the National Center of Biotechnology Information (NCBI) and UNITE (Koljalg et al. 2013) to identify the isolates. An ITS region with a shared identity of 97% was used as the criterion for the molecular identification of species. Sequence data have been submitted to DNA Data Bank of Japan (DDBJ) (Table S1). Selected isolates were stocked in the culture collection of Nite Biological Research Center (NBRC, Chiba, Japan) (Table S1).

Frequency (%) of each fungal species were calculated by the following equation:

$$\text{Frequency} = \text{number of disks with the species/total number of disks} \times 100.$$

Fungal species that showed > 10% frequency at least one DC were defined as the frequent species.

Physicochemical properties of wood

Wood samples were taken from each isolation point of each disk (Fig. 1) using an electric drill (drill bit diameter: 9 mm). Holes were drilled into each cross section, penetrating the disks such that all wood particles from the drilled-out holes were collected as samples. The depth of each drill hole was measured. Fresh wood samples were weighed and then dried to a constant weight at 40 °C. The water content of each wood sample was expressed as a percentage of the sample's dry weight. The relative density (g cm^{-3}) of the sample (also known as bulk density or apparent density, Yoneda 1975) was calculated as dry weight of the sample divided by the volume of the drill hole. Relative density is an adequate measure of the degree of decomposition in dead wood as long as the original shape and volume of the log was maintained (Yoneda 1975; Christensen 1984).

Dried wood samples were ground in a laboratory mill to pass through a 0.5 mm screen and then used for chemical analyses. The amount of acid-unhydrolyzable residue (AUR, also known as the acid-insoluble residue or 'Klason lignin' fraction) in the sample was estimated gravimetrically using hot sulphuric acid digestion (King and Heath 1967). Samples were extracted with alcohol–benzene at room temperature and the residues treated with 72% sulphuric acid (v v^{-1}) for 2 h at room temperature with occasional stirring. The mixture then was diluted with distilled water to make a 2.5% sulphuric acid solution and autoclaved at 120 °C for 60 min. After cooling, the residue was filtered and washed with distilled water through a porous crucible (G4), dried at 105 °C and weighed. The filtrate (autoclaved sulphuric acid solution) was used for total carbohydrate analysis by the phenol–sulphuric acid method (Dubois et al. 1956). Five percent phenol (v v^{-1}) and 98% sulphuric acid (v v^{-1}) were added to the solution. The optical density of the solution was then measured by spectrophotometer at 490 nm using the known concentrations of D-glucose as standards. Since wood contains very little carbohydrate other than holocellulose, measured amounts of total carbohydrate were regarded as holocellulose contents in this study.

After acid hydrolysis, the remaining residue contained a mixture of organic materials. Through nuclear magnetic resonance analysis, Preston et al. (1997) demonstrated that the organic residue remaining after acid hydrolysis of a variety of litter types contained a mixture of organic compounds, including not only lignin, but also condensed tannins and waxes. In the present study, we use the term AUR to refer to the organic residue remaining after chemical analysis.

Lignocellulose index (LCI) is a useful index of wood chemical quality (Fukasawa et al. 2010). LCI was calculated according to the following equation:

$$\text{LCI} = \text{holocellulose concentration}/(\text{AUR concentration} + \text{holocellulose concentration}).$$

This index is designed to equal 1 in the case wood contains only holocellulose but no AUR, and 0 if wood contains only AUR but no holocellulose.

Data analysis

Generalized liner models (GLM) with Gaussian error structure were used to detect significant effects among snag decay class, height from the ground, disk diameter, and wood position (sap or heart) in relation to density and water content of wood samples. Multicollinearity problems were not detected as the variance inflation factors of the explanatory variables were < 2 although a weak correlation was detected between decay class and diameter (Table S2). The Steel–Dwass test ($P < 0.05$) was performed to compare the mean values for wood density and water content between decay classes. GLM and Steel–Dwass tests were performed by R ver. 3.4.0 (R Core Team 2017).

A canonical ordination method was used to determine the relationships between the fungal community compositions and the wood physicochemical properties. A detrended correspondence analysis was performed as a preliminarily to analyse the length of the ordination axis, which was 8.231 (> 4 SD), suggesting that the response curve could be unimodal: thus, canonical correspondence analysis (CCA) was appropriate (Jongman et al. 1995). The occurrence data for frequent fungal species were binary transformed for each wood sample and used as species data. Two nominal variables (sapwood and heartwood) and five quantitative variables (decay class, height from the ground, disk diameter, relative density, and water content of wood) were used as environmental variables. All the quantitative variables were transformed into Z-scores using the following equation to eliminate the effects of different units:

$$Z = (x_0 - x') / \text{SD},$$

where x_0 is the value of variable x in a particular log and x' is the arithmetic mean of variable x in the sample.

All the ordination analyses were performed using Canoco 4.5 software (ter Braak and Šmilauer 2002), where the relationships between sets of environmental variables and ordination scores were plotted in ordination diagrams. In the diagrams, the arrows for the environmental variables depicted the direction and magnitude of the relationships between the environmental variables and the fungal community. The biplots focused on the inter-species distance. We tested the explanatory variables using the automatic forward selection procedure provided by Canoco 4.5 software (Monte Carlo permutation test with 9999 randomizations).

Results

Wood physicochemical properties

Wood density of DC 0 (living tree) was negatively associated with height from the ground and diameter, whereas no differences in wood density existed between sapwood and heartwood (Table 1). Water content of DC 0 wood was significantly higher in sapwood than in heartwood and positively associated with wood diameter, but negatively associated with height. AUR concentrations were positively associated with height (Table 1). Holocellulose concentrations and LCI were not associated with any variables tested.

Decay class, wood position, height from the ground, and diameter significantly affected wood density of the snags (Table 2). Negative estimate for the coefficient of height suggested that wood disks far from the ground have lower densities. Wood density significantly decreased as decay progressed both in sapwood and heartwood, but greater density loss was observed in sapwood than in heartwood (Fig. 2). Water content of wood also was affected significantly by all the variables tested (Table 2). Wood disks far from the ground had lower water content, whereas disks with larger diameters had higher water content. Water content increased as decay progressed, both in sapwood and heartwood, but was higher in sapwood compared to heartwood for all DCs, and there were different patterns of fluctuation between sapwood and heartwood (Fig. 2). In sapwood, live wood tissues (DC 0) contained great amounts of water exceeding 100% of the dry wood weight. Once the tree died, water content decreased significantly to approximately 70% of dry wood weight, then increased as decay progressed, reaching about 200% in DC IV. In contrast, heartwood contained little water, even in live wood (only about 30%). Although water content increased as decay progressed, it reached only about 110% of dry wood weight, even in DC IV.

AUR concentrations were positively associated with height from the ground and snag diameter, and increased with DC (Table 2). In particular, AUR concentrations in sapwood increased significantly from DC III to DC IV (Fig. 3). Such a significant increase in AUR concentration was not observed in heartwood. AUR concentrations were not significantly different between sapwood and heartwood (Table 2). In contrast to AUR, hollocellulose concentrations in sapwood decreased from DC III to DC IV (Fig. 3). Hollocellulose concentrations in heartwood did not differ among DCs. None of the tested variables had significant associations with holocellulose concentrations (Table 2). LCI in

Table 1 GLM results of the effects of variables on wood physicochemical properties in DC 0 living *Pinus densiflora* trees

Variable	Wood density	Water content	AUR	Holocelulose	LCI
Position (Sapwood)	− 0.013	85.178***	17.887	183.04	0.060
Height	− 0.013***	− 3.368*	17.281*	25.14	0.006
Diameter	− 0.004*	3.047***	− 0.706	− 11.48	− 0.008

$n = 75$ for wood density and water content, and 14 for AUR, holocellulose and LCI
AUR acid-unhydrolysable residue, *LCI* lignocellulose index = holocellulose conc./(holocellulose conc. + AUR conc.)
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

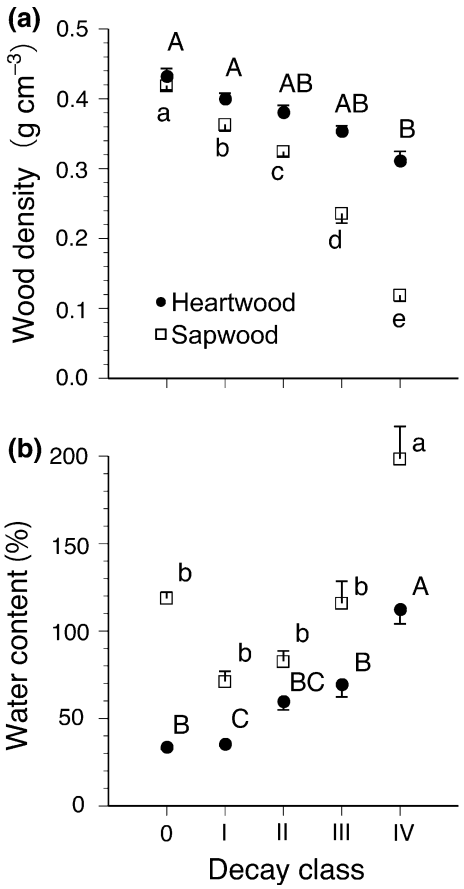

Fig. 2 Relative density (**a**) and water content (**b**) of pine snags in various decay classes. DC 0 represents living pine trees. Bars indicate standard errors ($n = 15$ for heartwood and 60 for sapwood). Symbols, closed circle heartwood; open square sapwood. The same letter above the symbols indicates no significant differences, uppercase heartwood; lowercase sapwood (Steel–Dwass test, $P < 0.05$). Criterion for decay class is after Fukasawa (2012)

sapwood remained constant from DC 0 to DC III, and decreased significantly thereafter (Fig. 3). LCI in heartwood fluctuated greatly among DCs, but did not decrease as decay progressed.

Fungal communities

From 375 wood chips, 74 fungal species were detected, including 53 ascomycetes, 16 basidiomycetes, and 5 zygomycetes (Table S1). Of the total wood chips, 19.2% were sterile even in later decay classes.

Ten fungal species were selected as the most frequently occurring species, with over 10% frequency observed in at least one DC. *Trichaptum abietinum* was selected as a frequently ooccurring species, even though its frequency was measured at only 8.3% because this fungus was the second-most frequent basidiomycota in the list. This species was included in the following analysis with respect to the mechanism of wood decomposition because of their wood decay abilities (Son et al. 2011). Other frequent species were *Ascocoryne cylichnium*, Helotiales sp. *Leptographium procerum*, *L. truncatum*, *Penicillium thomii*, *Phanerochaete sordida*, *Rhinocladiella atrovirens*, *Trichoderma harzianum*, and *T. viride* (Table S1, Fig. 4). They were separated into two groups: species dominating only in sapwood and species dominating in heartwood more than in sapwood: the first group included five species shown on the left side of Fig. 4; the second group is shown in the right side. In both sapwood and heartwood, fungal succession was observed (Fig. 4). In sapwood, the primary colonizer *T. abietinum*, dominated in DC I and II. The succession was followed by *P. sordida* and *T. viride* dominating in DC III, and *P. thomii* dominating in DC IV. *Trichoderma harzianum* frequently occurred in all DC, except for DC 0, and peaked in DC II. In heartwood, Helotiales sp. and *A. cylichnium* occurred even in DC 0 wood, suggesting they are latent inhabitants in heartwood (Fig. 4). Both maintained high frequencies greater than 10% throughout the decay process, and even increased for *A. cylichnium*. Two *Leptographium* species were the primary colonizers after the tree died, dominating in DC I and II. *Rhinocladiella atrovirens* was a secondary colonizer in heartwood and sapwood, dominating in DC IV.

Ordination analysis showed that wood density, height, position, and DC were significantly associated with the occurrence patterns of the ten frequent fungal species (Fig. 5). The first and the second axes explained 51.8 and 21.2% of the total variance found in the relationships between species and environmental variables, respectively. The occurrences of *A. cylichnium* and Helotiales sp. were associated with heartwood, while the occurrences of *T. abietinum* and *T. viride* were associated with sapwood. *A. cylichnium*, Helotiales sp., *L. truncatum*, *L. procerum*, and *T. abietinum* were associated with relatively early DCs of wood with high densities. In

Table 2 GLM results of the effects of variables on wood physicochemical properties of *Pinus densiflora* snags

Variables	Wood density	Water content	AUR	Holocelulose	LCI
Decay class	− 0.066***	23.101***	17.337***	− 20.02	− 0.017***
Position (Sapwood)	− 0.083***	55.385***	− 0.194	32.9	0.013
Height	− 0.007**	− 15.266***	7.166*	14.84	0.0002
Diameter	− 0.003**	4.154***	2.071*	− 1.29	− 0.002

n = 375 for wood density and water content, and 99 for AUR, holocellulose and LCI
AUR acid-unhydrolyzable residue, *LCI* lignocellulose index = holocellulose conc./(holocellulose conc. + AUR conc.)
*P < 0.05; **P < 0.01; ***P < 0.001

contrast, *R. atrovirens*, *T. viride*, *P. sordida*, and *P. thomii* associated with later DCs with lower wood densities. Water content and diameter had no significant associations with dominant fungal species.

Discussion

In the present study, wood density losses, chemical component changes, and fungal succession patterns in pine snags suggested that the decay processes in sapwood and heartwood are significantly different each other. Sapwood decayed faster than heartwood, even though the initial densities between the two were not different. Although wood density was negatively correlated with both height and diameter, it was not possible to confirm that wood located far from the ground and with a larger diameter decayed faster, because the initial wood density in DC 0 already was negatively correlated with height and diameter (Table 1). More rapid decomposition in sapwood versus heartwood also has been observed in oak (van der Wal et al. 2015). AUR concentrations are known to retard decay rates of dead plant tissues (Osono 2007). However, in the present study, AUR concentrations in DC 0 were not different between sapwood and heartwood (Table 1). Part of the difference in decay rates between sapwood and heartwood may have been due to their differences in water content. Heartwood water content at 50% of dry weight is too dry for most wood decaying fungi (Schmidt 2006). However, this was not the case for DC II and later DCs (Fig. 2). Other possible explanations are low oxygen content and/or high secondary metabolite content in heartwood; both are known to reduce fungal decay activities (Hintikka and Korhonen 1970; Hillis 1987). Effect of fungal communities on wood decay rate may be also possible and is discussed later.

In sapwood, wood decay processes can be divided into two phases according to LCI dynamics (Fig. 3). During the earlier decay phase, in DC 0 to III, LCI was constant (Fig. 3), even though wood density was significantly reduced (Fig. 2), suggesting that wood AUR and holocellulose were decayed simultaneously during this phase. This is a typical pattern for white rot (Fukasawa et al. 2009). The two basidiomycetes that dominated during this phase, *T. abietinum* and *P. sordida*, were both white rot fungi (Son et al. 2011; Ruttimann-Johnson et al. 1994). Even in low frequencies, other basidiomycetes recorded in this phase, such as *Hypochnicium albostramineum*, *Kuehneromyces mutabilis*, *Panellus stypticus*, *Scytinostroma odoratum*, and *Sistotrema brinkmannii* (Table S1) were white rot species (Tedersoo et al. 2010). Thus, it seems likely that the first phase of sapwood decay was driven by these white rot fungi. In contrast, LCI decreased significantly from DC III to DC IV (Fig. 3), suggesting a more selective decay of holocellulose took place during this phase. The *Trichoderma* species that dominated in this phase are known as soft rot fungi, and they selectively decay holocellulose under humid conditions (Nilsson 1973). In particular, *T. harzianum* has been reported to substantially decay holocellulose of delignified wood (Fukasawa et al. 2011). Other dominant ascomycetes, such as *Leptographium* species and *R. atrovirens*, are known as sapstain fungi without any ability for decaying wood structural components (Eaton and Hale 1993). *Penicillium thomii* also has little ability to decay lignocellulose (Osono et al. 2003).

In contrast to sapwood, decay rates in heartwood were relatively slow. Even in the late decay phase (DC IV), wood density of heartwood was similar to that of sapwood in only DC II (Fig. 2). The pattern of fungal succession in heartwood was quite different from that of sapwood. Two species of ascomycetes, Helotiales sp. and *A. cylichnium*, were dominant already in heartwood, even before the death of tree (Fig. 4). *Ascocoryne* species (such as *A. cylichnium* and closely related *A. sarcoides*) are well-known endophytes in the healthy heartwood of various tree species (Basham 1966, 1973, 1975; Rollhansen and Roll-hansen 1979a, b; Huse 1981; Kebli et al. 2014). Previous studies have reported that the abundance of *Ascocoryne* species tends to be larger in older stems and in those lower to the ground, but they seldom are detected in dead branches attached to living stems. As a result, they are expected to invade the heartwood of living stems at or near ground level, but not the dead branches on stems (Basham 1975). This genus was reported to cause only small weight losses in test blocks of pine (Basham 1966; Bourchier 1961), but is known to produce the strong antibiotic, Ascocorynin (Quack et al. 1980) and several volatile organic compounds (VOCs) (Griffin et al. 2010; Strobel et al. 2010) that likely inhibit the growth of heart rot fungi responsible for wood decay in the living stems of spruce and

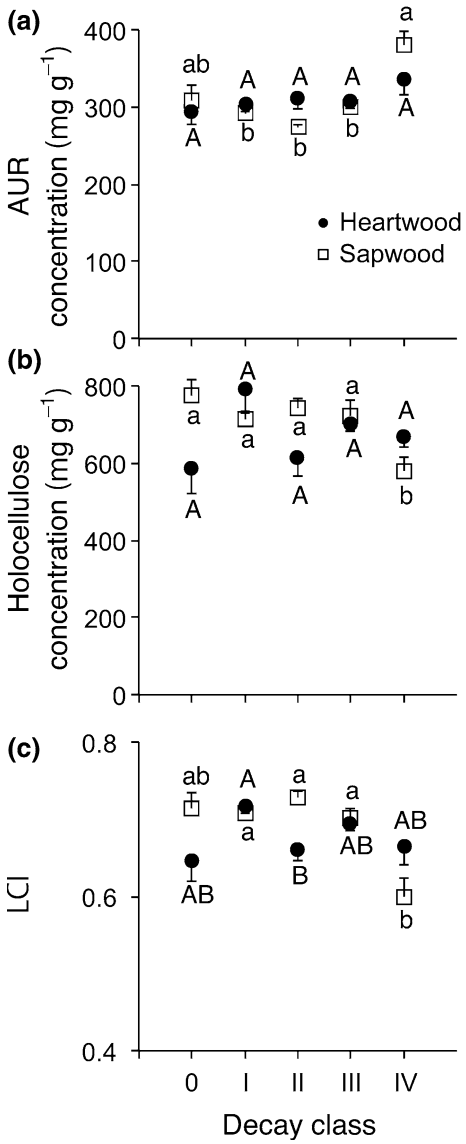

Fig. 3 Concentrations of acid-unhydrolysable residue (AUR, known as 'Klason lignin' fraction) (**a**) and holocellulose (**b**). Lignocellulose index (LCI) (**c**) was calculated from the above two concentrations (see text) in pine snags found in various decay classes. DC 0 represents living pine trees. Bars indicate standard errors (n = 5 for sapwood in DC 0, 9 for heartwood in DC 0, 14 for sapwood in DC I, 9 for heartwood in DC I, 10 for sapwood in DC II, 8 for heartwood in DC II, 15 for sapwood in DC III, 10 for heartwood in DC III, 14 for sapwood in DC IV, and 5 for heartwood in DC IV). Symbols are the same as Fig. 2. Criterion for decay class is after Fukasawa (2012)

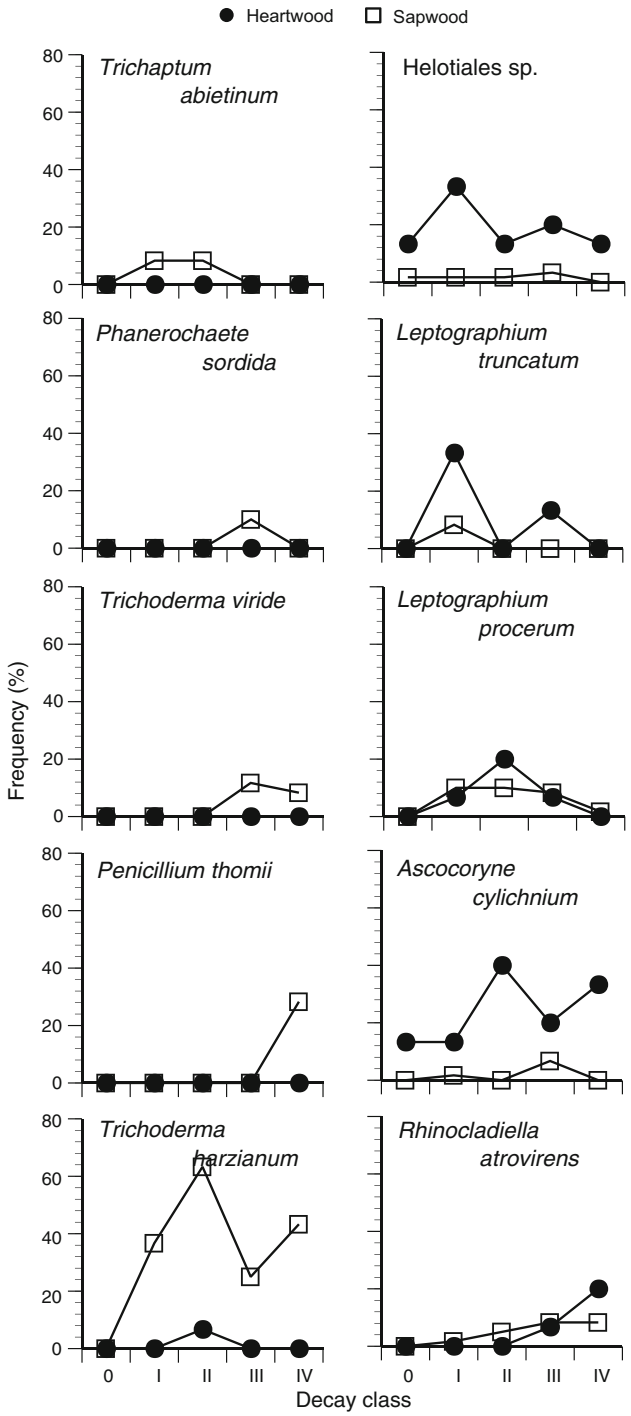

Fig. 4 Frequency of occurrence (%) for the 10 most frequent fungal species found on pine snags in various decay classes. DC 0 represents living pine trees. Symbols are the same as Fig. 2. Criterion for decay class is after Fukasawa (2012)

pine trees (Basham 1973, 1975). Interestingly, the present study found that abundance of *A. cylichnium* were not only maintained, but actually increased in pine heartwood, even after the tree died and into the later stages of snag decay. Kebli et al. (2014) also reported similar results during a 30-month decay experiment of aspen and fir wood blocks on the forest floor. These results suggest that the dominance of *A. cylichnium* in the present study may partially explain the observed reduced decay of pine heartwood. Wood decay and the antagonistic abilities of Helotiales sp. have not been tested at this point.

Two *Leptographium* species dominating in both sapwood and heartwood are known for their intimate associations with and vectored by particular species of

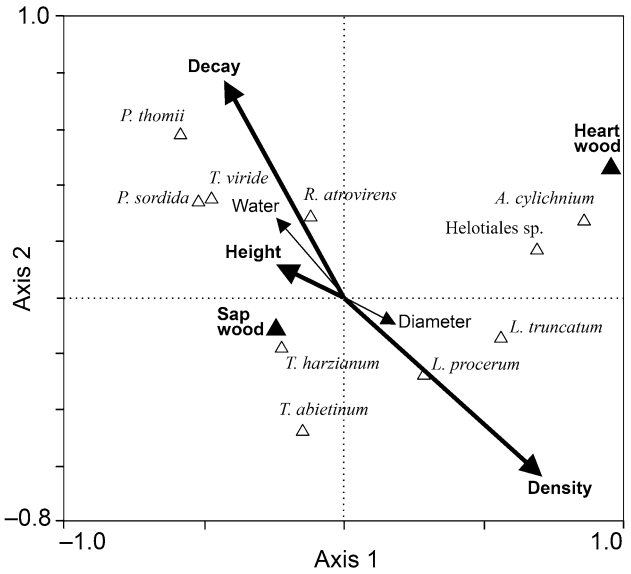

Fig. 5 Canonical correspondence analysis plots of the occurrences of the 10 most frequent fungal species found on pine snags, showing their associations with snag variables. Variables showing significant correlations with fungal communities (by Monte Carlo permutation test) are indicated by thick black arrows and filled triangles with boldface letters. The first and the second axes explain 51.8 and 21.2% of the total variance of the relationships between species and environmental variables, respectively

bark beetles (Coleoptera: Scolytdae) in the genera of *Hylurgops* and *Hylastes* that breed near the base of pine trunks (Masuya et al. 2009). Because these are wood-boring beetles, the distribution of the associated *Leptographium* fungal species within pine stems also is restricted near the base of trunk (Masuya et al. 2009). In line with this previous knowledge, the results from the CCA analysis in the present study showed that *Leptographium* species tend to occur in lower parts of pine stems (Fig. 5). Since the associated beetle species are saprotrophic to dead trees, the occurrence of *Leptographium* species were recorded only from dead trunks. *Rhinocladiella atrovirens* also is known to be a typical pine and spruce fungus in association with bark beetles that attack the trees (Jankowiak 2005; Jankowiak and Kurek 2006; Jankowiak et al. 2007). However, this fungus likely is not vectored by bark beetles because it is not found in the beetle bodies (Jankowiak et al. 2007). *Rhinocladiella atrovirens* has no wood decay abilities, but has a high tolerance to toxins (Zabel et al. 1985), and this may partially explain why they are frequently found in heartwood that is strongly protected by the toxin-producing *Ascocoryne* species.

Conclusions

In summary, this research found clear differences in the wood decaying processes and fungal community dynamics between the sapwood and heartwood of *P. densiflora* snags. Wood decay in sapwood is a typical two-phase pattern consisting of white rot and soft rot processes driven by the dominant white rot fungi, *T. abietinum* and *P. sordida*, and soft rot fungi, *Trichoderma* spp. On the other hand, heartwood was dominated by the latent inhabitants, *A. cylichnium* and Helotiales sp., which reside in the tree before tree dies and remain there until the late stages of decay. Most likely due to the antifungal activities of *A. cylichnium*, only toxin-tolerate stain fungi were able to colonize heartwood, thus restricting substantial decay in heartwood, even after the sapwood had decayed and become very fragile. Since *Ascocoryne* species abundance is known to increase with stem age and thickness in living conifer trees (Basham 1975; Roll-hansen and Roll-hansen 1979b), older and thicker pine tree stems may be more decay resistant than younger ones. Thus, the presence/absence of this species in heartwood of thick pine stems may be critical for defending heartwood from heart rot fungi. Actually, the frequency of *A. cylichnium* increased, even after the tree died, and no heart rot was recorded in wood disk samples in the present study. These results suggest the importance of naturally occurring heartwood endophytes, such as *A. cylichnium*, in thick stems of *P. densiflora* as biological agents to retard heartwood decomposition, and thus to create within-stem decay heterogeneity in forest ecosystems.

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Compliance with ethical standards

Conflict of interest The author declares no conflicts of interest. All the experiments undertaken in this study comply with the current laws of Japan.

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