Stem traits, compartments and tree species affect fungal communities on decaying wood

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Summary

Dead wood quantity and quality is important for forest biodiversity, by determining wood-inhabiting fungal assemblages. We therefore evaluated how fungal communities were regulated by stem traits and compartments (i.e. bark, outer- and inner wood) of 14 common temperate tree species. Fresh logs were incubated in a common garden experiment in a forest site in the Netherlands. After 1 and 4 years of decay, the fungal composition of different compartments was assessed using Internal Transcribed Spacer amplicon sequencing. We found that fungal alpha diversity differed significantly across tree species and stem compartments, with bark showing significantly higher fungal diversity than wood. Gymnosperms and Angiosperms hold different fungal communities, and distinct fungi were found between inner wood and other compartments. Stem traits showed significant afterlife effects on fungal communities; traits associated with accessibility (e.g. conduit diameter), stem chemistry (e.g. C, N, lignin) and physical defence (e.g. density) were important factors shaping fungal community structure in decaying stems. Overall, stem traits vary substantially across stem compartments and tree species, thus regulating fungal communities and the long-term carbon dynamics of dead trees.

Introduction

Forests play an important role in global carbon and nutrient cycling, and tree stems have significant afterlife effects on forest functioning (Stokland *et al*., 2012). A large part of forest carbon (∼8% of total C pool globally) is locked up in dead trees (Martin *et al*., 2020), which slowly becomes available as stems decompose (Yatskov *et al*., 2003). Moreover, dead wood provides a wide range of niches for diverse forest-dwelling species, therefore playing a vital role in forest biodiversity conservation (Stokland *et al*., 2012). Increasing evidence shows that biodiversity correlates with multiple ecosystem services (Brockerhoff *et al*., 2017). For example, previous studies reported that high diversity of wood-inhabiting fungi facilitate organic matter decay and enhance biochemical cycling (van der Wal *et al*., 2013) because of functional niche complementarity among fungal species (Hättenschwiler *et al*., 2011). However, negative impacts of fungal diversity on wood decomposition rates have also been reported and were linked to the competition among wood-rot fungi (Fukami *et al*., 2010; Wells and Boddy, 2002). The role of biodiversity in the processes underlying stem decay is thus still controversial.

Among the diverse dead wood inhabitants, wood-rotting fungi have gained special attention as they decompose the major polymers (i.e. cellulose, hemicellulose and lignin) encountered in dead tree logs (van der Wal *et al*., 2013). Wood-rotting fungi can generally be divided into three main functional groups; white-rot Basidiomycetes, brown-rot Basidiomycetes and soft-rot Ascomycetes. While all of these groups can degrade cellulose and hemicellulose, only white-rot fungi can degrade more

recalcitrant lignin (Blanchette, 2000). White-rot fungi use extracellular lignocellulolytic enzymes for degradation, brown-rot fungi use hydrogen peroxide and iron ions (Koenigs, 1974), while soft-rot fungi degrade wood through the excretion of cellulase (Schmidt, 2006). Other fungal functional groups such as ectomycorrhizal, lichenized and pathogenic fungi can also be associated with dead wood, although lichens do not penetrate into the bark or wood (Kharpukhaeva and Mukhortova, 2016). Stems of different tree species can support different fungal communities, with different fungal species showing a range of strategies to compete for limited resources such as nutrients, water, oxygen and space, and to degrade the wood matrix (Stokland et al., 2012; Brockerhoff et al., 2017). Moreover, wood-associated fungi may show strong temporal dynamics and priority effects; the first-arriving fungi may determine whether fungi compete with or facilitate other colonizing fungi during community assembly (Hiscox et al., 2015; Leopold et al., 2017). Fungal species also differ with respect to niche breadth, ranging from a high degree of specialization to more generalist lifestyles. The amount and variation of forest dead wood may therefore be an important driver of forest biodiversity (Kohout et al., 2018; Gómez-Brandón et al., 2020).

During the last decades, many studies have highlighted the importance of dead wood (Thorn et al., 2020) with forest biodiversity being positively correlated with the amount of dead wood (Heilmann-Clausen and Christensen, 2004; Meyer and Schmidt, 2011; Gossner et al., 2016). Recently, the focus has shifted from the quantity to the quality, i.e. the substrate characteristics of dead wood (Kahl et al., 2017; Andringa et al., 2019; Lee et al., 2020) and the relevance of various environmental drivers for decomposition (Krah et al., 2018; Müller et al., 2020). However, to date, it still remains unclear which stem traits are important for fungal communities and how these traits may contribute to the diversity and composition of wood-inhabiting fungal communities during the early decay stage.

Stem physical and chemical traits determine the accessibility and substrate quality of dead wood for different fungi, thereby impacting fungal diversity and composition (Fukasawa et al., 2009; Rajala et al., 2012; Purahong et al., 2017). For example, angiosperm tree species differ from gymnosperm species in anatomical structure and lignin composition, which can be related to different fungal community compositions (Cornwell et al., 2009; Kahl et al., 2017; Yang et al., 2022). Across tree species, defence traits (i.e. wood density, lignin concentration), nutritional quality (nitrogen concentration, C/N, C/P, and N/P ratios) and habitat condition (i.e. wood moisture content) have commonly been reported as determinants of fungal communities (Baldrian et al., 2016; Hoppe et al., 2016; Kielak et al., 2016; Krah et al., 2018). Yet, wood anatomical traits that are important for fungal access (such as conduit size and conduit wall thickness) and nutrient availability (such as radial and axial parenchyma fraction) have received less attention (Schwarze et al., 2003; Zanne et al., 2015; Lee et al., 2020). Moreover, few studies have evaluated variation in fungal communities with respect to stem compartment (i.e. bark, sapwood and inner wood) despite the fact that they can strongly differ in wood anatomical and chemical traits (Yang et al., 2022). Previous studies have generally been limited to 1–2 tree species (Kubartová et al., 2012; van der Wal et al., 2016) or yielded inconsistent results. For example, fungal diversity and composition differed significantly between sapwood and heartwood of decaying spruce logs (Kubartová et al., 2012), whereas no significant differences were detected between sap- and heartwood for 13 temperate tree species after 6 years of decay (Leonhardt et al., 2019). Such inconsistent findings between papers may be attributed to different target tree species and decay stages. It is clear that there is a need to expand knowledge to a much broader range of tree species and stem compartments, thereby allowing for a more systematic examination of wood traits with respect to fungal diversity and composition. By doing so, we can gain a better understanding on which factors are important for determining fungal community structure that is related to decomposition rates and C dynamics in forests.

In this study, we aim to examine how fungal richness, diversity and community composition vary between different stem compartments and how various stem traits affect these wood-inhabiting fungal communities. We hypothesize that, during the earlier phase of wood decay, (i) bark habitats would support a richer and more diverse fungal community than inner and outer wood because it has the greatest exposure to fungal colonization, (ii) fungal communities inhabiting chemically well-defended heartwood would be comprised of a greater proportion of specialized degraders compared to other compartments and (iii) differences in fungal communities between tree species would be linked to stem traits related to substrate quality and accessibility. To test these hypotheses, we used high-throughput amplicon sequencing to examine the fungal communities inhabiting different stem compartments (inner wood, sapwood and bark) of logs from a total of 14 gymnosperm and angiosperm species. All fresh logs were incubated in a common garden experiment in the same forest site in the Netherlands, thereby assuring that all logs were exposed to similar environmental conditions and were exposed to the same fungal community pool in their direct surroundings.

Results

Alpha diversity and its determining factors

The rarefaction curves indicated that the sequencing depth was enough to reach the diversity of all samples (Supporting Information Figs. S1 and S2). A total of 1466 (T1 – after 1 year decay) and 814 (T4 – after 4 years decay) fungal ASVs were obtained in samples from the three main stem compartments (inner wood, outer wood and bark) of the 14 temperate tree species. For samples collected after 1 year of decay, fungal richness and Shannon diversity index (H) values differed significantly among tree species and stem compartments (Fig. 2); bark samples had significantly higher fungal richness (54.5 ASVs) and Shannon diversity ($H = 2.1$) than samples from the inner wood (13.4 ASVs, $H = 1.4$) and outer wood (13.4 ASVs, $H = 1.1$) (Fig. 2A, Supporting Information Fig. S3a). Bark samples of *Fraxinus excelsior* had the highest fungal richness (137 ASVs) and Shannon diversity ($H = 3.1$), while the outer wood of *Chamaecyparis lawsoniana* had the lowest fungal richness and Shannon diversity (5.8 ASVs, 0.4 H). After 4 years (T4), no differences were observed among tree species in terms of each stem compartment (Fig. 2C, $P > 0.05$), but fungal richness still differed significantly among stem compartments (Fig. 2A, $P < 0.05$). Notably, the observed significant differences among stem compartments were mainly determined by the presence of bark samples; differences among stem compartments were less apparent or disappeared when bark samples were removed from the analyses (Supporting Information Fig. S4).

Pearson correlations showed the associations between physical–chemical traits of inner wood, outer wood and bark, and the fungal richness and Shannon diversity (Table 2). Variation in fungal alpha diversity can be explained by different stem traits, but such effects varied between stem compartments. Overall, inner wood traits have stronger afterlife effects on fungal alpha diversity compared to outer wood and bark as indicated by more significant traits. For the inner wood at T4, fungal richness and Shannon diversity correlated negatively with PC1 scores (i.e. weak physical defence and high nutrient levels, Supporting Information Fig. S5). Fungal richness and Shannon diversity correlated positively with carbon and lignin concentrations, but negatively with N, P concentrations and conduit diameter (Table 2; Fig. 3A). For outer wood, fungal richness and Shannon diversity tended to be higher in samples with high nutrient concentrations at T1 but tended to be higher in samples with high C/N ratio at T4 (Table 2; Fig. 3B). For bark, fungal richness and Shannon diversity tended to be lower for species with high lignin and cellulose concentrations after 1 year of bark decay (Table 2; Fig. 3C). These findings were supported and more comprehensively visualized by PCAs as shown in Supporting Information Fig. S5.

Fungal community composition

Among the ASVs detected at T1 (1466) and T4 (814), 806 (T1) and 512 (T4) were assigned to specific guilds according to primary lifestyles (described in Material and methods). A total of 469 (T1) and 359 (T4) ASVs belonged to saprotrophic fungi consisting of 79 (T1) and 94 (T4) white-rot fungi, eight (T1) and 36 (T4) brown-rot fungi, eight (T1) and seven (T4) soft-rot fungi, and 164 (T1) and 97 (T4) undefined saprotrophic fungi. Additionally, 146 (T1) and 50 (T4) fungi belonged to pathotrophs. The relative abundance of these fungal ecotypes differed among tree species and decay times (i.e. T1 and T4), but no clear pattern was found among stem compartments in terms of fungal ecotypes (Fig. 4). After 1 year of decay, the mean relative abundance of plant pathogens was higher in angiosperm species (27.8%) compared to gymnosperms species (10.4%),

Table 1. Information on the 14 studied tree species with their name, abbreviation, major phylogenetic group they belong to, collection site and wood structure.

Species	Abbreviation	Major taxa	Collection sites	Heartwood presence	Wood structure
Fraxinus excelsior	Fra.e	Angiosperm	Flevoland	No	Ring-porous
Betula pendula	Bet.p	Angiosperm	Flevoland	No	Diffuse-porous
Quercus robur	Que.r	Angiosperm	Schovenhorst	Yes	Ring-porous
Fagus sylvatica	Fag.s	Angiosperm	Flevoland	No	Diffuse-porous
Populus × *canadensis*	Pop.c	Angiosperm	Flevoland	No	Diffuse-porous
Populus tremula	Pop.t	Angiosperm	Schovenhorst	No	Diffuse-porous
Chamaecyparis lawsoniana	Cha.l	Gymnosperm	Schovenhorst	Yes	Tracheid
Thuja plicata	Thu.p	Gymnosperm	Schovenhorst	Yes	Tracheid
Cryptomeria japonica	Cry.j	Gymnosperm	Schovenhorst	Yes	Tracheid
Taxus baccata	Tax.b	Gymnosperm	Schovenhorst	Yes	Tracheid
Picea abies	Pic.a	Gymnosperm	Schovenhorst	Yes	Tracheid
Larix kaempferi	Lar.k	Gymnosperm	Schovenhorst	Yes	Tracheid
Pseudotsuga menziesii	Pse.m	Gymnosperm	Schovenhorst	Yes	Tracheid
Abies grandis	Abi.g	Gymnosperm	Schovenhorst	No	Tracheid
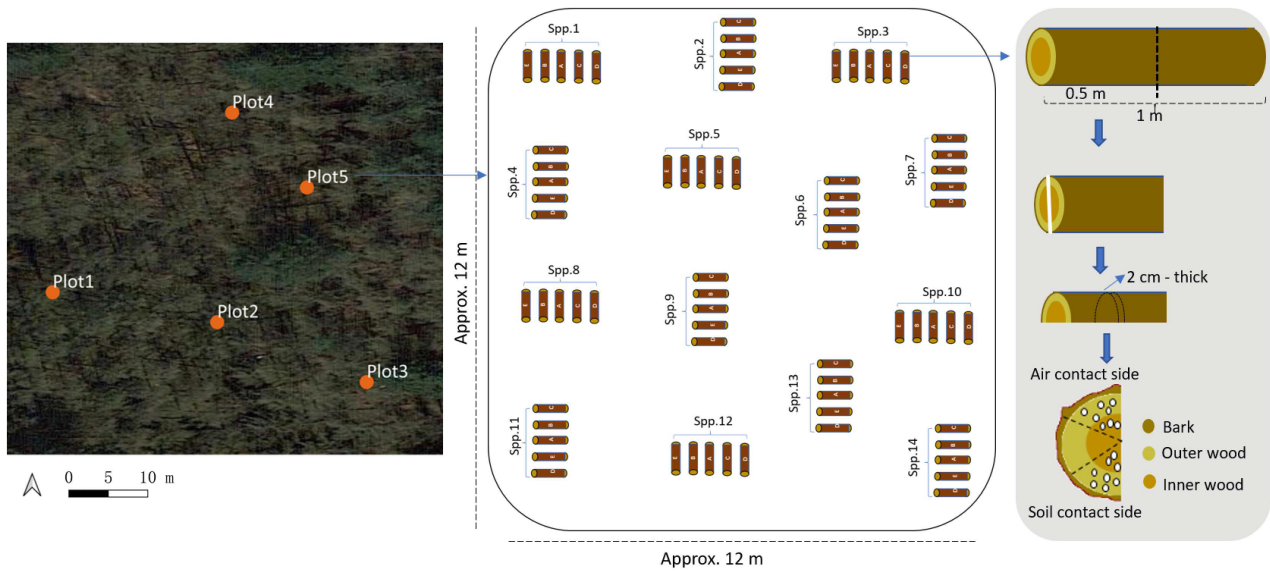
Fig. 1. Experimental design of the decomposition experiment in Schovenhorst. Five trees per species ($N = 14$) were incubated in five plots per site. Each plot measured approximately 12 by 12 m, with a minimum distance of 20 m between plots. Within each plot, 5 one-meter length tree logs (A–E) belonging to the same individual tree were left to decompose. Sampling for DNA extraction was shown in right panel as described in Materials and methods.

Table 2. Pearson correlations between fungal alpha diversity and traits of each stem compartment (inner wood, outer wood and bark).

Traits	Inner wood				Outer wood				Bark	
	ASVT1	ASVT4	HT1	HT4	ASVT1	ASVT4	HT1	HT4	ASVT1	HT1
Nitrogen	−0.14	−0.50	−0.04	−0.34	*0.50*	**−0.60**	0.43	−0.29	0.55	0.36
Phosphorus	−0.16	**−0.60**	−0.17	**−0.58**	0.42	−0.16	**0.59**	−0.09	0.01	−0.03
Carbon	0.43	**0.66**	0.19	**0.70**	−0.20	0.10	−0.16	0.04	−0.37	−0.16
Carbon/nitrogen	0.20	**0.58**	0.07	0.40	*−0.54*	**0.63**	−0.46	0.32	−0.53	−0.31
Carbon/phosphorus	0.18	**0.62**	0.17	**0.60**	−0.42	0.16	**−0.58**	0.09	−0.05	0.01
Nitrogen/phosphorus	0.10	0.42	0.18	*0.49*	−0.12	−0.24	−0.35	−0.10	0.27	0.20
Lignin	**0.61**	**0.82**	0.15	**0.73**	−0.19	0.32	−0.31	0.01	*−0.63*	*−0.57*
Cellulose	0.14	−0.35	0.08	**−0.55**	−0.03	0.06	0.09	0.05	−0.60	**−0.77**
Lignin/cellulose	*0.53*	**0.83**	0.12	**0.79**	−0.16	0.26	−0.29	0.00	−0.32	−0.16
Phenolics	0.06	0.44	0.21	**0.51**	0.11	−0.03	0.07	0.08	0.21	0.54
Tannins	0.03	0.41	0.18	0.46	0.03	0.08	0.02	0.12	0.17	0.47
pH	0.21	−0.41	−0.08	−0.43	0.13	0.14	0.30	0.12	−0.07	−0.13
Heartwood formation	*0.52*	**0.85**	0.04	**0.69**	−0.23	0.43	−0.41	0.09	−0.26	−0.21
Conduit fraction	0.26	**0.74**	−0.19	**0.59**	−0.45	0.39	−0.44	0.17		
Conduit diameter	*−0.49*	**−0.76**	0.08	−0.47	0.25	−0.38	0.41	−0.06		
Conduit density	0.43	**0.80**	−0.11	**0.57**	−0.33	0.39	−0.44	0.11		
Conduit wall thickness	−0.08	0.15	0.27	0.08	0.23	0.12	0.12	−0.33		
Conduit wall thickness/radius	0.45	**0.75**	−0.03	0.47	−0.19	0.39	−0.35	−0.01		
Ray fraction	−0.37	**−0.71**	−0.07	−0.43	0.33	−0.35	0.30	0.11		
Wood density	−0.29	**−0.57**	0.02	−0.32	0.17	−0.19	0.20	0.12	-	-
Bark density									−0.43	−0.54
Bark punch resistance									0.18	0.09
Bark thickness									−0.11	−0.08
PC1_inner wood	−0.39	**−0.84**	0.01	**−0.65**						
PC2_inner wood	−0.20	−0.02	0.23	0.18						
PC1_outer wood					0.33	−0.40	0.44	−0.10		
PC2_outer wood					0.12	−0.02	0.12	0.08		
PC1_bark									−0.06	−0.35
PC2_bark									0.53	0.49

Note: ASV indicates fungal richness and *H* indicates Shannon diversity after 1 (T1) and 4 (T4) years of stem decay. Correlations in bold have a significance of $P < 0.05$, correlations shown in bold and italics have a significance of $P < 0.001$, and correlations shown in italic show a tendency $0.05 < P < 0.1$. '.' means data are not applicable. Traits and fungal alpha diversity were log-transformed. PC1 and PC2 are the scores of first two PCA axes obtained from Fig. S5. Not enough species still had bark available at T4.

Table 3. Results of a redundancy analysis (RDA) showing the relationship between fungal taxonomic community composition and stem traits based on a forward selection procedure.

Factors	Inner wood		Outer wood		Bark
	T1	T4	T1	T4	T1
Tree major taxa	5.4	3.1	7.7	–	6.5
Conduit fraction	–	–	3.6	–	
Conduit diameter	3.2	3.6	5.3	–	
Conduit wall thickness	–	–	–	3.8	
Ray fraction	3.2	–	–	–	
N	–	–	2.7	–	3.9
P	–	–	–	–	4.0
C	3.0	3.5	5.1	–	3.0
Lignin/cellulose	–	–	–	–	5.2
Phenols	–	–	–	–	–
pH	–	3.1	4.9	–	–
Wood density	–	–	3.7	–	
Bark density					4.2
Bark punch resistance					4.3
Bark thickness					4.0
Total variation	14.8	13.3	33.0	3.8	35.1

Significance was assessed with 999 permutations. RDAs were conducted for two decay times (T1 and T4) and three stem compartments (inner wood, outer wood and bark) separately. '–' means non-significance, while '.' means data are not applicable. The percentage explained variation is shown. Not enough species still had bark available at T4.

while gymnosperm species were dominated by white-rot fungi (55.8%). The proportion of plant pathogens tended to decrease over time (from 19.1% at T1 to 3.6% at T4), while brown-rot fungi increased over time (from 0.2% at T1 to 7.0% at T4). Overall, most samples were dominated by saprotrophic fungi at both decay periods, especially for the white-rot fungi (Supporting Information Table S2). Similar results were found when we used the data with CLR transformation that can convert compositional data to scale-invariant data in real space (Supporting Information Fig. S6). We again found that most samples were dominated by saprotrophic fungi.

PCAs showed that fungal taxonomic composition differed among the three main stem compartments (inner wood, outer wood and bark) and across tree species. The variation in fungal community composition was mainly explained by the first PCA axis. More diverse fungal communities were found in inner wood (PC1 scores ranged from −1.8 to 2.3), compared to outer wood (−1.4 to 1.0) and bark (−1.5 to 1.1) (Figs. 5A and B, Supporting Information Fig. S7). After 1 year of decay, the first PCA axis showed a pattern that represented the gradient from the outermost stem part (bark) to the innermost stem part (inner wood). Inner wood tended to contain more distinct fungal communities from those in outer wood and bark at T1 (Fig. 5A), with such differences becoming less apparent at T4 (Fig. 5B). Gymnosperm and angiosperm species tended to contain different fungal communities since, as indicated by their separation along the first axis at T1 (Fig. 5A) and along the second axis at T4 (Fig. 5B). Moreover, a clear successional pattern

was observed in gymnosperm species; fungal communities tended to converge from T1 to T4 as indicated by the arrows shown in Fig. 5C, whereas no convergent succession pattern was found for angiosperm species, where fungal communities shifted consistently along the second PCA axis over time (Fig. 5D).

Factors linked with fungal community composition

Fungal community composition in each stem compartment was affected by tree major taxa (Gymnosperms vs. Angiosperms) and different physical–chemical traits. Overall, tree major taxa and stem traits were associated with accessibility (represented by conduit diameter, fraction and wall thickness), stem chemistry (i.e. N, P, C and lignin) and physical defence (i.e. wood and bark density, bark punch resistance and bark thickness). This finding indicated that tree major taxa and the afterlife effects of traits were important in regulating fungal community composition in decaying stems (Table 3, Supporting Information Fig. S8). Specifically, tree major taxa, carbon concentration and wood accessibility as indicated by conduit diameter significantly affected fungal composition of all stem compartments in both decay periods (except outer wood at T4). After 1 year of decay, traits could better explain variation in fungal composition of the bark (35.1%) and outer wood (33.0%) than the fungal composition of the inner wood (14.8%). Traits associated with stem chemistry (i.e. C, N, P, lignin/cellulose) and physical defence (i.e. bark density, thickness and punch resistance) significantly affected bark fungal communities. For wood samples at T1, traits associated with accessibility (e.g. conduit diameter), wood chemistry (i.e. C, N and pH) and physical defence (i.e. wood density) showed significant afterlife effects on fungal community composition. The afterlife effects of wood traits decreased over time and only conduit wall thickness showed significant effect on outer wood fungal community composition at T4.

Discussion

A unique combination of a multiple-species 'tree cemetery' experiment and sampling design enabled us to evaluate how fungal diversity and community composition vary among stem compartments and species of decaying temperate tree logs; and how this is driven by stem traits. Tree species and stem compartments differed in stem accessibility, nutrient levels and physical–chemical defence (Supporting Information Fig. S5), all of which related to differences in fungal diversity and community composition (Fig. 3; Tables 2 and 3) (Leonhardt *et al*., 2019; Lee *et al*., 2020). Across tree species, fungal richness in the inner wood increased with lignin concentration, which may reflect the differences between lignin chemistry in Gymnosperms and Angiosperms (Wagner *et al*., 2015). Fungal community
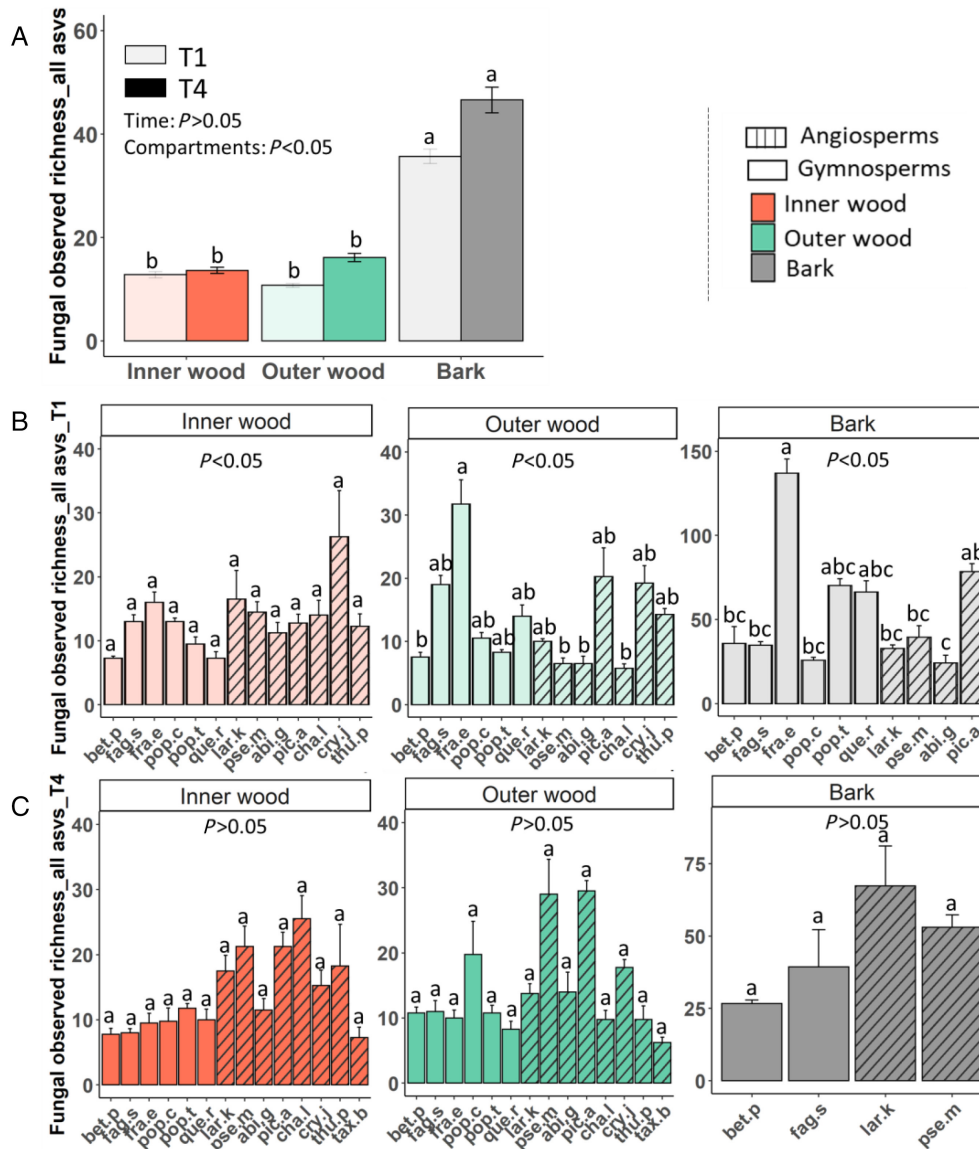
Fig. 2. Fungal community richness of different tree species. Bar plots illustrate the variation in fungal ASV richness among (A) different stem compartments, and among 14 tree species after (B) 1 year (T1) and (C) 4 years (T4) of decay for inner wood (orange), outer wood (green) and bark (dark grey) of angiosperm species (line bars) and gymnosperm species (open bars). Bars show the mean of three (bark at T4) or four replicates (others) per species, the error bars correspond to standard errors of the mean. Bar colours indicate different stem compartments, with light colours for T1 and dark colours for T4. Pairs of bars that do not share the same letters are significantly different (Tukey's HSD: *P* < 0.05). Please note that the *y*-axis scaling can vary across panels.

composition differed strongly between gymnosperm and angiosperm species, especially at T1. Overall, stem traits associated with wood accessibility, stem chemistry and physical defence had profound afterlife effects on fungal community composition.

Fungal alpha diversity varies across tree species and compartments and is associated with different stem traits

During the first year of decay, fungal diversity significantly varied across tree species, but such variation

disappeared after 4 years of decay, which may be because that wood decay can lead to a more homogenized substrate (Witkamp, 1966). As expected, bark had higher fungal diversity than wood (Fig. 2), likely because it is the outermost part of a tree and more exposed to fungal colonization. Bark has a highly complex heterogeneous structure consisting of periderm, cortex and the (secondary) phloem (Srivastava, 1964; Harmon *et al.*, 1986), thus creating a high heterogeneity of microhabitats and more niche opportunities for different fungal species. Moreover, bark is rich in nutrients compared to wood
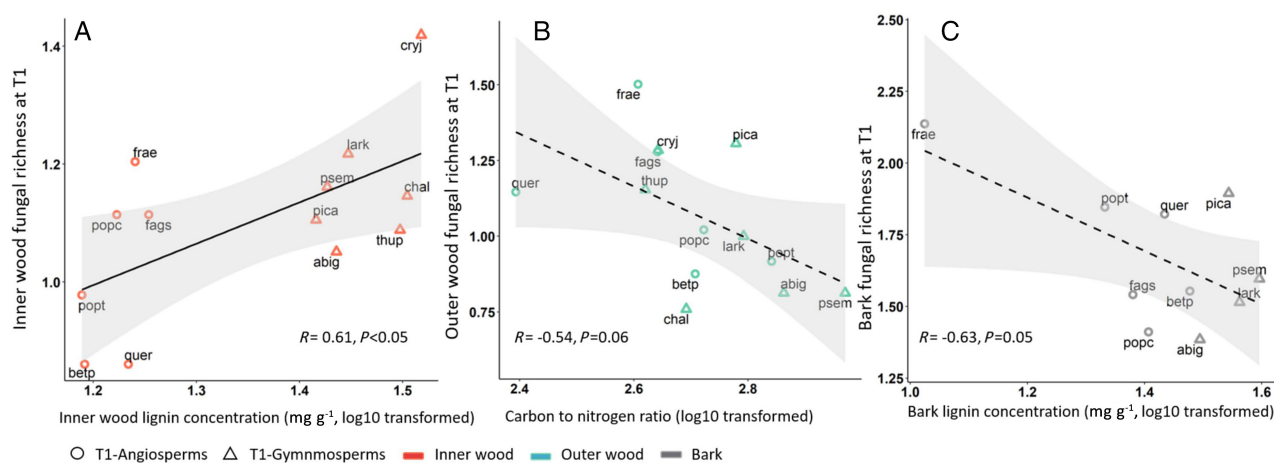
Fig. 3. Relationship between initial stem traits and mean fungal richness after 1 year (T1) of stem decay in A) inner wood (orange), B) outer wood (blue) and C) bark (grey). Data of gymnosperm species are shown as triangles and angiosperm species are shown as circle. The traits shown here are the most strongly correlated to fungal richness (Table 2). Solid line indicates a significant correlation, whereas dashed lines indicate marginally significant correlations (*P* < 0.05). Tree species are shown in different codes; frae: *Fraxinus excelsior*; betp: *Betula pendula*; quer: *Quercus robur*; fags: *Fagus sylvatica*; popc: *Populus × canadensis*; popt: *Populus tremula*; chal: *Chamaecyparis lawsoniana*; thup: *Thuja plicata*; cryj: *Cryptomeria japonica*; taxb: *Taxus baccata*; pica: *Picea abies*; lark: *Larix kaempferi*; psem: *Pseudotsuga menziesii*; abig: *Abies grandis*. Note: *Taxus baccata* was not available for wood samples at T1, so in total 13 tree species are shown in panels A and B, whereas only 10 tree species were available to collect bark samples as shown in panel C.

tissues (Yang *et al.*, 2022), thus resulting in a higher fungal species richness (Weedon *et al.*, 2009; Bebber *et al.*, 2011; Kazartsev *et al.*, 2018). Additionally, Scolytinae, including bark and ambrosia beetles, may act as a vector for transporting fungal species into bark habitats, thereby contributing to a high fungal diversity in this compartment (Stokland *et al.*, 2012). Fungal richness was similar for inner wood and outer wood, a similar result was found after a 6-year decay experiment in Germany with 13 tree species (Leonhardt *et al.*, 2019). Inner wood, especially heartwood, is less permeable and hence accessible and is often chemically protected with extractives (Noll *et al.*, 2016). But meanwhile, heartwood-forming species may require more fungal specialists to degrade extractives (Stokland *et al.*, 2012), and these opposing effects may explain the similar fungal richness of the two wood compartments.

Conduits and parenchyma are thought to facilitate fungal access to wood (Cornwell *et al.*, 2009; Zanne *et al.*, 2015). However, in our study we found that conduit diameter and ray fraction were negatively related to fungal richness in inner wood. Possibly this is the result of our species selection, as fungal richness of the inner wood was higher for gymnosperm species that not only have narrow conduits and little ray parenchyma but also often form (except *Abies grandis*) heartwood. In contrast, all our angiosperm species except one, *Quercus robur*, lack heartwood. Heartwood-forming species have higher fungal diversity (see above and Table 2); this may explain our counterintuitive result.

Altogether, fungal alpha diversity varied across tree species and stem compartments. Stem traits showed

significant afterlife effects on fungal alpha diversity, especially for inner wood at T4; traits associated with accessibility, wood chemistry and physical defence significantly affected fungal alpha diversity in decaying wood.

Fungal community composition differed among tree species, compartments and decay time

The abundance of fungal ecotypes differed with tree species, stem compartments and decay time (Fig. 4). In angiosperm species, pathotrophic fungi may stay active during early decay because angiosperm species have a higher proportion of ray parenchyma, which can provide a nutrient-rich radial infection route for the pathotrophic fungi. Alternatively, some of these fungi produce spores that stay dormant for a long time allowing them to benefit from unpredictable food source availability (i.e. dead wood substrates) (Campbell, 1985; Anthony *et al.*, 2017). Gymnosperm species, in contrast, were dominated by saprotrophs at T1, possibly because the endophytic and pathogenic fungi in freshly cut stems of gymnosperm species are rapidly replaced by saprotrophic fungi (Franceschi *et al.*, 2005; Stokland *et al.*, 2012). This interpretation is supported by a study in which *Larix* tree stems were dominated by endophytic and pathogenic fungi at the time of tree cutting, with their proportions diminishing after a year of decay (van der Wal *et al.*, 2016). We found that most samples were dominated by saprotrophic fungi, especially white-rot fungi (Fig. 4). White-rot fungi can decompose recalcitrant lignin substance (Blanchette, 2000; Cornwell *et al.*, 2009; Del Cerro *et al.*, 2021), thus facilitating the establishment of
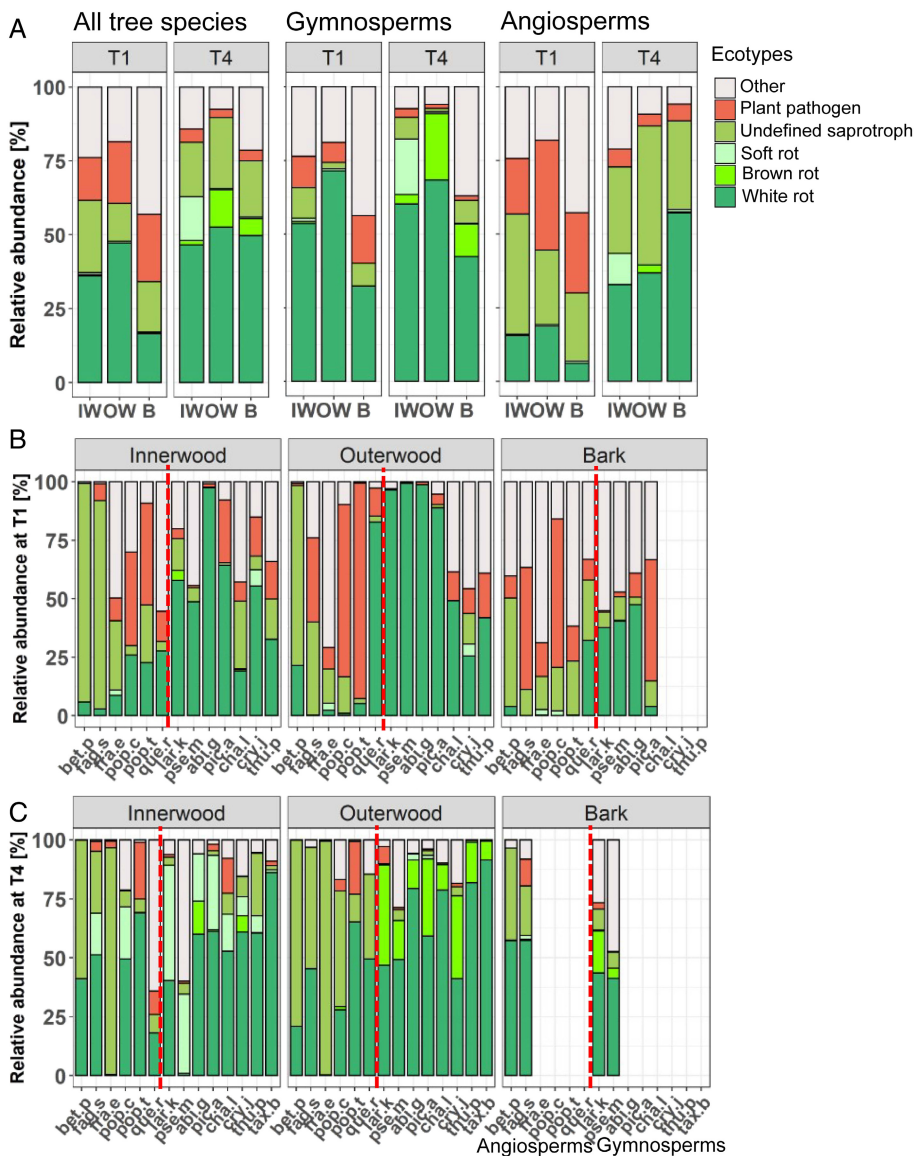
Fig. 4. Mean relative abundance of fungal ecotypes in different tree species and stem compartments after 1 year (T1) and 4 years (T4) of stem decay. The fungal ecotypes have been derived using the FungalTraits database and marked as white-rot fungi, brown-rot fungi, soft-rot fungi, undefined saprotrophs, plant pathogens and others. The abundance is given for three stem compartments (IW = inner wood, OW = outer wood and B = bark) of (A) different tree groups and of 14 temperate tree species after (B) 1 year (T1) and (C) 4 years (T4) of decay. For tree species abbreviations see Table 1.

brown-rot fungi that can only degrade cellulose and hemicellulose. This agrees with our observation that the brown-rot fungi increased in abundance over time, which is also consistent with results from an earlier study (Van der Wal *et al.*, 2013).

We found that gymnosperm and angiosperm species contained different fungal communities in different stem compartments during early decay stage (T1 in Fig. 5A and B). Inner wood differed in fungal community structure compared to outer wood and bark (Fig. 5A). Correspondingly, a previous study on two species only showed that heartwood and sapwood of *Larix* and *Quercus* trees had distinct fungal communities after 1 year of decay (van der Wal *et al.*, 2016). Such differences between inner wood and outer wood, however, became weaker or even disappeared over time (Fig. 5B) (van der Wal *et al.*, 2016;

Leonhardt *et al.*, 2019). A combination of exhaustion of the sugar and protein resources and the enzyme effects of fungal decomposers may result in a more homogeneous substrate (Witkamp, 1966), leading to more similar fungal communities over time. Moreover, there were clear shifts in fungal species composition over time, which were disparate for tree species from the two major taxa (Fig. 5); fungal communities in gymnosperm species tended to converge from T1 to T4 (Fig. 5C), while in angiosperm species they shifted along the second PCA axis over time (Fig. 5D).

In conclusion, there was a clear shift in functional group composition over time from lignin-decomposing white-rot fungi to cellulose-decomposing brown-rot fungi. Inner wood had a different fungal community than outer wood and bark, and gymnosperm and angiosperm
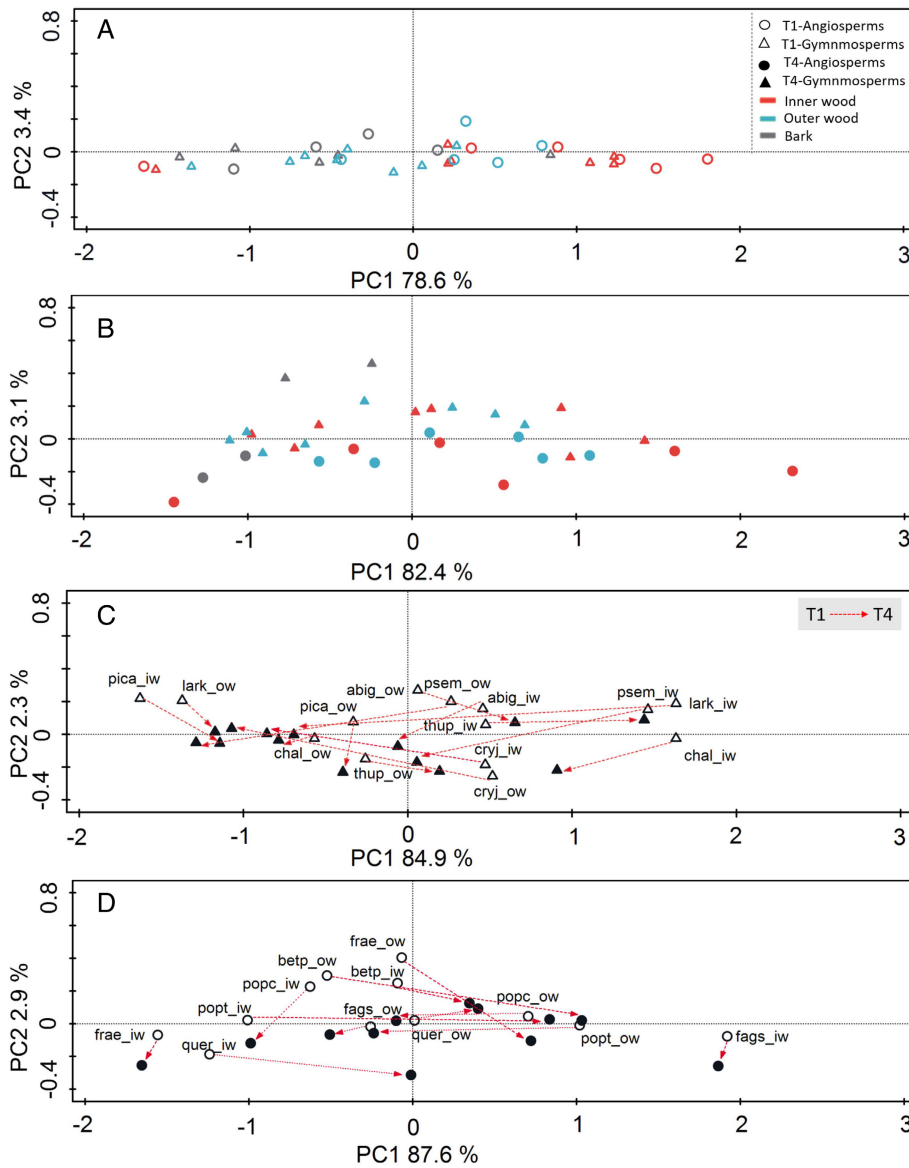
Fig. 5. Principal component analyses of fungal taxonomic community composition across different stem compartments of angiosperm species (circles) and gymnosperm species (triangles) at (A) T1 (open symbols) and (B) T4 (filled symbols). Different colours indicate different stem compartments; inner wood (orange), outer wood (blue) and bark (grey). Succession of fungal communities in wood samples from (C) gymnosperm species and (D) angiosperm species (circles) from T1 (open symbols) to T4 (filled symbols).

species showed different shifting patterns in fungal composition.

Stem traits have significant afterlife effects on modulating fungal community composition

We found that stem traits had significant afterlife effects on dead wood fungal communities (Table 3; Supporting Information Fig. S5). The RDAs showed that tree major taxa and stem traits associated with accessibility (i.e. conduit diameter, fraction and wall thickness) (Cornwell *et al*., 2009; Zanne *et al*., 2015; Lee *et al*., 2020), physical defence (i.e. wood and bark density, bark thickness and punch resistance) (Purahong *et al*., 2016; McDonald *et al*., 2017) and stem chemistry (e.g. C, N, lignin) (Rajala *et al*., 2010, 2012; Hoppe *et al*., 2016; Lee

et al., 2019) were important factors regulating fungal community composition in decaying stems. The distinct fungal communities between Gymnosperms and Angiosperms suggested a long adaptation between major plant lineages and their fungal partners, which may also be linked to the structure and the origin of the species. Most of the gymnosperm species tested in our study are heartwood-forming species and are exotic, potentially explaining their distinct fungal communities compared to angiosperm species. Conduit diameter showed strong afterlife effects on the fungal community composition in different stem compartments, suggesting its important role on fungal community assembly during decay. Conduits can serve as important pathways of wood to decay organisms, with larger conduit diameter facilitating wood accessibility (Cornwell *et al*., 2009; Zanne *et al*., 2015).

Moreover, carbon and nitrogen are major constituents of the proteins required for the synthesis and activities of degrading enzymes (Baldrian *et al*., 2016), therefore played important roles on fungal community assembly (Kielak *et al*., 2016; Kuramae *et al*., 2019; Lee *et al*., 2020). Other studies in a natural, beech-dominated forest also found that nitrogen was the key factor driving fungal community composition in deadwood (Baldrian *et al*., 2016; Purahong *et al*., 2016).

Inner wood traits showed strong afterlife effects on fungal community composition at both time points (i.e. T1 and T4), while the afterlife effects of outer sapwood traits largely decreased over succession. Heartwood is well protected as it is the innermost compartment of a stem and resistant to microbial decomposers due to its low permeability and high antifungal substances. However, sapwood is more vulnerable to attack and its properties can be largely altered by the enzyme activities of fungal decomposers, indicating that the traits of decayed wood, rather than those of initial wood, may play more important roles in impacting the fungal community assembly (Hoppe *et al*., 2016; Noll *et al*., 2016). Though the effects of deadwood traits on fungal communities were not tested in this study, our conclusion can be supported by a comparative study in Germany – the BELongDead (Biodiversity Exploratories Long-term Deadwood) experiment, where 13 temperate tree species were placed to decompose in the German Biodiversity Exploratories at 2009 (Kahl *et al*., 2017); initial wood traits failed to shape wood-inhabiting fungal communities of 11 temperate tree species after 3 years of decay (Purahong *et al*., 2018), but another study, based on two tree species (i.e. *Picea abies* and *Fagus sylvatica*), found physical–chemical traits (e.g. C, N, P, lignin) of decayed wood changed over time and significantly impacted fungal community composition (Hoppe *et al*., 2016).

In sum, stem traits associated with accessibility, stem chemistry and physical defence showed strong afterlife effects on fungal community composition. With prolonged decay, initial traits of well-protected heartwood but not sapwood are good predictors for fungal community structure.

Experimental procedures

Study site

A common garden experiment was carried out in a Dutch forest, located at the Schovenhorst Estate in the Veluwe region of the Netherlands (52.25 N, 5.63 E), with a mean annual temperature of 10.8°C and annual precipitation of 829 mm. This forest site has Pleistocene sandy soil that is well-drained and acidic (pH of c. 4). The tree log incubation plots are in a rather light-open *Larix kaempferi* stand with a low understory layer that is dominated by *Vaccinium myrtillus*, mosses and patches of *Deschampsia flexuosa*. Details of the study site are presented in Cornelissen *et al*. (2012).

Experimental design

We took advantage of the LOGLIFE project (Cornelissen *et al*., 2012), in which freshly cut stems of temperate tree species have been left to decompose in a common garden experiment. We included ring-porous and diffuse-porous angiosperm species and included heartwood and non-heartwood forming species to cover a broad range of wood qualities (Table 1). Ten tree species were incubated from February 2012, and four more tree species were added in February 2015. In total, six angiosperm and eight gymnosperm species were included (see tree species list in Table 1). Logs from the original 10 species were extracted from the local forest Schovenhorst, while logs for the other four tree species were extracted from a forest in Flevoland, the Netherlands (52.46N, 5.42E). Though the two forests differ strikingly in soil conditions, i.e. sand versus clay, the growing conditions in the two forests had little effect on stem trait variation in harvested trees (Yang *et al*., 2022). All tree logs were 100 cm long and had approximately the same diameter (c. 25 ± 3 cm), thus assuring a similar exposed surface area and substrate quantity. Before incubation, five individual trees were cut for each tree species. Each tree was cut into five logs (each with 1 m length) from the main trunk without major side branches. These tree logs were placed in five plots in the study site (see Fig. 1). In total, 350 logs (14 tree species × 5 individuals × 5 logs) were incubated.

Each plot measured approximately 12 m by 12 m, with a minimum distance of 20 m between plots. Each plot was surrounded by a 1.2-m high fence to exclude wild boars that are abundant in this area. Within each plot, tree logs and large branches already present in the plot were removed before log placement. The logs were positioned 30 cm apart on the soil surface, assuring good contact with the soil to harmonize micro-site conditions for all logs, while mimicking natural conditions and allowing fungal access, also from the soil exposed side. Logs from the same tree were placed together with the same compass orientation, but the location and orientation of different tree species within each plot were random (see Cornelissen *et al*., 2012 for more information).

Sample preparation

Samples for DNA extraction. Among all studied temperate tree species, 19% (range 9%–25%) of wood density loss was found after one and 27% wood density loss

(range 7%–47%) after 4 years of decay (i.e. marked as T1 and T4). One log of a tree species was extracted from each plot at each harvest, resulting in a total of five logs per tree species for each harvest time (i.e. T1 or T4) (Fig. 1). Each harvested tree log was cut into two equal parts, one part (c. 50 cm) was returned to its original location for later investigation, and the other part was used for fungal community analysis. It was vertically divided into two parts (each being one-quarter of the whole 1 m-length tree log) and disks from the middle of each half log were extracted (Fig. 1). For the 10 tree species incubated in 2012, the disk of each decaying log was extracted in 2013 (T1 – after 1 year of decay) and 2016 (T4 – after 4 years of decay). For the other four tree species incubated in 2015, the disks were taken in 2016 (T1) and 2019 (T4). All the extracted disks have been preserved in the freezer and used for further analyses. Sawdust samples of different stem compartments (heartwood/inner wood, sapwood/outer wood and bark) were collected using an electric drill in August 2019. Notably, the samples for the *Taxus baccata* at T1 were missing. Bark samples were available for 10 tree species at T1, but for only four species at T4 since the other species lost their bark during decay. A stem consists of wood and bark, and some species convert the inward part of the functional sapwood into heartwood, whereas other species do not form heartwood. To avoid confusion, hereafter we will use the terms inner wood, outer wood and bark to indicate the three stem compartments. To obtain representative samples of the fungal communities in the decaying logs, samples were collected from the top (upper side) and bottom (soil contact side) of the disks, pooled (see Fig. 1) and stored at −20°C prior to further molecular analysis.

Before extracting the DNA, the sawdust samples were further ground into a fine powder with a Retsch MM400 ball mill (Retsch, Haan, Germany). Specifically, sawdust samples and a metal ball (20 mm) were first put into a stainless steel beaker (50 ml) allowing the sawdust to fill 30%–40% of the beaker. We then closed the beaker, put it in liquid nitrogen until frozen, after that, the sawdust was ground for 3 min at 30 Hz. Finally, sample powder was stored at −20°C prior to further analysis. Notably, the drill bit used in the sawdust preparation process, and the beaker and metal ball used in the grinding process were all thoroughly disinfected with ethanol between samples to prevent sample cross-contamination.

Samples for physical–chemical analyses. To examine the relationship between stem trait properties and the fungal community in dead wood, we measured 18 stem traits of the 14 tree species (six angiosperm and eight gymnosperm species). The full list of measured stem traits is shown in Supporting Information Table S1. Two adjacent 2-cm thick basal stem disks of each individual tree were sawn out for initial stem trait analyses before tree log incubation. Subsamples (two 1.5 cm^3 blocks) were extracted from the inner wood and outer wood of one disk and used for physical trait measurements (i.e. wood density, conduit cell-wall thickness, conduit diameter and ray fraction). Chemical traits were measured using sawdust samples taken from the second disk with an electric drill (bit diameter 8 mm). Sawdust from inner wood, outer wood and bark was collected separately, then the sawdust samples were ground in an MM400 ball mill (Retsch) and used for measurements of pH, C/N, C/P, N/P ratios, lignin concentration, nitrogen (N) and phosphorus (P) concentrations. Sampling and measurements are described in detail in Yang *et al*. (2022).

DNA extraction, PCR amplification and sequencing

DNA was isolated from 0.10 to 0.25 g fresh weight of ground samples using the PowerSoil DNA Isolation Kit (MO BIO Laboratories). The total DNA quantity and quality was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). All DNA extractions were stored at −80°C prior to downstream analyses.

We used the primer pair ITS1F (5′-CTTGGTCATTTAG AGGAAGTAA-3′) (Gardes and Bruns, 1993) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) (White *et al*., 1990) to amplify the fungal Internal Transcribed Spacer (ITS) region. Amplifications were performed in 25 μl volumes with the Qiagen HotStar Taq master mix (Qiagen Laboratories) using the following conditions: denaturation period of 15 min at 96°C followed by 33 cycles of 96°C for 30 s, 52°C for 30 s, 72°C for 1 min and a final elongation step at 72°C for 10 min. Product quality was verified on a 2% agarose gel. Quantification of each amplicon was performed with Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies). The library was then generated by pooling the same quantity (ng) of each amplicon. The pool (or library) was cleaned up with sparQ PureMag Beads (from Quantabio). The library was quantified using Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems). Average fragment size was determined using a LabChip GX (PerkinElmer) instrument. Before sequencing, 15% of the Phix control library was added to the amplicon pool (loaded at a final concentration of 9 pM) to improve the unbalanced base composition on the flowcell. Subsequently, paired-end (2 × 250 bases) sequencing was performed with the MiSeq Reagent Kit v2 on an Illumina MiSeq System (Illumina).

Bioinformatics

We followed the standard ITS pipeline (https://benjjneb.github.io/dada2/ITS_workflow.html) with a modification: in 'filter and Trim' section, we changed 'multithread = TRUE' to 'multithread = 12' to avoid overloading the server. The fungal community was determined by filtering, denoising and assigning taxonomy to paired amplicons with the package DADA2 v.1.8 (Callahan *et al.*, 2016), which does not infer amplicon sequence variants (ASVs) that are only supported by a single read, because singletons are assumed too difficult to differentiate from errors. In brief, first, primers from the reads were removed using a specialized primer/adapter removal tool – cutadapt. After inspecting the read quality profiles, fungal reads with more than two expected errors (maxEE = 2) and shorter than the length of 50 base-pairs (minLen = 50) were discarded by the 'filterAndTrim' function. Then, ASVs, which can be utilized to classify groups of species based on DNA sequences, were inferred for each sample, forward and reverse reads were merged and a sequence frequency table was generated. After chimaera removal, the taxonomy of the ASV was assigned with the UNITE database, v. 8.2 (Abarenkov *et al.*, 2010). Ultimately, taxonomic identities were assigned to 100% of ASVs (i.e. 1987 fungal ASVs) at the kingdom level, 91.0% phylum, 79.1% class, 74.8% order, 64.8% family, 57.7% genus and 39.8% at species level.

FungalTraits is a user-friendly trait database of fungi (Põlme et al., 2020), which combines the information from the databases of FUNGuild (Nguyen *et al.*, 2016) and Fun^{Fun} (Zanne *et al.*, 2020) together with expert knowledge. Based on this FungalTraits database, we assigned the fungal guilds according to their primary lifestyles, among which saprotrophs and plant pathogens were the most common in terms of the number of genera. Therefore, in this study, all detected fungal communities (ASVs) were grouped into (i) saprotrophs – receiving nutrients by breaking down dead host cells, which was further divided into 'white-rot', 'brown-rot', 'soft-rot' and 'undefined saprotroph' based on decay types in FungalTraits database; (ii) 'pathotroph'- receiving nutrients by harming host cells; and (iii) other – other fungal primary lifestyles (e.g. lichenised, ectomycorrhizal, mycoparasite and animal/lichen parasite fungi).

Statistical analyses

In this study, all statistical analyses were performed using R v. 3.6.1 (Team, 2019) and CANOCO 5.0 (ter Braak and Smilauer, 2012).

Fungal ASVs richness and Shannon diversity were calculated using the *R* package 'phyloseq', function 'estimate_richness' (McMurdie and Holmes, 2013). To test how fungal alpha diversity (i.e. observed richness and Shannon diversity) differed between stem compartments and decay time, a two-way ANOVA was conducted with the alpha diversity indices as dependent variables followed by Tukey's HSD *post hoc* test. Notably, with the prolonged decay, the bark of some tree species peeled off and we were not able to collect their bark. Therefore, only four tree species are available for bark fungal detection after 4 years of decay. Therefore, to make better comparisons, we tested how mean fungal alpha diversity varied across compartments based on four tree species – two broadleaf species (*Betula pendula* and *Fagus sylvatica*) and two conifer species (*Larix kaempferi* and *Pseudotsuga menziesii*). To test how fungal alpha diversity varies across different tree species, one-way ANOVAs were conducted followed by Tukey's HSD *post hoc* test. The normality of the residuals was checked with the Shapiro test and Q-Q plots.

Because sequence data are compositional, we used centred-log ratio (CLR) transformation by converting compositional data to scale-invariant data in real space, thereby allowing application of multivariate analyses (Gloor *et al.*, 2017; Sisk-Hackworth and Kelley, 2020). Since CLR transformation requires the replacement of zeros, we replaced the zeros in the dataset by using the 'czm' method in the 'zCompositions' R package (Palarea-Albaladejo and Martín-Fernández, 2015) before CLR transformation (Gloor *et al.*, 2017). To test how fungal richness and Shannon diversity index values were associated with different physical–chemical traits of the three stem compartments, principal component analysis (PCA) was conducted with fungal observed richness and Shannon diversity of the different stem compartments as supplementary variables that did not influence the ordination. Then Pearson correlations were calculated to show the relationships between fungal alpha diversity and log-transformed stem traits, as well as the first two axes extracted from PCA of each stem compartment. Additional principal component analyses (PCAs) were performed to visualize how fungal community composition varied across the stem compartments of different tree species. We examined the relationship between stem traits and fungal community composition in the three main stem compartments across tree species after two decay periods (T1 and T4) using constrained linear ordination analyses (redundancy analyses, RDAs) as carried out in CANOCO 5.0 using a Monte Carlo permutation test (MCPT, number of permutations 4999). Resulting explanatory effects were evaluated in MCPT with a forward step-wise procedure, and only the variables with significant effects were included.

Conclusions

In this study, starting from a broadly similar pool of fungal taxa in the direct environment as dictated by our 'common garden' approach, we found that fungal alpha diversity and community composition varied across tree species, and specifically between Gymnosperms and Angiosperms, and between stem compartments, especially after 1 year of decay. Such variation was significantly linked to different stem traits; bark had significantly higher fungal richness compared to wood samples. Stem traits associated with accessibility, stem chemistry and physical defence were important factors regulating fungal alpha diversity and composition in decaying stems. Inner wood traits showed strong afterlife effects on fungal community composition at both decay periods, while the afterlife effects of outer sapwood traits largely decreased over succession. As decay proceeds, stem quality will be altered by the exhaustion of easy decomposable resources and enzyme activities of multiple decomposers, thereby impacting the composition of the fungal community. Therefore, to better understand fungal succession, one should consider dead stem traits and detailed assessments of the enzyme activities that reflect the activity of fungal decomposers (cf. Noll *et al*., 2016). By building on our key findings this way, we can gain a still deeper understanding of how various factors jointly determine fungal diversity and composition in dead stems, thereby providing opportunities to investigate potential cascading effects on dead wood decomposition rates as related to forest carbon and nutrient cycling.

Acknowledgements

Sequence data availability

Sequence data generated for this study have been deposited in the National Center for Biotechnology Information's Short Read Archive under BioProject PRJNA768246 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA768246).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting Information.