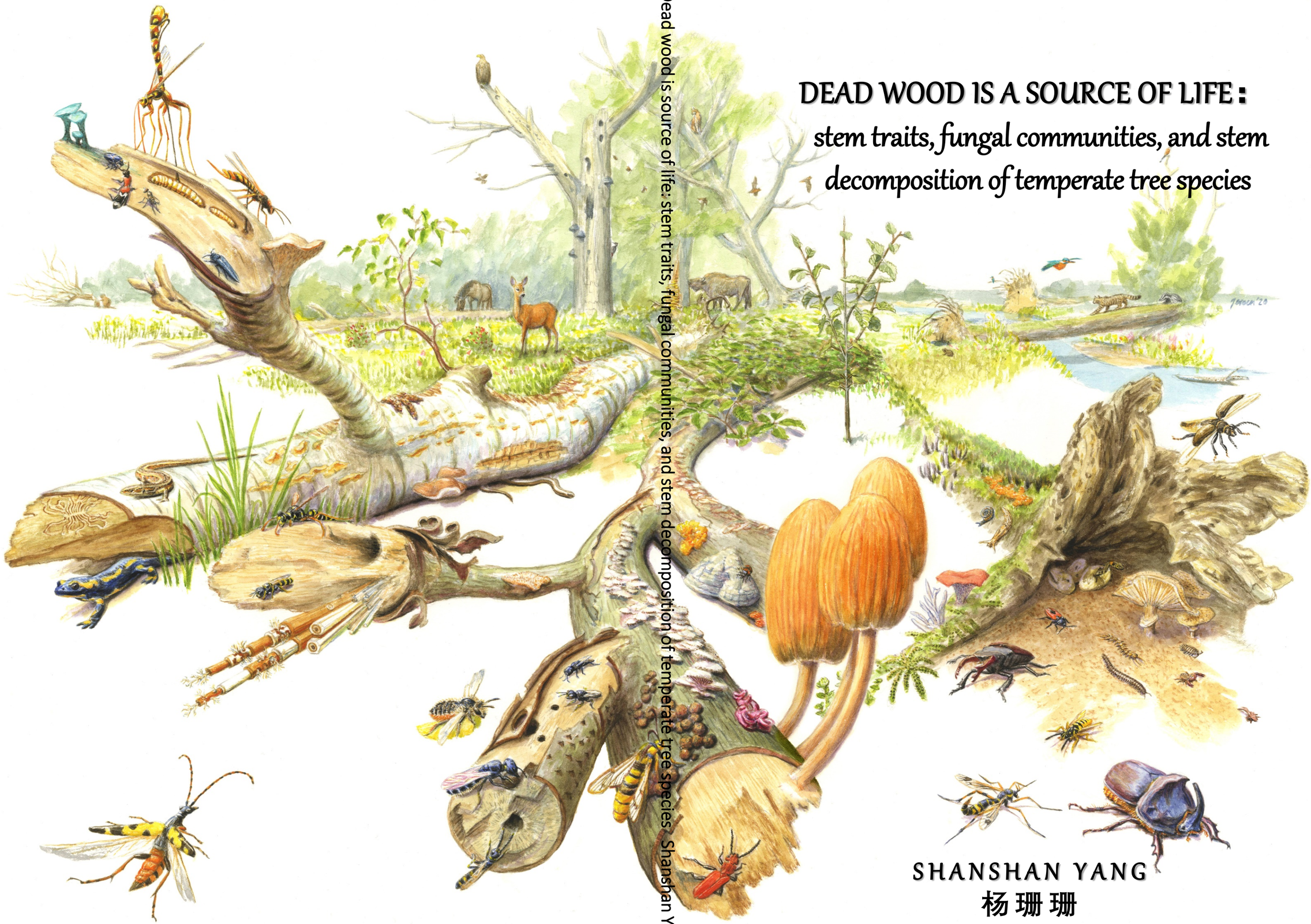
DEAD WOOD IS A SOURCE OF LIFE:

stem traits, fungal communities, and stem decomposition of temperate tree species



SHANSHAN YANG
杨珊珊

Dead wood is source of life: stem traits, fungal communities, and stem decomposition of temperate tree species. Shanshan Yang

Propositions

1. Dead wood diversity promotes fungal diversity.
   (this thesis)

2. Wood decomposition rate is most strongly driven by stem traits in early decay stages, and by fungal composition in later decay stages.
   (this thesis)

3. Science advances more by taking advantage of what exists and by continuing long-term studies than by setting up new studies.

4. Unexpected results are often puzzling but bring new ideas to science.

5. Without a large supervision team you may finish your PhD thesis faster but not better.

6. Online academic conferences are environment friendly but not communication friendly.

7. Deadlines are the primary drivers of productivity.

8. Happiness is not about how much you have but to what extent you achieve your expectations.

Propositions belonging to the thesis, entitled

Dead wood is a source of life:
stem traits, fungal communities, and wood decomposition of temperate tree species

Shanshan Yang
Wageningen, 3 November 2021

Dead wood is a source of life: stem traits, fungal communities, and stem decomposition of temperate tree species

Shanshan Yang

Thesis committee

Promotors

Prof. Dr L. Poorter

Personal chair at the Forest Ecology and Forest Management Group

Wageningen University & Research

Dr F. J. Sterck

Associate Professor, Forest Ecology and Forest Management Group

Wageningen University & Research

Co-promotors

Dr U. G. W. Sass-Klaassen

Associate Professor, Forest Ecology and Forest Management Group

Wageningen University & Research

Other members

Prof. Dr T. W. Kuyper, Wageningen University & Research

Dr M. J. Schelhaas, Wageningen University & Research

Dr G. F. Veen, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen

Dr B. Hoppe, Julius Kühn Institute (JKI), Braunschweig, Germany

This research was conducted under the auspices of the C. T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC).

Dead wood is a source of life: stem traits, fungal communities, and stem decomposition of temperate tree species

Shanshan Yang

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A. P. J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday 3 November 2021
at 11 a.m. in the Aula.

Shanshan Yang
Dead wood is a source of life: stem traits, fungal communities, and stem decomposition of temperate tree species,
198 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2021)
With references, with summary in English

ISBN: 978-94-6395-972-8
DOI: https://doi.org/10.18174/553439

TABLE OF CONTENTS

CHAPTER 1

General introduction

1.1 | Living trees and their afterlife effects

Forests play important roles in timber production, climate regulation, and global biogeochemical cycling (Bonan 2008). Moreover, forests host a huge proportion of terrestrial biodiversity, as they host diverse tree species and other associated organisms (Fichtner et al. 2021). This diversity in tree species is also reflected in their diversity in plant strategies and stem traits. For temperate tree species, stem traits vary from species that form well-defended heartwood to species without heartwood, and their water-transporting conduits vary from ring-porous broadleaf species with distinctly larger vessels in earlywood than in latewood to diffuse-porous broadleaf species with vessels of approximately the same diameter throughout the ring (and coniferous species that don't have vessels but small tracheids for conducting water (Fig. 1.1) (Wheeler and Sneden 1989). This creates a large variation in stem traits with potentially large implications for species-specific ecological strategies (Chave et al. 2009), and different afterlife effects (Cornelissen et al. 2012).

When trees die, a large part of forest carbon and nutrients is locked up in their dead stems, and becomes slowly available during decomposition (Yatskov et al. 2003). Dead trees are vital structural elements in forest ecosystems, as they play important roles in carbon retention, energy flow and long-term nutrient storage (Harmon et al. 1986, Christensen et al. 2005, Weedon et al. 2009) and provide habitats and food for a diversity of species. As much as one third of European forest-dwelling species (i.e., 6000 species) is associated with dead wood (Vogel et al. 2020), indicating that a large part of forest biodiversity is associated with dead trees. The relevance of dead trees for biodiversity and ecosystem functioning has been increasingly recognized by forest managers. For example, since the 1980s the number of dead trees and wood remains has increased steadily in Dutch and European forests as a consequence of management measures. However, up to now, most emphasis has been given to the quantity rather than the quality of dead wood if it comes to increasing forest biodiversity. Quality characteristics are linked to anatomical and chemical stem traits of tree species, which shape the diversity and structure of wood-colonizing fungal communities which, in turn, affect stem decomposition rate and biogeochemical cycling (Fig. 1.2). In this thesis, I study stems from a broad range of conifer and broadleaf tree species to show 1) how they differ in their stem traits and plant strategies, 2) how these traits have afterlife effects by affecting the diversity and composition of fungi, and 3) how stem traits and fungi together affect stem decomposition.

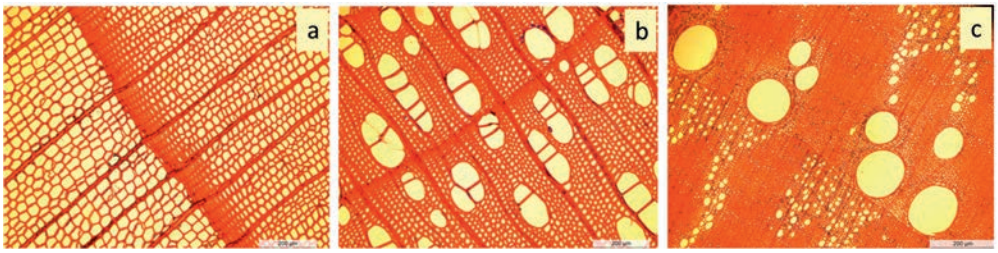


Fig. 1.1 Three main wood structure types of gymnosperm- and angiosperm tree species a: water transporting tracheids of larch (*Larix kaempferi*) with larger tracheids formed at the beginning of the growing season and small tracheids at the growth ring boundary (cross section, magnification 10×); b: diffuse-porous birch (*Betula pendula*) showing vessel of approximately the same diameter throughout the ring (magnification: 10×); c: ring-porous oak (*Quercus robur*) with distinctly larger vessels in earlywood and smaller vessels in latewood (magnification, 5×).

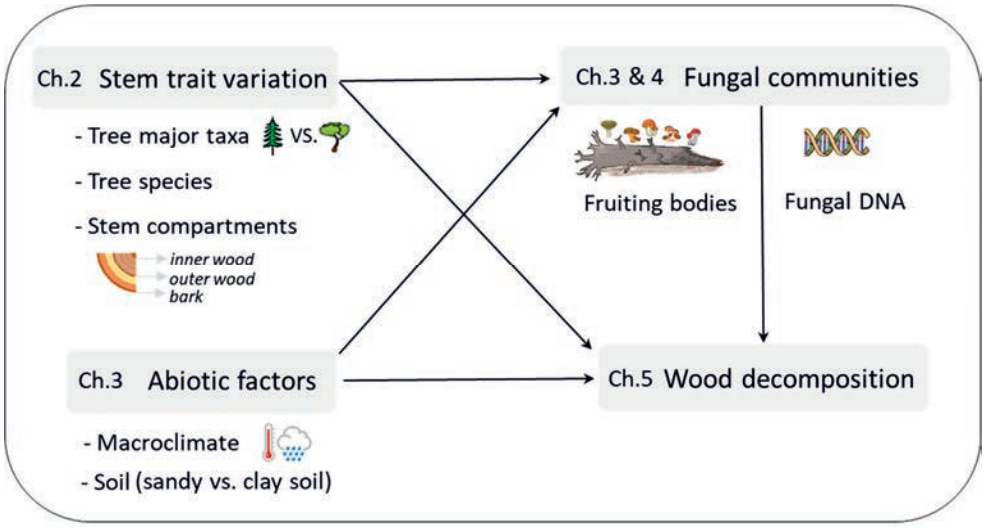


Fig. 1.2 Conceptual framework of this thesis showing how stem traits vary between two tree major taxa (Gymnosperms vs. Angiosperms), amongst tree species and stem compartments (chapter 2), how stem traits and abiotic factors affect the diversity and composition of fungi (chapter 3 & 4), and how stem traits, fungal decomposers and abiotic factors affect wood decomposition (chapter 5). Black arrows indicate the relationship between different research components of the thesis. Fungal communities in decaying stems were detected by two methods, with fungal fruiting body survey applied in chapter 3 and Internal Transcribe Spacer (ITS) amplicon sequencing applied in chapter 4.

1.2 | Stem traits of living trees

Tree species show a diversity of plant strategies, as indicated by associations between functional traits across species (Grime et al. 1997, Vile et al. 2006), which in turn influence the performance (growth, survival and reproduction) of a species in a given environment. Functional traits are defined as "morpho-physio phenological traits of an individual which impact the growth, reproduction and survival of an individual and, ultimately, its fitness" (Violle et al. 2007). Variation in root, stem, and leaf functional traits indicates that tree species coordinate their traits and functions in different ways (Hacke and Sperry 2001, Vleminckx et al. 2021), which can explain and predict species differences in performance (Poorter et al. 2010), distribution (Sterck et al. 2011) and their responses and adaptation potential to global climate change (Cornwell et al. 2009). Species sort out along a global economic strategy spectrum, ranging from slow (i.e., conserve carbon and nutrient resources) to fast (i.e., rapidly acquire carbon, water and nutrient resources) growth strategies (Reich 2014).

Most studies have focused on the leaf economics strategy spectrum (Wright et al. 2004, Freschet et al. 2010, Kerguntеuil et al. 2018), but an increasing number of studies is now also considering wood traits and, in fewer cases, bark traits too (Rosell et al. 2014). These stem traits largely vary across species, particularly between conifers and broadleaf species (e.g., tracheids vs. vessels) and between ring-porous vs. diffuse porous broadleaf species (Chave et al. 2009, Zanne et al. 2010). However, despite studies on structural variability among tree species, few studies focus on the distinction of the stem compartments. These stem compartments (i.e., heartwood, sapwood, and bark) vary in traits and have therefore different functional roles (Rosell et al. 2014, Rosell et al. 2017). For example, the sapwood transports water and nutrients from roots to leaves, whereas the inner bark transports sugars from leaves to other organs (Steppe et al. 2015), and the outer bark presents a defence barrier against insects and fire (Loram-Lourenço et al. 2020). Some species form heartwood, which is impermeable and well protected against pathogen attacks by accumulating anti-fungal substances (Hillis 2012). Most stem studies focus on only one stem compartment (e.g., sapwood or bark) (Hacke and Sperry 2001, Zheng and Martínez-Cabrera 2013, Loram-Lourenço et al. 2020), or on a relatively limited set of traits that are thought to integrate different stem functions, e.g., wood density (Swenson and Enquist 2007). Other studies focus on a specific set of traits depending on the focus of the study, e.g., anatomical traits to assess hydraulics (Olson and Rosell 2013) or nutrient traits to assess the nutrient balance of tree (Brant and Chen 2015). In my thesis, I take a holistic approach by analyzing stem traits from different stem compartments (inner wood,

outer wood and bark), and showing how these stem traits are associated and what trait trade-offs and plant strategies are found.

1.3 | Dead wood as a source of life

Dead wood, which refers to fallen logs, branches, bark, and standing dead trees, is a source of life since they provide habitats and substrates for a wide range of organisms, such as birds, mammals, plants, and saproxylic organisms (Stokland et al. 2012, Zuo et al. 2016, Andringa et al. 2019). Among these organisms, a vast array of invertebrates is associated with dead wood as essential decomposers that mechanically break down wood and bark tissues. Moreover, they make dead wood more accessible and facilitate the colonization of dead stems by other invertebrates and microorganisms. For example, bark beetles, as an early group of invertebrates colonizing dead wood facilitate the entrance by other invertebrates to spruce logs (Zuo et al. 2016) and facilitate the establishment wood decay fungi by opening up bark layers (Strid 2012).

Apart from saproxylic invertebrates, saproxylic fungi (i.e., wood-inhabiting fungi) represent a high proportion of dead wood biodiversity. For instance, Dutch forests contain only around 40 shrub and tree species, but as many as 1250 species of wood-inhabiting fungi (Wijdeven et al. 2010). The visible part of these fungi, i.e., their fruiting bodies, are attractive both in shape and colour, and highly appreciated for their aesthetic qualities (Fig. 1.3). Saprophytic fungi can decompose the major wood polymers, cellulose, hemicellulose and lignin in dead stems by the production of extracellular enzymes (Baldrian 2008, van der Wal et al. 2013), and play an important role in dead wood decomposition and nutrient cycling. Beyond that, saproxylic fungi can degrade stem polymers or defensive compounds and make substrates and food resources available for dead wood insects (Biedermann and Vega 2020), and therefore contribute to forest biodiversity.

The assembly process of dead wood fungi begins already in the living trees (Stokland et al. 2012). Some pathogenic fungi (e.g., *Ceratocystis* spp.) may be transported to fresh wounds of living trees by bark beetles, and cause lethal damage to the trees (Hayslett et al. 2008). Some endophytic fungi present in living trees can be latent primary colonizers after tree death and consume soluble carbohydrates and cell-content compounds (Parfitt et al. 2010, Stokland et al. 2012). Subsequently, the secondary colonizers may gradually outcompete and replace the primary colonizers via airborne spores, and intensively decay recalcitrant lignocellulose (Boddy 1994, Fukasawa et al. 2009). In the final decomposition stage,

mycorrhizal fungi may become the dominating fungal species, because when dead wood is disintegrated into small fragments in advanced decay wood, these wood fragments are buried in the soil and mycorrhizal fungi can access them with their mycelium (Kropp 1982, Stokland et al. 2012). To date, fungal community development on decaying wood has been widely studied, however, most of these studies have been based on fungal fruiting body surveys (Halme et al. 2012, Mueller et al. 2014). The advantage of such a macroscopic approach is that it is relatively cheap, fast and straightforward to describe fungal communities based on conspicuous fruit-bodies, and thus enable large scale comparative studies (Runnel et al. 2015). In addition, this approach allows for annual monitoring in order to evaluate long-term successional patterns. Nevertheless, such fungal fruiting body surveys fail to detect mycelia inside the wood, or to detect fungi that do not produce fruit bodies at all (Moore et al. 2004). Advanced molecular techniques solve this problem since they have the potential to show the full fungal community in different stem compartments (inner wood, outer wood, bark) that differ in accessibility and chemical properties (Saine et al. 2020). Here I used both, a fungal fruiting body survey to assess long-term successional trends in fungi communities, and molecular techniques to provide a full description of the fungal communities in bark, outer wood and inner wood and how these communities are related to anatomical and chemical traits of these stem compartments.

Fig. 1.3 Wood-inhabiting fungi present in dead wood. a-f: *Eriopezia caesia (*photo: Theo Westra). *b:Hypholoma fasciculare* (photo: Aldert Gutter). *Scutellinia scutellata* (photo: Gerben Winkel), *Dacrymyces stillatus* (photo: Henk Huijser), *Heterobasidion annosum* (photo: Dinant Wanningen), and *Gymnopus androsaceus* (photo: Nico Dam). All photos are obtained from https://www.verspreidingsatlas.nl/.

1.4 | Determinants of stem decomposition

Saprotrophic fungi are the main decomposers of the major wood polymers, i.e., cellulose, hemi-cellulose, and lignin (Kirk and Farrell 1987, van der Wal et al. 2013). These fungi are divided into three main functional groups: white-rot Basidiomycetes that degrade cellulose, hemicelluloses and recalcitrant lignin with the aid of extracellular lignocellulolytic enzymes; brown-rot Basidiomycetes that degrade cellulose and hemicelluloses in multiple steps: pre-cellulolytic and enzymatic (Koenigs 1974); and soft-rot Ascomycetes, degrading cellulose and hemicelluloses via secreting cellulase (Schmidt 2006). These main stem decomposers show preferences for different tree species and decay stages. For example, it was reported that the brown-rot fungi prefer to colonize in gymnosperm species (Ryvarden and Gilbertson 1993). In contrast, white-rot fungi are dominant decomposers of angiosperm stems (Ryvarden and Gilbertson 1993, Hatakka and Hammel 2011). They can occur as latent spores in living sapwood and start decomposition when water content drops in dead cells (Boddy 1994).

Stem decomposition is the outcome of complex interactions between stem traits and fungal decomposers (Weedon et al. 2009, Cornelissen et al. 2012), which are strongly controlled by various abiotic factors. For example, humidity, temperature, soil contact and low soil fertility are known to increase fungal diversity, and shape the composition of wood-inhabiting fungi (Rajala et al. 2012, Purahong et al. 2016). Water availability plays a key role, as below 20% wood moisture content most wood-inhabiting fungi are inactive, whereas above 80% wood moisture content enzymatic activity of fungi is restricted because of anoxic conditions (Schmidt 2006, Venugopal et al. 2016). Biotic factors, such as stem traits, inherited from the functional traits of living trees, define the accessibility and substrate quality of dead stems for different fungal decomposes, and determines therefore stem decomposition rate (Fukasawa et al. 2009, Rajala et al. 2012, Baldrian et al. 2016). Chemical defence traits such as phenols inhibit fungal growth (Kahl et al. 2017). whereas high nutritional quality may stimulate fungal growth (Sinsabaugh et al. 1993). Other traits, such as wood density, wood moisture content, lignin content and C/N ratio are also commonly determinants of fungal diversity and composition (Baldrian et al. 2016, Hoppe et al. 2016, Krah et al. 2018). Much less attention has been given to wood anatomical traits like parenchyma fraction, conduit size and conduit wall thickness. These traits may be important to consider too because they regulate

access of wood to decay organisms and they regulate nutrient storage, and in a later stage carbon availability (Schwarze et al. 2003, Zanne et al. 2015, Lee et al. 2020).

The identity of the first colonizing fungi (the "priority effect") plays an important role in fungal community assembly (Hiscox et al. 2015). Previously established saproxylic fungi may affect the colonization success of later arriving species via facilitation (Heilmann-Clausen and Boddy 2005). For example, the dominance of *Moristroma* sp. in early decaying *Quercus robur* logs increase the probability of colonization by *Pezicula* sp. in later decay stage (van der Wal et al. 2016). However, the early arriving fungi gain early access to food resources, occupy most of the space and therefore may inhibit the establishment of later arriving species, or even competitively exclude them. For example, decomposition by the basidiomycete *Stereum hirsutum* lowers the pH thus inhibiting the colonization of ascomycetes, which prefer slightly more alkaline conditions (Fukami et al. 2010, Tudor et al. 2013). During the past few decades, researchers have investigated how individual drivers affect dead tree decomposition, but how stem traits affect fungal infestation during the decay process in different stem compartments across a broad range of species, and how fungal decomposers respond to various biotic and abiotic factors, and jointly with stem traits contribute to stem decomposition remains unclear.

1.5 | Research aims and thesis outline

This thesis aims to investigate how stem traits and plant strategies differ among stem compartments (i.e., inner wood, out wood, and bark) of different temperate tree species, and how these stem traits interact with fungal decomposers affecting stem decomposition and add to forest biodiversity (Fig. 1.2). I take advantage of a unique long-term project (LOGLIFE), in which logs of 14 temperate tree species have been incubated in a common garden experiment in two Dutch forests. I first quantify how tree species differ in their physical and chemical stem traits (*chapter 2*), assess how these species traits relate to the diversity and composition of fungi fruiting bodies observed on stem surfaces over 8 years of decay (*chapter 3*), and study the succession of fungi in different stem compartments, including inner wood, outer wood and bark using Internal Transcribe Spacer (ITS) region amplicon next generation sequencing (*chapter 4*). Finally, I explain how stem traits and fungal composition affect stem decomposition rates of different tree species (*chapter 5*).

Chapter 2 addresses the question how stem traits differ across 14 temperate tree species and what trait trade-offs and plant strategies are found? To tackle this question, I measured for 18 anatomical, chemical and morphological stem traits that are important for

resource acquisition (hydraulic conductivity, hydraulic safety, nutrient storage, metabolism) and plant defence (chemical and physical). I expect large trait variation to be found between two major taxa and amongst three main stem compartments, which strikingly differing in xylem structure.

Chapter 3 addresses the question how fungal abundance, richness, and community composition depend on tree species, and especially, how fungal communities vary during long-term succession. Stems of 10 common European tree species were monitored annually for an 8-year period for fungal composition and on the succession of wood-inhabiting fungi that are related to the wood and bark traits of the tree species. I expect that fungal abundance and richness will be higher for stems with high nutritional quality and low chemical and physical defence. In addition, I expect that the fungal community composition will initially be very diverse because resident endophytic fungi may become saprotrophs and locally common generalists will arrive, but that the fungal community composition will converge in a later stage, because wood decay will lead to a more homogenised substrate with less influence of stem traits.

Chapter 4 addresses the question how fungal diversity and community composition vary across 14 temperate tree species, and especially among three main stem compartments (inner wood, outer wood, and bark)? Fungal communities were analysed using advanced molecular analysis after one and four years of decay. I expect that a higher fungal richness and diversity will be found in bark, because bark is most nutrient rich and exposed to fungi infestation than wood. In addition, I expect significant differences in fungal community composition amongst stem compartments (inner wood, outer wood, and bark) and host tree species, and such variation can be explained by different physical-chemical traits of the stem compartments.

Chapter 5 synthesizes the main findings of the previous chapters, and integrates all components by analysing how the stem traits of tree species and the characteristics of the fungal community drive wood decomposition rates. I then provide recommendations on how to manage dead wood in forest ecosystems and give suggestions for future research.

1.6 | LOGLIFE project

1.6.1 | The research sites

Research was carried out in two forests in the Netherlands; The Hollandse Hout site (52.46N, 5.42E) and the Schovenhorst forest estate (52.25N, 5.63E). Both sites experience a temperate maritime climate, with an annual mean rainfall of approximately 700 mm and an annual mean temperature of approximately 10 ℃. The sites were selected because they represent two contrasting soil types of forests located in the Netherlands. Hollandse Hout has young marine fertile clay soils reclaimed from the former Zuiderzee in the 1960s. In this site, plots were established within a relatively light-open *Populus x canadensis* plantation with a tall herb layer of *Urtica dioica.* The Schovenhorst forest estate is situated on Pleistocene sandy soil. In this site, plots were established within a light-open *Larix kaempferi* plantation with a ground layer of *Vaccinium myrtillus* and *Deschampsia flexuosa*

1.6.2 | The LOGLIFE experiment

The LOGLIFE project is a long-term common garden experiment on tree decomposition (Fig. 1.4) (Cornelissen et al. 2012). For this experiment, trees were harvested and 1-meter length stem parts were incubated in five plots per site. In these incubation plots, stem parts of different species were successively incubated in February 2012 (10 species), in January 2013 (10 species), and in February 2015 (5 species). Species selection was balanced for diversity in wood and bark traits. Because of time and budget limitation, not all tree species but 14 species were selected for this thesis, with a relatively balanced representation of six angiosperm species and eight gymnosperm species (see table 1.1). Most species were extracted either from the forest on clay soil or forest on sandy soil, but the angiosperm *Quercus robur* and gymnosperm *Picea abies* were extracted from and incubated in both sites to compare how growing conditions and incubation sites affect fungal communities and decomposition rate. Selected trees were well developed, mostly canopy trees had a similar stem diameter (25±3 cm) to reduce the possible confounding effects of stem size on decay and fungal colonization. For each tree species from either site, five trees were harvested, each individual was sawn in 5 logs (each with 1 m length, and without major side branches from the main trunk), so that each tree individual had its own subplot representing a statistical block. In each incubation site, five incubation plots, measured by 12 m ×12 m, were established, and one log per tree

was put in each of these plots. These incubation plots were spaced at least 20 m apart to assure independence. More details about the experimental design of LOGLIFE are given in Cornelissen et al. (2012). The LOGLIFE experiment has yielded information on leaf- and twig decomposition rates (Zuo et al. 2018), bark traits and their effects on bark inhabiting fauna communities (Zuo et al. 2016), and methodological approaches to analyze stem decomposition rate (Chang et al. 2020).

To test how stem traits differ across temperate tree species and what trait trade-offs and plant strategies can be found, two adjacent 2-cm chain-sawed disks of each individual tree were collected from the bottom part of the main stem before incubation, and used for stem trait measurement (***Chapter 2).*** To monitor the occurrences of fungal fruiting bodies on decaying logs, surveys were conducted annually in the peak fructification time from 2012 to 2019 (except in 2018 due to the extremely drought) **(*Chapter 3*)**. Logs were harvested after one and four years of decay, and subsamples from these logs were used for fungal molecular analysis (***Chapter 4***) and decomposition rate determination (***Chapter 5***).

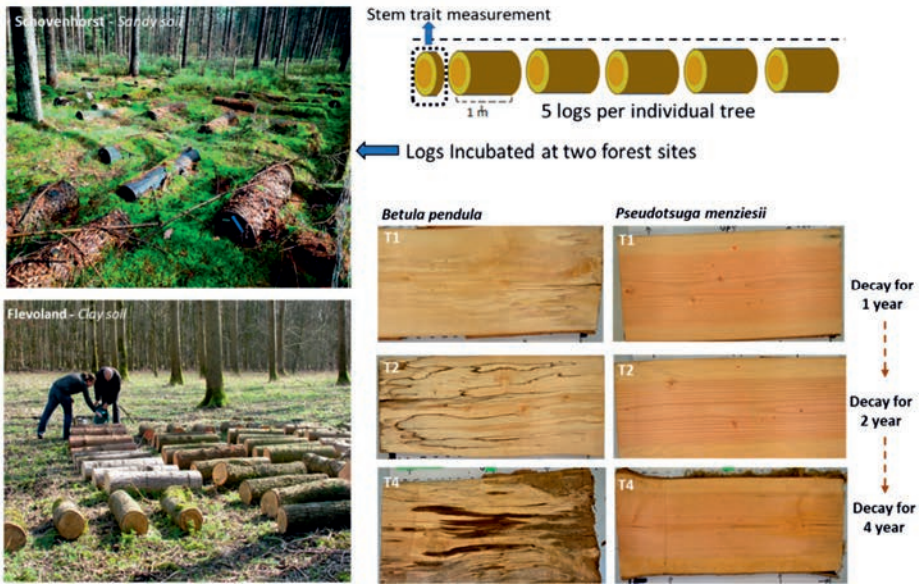


Fig. 1.4 Experimental design of LOGLIFE. Two photos in the left panel show the two incubation sites of our study: Schovenhorst forest estate (52.25N, 5.63E) and the Hollandse Hout site (52.46N, 5.42E). Sampling design for an individual tree is shown (top right), disk is sawn from bottom part of the main stem and used for initial trait measurement, and the five logs to be harvested in random order during

long-term decomposition process. Stem decay patterns are shown in six planks (bottom right), which are extracted from decaying logs of *Betula pendula* and *Pseudotsuga menziesii* after one year, two years and four years of decay.

Table 1.1 Characteristics of the 14 study tree species with species number, species name, family name, major phylogenetic group they belong to, collection site, occurrence of heartwood, and wood structure.

Species	Family	Major taxa	Leaf habit	Collection sites	Heartwood occurrence	Wood structure
Fraxinus excelsior	Oleaceae	Angiosperm	Deciduous	Flevoland	No	Ring-porous
Betula pendula	Betulaceae	Angiosperm	Deciduous	Flevoland	No	Diffuse-porous
Quercus robur.	Fagaceae	Angiosperm	Deciduous	Both sites	Yes	Ring-porous
Fagus sylvatica	Fagaceae	Angiosperm	Deciduous	Flevoland	No	Diffuse-porous
Populus×canadensis	Salicaceae	Angiosperm	Deciduous	Flevoland	No	Diffuse-porous
Populus tremula	Salicaceae	Angiosperm	Deciduous	Schovenhorst	No	Diffuse-porous
Chamaecyparis lawsoniana	Cupressaceae	Gymnosperm	Evergreen	Schovenhorst	Yes	Tracheid
Thuja plicata	Cupressaceae	Gymnosperm	Evergreen	Schovenhorst	Yes	Tracheid
Cryptomeria japonica	Cupressaceae	Gymnosperm	Evergreen	Schovenhorst	Yes	Tracheid
Taxus baccata	Taxaceae	Gymnosperm	Evergreen	Schovenhorst	Yes	Tracheid
Picea abies	Pinaceae	Gymnosperm	Evergreen	Both sites	Yes	Tracheid
Larix kaempferi	Pinaceae	Gymnosperm	Deciduous	Schovenhorst	Yes	Tracheid
Pseudotsuga menziesii	Pinaceae	Gymnosperm	Evergreen	Schovenhorst	Yes	Tracheid
Abies grandis	Pinaceae	Gymnosperm	Evergreen	Schovenhorst	No	Tracheid

1

CHAPTER 2

Stem trait spectra underpin multiple functions of temperate tree species

Shanshan Yang, Frank J. Sterck, Ute Sass-Klaassen, J. Hans C. Cornelissen, Richard S.P. van Logtestijn, Mariet Hefting, Leo Goudzwaard, Juan Zuo, Lourens Poorter

Submitted for publication

ABSTRACT

A central paradigm in comparative ecology is that species sort out along a slow-fast resource economy spectrum of plant strategies, but this has been rarely tested for a comprehensive set of stem traits and compartments. We tested how stem traits vary across wood and bark of temperate tree species, whether a slow-fast strategy spectrum exists, and what traits make up this plant strategy spectrum. For 14 temperate tree species, 20 anatomical, chemical and morphological traits belonging to six key stem functions were measured for three stem compartments (inner wood, outer wood and bark). Trait variation was explained by major taxa (38%), stem compartments (24%) and species within major taxa (19%). A continuous plant strategy gradient was found across and within taxa, running from hydraulic safe Gymnosperms to conductive Angiosperms. Both groups showed a second strategy gradient related to chemical defence. Gymnosperms strongly converged in their trait strategies because of their uniform tracheids. Angiosperms strongly diverged because of different vessel arrangement and tissue types. Bark had higher concentrations of nutrients and phenolics whereas wood had stronger physical defence. In conclusion, Gymnosperm species have a conservative "slow" strategy and a narrow trait spectrum which allows them to grow well in colder, drier and unproductive habitats. Angiosperm species show a wider trait variation in all stem compartments which makes them successful in marginal- and in mesic, more productive habitats. Different stem compartments fulfil different strategies; bark serves as storage organ and a first chemical defence layer, while wood is physically defended by tough tissues.

KEYWORDS

Stem economics spectra; plant strategies; trade-offs; physio-chemical traits; plant functions.

2.1 | INTRODUCTION

The unique feature that set trees apart from other life forms is their tall and lignified stem. The stem allows trees to grow for decades to centuries to a large size and compete successfully for light with other trees and life forms. Stems provide multiple functions (Sterck et al. 2005, Chave et al. 2009), which can be broadly grouped into transport and storage of resources and assimilates, mechanical support, and defence. These functions are partly delivered by different stem compartments such as heartwood, sapwood and bark, where for example the sapwood transports water and nutrients from roots to leaves, the inner bark transports sugars from leaves to other organs (Steppe et al. 2015), the outer bark presents a first defence line against insects, drought and fire (Loram-Lourenço et al. 2020), and the heartwood is protected against pathogen attacks (Hillis 2012). The large stem trait variation across species indicates that tree species coordinate their traits and functions in different ways (Hacke and Sperry 2001), with important implications for species performance (Poorter et al. 2010), species distribution within forests and across climates regions (Sterck et al. 2011) and species responses to global climate change (Cornwell et al. 2009).

2.1.1 | Is there a slow-fast stem spectrum?

One of the central paradigms in comparative plant ecology is that species sort out along a slow-fast resource economy spectrum of plant strategies (Reich 2014). This spectrum ranges from species that conserve carbon and nutrient resources and persist in resource-poor unproductive environments, to species that rapidly acquire light, carbon, water and nutrient resources, grow fast and thrive in resource-rich productive environments (Diaz et al. 2004, Lambers and Poorter 2004, Wright et al. 2004, Poorter et al. 2006). This “slow-fast” spectrum has been widely supported by empirical studies, but these studies are strongly biased towards leaf traits (Wright et al. 2004, Freschet et al. 2010, Kergunteuil et al. 2018). Fewer studies have analysed the fast-slow-spectrum for stem traits (Chave et al. 2009). A study on subarctic life forms found that leaf and stem traits are coupled and form part of an overall plant strategy spectrum (Freschet et al. 2010), whereas studies across tropical tree species (Baraloto et al. 2010) and across woody biomes (Pietsch et al. 2014) found them to be uncoupled. Yet, these studies included only a limited set of stem traits, such as wood density or nutrient concentrations. To our knowledge no studies have tested the stem economics spectrum using a comprehensive set of anatomical, chemical and morphological traits for different stem compartments. There is therefore a strong

need to further unpack the stem economics spectrum beyond wood density and its associated traits (Chave et al. 2009).

2.1.2 | Contrasting stem traits for Gymnosperms and Angiosperms

Gymnosperms and Angiosperms represent an old phylogenetic split with large consequences for wood structure and functioning (Feild and Arens 2007). Angiosperms can transport water efficiently through wide vessels and form thick-walled fibres for mechanical support, while Gymnosperms have narrow tracheids that combine both functions (Sperry et al. 2006). Angiosperms generally have higher xylem conductivity because their vessels can achieve much larger dimensions, but Gymnosperms can partly compensate for their limited conduit size due to their efficient torus-margo pits (Pittermann et al. 2006a). Many studies hypothesize that there is a general trade-off between hydraulic safety and water transport efficiency, but such a trade-off is not universal: only a quarter of the hydraulic studies have supported this trade-off hypothesis (Gleason et al. 2016). For example, no relationship between hydraulic safety and efficiency was found in a study comparing 14 angiosperm and gymnosperm species (Zhang et al. 2020).

Less attention has been paid to the role of parenchyma in ecological strategies. Radial and axial parenchyma are living cells coordinating physiological processes of plants, such as storage of water, non-structural carbohydrates, and production of chemical defence compounds. Angiosperms have in general more parenchyma than Gymnosperms, probably because of their different defence strategies; the antifungal substance produced in angiosperm parenchyma cells avoid the spread of fungi, whereas gymnosperm defence relies more on the occlusion of tracheids and resin-producing ducts synthesized by epithelial cells (Morris et al. 2016). In addition, it allows them to store non-structural carbohydrates that can be used for the production of new leaves in spring. Gymnosperms and Angiosperms also differ in their bark traits, for example, gymnosperm bark translocating cells offer greater resistance to sap flow than those in Angiosperms (Jensen et al. 2012).

Overall, the qualitative differences between angiosperm and gymnosperm species have been well established but the magnitude of these differences, and how that varies with traits is less clear. Similarly, it remains unclear how a comprehensive set of stem traits is coordinated to fulfil the main stem functions, what are the fundamental trade-offs, and whether the same “slow-fast” strategy spectrum can be found across and within these phylogenetic groups.

2.1.3 | Questions and hypotheses

In this study we examine how 20 anatomical, chemical, and morphological stem traits vary across a phylogenetically and ecologically diverse set of 14 temperate European tree species. We selected traits that are important for six main stem functions (hydraulic conductivity, hydraulic safety, storage, metabolism, chemical and physical strength) and measured traits for three stem compartments (inner wood, outer wood and bark) that have different developmental origins and partly have different functional roles. To minimize possible confounding ontogenetic or allometric effects of tree size on anatomical traits (Rungwattana and Hietz 2018, Fajardo et al. 2020), we selected stem sections of similar (25±3 cm) diameter and controlled statistically for stem length. We addressed the following questions and hypotheses:

1) How do stem traits vary amongst major taxa (Angiosperms vs. Gymnosperms), species within major taxa, and stem compartments? We expect the largest trait variation to be found amongst taxa mainly because of strikingly different xylem structure, followed by variation amongst stem compartments as they supposedly differ in chemical traits linked to protection and nutrient supply. Less trait variation is found within major taxa, especially within Gymnosperms due to their largely convergent xylem anatomy (Gleason et al. 2016).

2) How are traits coordinated and/or traded-off against one another between the two major taxa, species within major taxa, and stem compartments? For the major taxa and also for species within the taxa, we expect that trait trade-offs determine a stem strategy spectrum running from “slow” to “fast” species. For angiosperm species with large variation in conduit size, we expect the strongest trade-off between hydraulic conductivity and hydraulic safety in the wood. For Gymnosperms, we expect a trade-off between hydraulic conductivity and physical strength since both functions are determined by tracheids in Gymnosperms, while Angiosperm trees use vessels functioning in conductivity and fibres, respectively functioning in mechanical support (Zanne et al. 2010). Concerning traits in stem compartments, we expect coordination of traits and functions between wood and bark because they are partially derived from the same vascular cambium (Evert 2006).

2.2 | MATERIALS AND METHODS

2.2.1 | Study sites and species

Tree species were collected from wo forests in the Netherlands, located in the same temperate climate zone, with an annual mean rainfall of approximately 700 mm and an annual mean temperature of approximately 10 °C. (1) one site is the Hollandse Hout forest plantation in Flevoland (52.46N, 5.42E). It was reclaimed from the former Zuiderzee in the 1960s, and is calcareous, moist and fertile, with a pH close to neutrality and consists of marine clay, remarked as C; and (2) the other site is the Schovenhorst forest estate in the Veluwe region (52.25N, 5.63E). It is well-drained and consists of acidic sandy soil, remarked as S. More details about the study sites are given in Cornelissen et al. (2012).

Fourteen species were harvested from these two sites, of which six were angiosperm species and the other 8 were gymnosperm species (Table 2.1). All these species are common in Europe, and most species occur frequently in Dutch forests, but only six species are native. Because species differ in their distribution, we collected samples of most angiosperm species from the forest on clay, and of most gymnosperm species from the forest on sand. There were two exceptions: Individuals of two species (the angiosperm *Quercus robur* (Que.r) and gymnosperm *Picea abies* (Pic.a), were extracted from both sites (clay=C and sandy=S), and labelled them as Que.r_C, Que.r_S, Pic.a_C and Pic.a_S separately. These two species allowed us to quantify the site effects related to species on stem traits, and the results confirm our expectation that site effects were inferior to species effects. Detailed information can be found in Table 2.1.

Table 2.1 Basic information of 14 studied tree species. For *Quercus robur* (Que.r) and *Picea abies* (Pic.a), they were extracted from two contrasting sites and labelled as Que.r_C, Que.r_S, Pic.a_C and Pic.a_S.

Species	Abbreviation	Collecting sites	Heartwood occurrence	Taxa	Distribution of pores
Fraxinus excelsior	Fra.e	C (clay)	No	Angiosperm	Ring-porous
Betula pendula	Bet.p	C (clay)	No	Angiosperm	Diffuse-porous
Quercus robur	Que.r_C	C (clay)	Yes	Angiosperm	Ring-porous
Quercus robur	Que.r_S	S (sandy)	Yes	Angiosperm	Ring-porous
Fagus sylvatica	Fag.s	C (clay)	No	Angiosperm	Diffuse-porous
Populus×canadensis	Pop.c	C (clay)	No	Angiosperm	Diffuse-porous
Populus tremula	Pop.t	S (sandy)	No	Angiosperm	Diffuse-porous
Chamaecyparis lawsoniana	Cha.l	S (sandy)	Yes	Gymnosperm	Tracheids
Thuja plicata	Thu.p	S (sandy)	Yes	Gymnosperm	Tracheids
Cryptomeria japonica	Cry.j	S (sandy)	Yes	Gymnosperm	Tracheids
Taxus baccata	Tax.b	S (sandy)	Yes	Gymnosperm	Tracheids
Picea abies	Pic.a_C	F (fertile)	Yes	Gymnosperm	Tracheids
Picea abies	Pic.a_S	S (sandy)	Yes	Gymnosperm	Tracheids
Larix kaempferi	Lar.k	S (sandy)	Yes	Gymnosperm	Tracheids
Pseudotsuga menziesii	Pse.m	S (sandy)	Yes	Gymnosperm	Tracheids
Abies grandis	Abi.g	S (sandy)	No	Gymnosperm	Tracheids

2.2.2 | Sampling and variables

For each of the 14 species, five individual trees were sampled, providing 85 trees. Selected trees were well developed, and had an average stem diameter of 25±3 cm. Two adjacent 2-cm chain-sawed disks (disk 1 and 2) of each individual tree were collected from the base of the main stem. Disk 1 was used for physical trait measurement. In this study, we took samples from different stem compartments; stem consists of wood and bark, and may convert the inward part of the functional sapwood into heartwood, which is no longer functional in terms of transport but impermeable and chemically protected against pathogens by accumulation of antifungal substances (e.g., phenols and tannins) (Domec et al. 2005, Hillis 2012).

Wood density was measured based on four 1.5 cm^3 blocks extracted from inner wood and outer wood of disk 1 respectively. (Fig. 2.1). In addition, several 1.5 cm^3 bark pieces were extracted to measure bark punch resistance. Wood anatomical traits were measured based on one block of inner and outer wood respectively (Fig. 2.1). Chemical traits were measured based

on sawdust samples taken from disk 2 using an electric drill (bit diameter 8 mm). The plate and drill were cleaned between samples with 70% ethanol; sawdust from inner wood, outer wood and bark were collected separately for each disk and stored for chemical trait measurement.

In total, 20 traits (see Table S2.2) were measured in three compartments (inner wood, outer wood and bark) of 14 temperate species. Although it is acknowledged that traits have multiple functions, we assigned, for the sake of overview and synthesis, each trait to the stem function to which it contributes most. The six stem functions are: hydraulic conductivity, hydraulic safety, storage, metabolism, chemical defence. For a definition of the traits and their link to functions, see Table S2.3. We acknowledge that inner wood does not contribute to hydraulic conductivity and safety, but included the hydraulic trait values of inner wood to provide a complete and balanced overview of the traits in different stem compartments, and because it allows to evaluate to what extent hydraulic wood traits vary during tree development when trees increase in size, and whether the species ranking is maintained.

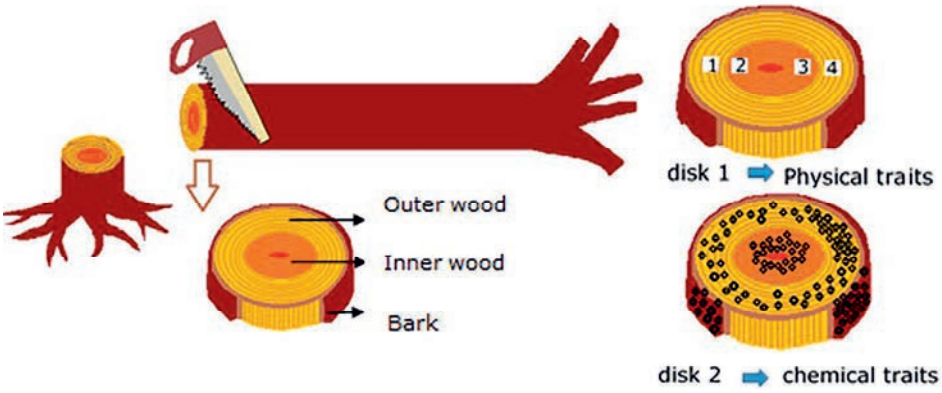


Fig. 2.1 Sampling design for measuring physical and chemical traits. Traits were measured for three stem compartments: inner wood, outer wood, and bark. Two disks (disk 1 and 2) of each tree were collected from the base of the main stem. Disk 1 was used for physical trait measurement, and disk 2 for chemical trait measurement.

2.2.3 | Physical traits

Wood density and heartwood proportion. Wood density and heartwood proportion were measured on five individual trees for each species. Four blocks (Fig. 2.1) were taken along the

diameter of disk 1. The wood density was determined by the water displacement method as mass after drying at 105°C divided by fresh volume. The average density of block 2 and 3 represented the inner wood (in some cases heartwood) density, while block 1 and 4 represented the outer wood (i.e., sapwood) density. All disks were assumed to be cylinders, therefore we calculated the heartwood proportion based on formula: $\frac{\pi r^2}{\pi R^2}$, when r is the width of heartwood and R is the radius of the selected disk. This calculation could only be done for heartwood forming tree species (Table S2.1).

Wood anatomical traits. Anatomical traits were measured for three individual trees per species. Transverse sections of wood samples were taken from inner wood (block 1 or 2) and outer wood (block 3 or 4, Fig. 2.1), and used for anatomical measurements.

To produce permanent samples, the thin sections were prepared in five steps: (1) samples were cut using a sliding microtome (wood samples that were too hard to be cut were first softened in boiling water); (2) a 5% hypochlorite solution was applied to bleach the samples, then samples were rinsed thoroughly using demi-water; (3) the samples were stained with a mixture of Astrablue and Safranin for at least 5 minutes; (4) samples were washed with demi-water and dehydrated in ethanol series (50%, 96% and 100%); (5) thin sections were dewaxed using Roticlear® (Carl Roth, Karlsruhe, Germany) and permanently embedded with Roti®-Mount (Carl Roth, Germany). High-resolution digital images of anatomical sections were made using a camera mounted on an optical microscope. Gymnosperm sections with narrow tracheids were measured using lens of 10× magnification, while sections of angiosperm species with wider vessels were measured using lens of 5× magnification. Digital images were calibrated with a slide-mounted micrometer and then analysed using Fiji/ImageJ (Schindelin et al. 2012), and conduit density, diameter, fraction, wall thickness and ray fraction, fibre wall thickness (for Angiosperms only), the ratio of conduit wall thickness to conduit radius were quantified.

Potential hydraulic conductivity (K_p) was calculated according to the Hagen-Poiseuille law (Sterck et al. 2008):

$$Kp = (\pi\rho_w/128\eta) \times CD \times D^4 \qquad \text{Equation 1}$$

where K_p is the potential specific stem conductivity (in kg m Mpa^{-1} s^{-1}), η is the viscosity of water at 20°C (1.002×10^{-3} Pa s at 20°C), ρ_w is the density of water at 20°C (998.2 kg $m10^{-3}$ at 20°C), CD is the conduit density and D is the conduit diameter (in m).

Bark punch resistance. Bark punch resistance was used as an indicator of bark toughness. A Mecmesin Ultra Test with AFG-1000N force gauge (Slinfold, West Sussex, UK) was applied and bark punch resistance (N) was determined as the maximum force needed to penetrate with a stainless-steel needle of 1 mm diameter the extracted bark pieces. The acceleration was standardized to 150 mm per minute for all samples. Bark toughness may be different along the circumference of the disk, therefore, three to five pieces (1.5 cm × 1.5 cm) of bark were extracted randomly along the circumference of disk 1.

2.2.4 | Chemical traits

Chemical traits were measured from sawdust samples taken from the inner wood, outer wood and bark. Samples were ground into fine powder with a Retsch MM400 ball mill (Retsch, Haan, Germany) and oven-dried (48h at 70°C).For samples from the three compartments we measured: concentrations of carbon, nitrogen, phosphorus, lignin, lignin/cellulose ratio, phenols and tannins; pH value was measured too, and the C/N ratio was calculated.

Carbon and nitrogen concentrations were determined by dry combustion using a Flash EA 1112 elemental analyser (Thermo Scientific, Rodana, Italy).

Phosphorus concentration was determined by digestion with HNO_3/HCl (1:4 mixture of 37% HCl and 65% HNO_3). P concentration was determined with spectrophotometry using the ammonium molybdate method (at a wavelength of 880 nm) as proposed by Murphy and Riley (1962).

Lignin and cellulose concentrations were determined following Poorter and Villar (1997). Samples were extracted with water, methanol and chloroform to remove the soluble sugars, soluble phenols and lipids. Then starch, fructan, pectin and a part of the hemi-cellulose were removed during acid hydrolysis. Finally, after correction for ash concentration (including silicates) and remaining proteins, the lignin and cellulose concentrations were calculated based on their difference in C concentration.

The Folin-Ciocalteu method was used to determine total phenols and tannins concentrations. A 50% methanol solution was used to extract the phenolic hydroxyl groups. Then the phenols were coloured with a Folin-Ciocalteu reagent and samples were measured at 760 nm on a spectrophotometer. Finally, the total phenols were calculated according to a tannic acid-based calibration curve, and non-tannin phenols were measured after binding the tannins in the 50% methanol extract with polyvinylpolypyrrolidone (PVPP).

The pH_{H2O} was measured according to (Cornelissen et al. 2006). Briefly, 0.15 ml sample was added to 1.2 ml demi-water in an Eppendorf tube and shaken for 1 h at 250 rotations per minute. Then the tubes were centrifuged for 5 min at 13,000 rpm and the supernatant was used to measure pH_{H2O} value with a WTW SenTix Mic electrode.

2.2.5 | Statistical analysis

Statistical analyses were performed using R v. 3.6.1 (R Core Team, 2016) and CANOCO 5.0 (ter Braak and Smilauer 2012). All trait variables with the exception of heartwood proportion were $\log_{10}$ transformed to increase normality and homoscedasticity.

To evaluate how much trait variation was explained by major taxa (Angiosperms vs Gymnosperms), species within major taxa, stem compartments (inner wood, outer wood, bark), a variance component analysis was performed using the R package "*variancePartition*" (Hoffman and Schadt 2016). This package uses a linear mixed model, built on top of the lme4 package (Bates et al. 2011), to partition the variance attributable to multiple variables in the data. The contribution of each variable was expressed as the fraction of explained variation. These categorical variables (major taxa, species nested in major taxa and stem compartments) are modelled as random effects to obtain statistically valid results as specified in "variancePartition" package.

To test which factor(s) significantly contribute to trait variation, a variance partitioning and multiple linear effect model were fitted with each trait as response variable, and major taxa, species within major taxa and stem compartments as fixed factors. We included stem length (i.e., the estimated vertical distance from stem disk to tree top) as a co-variate in these analyses to correct for its potential confounding effects (Table S2.4, Fig. S2.1). Detailed information about stem length calculations is shown in Fig. S2.2.

To evaluate how traits were associated, a principal-component analysis (PCA) was performed using species-mean trait values per compartment type (i.e., inner wood, outer wood, and bark) as data points. In total, 20 stem traits linked to six plant functions were included in the PCAs (for the full list of variables and their functionality, see Table S2.3). To evaluate whether the same trait associations are found within anatomically distinct groups, two additional PCAs were carried out for angiosperm and gymnosperm species separately. PCA analyses were performed in CANOCO 5.

2.3 | RESULTS

2.3.1 | Stem trait variation

Major taxa (Angiosperms vs. Gymnosperms), species and compartments differed in stem traits (Table 2.2, Fig. 2.3). All stem traits (100%) were significantly affected by species and major taxa and ca. 70% by stem compartments. Most of the variation was explained by major taxa (40%), followed by stem compartments (24%) and species within major taxa (19%) (Table 2.2 & Fig. 2.2). Corresponding values are shown in Table 2.2. Below, we first discuss major taxon and compartment effects (Tables 2.2 and 2.3) whereas species differences are discussed in detail based on the PCA analysis (Fig. 2. 4, 2.5 & 2.6).

Differences between Gymnosperms and Angiosperms largely explained differences in traits related to hydraulic conductivity, hydraulic safety, storage and physical strength (Table 2.2). Conduit diameters were on average 3-fold larger in angiosperm species than in Gymnosperms (Table 2.3 & Fig. 2.3). Despite the 20-fold larger conduit density in Gymnosperms, angiosperm species had a theoretical conductivity that was approximately 12-fold higher compared to Gymnosperms due to the strong effect of conduit diameter (Hagen-Poiseuille law, see Methods section). Also variation in our best proxy for hydraulic safety (conduit wall thickness to conduit radius ratio) was best explained by major taxon differences. Major taxa also explained most of the variation in ray parenchyma, with angiosperm species having a larger ray fraction (~10%) than Gymnosperms (~5%, Table 2.3 & Fig. 2.3). Finally, gymnosperm species had a higher lignin concentration but a lower wood density (0.42 g cm^{-3}) than angiosperm species (0.53 g cm^{-3}). Remarkably, these two major taxa hardly differed in other physical strength traits, neither in any of the traits related to metabolism or chemical defence.

Stem traits also varied significantly among different tree species (Table 2.3 & Fig. 2.3). Oak (*Quercus robur*) had the largest conduit diameter, high ray fraction, nitrogen and phenolics concentration, which allow it to perform well in terms of hydraulic conductivity, nutrient storage and chemical defence. Beech (*Fagus sylvatica*) had of all angiosperm species the largest storage capacity, as indicated by its highest ray fraction (Fig. 2.3C), but the lowest chemical anti-fungal defence, as indicated by the low concentration of phenols (Fig. 2.3E). Gymnosperms performed better than Angiosperms in terms of hydraulic safety and physical-chemical defences. Among the eight gymnosperm species, *Thuja* had the thickest relative

conduit wall thickness (Fig. 2.3B), *Larix* had the highest phenolics concentration though its relative conduit wall thickness was smaller compared to other gymnosperm species (Fig. 2. 3E & B). *Cryptomeria* performed better in physical defence as indicated by its highest lignin concentration, while *Fraxinus* had the lowest lignin concentration (Fig. 2.3F).

Stem compartments (i.e., inner-, outer wood and bark) strongly varied in traits related to metabolism (53%), chemical defence (56%) and, partially, physical strength (30%). For metabolic traits, the N and P concentrations were significantly higher in bark than in both wood components (Table 2.3 & Fig. 2.3), and higher in the younger outer wood compared to the older inner wood. With respect to chemical defence, bark was best defended by having the highest concentration of phenols and tannins, followed by inner wood, and then outer wood (Table 2.3). The opposite was true for physical strength; the C/N ratio was higher in both wood compartments compared to bark. Inner- and outer wood compartments did not vary significant in traits related to hydraulics and storage (bark was not included in these measurements).

We checked for the potential confounding effect of stem length on trait values (Table S2.4, Fig. S2.1). Stem length had a significant effect on 50% of the stem traits, but those traits explained only 0.56% of the trait variation (range 0.04% to 2.58%). We conclude therefore that stem length has little confounding effect on the investigated traits.

Table 2.2 The percentage of trait variation explained by major taxa (Angiosperms vs Gymnosperms), species within major taxa, and compartments (inner wood, outer wood and bark). The variance explained by the most important factor is given in bold. Asterisks indicate the significance level. At the bottom of the table, the % significance indicates what percentage of measured traits was significantly affected by each factor. †: Traits were $\log_{10}$-transformed; ***: P<0.001, **: P<0.001, *: P<0.05, ns: not significant.

Ecological function	Stem traits	Unit	Major taxa	Species	Compartments
Hydraulic conductivity	Conduit fraction	%	83.7***	7.32***	0.00^{ns}
	Conduit diameter†	μm^2	88.5***	7.99***	0.04^{ns}
	theoretical hydraulic Conductivity (K_p)†	kg m $MPa^{-1}s^{-1}$	69.2***	19.3***	0.09^{ns}
	Average		**80.5**	11.5	0.04
Hydraulic safety	Conduit density†	cm^{-2}	93.1***	5.09***	0.00^{ns}
	Conduit wall thickness	µm	10.4**	17.5**	4.98**
	Conduit wall thick/radius†	µm/µm	90.9***	4.84***	0.00^{ns}
	Fiber wall thickness	µm	NA	64.3***	5.11*
	Average		**64.8**	22.9	2.52
Storage	Ray fraction	%	**48.2****	26.1***	2.71*
Metabolism	Nitrogen†	%	0.93***	3.58***	91.4***
	Phosphorus	%	1.61***	11.1***	65.1***
	pH†	NA	6.25***	47.9***	1.30*
	Average		2.93	20.9	**52.6**
Chemical defence	Phenols†	%	0.38**	15.8***	61.6***
	Tannins†	%	1.22***	22.2***	51.0***
	Average		0.80	19.0	**56.3**
Physical strength	Wood density	%	34.3***	48.8***	2.16**
	Carbon	%	30.1***	10.2***	11.5***
	Carbon/nitrogen	NA	3.90***	7.00***	71.9***
	Lignin	%	55.9***	7.94***	9.59***
	Lignin/cellulose†	NA	29.4***	4.55***	42.1***
	Average		30.7	15.2	**29.6**
Average			**38.0**	19.3	24.0
% significance			***100***	***100***	***77.2***

Table 2.3 Stem trait variation in % among compartments of Angiosperms and Gymnosperms. Mean trait values of inner wood, outer wood and bark are shown for Angiosperms (N=6 species) and Gymnosperms (N=8).

Ecological function	Stem traits	Unit	Angiosperms			Gymnosperms		
			Inner wood	Outer wood	Bark	Inner wood	Outer wood	Bark
Hydraulic conductivity	Conduit fraction	%	17.7	18.7	-	39.3	39.6	-
	Conduit diameter	μm^2	110	122	-	31.2	33.7	-
	Theoretical hydraulic conductivity (K*p*)	kg m $MPa^{-1}s^{-1}$	62.5	76.9	-	5.00	5.78	-
Hydraulic safety	Conduit density	cm^{-2}	69.5	59.8	-	1471	1334	-
	Conduit wall thickness	µm	2.50	2.57	-	2.68	2.88	-
	Conduit wall thick/radius	µm/µm	0.07	0.07	-	0.29	0.29	-
	Fiber wall thickness	µm	2.67	2.83	-	-	-	-
Storage	Ray fraction	%	9.53	7.80	-	4.84	4.54	-
Metabolism	Nitrogen	%	0.09	0.12	0.66	0.07	0.09	0.61
	Phosphorus	%	0.006	0.015	0.05	0.002	0.007	0.04
	pH	NA	5.3	5.11	5.07	4.83	4.95	4.63
Chemical defence	Phenols	%	2.14	0.74	4.43	2.31	0.49	5.49
	Tannins	%	1.84	0.51	2.93	1.73	0.33	3.86
Physical strength	Wood density	%	0.53	0.53	-	0.40	0.44	-
	Carbon	%	46.2	46.0	48.1	48.8	48.4	49.5
	Carbon/nitrogen	NA	550	445	75.1	806	578	93.5
	Lignin	%	16.7	16.7	23.6	29.0	30.0	32.0
	Lignin/cellulose	NA	0.52	0.48	1.26	0.95	0.96	1.44

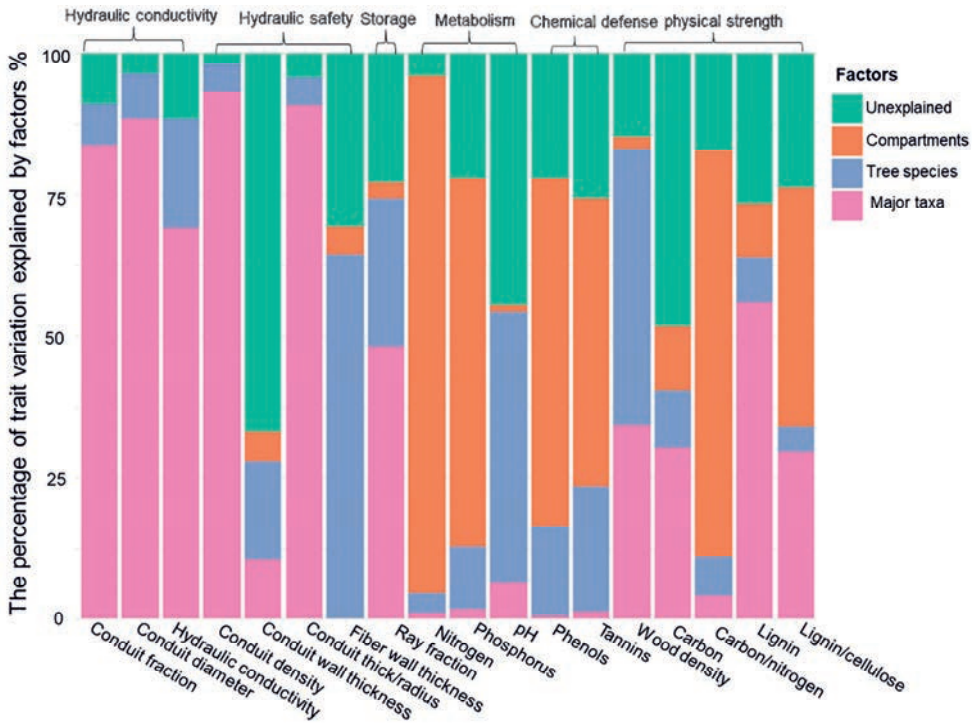


Fig. 2.2 The percentage of trait variation explained by major taxa (Angiosperms vs Gymnosperms; pink), species within major taxa (blue), and compartments (inner wood, outer wood and bark, orange), and the unexplained variation (green). The traits are grouped into six stem functions.

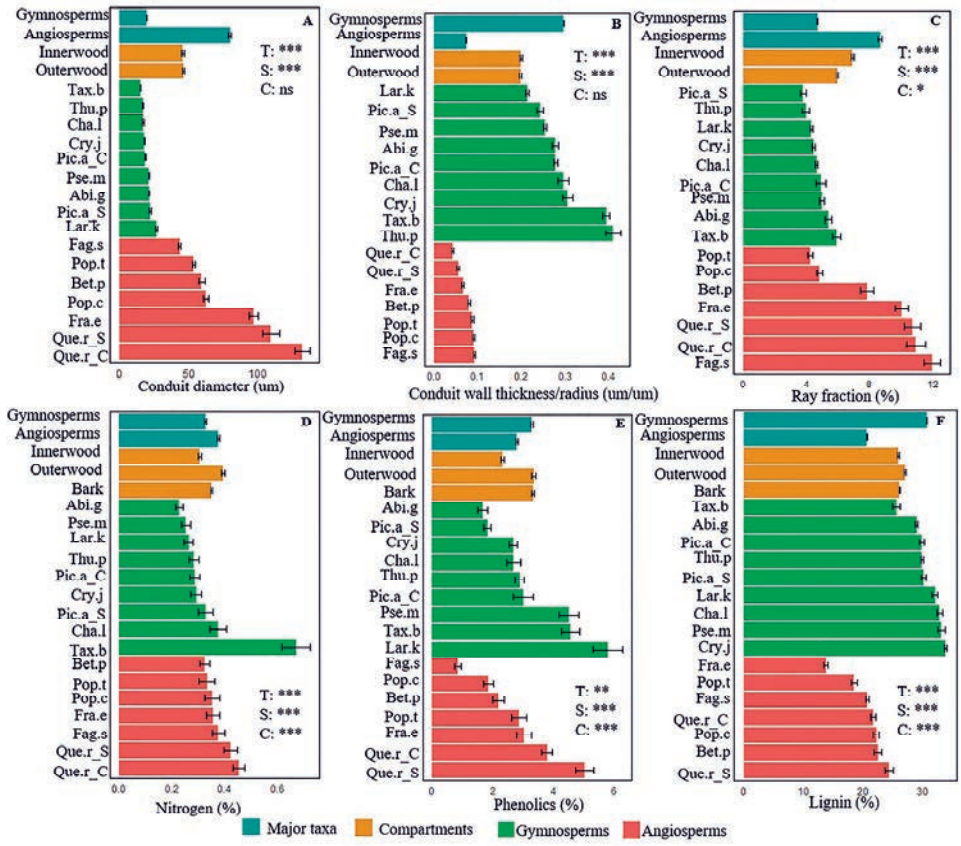


Fig. 2.3 Trait variation between two major taxa (Gymnosperms vs. Angiosperms; blue-green), across different stem compartments (inner wood, outer wood and bark; brown) and species nested in major taxa (Gymnosperms dark green, Angiosperms reddish brown). For each of the six stem functions, one representative trait is shown: conduit diameter, conduit wall thickness/radius ratio, ray fraction, nitrogen, phenolics and lignin concentrations. Means and standard error of the mean are shown. T: Major taxa; S: Species nested within taxa; C: Compartments. Triple asterisks: P<0.001, double asterisks: P<0.001, single asterisks: P<0.05, ns: not significant. For species abbreviations see Table S2.3 in Supplementary.

2.3.2 | Associations amongst stem traits

For all species, the first two PCA axes explained almost 86% of the variation in stem traits (Fig. 2.4). Strong major taxon differences determined the variation explained by the first PCA axis, running from gymnosperm species with strong physical strength (e.g., lignin) and hydraulic safety (e.g., conduit wall thickness/radius ratio), to angiosperm species with the opposite suite of traits, as well as high conductance traits (e.g., conduit diameter and K_p) and large storage capacity (i.e., ray fraction). Perpendicularly, independent of the major taxon effect, an apparent trade-off was found between chemical defences (e.g., phenols) and pH, which at least partially ran parallel for both Gymnosperms and Angiosperms.

Large trait variation was observed within angiosperm species; ring-porous oak (*Quercus*) and ash (*Fraxinus*) had stronger hydraulic conductivity (larger conduit diameter) and higher wood density, while diffuse-porous birch (*Betula*), beech (*Fagus*) and both poplar species (*Populus*) were clustered with higher pH. In contrast to the large trait variation within angiosperm species, there was convergence of trait variation within gymnosperm species.

Subsequently separate PCAs (Fig. 2.5 & Fig. 2.6) for angiosperm and gymnosperm species, to remove the dominant major taxon effect, showed rather distinct trait associations within these two major taxa.

For Angiosperms only, the first two axes of the PCA explained ca. 82% of the variation in stem traits (Fig. 2.5). The first PCA axis explained 70.5% of the trait variation, running from beech (*Fagus*) with small conduits and low pH through the other diffuse-porous species, to ring-porous oak (*Quercus*) with heartwood. Hence, this first axis ran from relatively high hydraulic safety (e.g., relatively thicker vessel walls) and low conductance to wood that was more conductive and better chemically defended by heartwood formation. The second axis ran from *Populus tremula* with strong physically defended bark and weaker defended wood, to ring-porous ash (*Fraxinus*) with relatively strong physical strength (e.g., high wood density) to compensate for a lack of chemical defence.

For Gymnosperms only, the first two axes explained 46% of the trait variation (Fig. 2.6). The first PCA axis indicates a phylogenetic split between the Pinaceae (*Larix*, *Pseudotsuga*, *Picea*) versus the Cupressaceae (*Chamaecyparis*, *Cryptomeria and Thuja*) together with the closely phylogenetically related yew (*Taxus)* from the Taxaceae. Accordingly, we found that this axis ran from higher hydraulic conductivity (e.g., conduit diameter, and K_p) to higher hydraulic safety (e.g., thicker conduit walls), metabolism (e.g., N & P) and storage ability (i.e., ray fraction). Additionally, an interesting apparent trade-off was observed between bark

physical strength (e.g., bark lignin) and wood physical strength (e.g., wood lignin). The second axis was mainly driven by bark chemical defence and pH, running from *Chamaecyparis* with high pH to *Larix* and *Pseudotsuga* with denser and better chemically protected bark.

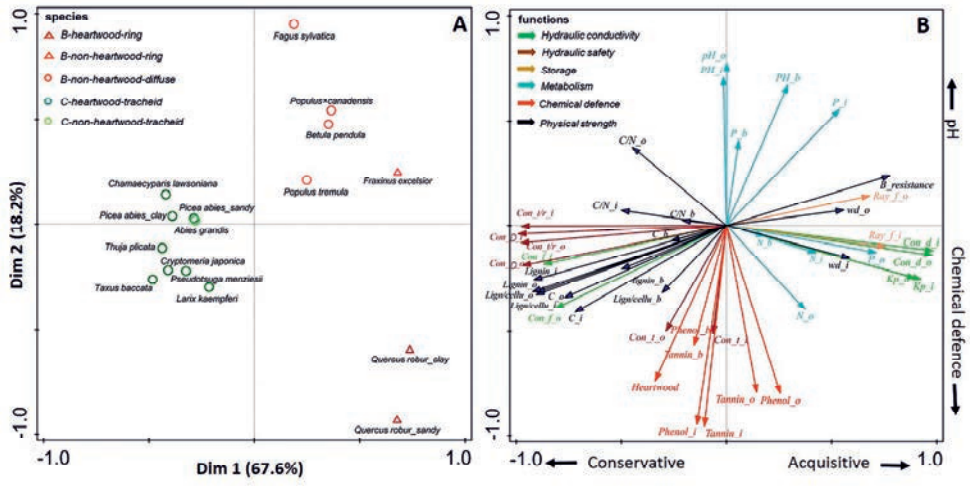


Fig. 2.4 Principal-component analysis of 20 stem traits of three compartments (inner wood, outer wood, bark) of all tree species. Dark green circles show gymnosperm species with heartwood; light green circles show gymnosperm species without heartwood; dark red triangles show ring-porous angiosperm species with heartwood; light red triangles show ring-porous angiosperm species without heartwood; light red circles show diffuse-porous angiosperm species without heartwood. The traits (indicated with arrows) are grouped according to six functions:hydraulic conductivity (in green), hydraulic safety (in dark brown), storage (in light brown), metabolism (in blue), chemical defence (in red) and physical strength (in black).

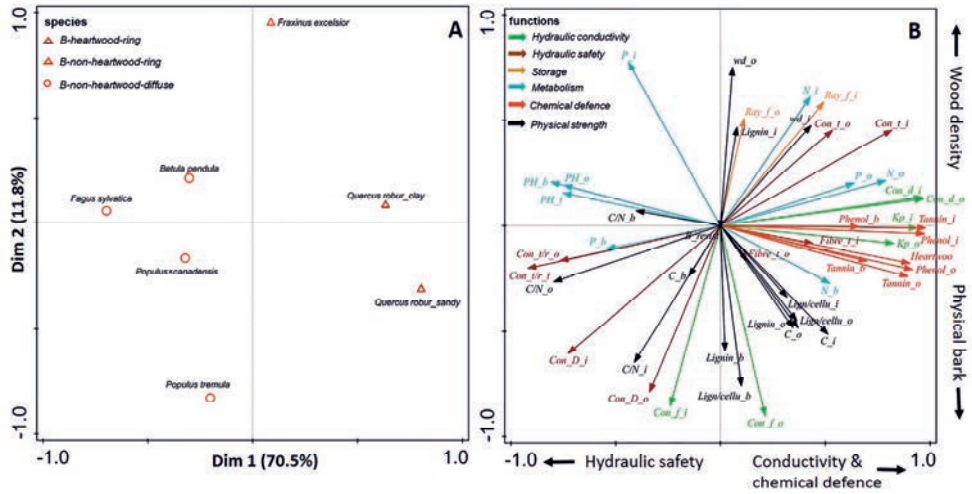


Fig. 2.5 Principal-component analysis based on stem traits assessed in of three compartments (inner wood, outer wood, bark) for six angiosperm species.

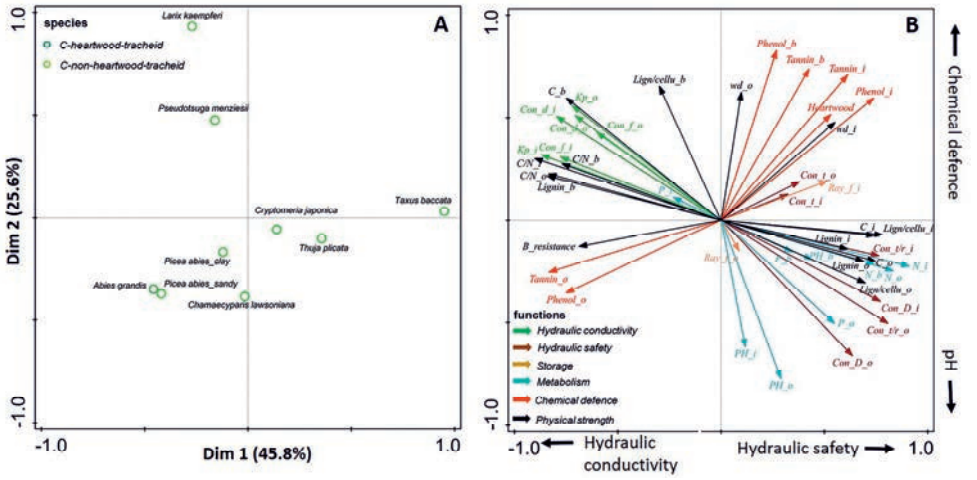


Fig. 2.6 Principal-component analysis based on stem traits assessed in of three compartments (inner wood, outer wood, bark) for eight gymnosperm species.

2.4 | DISCUSSION

We evaluated 1) how strong traits differ between major taxa (gymnosperm vs. angiosperm), species within major taxa, and stem compartments, and 2) how traits are coordinated and/or traded-off against one another. As expected, most of the trait variation was explained by major taxa (38%), followed by compartments (24%) and species within major taxa (19%). Stem trait variation in temperate tree species is therefore mainly explained by an old phylogenetic split which has led to a fundamentally different anatomy and characteristics of wood compartments between gymnosperm species (with narrow tracheids, lack of fibres, but higher hydraulic safety and chemically protected heartwood formed in the majority of species) and angiosperm species (with wide vessels embedded in fibres enabling high conductivity, and with lack of chemically protected heartwood in most, i.e., diffuse porous species). Trade-offs between hydraulic traits were less marked between angiosperm species and specially between gymnosperm species. Angiosperm species nevertheless largely diverged in their trait values whereas gymnosperm species tended to converge in their trait values. As expected, stem compartments differ in traits with bark serving as storage organ and a first defence layer, whereas the wood components were protected by physical and chemical (heartwood) defence. In the discussion, we will focus first on trait variation and associations between major taxa, then within each major taxon and finally discuss coordination across stem compartments.

2.4 1 | Conservative gymnosperm versus acquisitive angiosperm species

We first asked how stem traits vary between gymnosperm and angiosperm species. Thirty-eight percent of the trait variation was explained by these two taxa, particularly for traits related to hydraulic conductivity, hydraulic safety and storage, but less for chemical traits (Table 2.2).

Angiosperm species achieved a 10 times higher theoretical hydraulic conductivity than gymnosperm species (Table 2.3) because they had ca. 4 times wider conduit diameters (Tyree and Zimmermann 2002). High water conductivity allows angiosperm species to sustain the high transpiration rates of their thin leaves, attain high photosynthetic rates and become successful also in mesic, productive habitats. This high productivity allows these winter-deciduous Angiosperms to partly compensate for their shorter growing season (Reich et al. 1997).

Instead, gymnosperm species had a higher resource investment in hydraulic safety, as they had ca. 4-fold higher ratio of conduit wall thickness to conduit radius, compared to angiosperm

species (Table 2.3 & Fig. 2.3). Such reinforced conduits can better resist implosion when drought causes strong water tension (Pittermann et al. 2006b, Blackman et al. 2010). Gymnosperms also had a higher conduit density which may increase hydraulic safety; when some conduits cavitate sufficient others are left to continue water transport. Angiosperm species had a 2-fold higher ray fraction compared to gymnosperm species (Table 2.3 & Fig. 2. 3) (Morris et al. 2016, Zhang et al. 2020), indicating a larger capacity for carbohydrate, water, and nutrient storage. Ray parenchyma has multiple functions as it contributes to radial strength (Reiterer et al. 2002), defence against pathogens (Jupa et al. 2016), transition of sapwood into heartwood (Spicer and Holbrook 2007), and storage of non-structural carbohydrates that allow for cell metabolism and cavitation repair, and can also fuel new growth of trees (Rosas et al. 2013). Winter-deciduous angiosperm species may require a high carbohydrate storage capacity to survive winter and flush new leaves in spring (Epron et al. 2012), whereas the evergreen gymnosperm species can flush new leaves later in spring using photosynthates coming from their preserved needles rather than stored carbohydrates (Lusk 2001). Parenchyma cells in angiosperm species are also important to produce a large amount of extractives to avoid the spread of decay (Sun et al. 2015, Morris et al. 2016), whereas gymnosperm defence more relies on the occlusion of tracheids and abundant anti-fungal compounds produced by resin ducts (Hudgins et al. 2004, Fuhr et al. 2013).

Interestingly, most angiosperm species physically protect their wood by having a 1.3-fold higher wood density, mainly realised through thick-walled fibres than gymnosperm species, whereas gymnosperm species protect their wood by having a 2-fold higher lignin concentration than angiosperm species (Lamlom and Savidge 2003). In addition, the lignin of gymnosperm species has more condensed carbon-carbon inter-unit bonds that can enhance wood stiffness (Lourenço et al. 2015).

The observed trait variation between these two major taxa may be potentially affected by the different sites and soil types (clay vs. soil) from which they were collected. We analysed the potential site effect for spruce and oak that were sampled at both sites (Fig S3). Eighty-three percent of the traits were not affected by site conditions, and for the three traits with a significant site effect observed difference between Gymnosperms and Angiosperms was much larger than the observed site difference. We believe therefore that differences in soil type cannot explain the observed differences between Gymnosperms and Angiosperms.

In sum, Gymnosperms have many narrow reinforced tracheids which increase hydraulic safety, and high lignin concentrations that increases physical defence. Such a “slow and safe” strategy may allow Gymnosperms to avoid freezing- and drought induced cavitation, have

persistent evergreen leaves, and occupy colder, drier, unproductive habitats. Angiosperms are more diverse in stem traits and combine high hydraulic conductivity with storage parenchyma. Paradoxically, angiosperm species also have a high wood density, as they have (thick-walled) fibres that are needed to strengthen the larger conduits. Such a "fast and efficient" strategy allows them to be winter deciduous and occupy a large variety of habitats including mesic, seasonally productive habitats.

2.4.2 | Coordination and trade-offs amongst stem traits of temperate tree species

A multivariate trait analysis of all species combined (Fig. 2.4) confirmed that trait variation was largely driven by major taxon differences and reflected possible coordination and trade-offs amongst stem traits.

Hydraulic and physical strength strategies. The first axis showed the trade-off between high hydraulic safety in gymnosperm species versus high hydraulic conductivity and storage capacity of angiosperm species (see also Fig. 2.4). This axis was paralleled by a gradient in wood defence where angiosperm species physically protected their wood by having a higher wood density (Barnett and Jeronimidis 2009) (Fig. 2.4). A unique anatomical feature of angiosperm species is that they can free up a larger part of cross-sectional area for thick-walled fibres, as they possess fewer but wider vessels. As a result, they can maintain both high conductivity and dense wood (Fig. 2.4). In contrast, gymnosperm species protect their soft wood against insects and pathogens through higher lignin concentration and heartwood formation.

Chemical defence strategies. The second strategy axis showed a gradient in chemical defences in stem and bark, with slower-growing species having high concentrations of slightly acidic and toxic phenols and tannins, as well as chemically-defended heartwood at the bottom (e.g., oak), and faster-growing species with high stem quality (e.g., high P concentration) at the top (e.g., birch, poplar and beech). Both angiosperm and gymnosperm species sorted out along this second defence axis.

Diverging angiosperm versus converging gymnosperm trait values. Interestingly, a larger trait variation and a range of strategies was observed for angiosperm species whereas convergence of trait values and more similar strategies were found for gymnosperm species. This is probably

caused by the different anatomical features of angiosperm species and gymnosperm species. Angiosperm species mainly have three tissues types (vessels, parenchyma, and fibres) in the wood, whereas gymnosperm species only have two (tracheids and parenchyma). Moreover, compared to tracheids, which are relatively small and homogenously distributed, there is a wider range of possibilities for angiosperm species to make their stems as their vessels can vary largely in size, distribution (ring-porous and diffuse porous) and therefore in plant strategies (Olson et al. 2020). This larger variation in stem traits is also paralleled by a larger variation in leaf and crown traits (Wright et al. 2004), where leaves of angiosperm species vary largely in size and shape, whereas gymnosperm species make small needles and scale leaves, have mostly an evergreen leaf habit, and they make regular horizontal branches that are under stronger apical control.

In sum, a major plant strategy gradient was found running from hydraulic safe Gymnosperms to conductive Angiosperms suggesting that abiotic conditions (drought, freezing) present a major selective force to trees. This gradient was mainly caused by the different conduit types of gymnosperm- and angiosperm species, but within these group a continuous variation was found due to variation in conduit size. A second strategy gradient was formed by chemical defence gradient shown by both groups, indicating that defence against biotic pests is universally important. Gymnosperms showed strong convergence in their trait strategies because of less tissue types, which may result in a similar ecological behaviour.

2.4.3 | Angiosperm species show a trade-off between diffuse and ring-porous species

Within angiosperm species, the diffuse porous trees have a higher hydraulic safety (e.g., narrow vessels and relatively thicker vessel walls), which allows them to avoid drought and freezing-induced cavitation and flush therefore earlier in springtime with the benefit of a longer growing season. In contrast, ring-porous species avoid the risk of freezing induced cavitation by flushing later, but the shorter growing season is compensated by the higher hydraulic conductivity and chemical defence due to their wider vessels and higher phenols concentrations.

Ring-porous ash had a higher wood density compared to diffuse-porous poplar (Fig. 2.5) (Zobel and Van Buijtenen 2012), probably because the large vessels of ring-porous species are embedded in a matrix of thick-walled fibres that increase wood density (Zobel and Van Buijtenen 2012). In contrast, poplar compensates for its low wood density and weakly defended wood by strengthening bark physical defence (e.g., high bark lignin). Oak also stood out in

chemical defence by high phenols and tannin concentrations in its heartwood, which contributes to its longevity.

In sum, simple qualitative anatomical features such as vessel arrangement and size (diffuse- vs. ring-porous wood) are associated with as much as 71% of the quantitative variation in anatomical, chemical and morphological traits (Fig. 2.5).

2.4.4 | Gymnosperms show a trade-off between efficient Pinaceae and safe Cupressaceae

For gymnosperm species, Cupressaceae produce more tracheids and thicker walls that increase hydraulic safety, whereas Pinaceae produce wider tracheids that increase conductivity, growth and productivity (Cochard et al. 2004, Pittermann et al. 2006a). Counterintuitively, the cavitation resistant Cupressaceae in our study (*Chamaecyparis*, *Cryptomeria and Thuja)* tend to come from warm and wet areas, where they are known as fast growing species. Such high growth rates may be facilitated by high metabolic rates, as indicated by the high stem nutrient concentrations.

Gymnosperms showed coordinated hydraulic conductivity and physical strength (Fig. 2. 6), as they combined high hydraulic conductivity with well defended bark (e.g., high bark resistance and lignin concentration). This indicated that gymnosperm species, especially the Pinaceae, may combine the best of both worlds, by being both productive and well defended.

In sum, strategy variation in Gymnosperms is determined by a phylogenetic split, which generally runs from Cupressaceae with high hydraulic safety, metabolic rate and storage capacity, to Pinaceae with efficient water transport and well-defended bark.

2.4.5 | Trait variation and associations among stem compartments

Stem compartments showed a distinct radial allocation pattern in traits related to metabolism and defence. The on average 5-fold higher nitrogen and phosphorus concentrations in bark compared to wood tissues of the studied species are consistent with the literature (Harmon et al. 1986, Adler et al. 2005), and reflect that bark can be photosynthetically active and plays an important role in assimilating transport and nutrient storage (Heilman and Stettler 1986). The 4-fold higher phenol and tannin concentrations in the bark compared to wood were also consistent with earlier finding (Fengel and Wegener 2011, Lamounier et al. 2012, Navarro Hoyos et al. 2015), and indicate that bark plays an important role as a first layer of chemical defence, as phenols and tannins can hamper fungal growth and insects attack (Kahl et al. 2017).

Significantly higher phenol concentrations were found in the inner wood (2.2%) compared to the outer wood (0.6%) (Morais and Pereira 2012) which is due to heartwood production in all but one (*Abies*) of the gymnosperm species and *Quercus* among the angiosperm species. Heartwood is chemically protected against pathogens (Hillis 1971). The C/N ratio was higher in wood than in the bark (Table 2.3), which reduces wood palatability for microorganisms.

Interestingly, we found wood-bark trade-offs in terms of defence traits though most traits were uncoupled between wood and bark (Fig. S2.4-S2.6). This is contrary to our prediction that wood and bark traits are coordinated because they partly share the same ontogenetic origin (Rosell et al. 2014). Bark punch resistance and wood lignin concentration, as components of the same defence syndrome, were generally negatively correlated, implying that reinforced bark can compensate the weak physical strength of wood. Differing from angiosperm species with coordinated chemical defences in three compartments, gymnosperm species showed a trade-off between outer wood and bark, indicating that outer wood may invest more resources in other functions (e.g., physical strength and conductivity) when bark is well chemically defended.

In sum, large trait variation was observed amongst stem compartments and showed a distinct radial allocation pattern from outer-most bark to inner-most wood. Bark is photosynthetically active and serves as a first layer of chemical defence as indicated by a high nutrient level and presence of anti-fungal substances, whereas sapwood is well physically defended by possessing a higher carbon to nitrogen ratio. Heartwood was mainly present in the in gymnosperm species, and ring-porous oak; it provides additional chemical defences and increases stem longevity.

2.4.6 | Conclusions

Gymnosperms and Angiosperms can both dominate temperate and boreal forests, and across and within these taxa, a continuous slow-fast stem strategy spectrum is found. This spectrum runs from slow hydraulically safe Gymnosperms to more diverse, but potentially also fast and hydraulically efficient Angiosperms. This indicates that abiotic conditions (drought, freezing) can present a major selective force to trees. Both groups showed a second strategy gradient related to chemical defence, indicating that defence against biotic pests is universally important. Gymnosperms showed strong convergence in their trait strategies because of their uniform tracheids, explaining why they may have a more similar ecological behaviour compared to Angiosperms.

Stem traits differed significantly among stem compartments; bark had higher concentrations of nutrients and phenolics whereas sapwood has stronger physical defence as indicated by high carbon to nitrogen ratio. Inner wood of specifically conifers and some ring-porous species can be additionally chemically strengthened by heartwood formation. This supports that stem compartments reflected different strategies; bark served as storage organ and a first physical and chemical defence layer, while wood was well physically defended by having stronger tissues. Specific wood-bark trade-offs in terms of physical defence traits were found, implying reinforced bark can compensate the weak physical strength of wood. Yet, most traits were uncoupled between wood and bark partly indicating different ways how species optimize growth and defence strategies. Our generalizations are based on many (20) stem traits of important temperate tree species, but relatively few species per major taxa (eight Gymnosperms and six Angiosperms), hence, it would be good to test our findings for a wider range of tree species.

ACKNOWLEDGEMENTS

The authors thank many participants of the LOGLIFE Team in various years for help with setting up, maintenance and harvesting. The Schovenhorst estate and Staatsbosbeheer Flevoland are very hospitable and helpful with establishing and in hosting LOGLIFE throughout the experimental period. Financial support was provided by the Nora Croin Michielsen Fonds, and the Chinese Scholarship Council (no. 201706910085).

SUPPLEMENTARY MATERIALS

Methods

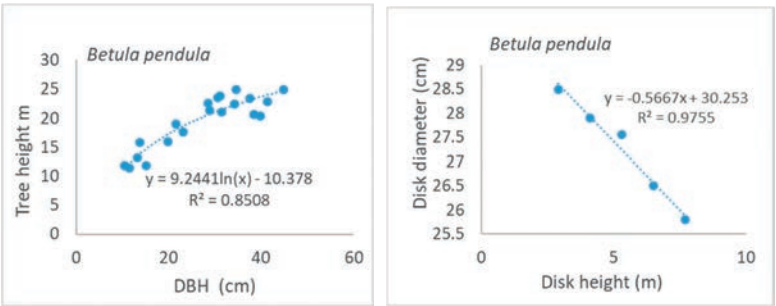
Testing for possible confounding site effects

Since only *Quercus robur* and *Picea abies* occurred in both sites (Flevoland and Schovenhorst), site effects could only be tested directly for these 2 species. A two-way ANOVA was conducted to show how species and site contribute to stem trait variation; among the 18 stem traits, only two (conduit fraction and carbon concentration) were significantly, but only marginally, affected by site (Table S2.1).

Furthermore, for each stem trait, group differences were calculated using the formula (angiosperm-gymnosperm)/(angiosperm+gymnosperm)*100% and site differences using the formula (clay-sand)/(clay+sand)*100%, where the terms angiosperm, gymnosperm, clay and sand refer to averaged traits values for species group or site. For all stem traits, the difference between major taxa outweighed site-specific differences, thus confirming that sites hardly affected stem trait variation. This analysis thus indirectly support our claims on the effect of major taxa effects on traits variation, irrespective of the confounding (but inferior) site effect.

Estimating xylem conductive length

The xylem conductive length between the sampled stem disk and the top of the tree may potentially influence the variation in xylem anatomical traits, therefore we incorporate stem length (i.e., tree height above the disk sampling position) as co-variate in our analysis. Given that individual tree height was not measured at the beginning of the experiment, we estimated the tree length for all trees using a step wise approach. We illustrate this approach with results for *Betula pendula* as example:



Step 1: tree height-DBH curve

We measured the height and diameter at 1.3 m (DBH) of 10-33 individuals per tree species in their site and fit the tree height and DBH relationship using the equation:
$y=9.2441 \ln(x)-10.378$,
where y is tree height and x is the DBH.

Step 2: DBH of our sampling individuals

The diameter of disks used in our study is known but we sampled these disks at different height or vertical distance from the soil, ranging from 0 to 10.43 m, in order to get disks of similar diameter. To test for possible stem tapering over this distance, we took advantage of LOGLIFE project in which five logs (A-E) each of 1 m length were sawn from the main trunk. Adjacent to each log, a 2-cm thick disk was sawn out for other analyses in LOGLIFE project (Cornelissen et al., 2012). The diameter and height of the disks were known and in total five disks were used for the diameter tapering rate calculation,
using equation:
$y' = -0.5667x' + 30.253$,
where y' is the disk height and x is the disk diameter. This allows us the estimate the stem diameter at 1.3 m (DBH), which is 29.5 cm in the shown example.

Step 3: tree height of our sampling individuals

Given the DBH obtained from step 2, the height of each individual tree was calculated based on formula 1. As the example shown above: with DBH =29.5 cm, the tree height of that *Betula pendula* tree is estimated 20.9 m.

Step 4: stem length (xylem conductive length)

The height above the base disk (used in our study) is considered a more important predictor for stem trait variation than tree height, since it indicates the conductive length between sample disk and tree top. We therefore used this length as the co-variate in our analysis, calculated from the difference between total tree height minus the vertical distance from soil surface to the sampled disk height.

Table S2.1 Trait variation between major taxa (Angiosperms vs Gymnosperms) and two tree growing sites. Group differences are shown as percentage (%). Two-way ANOVA was applied to test if species, growing sites conditions and interaction between species and sites can significantly contribute to trait variation. Asterisks indicate the significance level of each factor. ***: P<0.001, **: P<0.001, *: P<0.05, ns: not significant.

Ecological function	Stem traits	Angiosperms	Gymnosperms	Group difference %	Significance test (two-way ANOVA)			Site difference %
					species	sites	Species*sites	
Hydraulic conductivity	Conduit fraction	18.42	39.46	36.35	***	*	0.64	7.31
	Conduit diameter	79.53	19.70	60.29	***	0.40	0.21	6.66
	theoretical hydraulic Conductivity (K_p)	73.21	5.39	86.28	***	0.64	0.26	14.65
Hydraulic safety	Conduit density	64.17	1402.28	91.25	***	0.27	*	12.25
	Conduit wall thickness	2.54	2.78	4.44	0.41	0.44	0.74	1.38
	Conduit wall thick/radius	0.07	0.30	60.33	***	0.69	0.08	3.70
	Fiber wall thickness	2.74	NA	NA	NA	0.18	NA	NA
Storage	Ray fraction	8.64	4.69	29.61	***	0.54	0.67	4.35
Metabolism	Nitrogen	0.37	0.33	6.84	*	0.77	0.79	5.46
	Phosphorus	0.03	0.02	13.19	0.34	0.94	0.16	0.90
	pH	5.10	4.75	3.54	***	0.08	0.93	2.15
Chemical defence	Phenols	2.79	3.28	8.06	*	0.96	0.50	0.46
	Tannins	1.98	2.34	8.35	*	0.90	0.52	4.39
Physical strength	Wood density	0.55	0.44	11.84	***	0.80	0.72	0.61
	Carbon	47.04	49.07	2.11	**	**	**	1.15
	Carbon/nitrogen	286.31	417.99	18.70	**	0.45	0.45	2.20
	Lignin	20.57	30.71	19.78	***	0.39	0.51	2.82
	Lignin/cellulose	0.87	1.20	15.70	*	0.68	0.52	3.00

Table S2.2 Basic information of measured stem traits. Name of traits, related ecological functions, measured tissues and unit are shown.

Ecological functions	Functional traits	Abbreviation	Measured tissues	Unit
Hydraulic efficiency	Conduit fraction	Con_f_i	Inner wood	%
		Con_f_o	Outer wood	%
	Conduit diameter	Con_d_i	Inner wood	μm^2
		Con_d_o	Outer wood	μm^3
	Theoretical hydraulic conductivity	K*p*_i	Inner wood	kg m $Mpa^{-1}s^{-1}$
		K*p*_o	Outer wood	kg m $Mpa^{-1}s^{-2}$
Hydraulic safety	Conduit density	Con_D_i	Inner wood	cm^{-2}
		Con_D_o	Outer wood	cm^{-2}
	Conduit wall thick	Con_t_i	Inner wood	µm
		Con_t_o	Outer wood	µm
	Conduit wall thick/radius	Con_t/r_i	Inner wood	µm/µm
		Con_t/r_o	Outer wood	µm/µm
	Fibre wall thick	Fibre_t_i	Inner wood	µm
		Fibre_t_o	Outer wood	µm
Storage	Ray fraction	Ray_f_i	Inner wood	%
		Ray_f_o	Outer wood	%
Metabolism	Nitrogen fraction	N_i	Inner wood	%
		N_o	Outer wood	%
		N_B	Bark	%
	Phosphorus fraction	P_i	Inner wood	%
		P_o	Outer wood	%
		P_b	Bark	%
	pH	pH_i	Inner wood	NA
		pH_o	Outer wood	NA
		pH_b	Bark	NA
chemical defence	Phenols	Phenol_i	Inner wood	%
		Phenol_o	Outer wood	%
		Phenol_b	Bark	%
	Tannins fraction	Tannin_i	Inner wood	%
		Tannin_o	Outer wood	%
		Tannin_b	Bark	%
	Heartwood proportion	Heartwood	Disk	%
physical strength	Wood density	Wd_i	Inner wood	g/cm^3
		Wd_o	Outer wood	g/cm^3
	Carbon concentration	C_i	Inner wood	%
		C_o	Outer wood	%
		C_b	Bark	%
	Carbon/nitrogen	C/N_i	Inner wood	NA
		C/N_o	Outer wood	NA
		C/N_b	Bark	NA
	Lignin fraction	Lignin_i	Inner wood	%
		Lignin_o	Outer wood	%
		Lignin_b	Bark	%
	Lignin/cellulose	Lign/cellu_i	Inner wood	NA
		Lign/cellu_o	Outer wood	NA
		Lign/cellu_b	Bark	NA
	Bark punch resistance	B_resistance	Bark	N

Table S2.3 Stem traits with the information of definition, related stem functions, and supporting literature..

Stem traits	Definition	Ecological function	Supporting literature
Conduit fraction	Cross-sectional area of all conduit lumen divided by the total cross-sectional area	Hydraulic conductivity	Dimond, 1966; Chave *et al*., 2009; Martínez-Cabrera, and Estrada-Ruiz, 2014
Conduit diameter	Conduits were assumed to be round and the diameter was calculated based on conduit area measured in ImageJ.		
theoretical hydraulic Conductivity (K_p)	K*p* was calculated according to the Hagen-Poiseuille law (Sterck *et al*. 2008), see "Materials and Methods"		
Conduit density	The number of conduits per cross-sectional area	Hydraulic safety	Jacobsen, 2005; Chave *et al*, 2009; Janssen *et al*., 2020
Conduit wall thickness	Average wall thickness was measured based on all vessels and multiple tracheids per individual species		
Conduit wall thick/radius	Conduit wall thickness divided by conduit radius		
Fiber wall thickness	Fibre wall thickness was measured only in angiosperm species		
Ray fraction	Cross-sectional area of all ray parenchyma divided by the total cross-sectional area	Storage	Morris, 2016
Nitrogen	Mass-based nitrogen (N) concentration	Metabolism	Rektorschek *et al.*, 1998; Zhong *et al*., 2017
Phosphorus	Mass-based Phosphorous (P) concentration		
pH	pH value in distilled water, pH_{H2O}		
Phenols	Mass-based phenolics concentration	Chemical defence	Mounguengui, 2016; Valette, 2017
Tannins	Mass-based tannins concentration		
Wood density	Oven dry mass divided by fresh volume	Physical strength	Zanne *et al*., 2010; Ishida *et al.*, 2008; Sattler and Funnell-Harris, 2013; Wainhouse *et al*., 1990; Rasmann *et al.*, 2011
Carbon	Mass-based carbon (C) concentration		
Carbon/nitrogen	Carbon concentration divided by nitrogen concentration		
Lignin	Mass-based lignin concentration		
Lignin/cellulose	Lignin concentration divided by cellulose concentration		

Note: one trait may link to several functions, but we only group it to specific function, which we think is most related and important in this study.

Table S2.4 Percentage trait variation explained by major taxa (Angiosperms vs Gymnosperms), species within major taxa compartments (innerwood, outerwood, bark) and stem length (between sampled disk and tree top). Asterisks indicate significance levels. †: traits were log-transformed. ***: P<0.001, **: P<0.001, *: P<0.05, ns: not significant.

Ecological function	Stem traits	Unit	Major taxa	Species	Compartments	Length
Hydraulic conductivity	Conduit fraction	%	83.7***	7.32***	0.00^{ns}	ns
	Conduit diameter†	μm^2	88.5***	7.99***	0.04^{ns}	0.25(+)***
	theoretical hydraulic Conductivity (K_p)†	kg m $MPa^{-1}s^{-1}$	69.2***	19.3***	0.09^{ns}	ns
Hydraulic safety	Conduit density†	cm^{-2}	93.1***	5.09***	0.00^{ns}	0.20(-)***
	Conduit wall thickness	µm	10.4**	17.5**	4.98**	ns
	Conduit wall thick/radius†	µm/µm	90.9***	4.84***	0.00^{ns}	0.44(-)***
	Fiber wall thickness	µm	NA	64.3***	5.11*	ns
Storage	Ray fraction	%	48.2***	26.1***	2.71*	ns
Metabolism	Nitrogen†	%	0.93***	3.58***	91.4***	0.44(-)***
	Phosphorus	%	1.61***	11.1***	65.1***	2.58(-)***
	pH†	NA	6.25***	47.9***	1.30*	ns
Chemical defence	Phenols †	%	0.38**	15.8***	61.6***	0.33(-)**
	Tannins†	%	1.22***	22.2***	51.0***	0.04(+)**
Physical strength	Wood density	%	34.3***	48.8***	2.16**	ns
	Carbon	%	30.1***	10.2***	11.5***	ns
	Carbon/nitrogen	NA	3.90***	7.00***	71.9***	0.52(+)***
	Lignin	%	55.9***	7.94***	9.59***	ns
	Lignin/cellulose†	NA	29.4***	4.55***	42.1***	0.22(+)**
% significance			***100***	***100***	***72.2***	***50.0***

Fig. S2.1 The percentage of trait variation explained by major taxa (Angiosperms vs. Gymnosperms), species within major taxa, compartments (innerwood, outerwood and bark) and stem length between sampled disk and tree top (yellow).

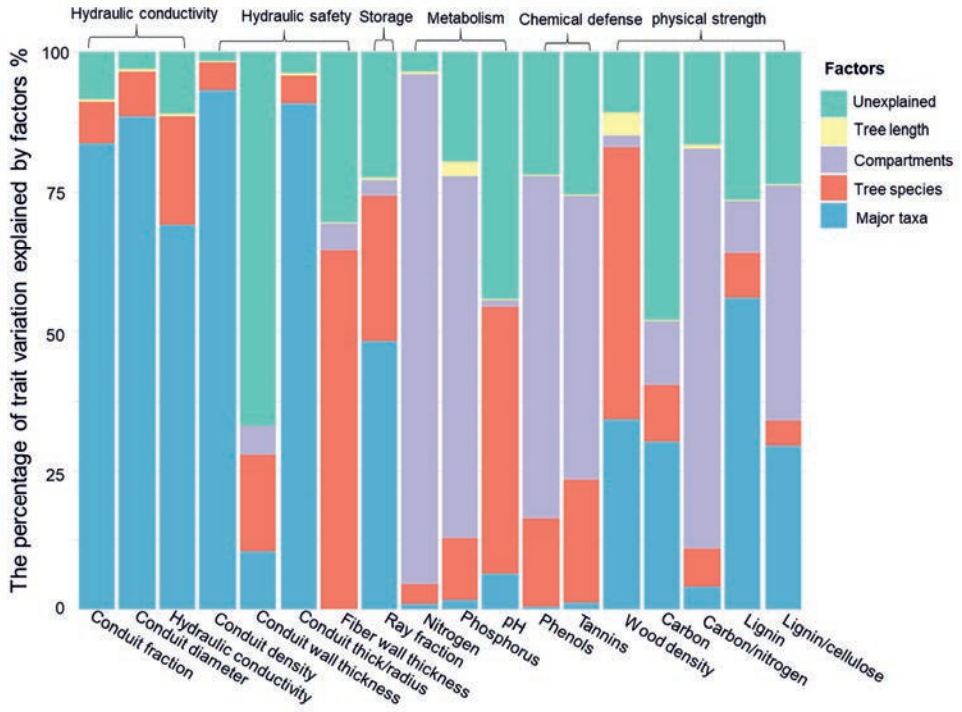


Fig. S2.2 Covariance matrix showing the associations of all stem traits in different tissues of **all species**. Only significant correlations ($p<0.05$) were shown here. Red dots indicate negative correlations, blue dots refer to positive correlations, and the darker of the colour the stronger the correlation. See Table S2.2 for trait abbreviations.

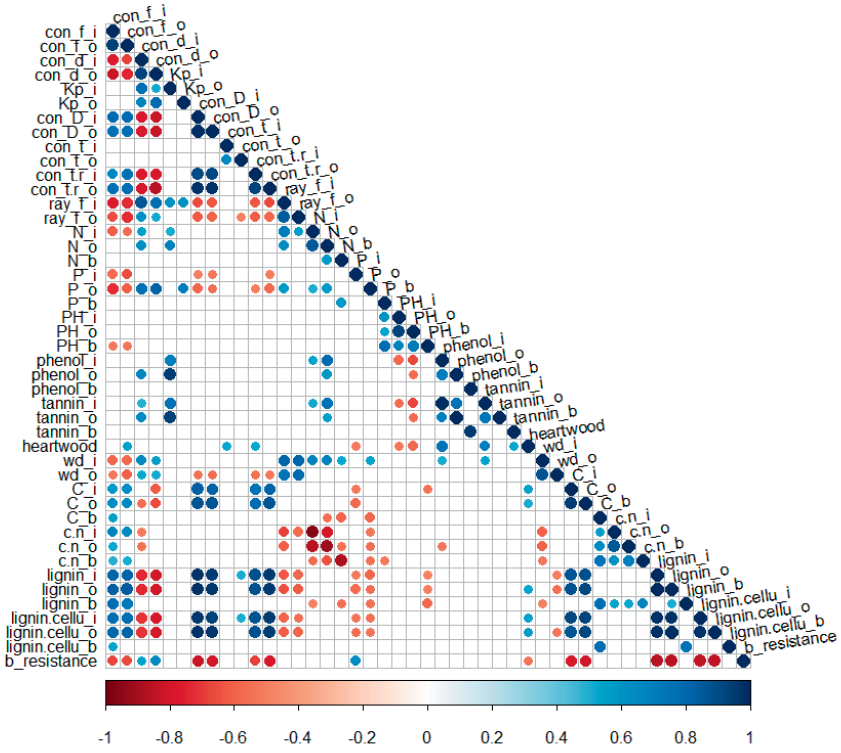


Fig. S2.3 Covariance matrix showing the associations of all stem traits in different tissues of **Angiosperms**. Only significant correlations ($p<0.05$) were shown here. Red dots indicate negative correlations and blue dots refer to positive correlations. The darker of the colour, the stronger the correlation. See Table S2.2 for trait abbreviations.

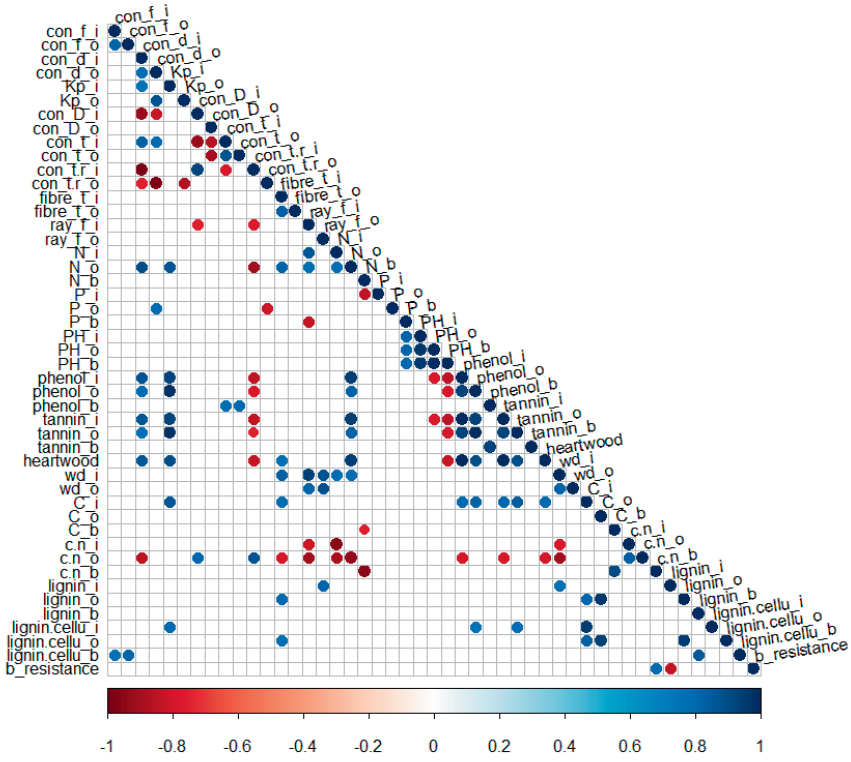
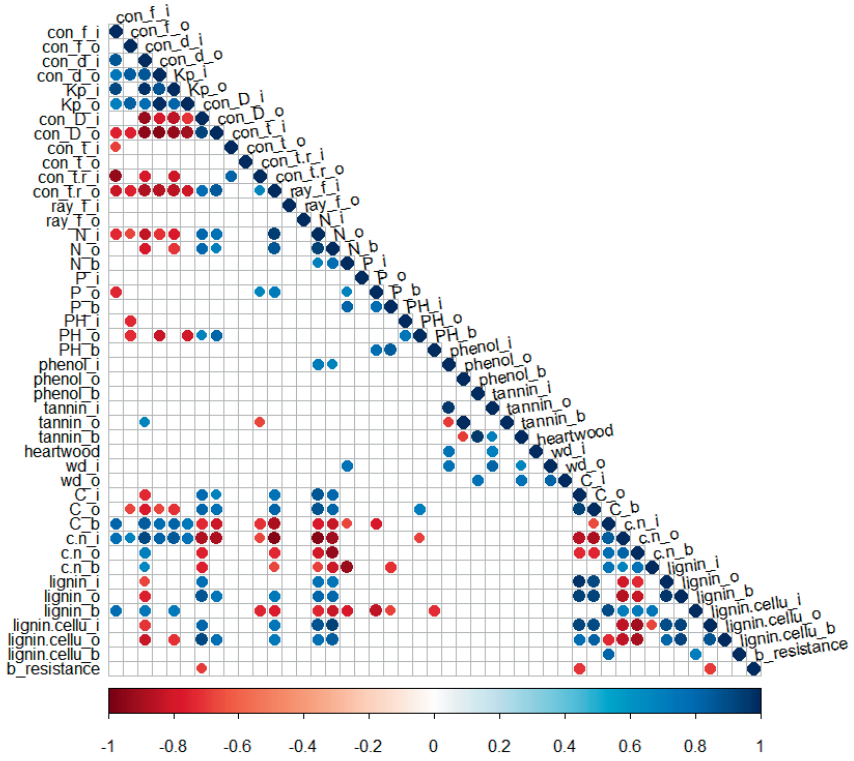
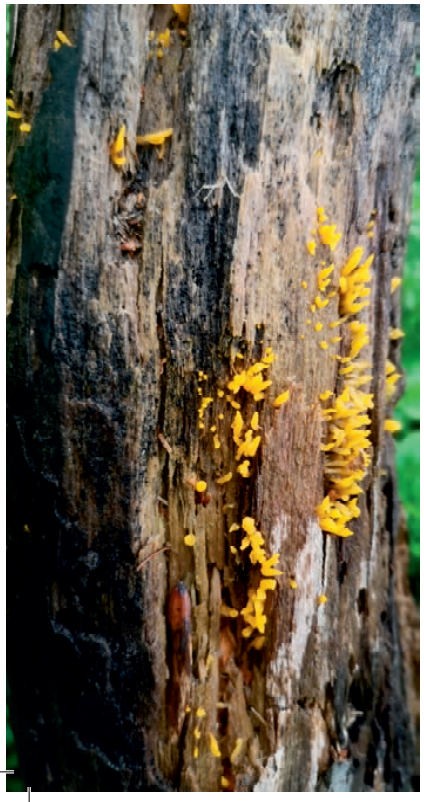


Fig. S2.4 Covariance matrix showing the associations of all stem traits in different tissues of **Gymnosperms**. Only significant correlations ($p<0.05$) were shown here. Red dots indicate negative correlations and blue dots refer to positive correlations. The darker of the colour, the stronger the correlation. See Table S2.2 for trait abbreviations.





CHAPTER 3

Dead wood diversity promotes fungal diversity

Shanshan Yang, Juul Limpens, Frank J. Sterck, Ute Sass-Klaassen, J. Hans C. Cornelissen, Mariet Hefting, Richard S.P. van Logtestijn, Leo Goudzwaard, Nico Dam, Marjo Dam, Mirjam T. Veerkamp, Bart van den Berg, Emiel Brouwer, Chenghui Chang, Lourens Poorter

Oikos (2021), doi: 10.1111/oik.08388

ABSTRACT

Dead wood is a source of life as it provides habitat and substrate for a wide range of fungal species. Biodiversity policies aim to increase the quantity of dead wood in forests, but few studies have assessed the effect of the quality of dead wood on fungal diversity. This study evaluates how abiotic and biotic factors affect the abundance, richness and composition of fungi during the process of dead wood decomposition. For 10 common European tree species, fresh similar-sized logs were incubated simultaneously in two Dutch forests. Annual surveys of fungal fruiting bodies were made for an 8-year period. For each tree species, 20 fresh stem traits were measured that are important for chemical and physical defence and for nutritional quality. Throughout 8 years, 4,644 fruiting bodies belonging to 255 species and 90 genera were recorded on the logs of 10 tree species. Fungal abundance and richness were higher for Angiosperms than for Gymnosperms, higher for tree species with more acquisitive stem trait strategies (i.e., high nutritional value and low physical defence), and peaked after four years of decay. Differences in fungal communities were strongly driven by phylogenetic group (Gymnosperms vs. Angiosperms), stem traits, decay time and forest sites, together explaining 23% of the variation. Fungal communities diverged early in the decay process but converged later because of substrate homogenization. Of the 128 fungal species included in analyses, 41% showed a preference for specific tree species, and 34% for a specific successional decay year. In conclusion, dead wood quality, determined by tree species and decay stage, is an important driver of fungal diversity. For forest management, promoting a wide array of dead wood tree species (especially angiosperm species), a range of tree economic strategies (from conservative to acquisitive) and decay stages will increase fungal and, thereby, forest biodiversity.

KEYWORDS

Fungal richness, fungal abundance, fungal composition, dead wood, decay time, stem traits

3.1 | INTRODUCTION

3.1.1 | Dead wood promotes forest biodiversity

Dead wood is a source of life as it provides habitat and substrate for a wide range of organisms (Stokland et al. 2012), ranging from bacteria, fungi, plants and insects to mammals. Saproxylic fungi, which are entirely confined to dead wood, form a very conspicuous and diverse group. For example, Dutch forests contain only around 40 shrub and tree species, but as many as 1250 wood inhabiting fungal species (Wijdeven et al. 2010), and in Denmark, as many as 50 fruiting fungal species have been identified on a single beech tree (Heilmann-Clausen and Christensen 2004). As much as one third of all European forest-dwelling species (i.e., 6000 species) is associated with dead wood (Vogel et al. 2020), which emphasizes the importance of dead wood for the diversity of entire forests.

The importance of dead wood for biodiversity and ecosystem functioning (i.e., long-term carbon storage and nutrient cycling) has recently been recognized by forest managers. Where dead wood was often removed from forests to reduce insect outbreaks and pests before the 1980s (Wijdeven et al. 2010), it has more often been left in the forest since the 2000s, which in European forests has led to an increase in the average amount of dead wood from ~ 8.0 (1990) to ~ 10.0 m^3 (2015) per hectare (Europe 2020). However, up to now, most emphasis has been given to the quantity rather than the quality of dead wood (Bauhus et al. 2009, Lindenmayer and Laurance 2017, Vítková et al. 2018).

3.1.2 | Drivers of fungal biodiversity

Various abiotic and biotic factors influence the abundance (number of fruiting bodies) and richness (number of species) of wood-inhabiting fungi, shaping fungal community structure (Kahl et al. 2017, Lee et al. 2020). A larger contact area between wood and soil may increase fungal access to the log (Rajala et al. 2012), while climate factors like relatively high temperature and humidity may facilitate fungal growth (Purahong et al. 2017). Community assembly of saproxylic fungi is affected by site location and history (Kubartová et al. 2012), soil fertility (Rühling and Tyler 1991, Wallander 1995), microclimates (Seibold et al. 2016, Müller et al. 2020) and the surroundings of dead wood (e.g., vegetation cover and composition) (Kubartová et al. 2012, Juutilainen et al. 2017). The stem traits of the tree species are also important, as they leave their legacy in the properties of dead wood; physical defence traits

such as a thick bark reduce the accessibility of the log to fungi (Lee et al. 2019), chemical defence traits such as phenols inhibit fungal growth (Kahl et al. 2017); but on the other hand, high nutritional stem quality may stimulate fungal growth (Sinsabaugh et al. 1993). Stems with larger diameter are particularly important for fungal biodiversity because they can provide more resources and serve as long-term substrate for saproxylic fungi (Heilmann-Clausen and Christensen 2004). In general, species with a relatively strong investment of assimilated carbon into physical and/or chemical defences and associated slow growth tend to be referred to as resource conservative, while less defended species investing carbon and nutrients into tissues promoting fast photo-assimilation, transport and growth are considered as resource acquisitive (Díaz et al. 2016, Poorter et al. 2018).

Gymnosperm species are relatively homogeneous in structure and their conductive and physical support tissues mainly consist of tracheids, whereas angiosperm species are more heterogeneous and have wider xylem conduits (i.e., vessels), and often wider rays (Pittermann et al. 2006a). The large wood trait variation found between Angiosperms and Gymnosperms, but also amongst different tree species within these clades, results in different substrate conditions and therefore different fungal richness and composition (Hoppe et al. 2016). Angiosperm species may be more attractive to saproxylic fungi than gymnosperm species, because they produce less resistant lignin, have lower lignin concentrations and higher nutrient concentrations (Weedon et al. 2009). This may facilitate fungal growth by increasing fungal access to the wood and providing more resources.

3.1.3 | Colonization and successional patterns

Fungal community assembly on dead wood is not only determined by initial stem traits, but also by decay stages (van der Wal et al. 2016, Ruokolainen et al. 2018), dispersal limitation (i.e., whether a species has been able to arrive at the site) (Peay et al. 2010), and the ”priority effect” (Fukami et al. 2010, Ottosson et al. 2015); the identity of the first colonizing fungi may determine whether fungi compete with and inhibit other colonizing fungal species, or whether they facilitate succession through substrate modification (i.e., degradative succession) (Hiscox et al. 2015). As dead wood decays, substrate conditions change over time (Rajala et al. 2015, Oberle et al. 2019). When wood decay proceeds, a clear shift can be found with (semi)parasitic species (endophytic fungi already present in the living trees) being the first colonizers, followed by polypores (common mushrooms with pores or tubes on the lower side of the cap) and agarics (common mushrooms with gills on the underside of the cap) (Stokland et al. 2012). Pioneer

colonizers may disappear in later decay stages, whereas other species (e.g., *Armillaria cepistipes, Fomitopsis pinicola* and *Ganoderma lipsiense*) can persist during different decay stages because of their diverse enzyme complexes (Rayner and Boddy 1988, Ruokolainen et al. 2018).

Many studies find that fungal species richness peaks during succession at intermediate decay stages (Renvall and Niemelä 1993, Rosenzweig and Abramsky 1993), probably because at the start of decay, different substrates are available to all kind of pioneers, whereas later on, the substrate becomes more homogenized, leading to intense interspecific competition (Huston and DeAngelis 1994) and species loss. Other studies, focusing on spruce and beech logs, found that fungal richness increases and peak in the most decayed logs (Fukasawa et al. 2009, Rajala et al. 2012), probably because soil fungi start to colonize the heavily degraded logs (Buee et al. 2009). How fungal richness varies during the wood decay process is still under debate, and more tree species should be studied to assess successional patterns in fungal communities.

Previous studies have advanced our knowledge by showing that fungal diversity is affected by deadwood characteristics, but virtually all of them included only between one to three tree species (Baldrian et al. 2016, Hoppe et al. 2016, Purahong et al. 2016, van der Wal et al. 2016, Purahong et al. 2017). The only study known to us with more (i.e., 11) tree species, only studied fungal diversity during initial decomposition (Purahong et al. 2018). Moreover, previous studies have only measured a limited number of three to five wood traits (e.g., wood density, lignin, C/N ratio) (Purahong et al. 2018, Leonhardt et al. 2019, Dossa et al. 2020). This makes it difficult to draw generalizations. Even more importantly, the traits of bark, with its structural and chemical defences still shielding logs from fungal attack (see above), thereby affecting not only fungal composition in the bark itself but also the colonization of the wood below it (Dossa et al. 2018), have been all but ignored in previous studies. In addition, many studies have compared fungal communities across tree species using uncontrolled field studies (Kwaśna et al. 2017, Ruokolainen et al. 2018), which makes it difficult to tease apart whether tree species differ in their fungal communities because of inherent tree species differences, or simply because they differ in log size and accessibility, the metacommunity of fungal spores around the logs, or the environmental conditions where the logs decompose. Finally, most studies concern only the first 3-5 years of decomposition (Purahong et al. 2018, Lee et al. 2020), which makes it difficult to infer longer-term patterns in community assembly. We therefore address these knowledge gaps and advance on previous studies by using a common garden experiment, in which we simultaneously incubate and monitor big (25 cm diameter) logs of a comprehensive set of 10 tree species in each of two contrasting forest types, provide a long-

term perspective on fungal succession and diversity over an 8-year period. Another unique feature of our study is that we relate fungal composition and diversity to 20 wide-ranging structural and chemical traits, with a relatively balanced representation of eleven wood and nine bark traits.

To address our questions, similar-sized (1 m length, 25 ± 3 cm diameter) fresh logs of 10 common European tree species (two of which were extracted from two forest sites) were incubated in each of two Dutch temperate forests contrasting greatly in soil properties and microclimate (Cornelissen et al. 2012). At each site, all logs experienced similar site conditions and fungal species pool, which reduced the confounding effect of dispersal limitation and abiotic conditions, allowed for a better assessment how tree species affect fungal community assembly. The initial stem traits including both wood and bark properties were measured, and the abundance, richness and composition of wood inhabiting fungi were monitored based on fruiting bodies forming during eight years of decay.

3.1.4 | Questions and hypotheses

In this study, we address two main research questions:

1) How do fungal abundance and richness depend on tree species and decay time? We expect that i) angiosperm species have a higher fungal abundance and richness than gymnosperm species, ii) fungal abundance and richness will be higher for tree species with high nutritional quality (i.e., high N and P concentrations) and low chemical defences (i.e., phenols, acidity) and physical defences (i.e., cell wall thickness, lignin concentration) both in wood and in bark, iii) fungal abundance and richness may increase initially because more resources become available during decay, but will decrease in more advanced decay stage because of substrate mass loss leading to resource limitation.

2) How does fungal community composition and diversity depend on tree species diversity and decay time? We expect fungal species will show preference for different substrates as determined by tree species and decay time. We expect fungal community composition will initially be very diverse because resident endophytic fungi may become saproxylic, and locally common generalists will arrive. Fungal community

composition will converge in a later stage, because wood decay will lead to a more homogenised substrate with less influence of bark traits.

3.2 | MATERIALS AND METHODS

3.2.1 | Study site

A unique long-term "common garden" experiment on dead tree decomposition (LOGLIFE) was carried out in two forests in the Netherlands, located in the same temperate climate zone, with an annual mean rainfall of approximately 700 mm and an annual mean temperature of approximately 10 °C. (1) The Hollandse Hout site (52.46N, 5.42E) has young marine clay soils reclaimed from the former Zuiderzee in the 1960s. The site is henceforth referred to as "clay site". The log incubation plots in this site are within a relatively light-open *Populus x canadensis* plantation with a tall herb layer of *Urtica dioica*. (2) The other site is the Schovenhorst forest estate (52.25N, 5.63E) on Pleistocene sandy soil. The site is henceforth referred to as "sandy site". The log incubation plots are within a light-open *Larix kaempferi* plantation with a ground layer of *Vaccinium myrtillus and Deschampsia flexuosa*. The soil type ("clay" and "sandy") represents a strong contrast between the two studied sites, which leads to concomitant changes in vegetation cover, vegetation composition, canopy openness, and microclimate that can all have a potential impact on fungal community assembly (Wallander 1995, Kubartová et al. 2012, Müller et al. 2020). Because we did not replicate our study to several stands on the same soil type, we can not prove that soil type and associated (a)biotic conditions affect fungal assembly. Yet, given the striking soil contrast, it at least allows to test for a possible impact of contrasting site conditions. More details about these two study sites are given in Cornelissen et al. (2012).

3.2.2 | Experimental design

Logs of 10 tree species (see tree species list in Supplementary material, Table S3.1) were placed in two contrasting forest sites. The experiment started in February 2012 when 10 common and representative temperate tree species were harvested, comprising four gymnosperm species and six angiosperm species. Most species were extracted either from the clay forest or sandy forest site, but the angiosperm *Quercus robur* and gymnosperm *Picea abies* were extracted from both sites to compare how growing conditions affect tree properties and fungal communities. Stems

of equal diameter (25 ± 3 cm) were selected, to reduce the possible confounding effects of stem size on decay and fungal colonization. Trees harvested from the sandy site were however growing slower and were thus older than trees harvested from the clay site, presumably because of the poorer growth conditions in the former. As different site conditions and tree ages may lead to differences in trait values and fungal communities, oak and spruce growing at different sites were treated as different tree "species" in this study. For each tree species, 10 trees were harvested. Each individual tree was cut into five sections (each with 1 m length) and without major side branches from the main trunk. Logs sawn from five individual trees were placed in five plots in the clay site and logs sawn from the other five individuals were placed in five plots in the sandy site. Each plot measured 12 m by 12 m, with a minimum distance of 20 m between plots. In total, 600 logs (12 tree "species"×two sites×5 individuals×5 logs) were incubated.

Within each plot, the logs were positioned 30 cm apart on the soil surface, making good contact with the soil to harmonize micro-site conditions for all logs, while mimicking natural conditions and allowing fungal access. The five logs from each individual tree were placed together with the same compass orientation, but the location and orientation of each tree species within each plot were random; more information is shown in Cornelissen et al. (2012).

To compare temporal changes in fungal community abundance and composition, mycological surveys were conducted on two of the logs per individual tree that were consistently assessed throughout the whole monitoring period from 2012 to 2019.

3.2.3 | Mycological surveys

We surveyed visible sporocarps as they are easy to detect and monitor, and are a conspicuous component of the fungal community and appreciated by naturalists and conservationist. Ecologically, sporocarps represent spore production and dispersal, which is a key event in the life cycle of fungi, critical to the distribution and long-term success of the species. We acknowledge that sporocarp production may vary over the year, and do not reflect the whole fungal community, as many fungi do not make sporocarps. Mycological surveys were conducted annually in the peak fructification time (October/November) from 2012 to 2019. No survey was made in 2018 due to the extremely dry conditions and lack of fungal fruiting bodies. In total, fungal fruiting body data were collected 1680 times (12 tree "species"×2 sites×2 individuals×2 logs×7 surveys). Fungal species diversity is likely to have been underestimated as some fungi were not visible at the time of inventory (Abrego et al. 2016).

During each visit, all fruiting bodies that were visible by eye were identified to species, but in a few cases only to genus, following the nomenclature from Arnolds and van den Berg (2013). When macroscopic identification was uncertain, species were microscopically identified. The logs were left undisturbed.

Mean fungal abundance and richness were calculated as the number of fungal species and the fungal fruiting body number respectively, found on each individual tree in the respective survey year. Total fungal abundance and richness were defined as the total number of fungal species and individual fruiting bodies found on all dead wood objects at each survey year.

We quantified the fungal community composition using abundance data. Before analysis, rare species with only one or two records in all survey years were omitted (only for analysis of fungal composition, for fungal abundance and richness, all observed fungi were considered) (Buée et al. 2011), because they might introduce high levels of noise and have a strong effect on the results. If a taxon could be identified to genus level only, then the taxon was either omitted when other taxa of this genus could be identified to species level (to avoid inflating the species number per genus), or treated as a species (if no other members of this genus were found). As a result, 14 non-unique genus-level data identifications (i.e., *Armillaria sp.*, *Ganoderma sp., Hypoxylon sp., Lasiosphaeria sp., Mollisia sp., Mycena sp., Mycoacia sp., Nectria sp., Pluteus sp., Postia sp., Schizopora sp., Stereum sp., Schizopora sp.*, *Trametes sp.*) were omitted. After this, a total of 128 fungal species were included in the analysis with 13 unique genus-level identifications treated as species.

To better interpret how fungal communities vary with different decay stages, we define year 2012 to 2013 as early decay stage (after 1-2 years' decay) for incubated logs, 2014-2016 (after 3-5 years' decay) as middle decay stage and 2017-2019 (after 6-8 years' decay) as late decay stage based on our current study, though dead wood of some tree species may take decades to reach their late decay stage.

The decay stage can potentially be confounded with climate conditions of that specific year. To address this issue, regression analysis and a constrained unimodal ordination analysis were conducted to test whether climate variables significantly explain the differences in fungal abundance and composition respectively. Three important climate variables were chosen that are thought to affect fungal fruiting bodies; mean temperature (°C), amount of precipitation (mm) and frost days (d) (Barge et al. 2019). Climatic data were collected from August to November of each survey year. We found no significant relationship between climatic variation and fungal abundance (Supplementary material, Fig. S3.5) and only mean temperature had a

small effect (accounting for 2.7% of total variation) on fungal community composition (Supplementary material, Fig. S3.6). Therefore, we conclude that the dead wood decay stage is not confounded by the climatic conditions in that specific survey year, and we did not include climatic conditions in the further analyses.

3.2.4 | Sample preparation and measurements

Stem traits of different tree species and compartments (wood and bark) result in different substrates for microbial decomposers. To test how stem traits affect fungal communities on dead wood, two adjacent 2-cm base disks of each individual tree were sawn out for initial trait analyses before log incubation, then dried and stored for further analyses. Subsamples (two 1.5 cm^3 blocks) were extracted from the sapwood of one disk and used for physical trait measurements (i.e., wood density, conduit cell wall thickness, conduit diameter and ray fraction). In addition, total, inner and outer bark thickness (in mm) were measured separately. Chemical traits were measured using sawdust samples taken from the second disk with an electric drill (bit diameter 8 mm). Between samples, the plate and drill were cleaned with 70% ethanol. Sawdust from wood and bark was collected separately, ground in a MM400 ball mill (Retsch, Haan, Germany) and used for measurements of pH value, C/N ratio, lignin concentration, nitrogen (N) and phosphorus (P) concentrations. Since different numbers of replicates were used for different variables, we used the mean values per tree species in the analyses. More information about stem trait measurements is given in Supplementary material, Table S3.2.

Physical traits. Wood density and heartwood proportion in the stem cross-section area were measured on five individual trees for each tree species. Wood density was calculated as dry mass of oven-dried wood samples per fresh volume (g cm^{-3}), which was measured by the water displacement method. The heartwood proportion was calculated using heartwood area divided by total disk area (excluding bark); for non-heartwood forming species, the heartwood proportion was set to zero.

Wood anatomical traits (ray fraction, conduit density and conduit diameter) were measured for three individual trees per tree species. Micro-thin sections were cut from the cross-section using a sledge microtome and stained with a mixture of Astrablue and Safranin for at least 5 minutes; then samples were washed with demi-water and dehydrated in ethanol series (50%, 96% and 100%); finally, thin sections were dewaxed using

Roticlear® (Carl Roth, Karlsruhe, Germany) and permanently embedded with Roti®-Mount (Carl Roth, Germany). High-resolution digital images of anatomical sections were made using a camera mounted on an optical microscope. Gymnosperm sections with narrow tracheids were measured using a 10× magnification, while angiosperm sections with wider vessels were measured using a 5× magnification. Digital images were calibrated with a slide-mounted micrometer and then analysed using Fiji/ImageJ (Schindelin et al. 2012); manual adjustments were added if necessary.

Chemical traits. Chemical traits of wood and bark were determined from stem disks of five individual trees per tree species (n =5). Carbon and nitrogen concentrations were determined by dry combustion using a Flash EA 1112 elemental analyser (Thermo Scientific, Rodana, Italy). Phosphorus was determined from sawdust samples, those samples were first digested with HNO3/HCl (1:4 mixture of 37% HCl and 65% HNO3), and then phosphorus concentration was determined with spectrophotometry using the ammonium molybdate method (at a wavelength of 880 nm) (Murphy and Riley 1962). Lignin concentration was determined following Poorter and Villar (1997). Samples were extracted with water, methanol and chloroform to remove the soluble sugars, soluble phenols and lipids. Then starch, fructan, pectin and a part of the hemi-cellulose were removed during acid hydrolysis. Finally, after correction for ash concentration (including silicates) and remaining proteins, the lignin concentrations were calculated based on C concentration. Phenol concentrations were measured using the Folin-Ciocalteu method. A 50% methanol solution was used to extract the phenolic hydroxyl groups. Then the phenols were coloured with Folin-Ciocalteu reagent and measured at 760 nm on a spectrophotometer. Finally, total phenol concentration was calculated according to a tannic acid-based calibration curve. Finally, the pHH2O was measured following Cornelissen et al. (2012). A 0.15 ml sawdust sample and 1.2 ml demi-water were added to an Eppendorf tube and shaken for 1 h at 250 rotations per minute. Then the tubes were centrifuged for 5 min at 13,000 rpm and the supernatant was used to measure pHH2O with a WTW SenTix Mic electrode.

3.2.5 | Statistical analyses

Statistical analyses were performed using R v. 3.6.1 (R Core Team, 2016) and CANOCO 5.0 (ter Braak and Smilauer 2012). All trait variables with the exception of heartwood proportion were $\log_{10}$ transformed to increase normality and homoscedasticity.

Fungal abundance and richness. To evaluate how fungal abundance and richness were affected by underlying drivers, we used linear mixed-effects models (package "lme4") (Douglas Bates et al. 2015) with mean fungal abundance or richness per individual tree (two logs; i.e., 2 m length) as dependent variables, plot as a random factor, and tree phylogenetic group (Angiosperms vs. Gymnosperms), tree species nested within group, decay year (2012-2019), incubation sites (clay vs. sandy) and interaction of time and incubation sites as fixed factors.

To analyze how fungal abundance and richness per individual tree vary amongst the 10 tree species in early and late decay years, one-way ANOVAs were carried out for the beginning (2012) and the end (2019) of the monitoring period.

Stems of different tree species may provide different substrate quality to different fungal communities. To analyze how the stem traits of tree species are associated, a principal component analysis (PCA) was performed in CANOCO 5.0 using mean species values of 20 traits as data points. To quantify how tree species differ in their multivariate stem traits, we used the species ordination scores of the first and second PCA axis. To evaluate how mean fungal abundance and richness depend on the multivariate tree species traits, a multiple regression was conducted with mean fungal richness and abundance per stem per survey year as dependent variables, the tree species ordination scores on the first two PCA axes and decay time as fixed factors.

Bivariate relationships between mean fungal abundance/richness and ordination scores on the first PCA axis were evaluated using Pearson correlation and ordinary least squares regression. Correlations were considered to be significant at a probability level of $P < 0.05$.

Fungal composition. To visualize how the total fungal community composition shifts over time for the different tree species, a detrended correspondence analysis (DCA) was performed, using CANOCO 5.0. We used all fungal species that occurred at a single census period on a single tree species as data points.

To test the effects of phylogenetic group, decay time, forest sites and compound stem traits (pc1 & pc2) on fungal community composition, a constrained unimodal ordination analysis (canonical correspondence analysis, CCA) was conducted in CANOCO 5.0 using a Monte Carlo permutation test (MCPT, number of permutations 4999). These explanatory effects were evaluated in MCPT with a step-wise procedure selecting variables with the best fit to species data.

To test if fungal species show preferences for specific host tree species, indicator species analysis (ISA) was applied using the "multipatt" function from the "indicspecies" package in R, and using the indicator statistic "IndVal.g" (Cáceres and Legendre 2009). This indicator statistic varies from 0 (no association) to 1 (complete association) and is determined by two probabilities, *P*(A) and *P*(B) (Dufrêne and Legendre 1997); "A" is sample estimate of the probability that the surveyed fungal species is only found in the target tree species (e.g., "A"=1 indicates that the observed fungus is completely restricted to the target tree species); "B" is the probability of finding the fungal species in a tree belonging to the target tree species (e.g., "B"=1 indicates that this fungus appears in all logs belonging to this tree species). Significance was tested, based on a permutation test with 9999 permutations. ISA was also applied to test if different decay years, phylogenetic group (Gymnosperms vs. Angiosperms) and incubation sites have their own specific fungal specialists.

3.3 | RESULTS

3.3.1 | Taxonomic fungal diversity

A total of 4644 fungal records representing 255 fungal species in 90 genera were scored for the 10 tree species over a period of 8 years from 2012 to 2019. Most fungi belonged to Basidiomycetes (76%) and 24% were Ascomycetes. The most species-rich genera were *Mycena* (with 21 species), *Pluteus* (9), *Galerina* (8), *Hypoxylon s.l.* (7), a, and *Hyphodontia* (7).

Across all fungal species, 43% (110 fungal species) were only present on the logs of angiosperm species, 19% (48) only on gymnosperm species and the remaining 38% (97) on the logs of both phylogenetic groups (Supplementary material, Fig. S3.7). The average number of fungal species observed per tree species was 73, with *Populus tremula* holding the highest number of fungal species (93) and *Abies grandis* holding the lowest number of species (55). Species richness increased over time and reached its maximum after 5 years' decay in 2017. The fungal abundance also increased but reached its peak after 3 years' decay in 2015 (777 records), and then declined (Supplementary material, Fig. S3.1). We found that fungal species confined to early (2012-2013), middle (2014-2016) and late decay stages (2017-2019) accounted for 12%, 22% and 32% of total fungal species respectively, while only 10% of the fungal species were ubiquitous and occurred in all studied decay stages. Fungal richness and abundance were similar in both forest sites; 181 fungal species and 2302 records were scored

for the clay forest site and 180 fungal species and 2342 records for sandy forest site. Yet, the two forest sites had only 42% of the fungal species in common.

3.3.2 | Factors influencing fungal abundance and richness

Fungal abundance and richness for both stem logs per sampled tree were significantly affected by tree phylogenetic group, tree species, decay time and interaction between time and incubation sites (Table 3.1). Angiosperm species had on average more fungal species (4.5 fungi per tree) than gymnosperm species (3.7 fungi per tree) (mixed linear model, $P<0.05$). Within angiosperm species, *Betula* had significantly higher, but *Fraxinus* had significantly lower fungal richness compared to *Quercus* collected from the forest with sandy soil (Table 3.1). *Quercus* that was initially growing in clay soil forest had a higher fungal abundance and richness than *Quercus* growing in the forest with sandy soil, indicating that trees originating from different forests and hence holding different stem traits can also differ in fungal richness. Within gymnosperm species, *Picea abies* growing in the forest with sandy soil showed the highest fungal abundance and richness, whereas *Pseudotsuga menziesii* held the least fungal species (Table 3.1). Fungal abundance and richness tended to increase and were significantly higher in 2014, 2015, 2016 and 2019 (richness only), than in 2012 (Table 3.1, Table S3.3). Overall, fungal abundance and richness did not significantly differ on the wood incubated in the two contrasting forest sites. Dead wood on the clay soil tended to have more fungi in early decay years, whereas fungal species number on wood in the forest with sandy soil increased in later decay years, and was eventually higher than that in the clay soil site in later decay years (Supplementary material, Fig. S3.2). After one year of decay in 2012, fungal abundance and richness varied significantly amongst tree species (Fig. 3.1); *Betula* had the highest and *Fraxinus* the lowest fungal abundance and richness. Such species differences disappeared later during decay, as shown for 2019.

Additionally, total fungal abundance and total richness on angiosperm and gymnosperm species varied between phylogenetic groups and across different survey years (Supplementary material, Fig. S3.1); while total fungal abundance increased till 2015 and thereafter gradually decreased, total fungal richness increased over time till 2017. In this analysis, total fungal abundance was significantly higher in angiosperm species compared to gymnosperm species.

The first PCA axis explained 55% of the total trait variation, and ran from gymnosperm species with strong physically defended stem (e.g., lignin%, C/N ratio), to angiosperm species with the opposite suite of traits and high nutrient concentrations. Hence, this axis showed a

transition from more resource conservative, well defended species to more resource acquisitive, metabolically active species. The second axis explained 25% of the variation and ran from species with strong chemically defended stem to species with poorly defended stem and more base-rich tissues (high pH) (Fig. 3.2).

The first trait axis (PC1) had a strong positive effect on fungal abundance and richness (Table 3.2; Fig. 3.3) and explained 44-47% of the variation. This indicates that angiosperm tree species with weak physical defences and high nutrient concentrations (especially low conduit density and lignin concentration, see Supplementary material, Table S3.4) are colonized by more species of fungi. The second trait axis (PC2) had no significant effect on fungal abundance or richness, indicating that wood chemical defence did not affect the amount or richness of colonizing fungi.

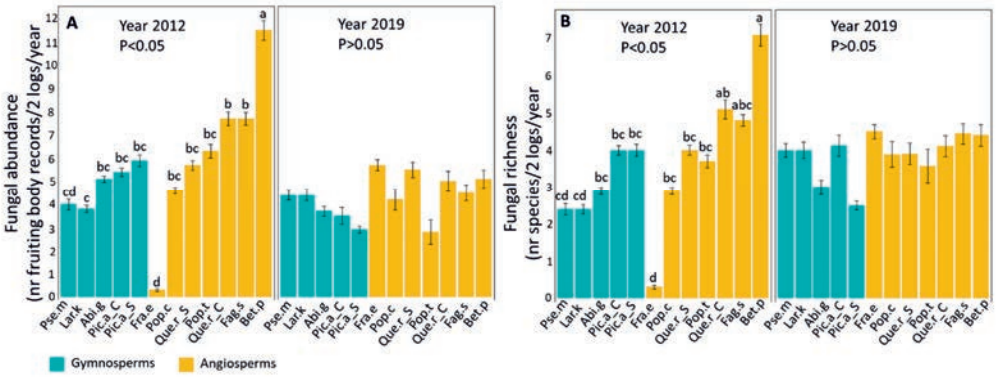


Fig. 3.1 Comparisons of fungal fruiting body abundance and richness for different years, phylogenetic groups (angiosperm vs. gymnosperm) and tree species. A&B-height of each bar represents mean fungal abundance and richness per individual tree (i.e., per 2 logs of 2 m length together). Error bars represent the standard error of the mean (N=10, stems per tree species). Within each phylogenetic group, bars that accompanied by different letters are significantly different (P<0.05). Full names of each tree species are listed in Supplementary material, Table S1.

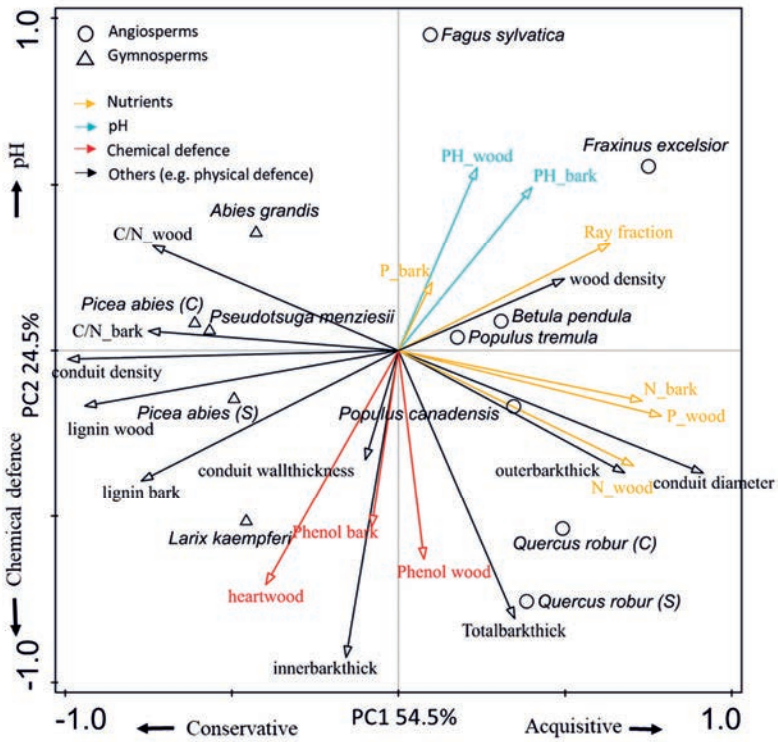


Fig. 3.2 Principle component analysis showing the associations amongst four groups of initial stem traits (nutrients, pH, chemical defence, and others) as indicated by arrows, for five gymnosperm species (triangles) and seven angiosperm species (circles), where two species (*Quercus robur* and *Picea abies*) were extracted from two different sites (sandy vs. clay soil).

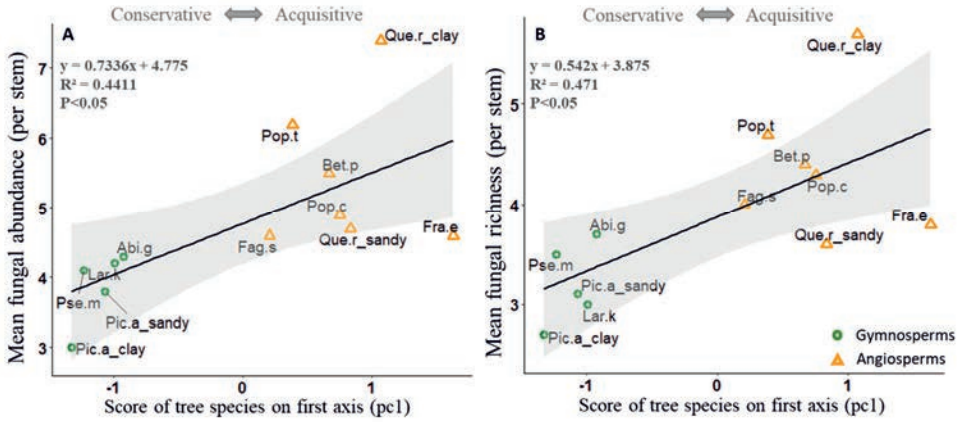


Fig. 3.3 Score of tree species on first axis of PCA graph (Fig. 3.2) in relation to fungal abundance and richness in year 2017 of 10 tree species. Data of gymnosperm species are shown as dark green circles (n = 5) and angiosperm species are shown as orange triangles (n = 7). Relationship between pc1 and mean fungal abundance/richness in other survey years are shown in Supplementary material, Fig. S3. Two species (*Quercus robur* and *Picea abies*) were extracted from two different sites (sandy vs. clay).

Table 3.1 Summary of the linear mixed models (LMMs) of fungal abundance and richness with plot as random factor, and phylogenetic group (Angiosperms vs. Gymnosperms), species nested within group, decay time, incubation sites (clay vs. sandy), and the interaction between time and site as fixed factors. The model coefficients, t-values and significance levels for each explanatory variables are given. In the analyses, gymnosperm species, the *Quercus robur* collected in the sandy site (Que.r_S), and the *Pseudotsuga menziesii* (Pse.m), the year 2012 and the sandy forest site were set as references (i.e., their value is zero). *p<0.05; **p<0.01; ***p<0.001.

Factors	Fungal abundance			Fungal richness		
	Estimate	t-value	*p*	Estimate	t-value	*p*
Intercept	3.91	7.31	***	2.51	6.18	***
Phylogenetic group						
Angiosperm species	1.37	2.91	**	0.77	2.14	*
Angiosperm only						
Angiosperm-Bet.p	1.90	4.04	***	1.25	3.47	***
Angiosperm-Fag.s	0.59	1.26	0.21	0.59	1.66	0.10
Angiosperm-Fra.e	-1.54	-3.26	**	-1.03	-2.85	**
Angiosperm-Pop.c	-0.69	-1.47	0.14	-0.20	-0.56	0.58
Angiosperm-Pop.t	0.55	1.17	0.24	0.64	1.78	0.08
Angiosperm-Que.r_C	1.92	4.06	***	1.55	4.32	***
Gymnosperm only						
Gymnosperm-Lar.k	0.72	1.55	0.12	0.33	0.93	0.35
Gymnosperm-Pic.a_C	0.57	1.20	0.23	0.63	1.76	0.08
Gymnosperm-Pica_S	1.32	2.81	**	0.80	2.23	*
Gymnosperm-Abi.g	0.39	0.84	0.40	0.03	0.08	0.93
time						
2013	-1.26	-2.25	*	-0.66	-1.55	0.12
2014	1.60	3.11	**	1.72	4.38	***
2015	2.20	4.28	***	2.12	5.40	***
2016	1.31	2.56	*	1.52	3.87	***
2017	-0.02	-0.04	0.97	0.75	1.91	0.06
2019	0.24	0.45	0.65	1.16	2.88	**
Site						
Clay forest	0.93	1.84	0.07	0.62	1.62	0.11
Time*site						
2013*clay forest	2.44	3.29	**	1.95	3.44	***
2014*clay forest	-2.10	-2.94	**	-1.59	-2.92	**
2015*clay forest	-2.72	-3.80	***	-2.06	-3.77	***
2016*clay forest	-1.87	-2.61	**	-1.06	-1.94	0.05
2017*clay forest	-1.68	-2.35	*	-0.97	-1.79	0.07
2019*clay forest	-3.16	-4.34	***	-2.41	-4.34	***

Table 3.2 Summary of the multiple regression analyses showing how fungal abundance and richness vary with the multivariate trait strategy axes (PC1, and PC2) of the tree species (see Fig. 2) and decay time. The model coefficients, t-values and significance levels (*p*) for each explanatory variable are given; *p<0.05; **p<0.01; ***p<0.001.

Fixed effects	Fungal abundance			Fungal richness		
	estimate	t-value	*p*	estimate	t-value	*p*
Intercept	5.67	16.4	***	3.64	14.4	***
PC1	0.46	4.28	***	0.30	3.71	***
PC2	-0.16	-1.48	0.14	-0.13	-1.57	0.12
2013	0.25	0.62	0.53	0.54	1.75	0.08
2014	0.52	1.32	0.19	0.90	3.03	**
2015	0.81	2.06	*	1.07	3.59	***
2016	0.35	0.89	0.37	0.97	3.26	**
2017	-0.89	-2.27	*	0.24	0.81	0.42
2019	-1.38	-3.39	***	-0.05	-0.16	0.88

3.3.3 | Factors influencing fungal community composition

A DCA on the fungal community composition in different years (Fig. 4S) showed that the first axis was mainly driven by decay time, running from fungal communities in 2012 (in green) toward 2019 (in brown). The second axis showed a split in the fungal composition between gymnosperm and angiosperm species. In the sandy the fungal communities were divergent during early decay years, but tended to converge during later decay years, while such a convergent pattern was not found in the clay site (Fig. 3.4).

A Canonical Correspondence analysis showed that phylogenetic group (Gymnosperms vs. Angiosperms), decay time and forest sites had strong and significant effects on fungal species composition (Fig. 3.5). Fungal community composition was also significantly affected by the initial traits of different tree species, as captured by the two PC strategy axes shown in Fig. 3.2. In total, 22.8% of the differences in fungal composition was explained.

Indicator species analysis shows that 52 out of the 128 analyzed fungal species (41 %), had significant preferences for specific tree species (Supplementary material, Table S3.5). For example, the best indicator for *Betula pendula* was *Annulohypoxylon multiforme* (stat=0.788), for *Quercus robur* growing at sandy site it was *Hypholoma fasciculare* (stat=0.717) and for *Fagus sylvatica* it was *Hypoxylon fragiforme* (stat=0.786). Additionally, three fungi turned out to be specialists as they were restricted to specific tree species; *Eriopezia caesia* only on oak species, the polyporoid *Oxyporus obducens* only occurred on P*opulus*×*canadensis* and the

tremelloid *Myxarium nucleatum* only occurred on *Fraxinus excelsior* as indicated by *P*(A)=1 (Supplementary material, Table S3.5).

Some fungi showed strong preference for different decay years (Supplementary material, Table S3.6). In total, 43 fungal species (34%) were detected as indicator species ($p<0.05$) for specific decay years from 2012-2019. The best indicator fungus for year 2012 was the corticioid *Cylindrobasidium laeve* (stat=0.617). Interestingly, some fungi were restricted to specific years as indicated by *P*(A)=1. The necrotrophic parasite *Nectria cinnabarina*, the biotrophic parasite *Gibberella* species, and the small saprotrophic *Bulgaria inquinans* only occurred in the early decay year (2012). *Stigmatolemma sp*ecies and the ectomycorrhizal corticoid *Tomentellopsis echinospora* only occurred in middle decay year (2014). More specialist fungi were only found in the late decay year (2019); the necrotrophic parasitic polyporoid *Ganoderma lipsiense,* the saprotrophic cortocoid *Gloiothele lactescens,* the litter saprophyte *Typhula erythropus* that is often found on leaf petioles*,* the saprotrophic polyporoid *Ceriporia* spec. and the saprotrophic corticoid *Peniophorella pubera* only fructified in this stage.

Mollisia cinerea s.l. (stat=0.669) was the best indicator fungus for angiosperm trees, while the corticoid saprotrophic *Amylostereum* species (stat=0.818) were the best indicators for gymnosperm trees (Supplementary material, Table S3.7). *Ascocoryne sarcoides s.l.* (stat=0.711) was the best indicator for the clay forest site while the corticoid *Serpula himantioides* (stat=0.663) showed the strongest preference for the sandy site (Supplementary material, Table S3.8). The colonization of logs by fungal species thus largely depended on tree species, decay time and site environmental conditions.

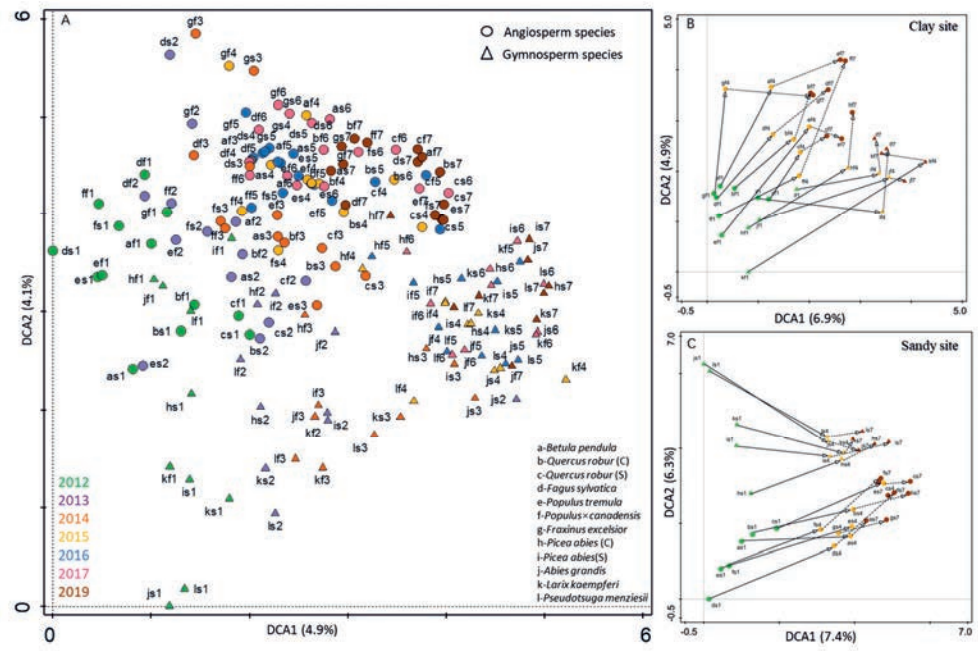


Fig. 3.4 Detrended correspondence analysis (DCA) showing how fungal species composition varies over time for five gymnosperm and seven angiosperm tree species. Years are indicated by symbol colours, and species by letters. (A) All fungal communities observed in all tree species and both forest sites during 2012-2019. (B and C) Fungal communities observed at forests with clay and sandy soil, respectively, 2012-2015 (continuous arrows) and 2015-2019 (broken arrows). Letters a-l: tree species; number 1-7: decay years 2012-2019 (we did not monitor year 2018 because of drought); letter s or c: sandy site vs. clay site. For example, "a1s" indicates *Betula* logs incubated at sandy site and in the first year of decay. Tree species on which no fungi were found in a particular year have been left out (e.g., g1s and g2s are lacking).

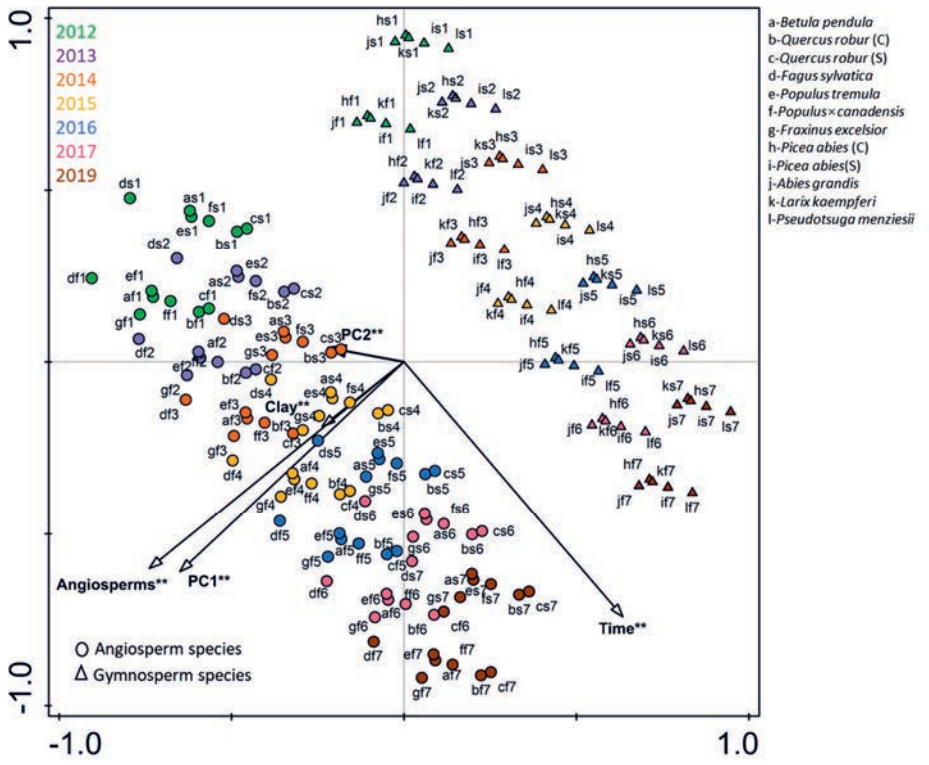


Fig. 3.5 Canonical Correspondence Analysis (CCA) ordination diagram showing how fungal composition is significantly affected by decay time (2012-2019), forest incubation site (clay vs. sandy), phylogenetic group (Gymnosperms vs. Angiosperms) and stem traits (i.e., species scores on pc1, pc2, Fig. 2). The first and second axes are shown, explaining 7.9 % of the total variability in fungal community composition. Asterisks denote environmental variables with significant effects in constraining community composition (*p<0.05; ** p<0.01; ***p<0.001).

Fig. 3.6 Wood-inhabiting fungi that are indicator species for angiosperm (a-c) and gymnosperm (d-f) tree species. *Eriopezia caesia* (photo: Theo Westra)., *Hypholoma fasciculare* (photo: Aldert Gutter) are indicators for angiosperm oak growing at forest in sandy site, *Scutellinia scutellata* (photo: Gerben Winkel) is an indicator for angiosperm poplar, *Dacrymyces stillatus* (photo: Henk Huijser) and *Heterobasidion annosum* (photo: Dinant Wanningen) are indicators for gymnosperm species, and *Gymnopus androsaceus* (photo: Nico Dam) is an indicator for gymnosperm douglas fir, late decay stage, as well as sandy soil site. All photos are obtained from https://www.verspreidingsatlas.nl/.

3.4 | DISCUSSION

We evaluated how fungal diversity in dead wood depends on a wide array of wood and bark traits of different tree species as well as on decay time. Particularly, we showed that fungal abundance and richness were higher on angiosperm species than on gymnosperm species, as explained by differences in wood and bark traits, and that the number of fungal species initially increased but declined after approximately 5 decay years (Table 3.1). Fungal communities differed between tree species in early decay stages; they converged during later years in the sandy site but not in the clay site. A clear division in fungal community composition was found between logs from gymnosperm and angiosperm species (Fig. 3.4 & 3.5).

3.4.1 | How do fungal abundance and richness depend on tree species and decay time?

Fungal abundance and richness of host trees. We hypothesized that fungal abundance and richness would be higher for tree species with high nutritional quality (i.e., high N and P concentrations) favoring fungal fructification (Lee et al. 2019), and lower for tree species with more chemical defences (i.e., higher phenol concentrations) and stronger physical defences (thicker cell walls and higher lignin concentration) that impede fungal growth (Hatakka and Hammel 2011, Lourenço et al. 2015). As expected, stems from angiosperm species had weaker defences and higher nutrient levels (Fig. 3.2) and, hence, had a higher fungal abundance and richness than gymnosperm species.

Betula, Fagus and *Quercus* had the highest fungal richness and *Pseudotsuga* the lowest. This must be due to specific characteristics of the host tree species (Vogel et al. 2020); colonization of fungi may be facilitated by thin cell walls and high proportion of ray parenchyma in *Betula*, low chemical defence in *Fagus* and high nutrient concentrations in *Quercus* (Kahl et al. 2017). *Quercus* logs extracted from the clay soil site had higher fungal abundance and richness than *Quercus* logs extracted from the sandy soil site. This may be because the *Quercus* trees originating from clay soils have a higher proportion of more permeable and nutrient rich sapwood whereas the oaks from sandy soils contain more heartwood (Fig. 3.2), in which anti-fungal metabolites are present (Noll et al. 2016), inhibiting colonization of fungi. *Pseudotsuga* held fewer fungal species than other gymnosperm species, which may because of its strong physical defence and low nutrient levels (Fig. 3.2). Moreover, *Pseudotsuga* was introduced from North America into Europe about 150 years ago (Schmid et al., 2014) and it may still lack a well-adapted European fungal species pool (Buée et al. 2011).

In conclusion, tree species with high nutrition levels may attract more wood-inhabiting fungal species than tree species with strong defence systems, which is also indicated by a strong increase in fungal richness going from resource conservative to resource acquisitive tree strategies (Table 3.2; Fig. 3.3).

Successional changes in fungal abundance and richness. Mean fungal abundance and richness per individual tree firstly increased over time and then declined in later decay stages (Heilmann-Clausen and Christensen 2004, Ovaskainen et al. 2013) (Table 3.1). The initial increase may reflect the greater variety of macrohabitats in decaying wood over years, with more nutrients becoming available following the enzyme activities of early decomposers. In addition, the enrichment of bioavailable metals during early decay years can also facilitate fungal fructification (Lindahl and Finlay 2006, Arnstadt et al. 2016). The large proportion of dead wood mass already lost in later decay phases may result in resource limitation and inhibit fungal fruiting body production (Juutilainen et al. 2011), thus explaining the later decline in fungal abundance. In contrast, several molecular studies have shown that fungal richness of spruce trees continued to increase over time (Rajala et al. 2011, Ovaskainen et al. 2013); the possible reason could be that many species survive as inconspicuous mycelia for a long time after fruiting (Kubartová et al. 2012) and numerous soil fungi may colonize on heavily decayed logs (Buee et al. 2009). Tree species with different physical-chemical traits were indicated to have different decay rates (Kahl et al. 2017), implying that later survey years include more diverse decay stages, thus the heterogeneity of the decaying wood increases. This point can explain our finding that total fungal richness of all dead wood objects increased over years.

In conclusion, long-term decomposition helps to maintain high forest fungal richness because more diverse substrates are created by dead wood of different decay stages, but fungal abundance declines in later decay years, probably because of energy limitation.

3.4.2 | How does fungal community composition depend on tree species and decay time?

Fungal communities of host tree species. Wood-inhabiting fungi are mainly substrate restricted and therefore they are often specialized on a certain tree species or phylogenetic group (Boddy and Heilmann-Clausen 2008). As expected, fungal community composition largely differed amongst tree species and a clear split in the fungal composition between gymnosperm and angiosperm species showed strong substrate specialization (Fig 3.4) (Schmidt 2006, Küffer et al. 2008), whereas multivariate tree strategies also determined the fungal composition (Fig.

3.5). Our result was supported by the findings of other authors that some white-rot fungi were typical decomposers of angiosperm species (Hatakka and Hammel 2011), whereas brown-rot fungi preferred to colonize gymnosperm species (Ryvarden and Gilbertson 1993). Indicator species analysis showed that angiosperm species held more fungal specialists (27) than gymnosperm species (20) (Supplementary material, Table S3.7), possibly because larger trait variation within angiosperm species reflects a wide range of substrates compared to gymnosperm species (cf. Fig. 3.2). Moreover, more angiosperm species (seven species) were analyzed in this study than gymnosperm species (five species). Within angiosperm species, *Trametes gibbosa* was exclusively found in angiosperm species and preferred to colonize birch species, while this fungus was previously also found on beech and poplar species (Ryvarden and Melo 2017), which may be due to their similar wood properties - high nutrition level and high pH (Fig. 3.2).

In summary, tree species with different traits in terms of the resource acquisitive versus conservation trade-off tend to host specific fungal communities; however, such host-dependent specialization often varies with decay stage (Parisi et al. 2018).

Successional patterns in fungal composition. We predicted that fungal community composition on dead wood would initially diverges and then converges in a later stage, because well-dispersed generalists will colonize the logs first together with endophytes, followed by tree species-specific specialists at a later stage, and ultimately by a more similar fungal community because decayed wood becomes more similar across species when decay proceeds (Rajala et al. 2011). We found support for this trend for the fungal community in the sandy forest site (Fig 4b), but not in the clay forest site (Fig. 3.4c). Such a convergent pattern during succession also occurs for wood-inhabiting invertebrates (Zuo et al. 2021). Possible reasons for the differences between clay and sandy sites could be that: i) species-specific endophytes present in living trees turned to be visible saprotrophs during early decay years (Parfitt et al. 2010); ii) most gymnosperm species harvested from old sandy forest may have more endophytes than those angiosperm species from the young clay forest during long term succession (Frank and Pirttilä 2018); iii) the endophytes may switch to a pioneer strategy when logs are incubated in their home site (home-field advantage) (Baber et al. 2016), while strong competition may occur when logs are incubated in the other site. With the decay process, dead wood properties generally change by the enzyme activities of multiple decomposers, hence results in continuous variation in substrate conditions for fungi (Bantle et al. 2014, Hiscox et al. 2015). This may be the reason for strong fungal specificity for different decay years. *Peniophora incarnata,*

assumed to be a pioneer fungus capable of colonizing living or recently dead wood (Lambevska et al. 2013), was strongly associated with the first survey years, while most fungal specialists were found in advanced decay stage (after 8 years' decay), with *Ganoderma lipsiense*, *Gloiothele lactescens*, *Typhula erythropus* and *Peniophorella pubera* exclusively occurring in this decay stage.

In conclusion, fungal succession occurs at very short time scales (<8 years) compared to the lifespan of a tree and the overall dead wood decay trajectory, and is driven by substrate specialization, site conditions and substrate homogenization during degradative succession .

We surveyed visible sporocarps as they are critical to the dispersal and long-term success of the species, easy to detect and monitor, conspicuous components of the fungal community and appreciated by naturalists and conservationist. Yet, many fungi do not make sporocarps, sporocarp production may vary over the years. Although we measured in the autumn peak fruiting period, we may not have captured the whole sporocarp community (Boddy et al. 2014) and describe the non-sporocarp community. Therefore, our research provides a conservative estimate of the abundance and diversity of fungal communities and more advanced molecular DNA techniques allow to capture the whole community. But some studies compared the fungal fruiting body survey and fungal DNA-based methods, and found that the effects of dead wood properties on wood-inhabiting fungal communities (Ovaskainen et al. 2013) and association networks of wood-inhabiting fungi are highly consistent (Saine et al. 2020), moreover, our fruit-body survey has the advantages as it infers the most active fungal species and is relatively low-cost, and thus enables large scale comparative studies (Runnel et al. 2015). Therefore, combining both fruit-body survey and molecular technique in future studies can obtain a more comprehensive insights of fungal communities on dead wood.

3.4.3 | Management implications

Dead wood is an important ecosystem component that increases forest biodiversity (Stokland et al. 2012). Most forest management efforts have so far focused on increasing the quantity and spatial distribution of dead wood (Lombardi et al. 2008, Bauhus et al. 2009, Vítková et al. 2018). There has been less focus on the quality of dead wood (Lassauce et al. 2011). Our study shows that fungal richness and composition are driven by phylogenetic group (angiosperm vs. gymnosperm species), species traits, and decay time (Table 3.1). Fungal richness is higher for angiosperm species than gymnosperm species, increases with resource acquisitive trait values

(Table 3.2; Fig. 3.3) and peaks after four years (Table 3.1; Supplementary material, Fig. S3.1). Of the 128 fungal species analyzed, 52 fungi (41 %) showed a preference for specific tree species, and 43 fungi (34%) for a specific decay time. This means that fungal alpha diversity can be increased by having dead wood of especially angiosperm species, and fungal diversity can be increased by having a diversity of tree species, a range of tree strategies (from resource conservative to acquisitive) and a range of decay stages. This means that forest managers, when they aim to increase biodiversity, should increase tree species diversity at the stand level (by having mixed species stand) (Schelhaas et al. 2003) or the forest level (by having a mixture of species stands). Many managed forest stands are young and natural tree mortality rates is low. Forest managers could rely on natural mortality, but also actively increase the volume of dead wood of desired tree species, through girdling, cutting or thinning to promote the diversity of wood-inhabiting fungi and invertebrates (Andringa et al. 2019). Based on our current study, a continuous flow of dead wood of different tree species could be assured by repeating these treatments every four years, so that the decay stage with optimal diversity (i.e., four years) is always present in the forest.

3.4.4 | Conclusions

In summary, the richness and composition of deadwood-inhabiting fungi is strongly determined by the characteristics of the host tree (i.e., phylogenetic group, strategy, and tree species) and decay time. Tree species with acquisitive traits (i.e., high nutrient concentrations and low defences) , both in their wood and their bark, have high fungal abundance and richness. Fungal communities diverge early in dead wood degradative succession with the arrival of fungal specialists, which indicates that dispersal limitation is important, but converge later in succession, which indicates that the wood substrate becomes more similar during succession. Hence, a high tree diversity of deadwood facilitates a high fungal diversity. However, the fungal fruiting body surveys should be complemented with molecular analyses of the invisible mycelia inside dead wood in the future, to obtain a comprehensive and high-resolution profile of fungal communities on dead wood.

ACKNOWLEDGEMENTS

The authors thank many participants of the LOGLIFE Team in various years for help with setting up, maintenance and harvesting. The Schovenhorst estate and Staatsbosbeheer Flevoland are very hospitable and helpful with establishing and in hosting LOGLIFE throughout the experimental period. Financial support was provided by the Nora Croin Michielsen Fonds, and the Chinese Scholarship Council (no. 201706910085).

SUPPLEMENTARY MATERIALS

Table S3.1 Basic information of 10 studied tree species. *Quercus robur* (Que.r) and *Picea abies* (Pic.a) were extracted from two forest sites (clay vs. sandy) and treated as four different tree "species" (Que.r_C, Que.r_S, Pic.a_C and Pic.a_S).

No.	Species	Abbreviation	Collecting sites	Family	Phylogenetic group
1	*Fraxinus excelsior*	Fre.e	C (clay)	Oleaceae	Angiosperm
2	*Betula pendula*	Bet.p	C (clay)	Betulaceae	Angiosperm
3	*Quercus robur*	Que.r_C	C (clay)	Fagaceae	Angiosperm
4	*Quercus robur*	Que.r_S	S (sandy)	Fagaceae	Angiosperm
5	*Fagus sylvatica*	Fag.s	C (clay)	Fagaceae	Angiosperm
6	*Populus×canadensis*	Pop.c	C (clay)	Salicaceae	Angiosperm
7	*Populus tremula*	Pop.t	S (sandy)	Salicaceae	Angiosperm
8	*Picea abies*	Pic.a_C	C (clay)	Pinaceae	Gymnosperm
9	*Picea abies*	Pic.a_S	S (sandy)	Pinaceae	Gymnosperm
10	*Larix kaempferi*	Lar.k	S (sandy)	Pinaceae	Gymnosperm
11	*Pseudotsuga menziesii*	Pse.m	S (sandy)	Pinaceae	Gymnosperm
12	*Abies grandis*	Abi.g	S (sandy)	Pinaceae	Gymnosperm

Table S3.2 Basic information on measured stem traits. Name of traits, measured compartments, number of replicates and unit are shown.

Functional traits	Measured compartments	Replicates	Unit
Conduit density	Sapwood	3	$\#cm^{-2}$
Conduit wall thick	Sapwood	3	µm
Ray fraction	Sapwood	3	%
Nitrogen fraction	Sapwood	5	%
	Bark	5	%
Phosphorus fraction	Sapwood	5	%
	Bark	5	%
pH	Sapwood	5	NA
	Bark	5	NA
Heartwood proportion	Wood	5	cm^2/cm^2
Phenols	Sapwood	5	%
	Bark	5	%
Wood density	Sapwood	5	$g\ cm^{-3}$
Carbon/nitrogen ratio	Sapwood	5	NA
	Bark	5	NA
Lignin fraction	Sapwood	5	%
	Bark	5	%
Bark thickness	Inner bark	10	mm
	Outer bark	10	mm
	total bark	10	mm

Table S3.3 Summary of the mixed linear models (LMMs) with plot as random factor. The model coefficients, t-values and significance levels for each explanatory variables are given. Group effect was removed and species *Pseudotsuga menziesii* (Pse.m), year 2012 and sandy forest site were set as references. *p<0.05; **p<0.01; ***p<0.001. For *Quercus robur* (Que.r) and *Picea abies* (Pic.a) were extracted from two forest sites (clay vs. sandy) and treated as four different tree "species" (Que.r_C, Que.r_S, Pic.a_C and Pic.a_S)

Factors	Fungal abundance			Fungal richness		
	Estimate	t-value	*p*	Estimate	t-value	*p*
Intercept	3.92	7.30	***	2.52	6.17	***
Tree species						
Abi.g	0.39	0.84	0.40	0.03	0.08	0.93
Fra.e	-0.06	-0.34	0.73	-0.25	-0.69	0.49
Lar.k	0.72	1.54	0.12	0.33	0.93	0.35
Pop.c	0.68	1.44	0.15	0.58	1.59	0.11
Pic.a_C	0.57	1.21	0.23	0.64	1.77	0.08
Que.r_S	1.38	2.91	**	0.77	2.15	*
Pic.a_S	1.32	2.81	**	0.80	2.23	*
Pop.t	1.93	4.08	***	1.41	3.92	***
Fag.s	1.97	4.17	***	1.37	3.80	***
Bet.p	3.28	6.93	***	2.02	5.61	***
Que.r_C	3.22	6.81	***	2.27	6.29	***
Decay time						
2013	-1.25	-2.25	*	-0.66	-1.54	0.12
2014	1.60	3.10	**	1.72	4.37	***
2015	2.20	4.27	***	2.12	5.39	***
2016	1.31	2.55	*	1.52	3.86	***
2017	-0.02	-0.04	0.97	0.75	1.91	0.06
2019	0.23	0.44	0.66	1.16	2.86	**
Incubation site						
Clay forest	0.93	1.83	0.07	0.62	1.61	0.11
Time*site						
2013*clay forest	2.44	3.28	**	1.94	3.43	***
2014*clay forest	-2.10	-2.93	**	-1.59	-2.91	**
2015*clay forest	-2.72	-3.79	***	-2.06	-3.76	***
2016*clay forest	-1.87	-2.60	**	-1.06	-1.93	0.05
2017*clay forest	-1.68	-2.35	*	-0.97	-1.78	0.08
2019*clay forest	-3.25	-4.48	***	-2.49	-4.48	***

Table S3.4 Summary of the multiple regression analyses showing how fungal abundance and richness vary with stem traits of the tree species and decay time. The model coefficients, t-values and significance levels (*p*) for each explanatory variable are given. *p<0.05; **p<0.01; ***p<0.001. Twelve traits were selected based on contribution to pc1 and pc2 axes (Fig. 3.2), then forward selection was conducted and final included variables are conduit density, conduit diameter, p wood, C/N bark, N bark and N wood.

Fixed effects	**Fungal abundance**			**Fungal richness**		
	Estimate	**t-value**	***p***	**Estimate**	**t-value**	***p***
Intercept	15.58	1.35	**0.18**	14.53	1.65	**0.10**
Conduit density	3.10	4.54	***	2.20	4.24	***
Lignin wood	-17.44	-4.64	***	-11.6	-4.06	***
Conduit diameter	2.96	1.75	0.08	2.18	1.69	0.09
P wood	-1.62	-1.49	0.14	-1.16	-1.40	0.16
C/N bark	0.05	0.01	0.99	-3.21	-0.44	0.66
N bark	1.77	0.18	0.86	-0.65	-0.09	0.93
N wood	2.25	1.24	0.22	0.84	0.61	0.54
2013	0.25	0.62	0.53	0.53	1.78	0.08
2014	0.52	1.35	0.18	0.90	3.09	**
2015	0.81	2.11	*	1.07	3.66	***
2016	0.35	0.91	0.36	0.97	3.31	***
2017	-0.89	-2.32	*	0.24	0.83	0.41
2019	-1.41	-3.53	***	-0.07	-0.23	0.82

Table S3.5 Tree host specific..ity of wood-inhabiting fungi tested by indicator species analysis. "A" is sample estimate of the probability of the surveyed fungi being found on the target tree species; "B" is the probability of finding the fungal species on the tree belonging to the target tree species. "stat" is the indicator statistic and P-value is derived from a permutation test with 9999 permutations. *p<0.05; **p<0.01; ***p<0.001.

Host tree species	Fungal species	Indicator tree species			
		A	B	stat	*p*
Betula Pendula	*Annulohypoxylon multiforme*	0.724	0.857	0.788	***
	Trametes gibbosa	0.833	0.500	0.645	***
	Trametes versicolor	0.322	0.571	0.429	**
	Bjerkandera adusta	0.220	0.643	0.376	*
	Nectria episphaeria	0.571	0.214	0.350	*
Quercus robur_clay	*Mollisia cinerea s.l.*	0.292	0.786	0.479	***
	Phlebia radiata	0.400	0.429	0.414	**
	Panellus stipticus	0.563	0.286	0.401	**
	Galerina marginata s.l.	0.500	0.286	0.378	*
	Mycena adscendens	0.170	0.786	0.366	*
	Mycoacia aurea	0.571	0.214	0.350	*
	Mycena galopus	0.338	0.357	0.348	*
Quercus robur_sandy	*Hypholoma fasciculare*	0.599	0.857	0.717	***
	Eriopezia caesia	0.757	0.643	0.698	***
	Stereum hirsutum	0.672	0.571	0.620	***
	Mycena galericulata	0.667	0.500	0.577	***
	Stereum gausapatum	0.833	0.357	0.546	***
Fagus sylvatica	*Hypoxylon fragiforme*	0.960	0.643	0.786	***
	Polydesmia pruinosa	0.486	0.571	0.527	***
	Flammulina velutipes s.l.	0.538	0.429	0.480	***
	Coprinellus domesticus	0.464	0.500	0.482	***
	Coprinellus micaceus	0.348	0.357	0.353	*
Populus tremula	*Trametes ochracea*	0.882	0.286	0.502	***
Populus×canadensis	*Scutellinia scutellata*	0.419	0.857	0.600	***
	Oxyporus obducens	1.000	0.286	0.535	***
	Bjerkandera fumosa	0.442	0.429	0.435	**
	Mycena hiemalis	0.331	0.500	0.407	**
	Lasiosphaeria ovina	0.379	0.286	0.329	*
Fraxinus excelsior	*Crepidotus mollis*	0.800	0.583	0.681	***
	Hypoxylon rubiginosum	0.745	0.583	0.659	***
	Bombardia bombarda	0.671	0.417	0.529	***
	Bisporella sp.	0.364	0.750	0.522	***
	Myxarium nucleatum	1.000	0.167	0.408	**
	Stypella dubia	0.539	0.167	0.300	*
Picea abies_clay	*Postia caesia*	0.303	0.571	0.416	**
	Mycena speirea	0.300	0.500	0.386	*
	Postia tephroleuca	0.400	0.286	0.336	*
	Heterobasidion annosum	0.316	0.357	0.336	*
Picea abies_sandy	*Resinicium bicolor*	*0.469*	*0.357*	0.409	**

	Botryobasidium subcoronatum	*0.333*	*0.429*	0.378	*
Abies grandis	*Amylostereum sp.*	0.366	0.857	0.560	***
Larix kaempferi	*Leptosporomyces mutabilis*	0.731	0.571	0.646	***
	Leptosporomyces fuscostratus	0.910	0.286	0.508	***
	Stereum sanguinolentum	0.340	0.429	0.382	*
	Hypholoma capnoides	0.286	0.500	0.378	*
Pseudotsuga menziesii	*Hyaloscypha sp.*	0.684	0.500	0.585	***
	Schizopora paradoxa s.l.	0.389	0.357	0.373	*
	Gymnopus androsaceus	*0.364*	*0.357*	0.360	*
	Phlebiella vaga	0.533	0.214	0.338	*
	Boidinia furfuracea	0.500	0.214	0.327	*

Table S3.6 Decay time specificity of wood-inhabiting fungi tested by indicator species analysis. Component "A" the sample estimate of the probability that of the surveyed fungi being found on the target tree species; "B" is the probability of finding the fungal species ion the tree belonging to the target tree species. "stat" is the indicator statistic and *P*-value is derived from a permutation test with 9999 permutations. *p<0.05; **p<0.01; ***p<0.001.

Decay time	Fungal species	Indicator species			
		A	B	stat	*p*
2012	*Peniophora incarnata*	0.675	0.609	0.641	***
	Cylindrobasidium laeve	0.973	0.391	0.617	***
	Chondrostereum purpureum	0.738	0.478	0.594	***
	Pezicula sp.	0.967	0.348	0.580	***
	Nectria cinnabarina	1.000	0.217	0.466	***
	Byssomerulius corium	0.700	0.217	0.390	**
	Bulgaria inquinans	1.000	0.130	0.361	**
	Gibberella.sp.	1.000	0.087	0.295	*
2013	*Ascocoryne.sarcoides s.l.*	0.305	0.696	0.461	**
	Merismodes anomala	0.514	0.261	0.366	**
	Bisporella sp.	0.323	0.391	0.355	*
	Stereum sanguinolentum	0.312	0.391	0.349	*
	Mycena acicula	0.688	0.174	0.346	*
2014	*Bjerkandera adusta*	0.398	0.750	0.546	***
	Pluteus plautus	0.870	0.208	0.426	**
	Stigmatolemma.sp.	1.000	0.125	0.354	*
	Tomentellopsis echinospora	1.000	0.125	0.354	*
	Mycena vitilis	0.529	0.208	0.332	*
2015	*Calocera cornea*	0.430	0.417	0.423	**
	Trametes versicolor	0.387	0.458	0.421	**
	Mycoacia aurea	0.714	0.167	0.345	*
2016	*Physisporinus sanguinolentus*	0.817	0.458	0.612	***
	Mycena hiemalis	0.425	0.458	0.441	**
	Botryobasidium subcoronatum	0.465	0.250	0.341	*
	Coprinellus micaceus	0.400	0.250	0.316	*
2017	*Orbilia sp.*	0.384	0.333	0.358	*
	Castinella olivacea	0.667	0.167	0.333	*
	Postia tephroleuca	0.462	0.208	0.310	*
2019	*Paxillus involutus*	0.733	0.292	0.462	***
	Ganoderma lipsiense	1.000	0.167	0.408	**
	Gloiothele lactescens	1.000	0.167	0.408	**
	Typhula erythropus	1.000	0.167	0.408	**
	Serpula himantioides	0.344	0.458	0.397	**
	Ceriporia sp.	1.000	0.125	0.354	*
	Gymnopus androsaceus	0.500	0.250	0.354	**
	Peniophorella pubera	1.000	0.125	0.354	*
	Rickenella fibula	0.419	0.292	0.350	*
	Boidinia furfuracea	0.571	0.208	0.345	**
	Gyrophanopsis polonensis	0.667	0.167	0.333	*
	Mucronella calva	0.625	0.167	0.323	*

Kuehneromyces mutabilis	0.571	0.167	0.309	*
Pluteus cervinus	0.750	0.125	0.306	*
Coprinellus disseminatus	0.417	0.208	0.295	*

Table S3.7 Indicator fungal species for two phylogenetic groups (Angiosperms vs Gymnosperms). Component "A" is sample estimate of the probability that the surveyed fungi being in the target tree species; "B" is the probability of finding the fungal species in tree belonging to the target tree species. "stat" is the indicator statistic and *P*-value is derived from a permutation test with 9999 permutations. **p*<0.05; ***p*<0.01; ****p*<0.001.

Phylogenetic groups	Fungal species	Indicator species			
		A	B	stat	*p*
Angiosperm species	*Mollisia cinerea s.l.*	0.955	0.469	0.669	***
	Annulohypoxylon multiforme	1.000	0.240	0.489	***
	Bjerkandera adusta	0.675	0.354	0.489	*
	Trametes versicolor	0.791	0.292	0.480	**
	Hypholom fasciculare	0.784	0.281	0.470	*
	Coprinellus domesticus	1.000	0.198	0.445	***
	Polydesmia pruinosa	1.000	0.198	0.445	***
	Bisporella sp.	0.821	0.240	0.444	**
	Mycena hiemalis	0.773	0.240	0.430	*
	Bjerkandera fumosa	0.974	0.188	0.427	***
	Eriopezia caesia	1.000	0.177	0.421	***
	Stereum hirsutum	1.000	0.177	0.421	***
	Lachnum sp.	1.000	0.167	0.408	***
	Scutellinia scutellata	0.691	0.240	0.407	*
	Hypoxylon rubiginosum	1.000	0.146	0.382	**
	Phlebia radiata	0.911	0.146	0.364	**
	Chondrostereum purpureum	0.910	0.146	0.364	*
	Rickenella fibula	0.956	0.135	0.360	**
	Coprinellus micaceus	0.793	0.156	0.352	*
	Crepidotus mollis	1.000	0.114	0.339	**
	Flammulina velutipes s.l.	0.836	0.135	0.337	*
	Hymenocyphus sp.	0.879	0.125	0.332	*
	Hypoxylon fragiforme	1.000	0.104	0.323	**
	Lasiosphaeria ovina	1.000	0.104	0.323	**
	Trametes gibbosa	1.000	0.104	0.323	**
	Coprinellus disseminatus	0.889	0.094	0.289	*
	Bombardia bombarda	1.000	0.083	0.289	*
Gymnosperm species	*Amylostereum sp.*	0.997	0.671	0.818	***
	Postia caesia	1.000	0.414	0.644	***
	Stereum sanguinolentum	0.995	0.386	0.620	***
	Hypholoma capnoides	1.000	0.371	0.609	***
	Gymnopilus penetrans	0.912	0.343	0.559	***
	Dacrymyces stillatus s.l.	0.832	0.371	0.556	***
	Resinicium bicolor	0.906	0.229	0.455	***
	Schizopora paradoxa s.l.	0.837	0.214	0.424	**
	Heterobasidion annosum	0.921	0.186	0.414	***
	Botryobasidium subcoronatum	0.818	0.200	0.405	**
	Leptosporomyces mutabilis	0.943	0.171	0.402	***
	Gymnopus androsaceus	0.943	0.171	0.394	***
	Hyaloscypha sp.	0.880	0.143	0.355	**

Postia tephroleuca	0.846	0.143	0.348	**
Physisporinus sanguinolenta	0.785	0.143	0.335	*
Boidinia furfuracea	0.947	0.100	0.308	**
Leptosporomyces fuscostratus	1.000	0.086	0.293	**
Pluteus plautus	1.000	0.086	0.293	**
Kuehneromyces mutabilis	0.892	0.086	0.276	*
Calocera viscosa	0.916	0.071	0.256	*

Table S3.8 Indicator fungal species for two forest sites (clay vs. sandy). Component "A" is sample estimate of the probability that the surveyed fungi being in the target tree species; "B" is the fidelity probability of finding the fungal species in tree belonging to the target tree species. "stat" is the indicator statistic and *P*-value is derived from a permutation test with 9999 permutations. **p*<0.05; ***p*<0.01; ****p*<0.001.

Incubation sites	**Fungal species**	**Indicator species**			
		A	**B**	**stat**	***p***
Clay	*Ascocoryne sarcoides s.l.*	0.696	0.726	0.711	***
	Mycena adscendens	0.743	0.488	0.602	***
	Mycena speirea	0.815	0.417	0.583	***
	Mycena hiemalis	0.800	0.262	0.458	**
	Scutellinia scutellata	0.799	0.262	0.458	**
	Schizopora paradoxa s.l.	0.886	0.202	0.424	**
	Bjerkandera fumosa	0.941	0.190	0.423	***
	Heterobasidion annosum	1.000	0.179	0.423	***
	Lachnum sp.	0.953	0.179	0.413	***
	Hymenocyphus sp.	0.861	0.143	0.351	**
	Merismodes anomala	1.000	0.119	0.345	**
	Physisporinus sanguinolenta	0.815	0.131	0.327	*
	Coprinellus disseminatus	0.940	0.143	0.366	**
	Mycena acicula	0.891	0.107	0.309	*
	Hyaloscypha sp.	0.732	0.119	0.295	*
	Kuehneromyces mutabilis	1.000	0.083	0.289	*
	Castinella olivacea	1.000	0.071	0.267	*
	Gyrophanopsis polonensis	1.000	0.071	0.267	*
	Lasiobelonium variegatum	1.000	0.071	0.267	*
Sandy	*Serpula himantioides*	1.000	0.439	0.663	***
	Dacrymyces stillatus s.l.	0.945	0.402	0.617	***
	Gymnopilus penetrans	0.984	0.354	0.590	***
	Mycena galopus	0.948	0.329	0.559	***
	Postia caesia	0.910	0.268	0.494	***
	Orbilia sp.	0.849	0.244	0.455	***
	Botryobasidium subcoronatum	0.935	0.220	0.453	***
	Resinicium bicolor	0.878	0.207	0.427	***
	Gymnopus androsaceus	1.000	0.170	0.413	***
	Rickenella fibula	0.968	0.159	0.392	***
	Galerina atkinsoniana	1.000	0.134	0.366	***
	Paxillus involutus	1.000	0.134	0.366	***
	Postia tephroleuca	1.000	0.134	0.366	***
	Hypoxylon rubiginosum	0.755	0.146	0.332	*
	Mycena epipterygia	1.000	0.098	0.312	**
	Panellus stipticus	0.878	0.098	0.293	*
	Phlebiella vaga	1.000	0.085	0.292	**
	Amphinema byssoides	1.000	0.073	0.271	*
	Nemania serpens	1.000	0.061	0.247	*
	Trichaptum abietinum	1.000	0.061	0.247	*

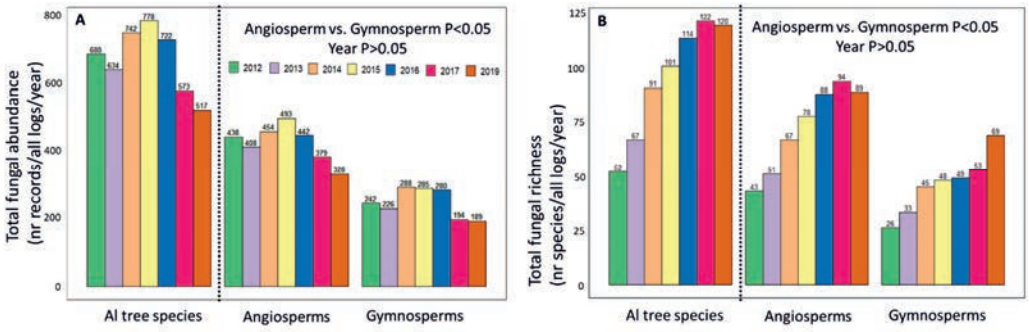


Fig. S3.1. Comparison of fungal abundance and richness for different years, phylogenetic groups (Angiosperms vs. Gymnosperms) and tree species. A&B-height of each bars represents total fungal abundance and total richness per year, respectively. A two-way ANOVA was carried out to analyse how total fungal abundance and richness vary between phylogenetic group and across different survey years. To correct for the different number of tree species within the phylogenetic groups (five gymnosperm tree species and seven angiosperm tree species), the total fungal abundance and richness of Gymnosperms and Angiosperms were divided by their tree species number (i.e., five and seven respectively) prior to analysis to have the same sample size for the two tree phylogenetic groups.

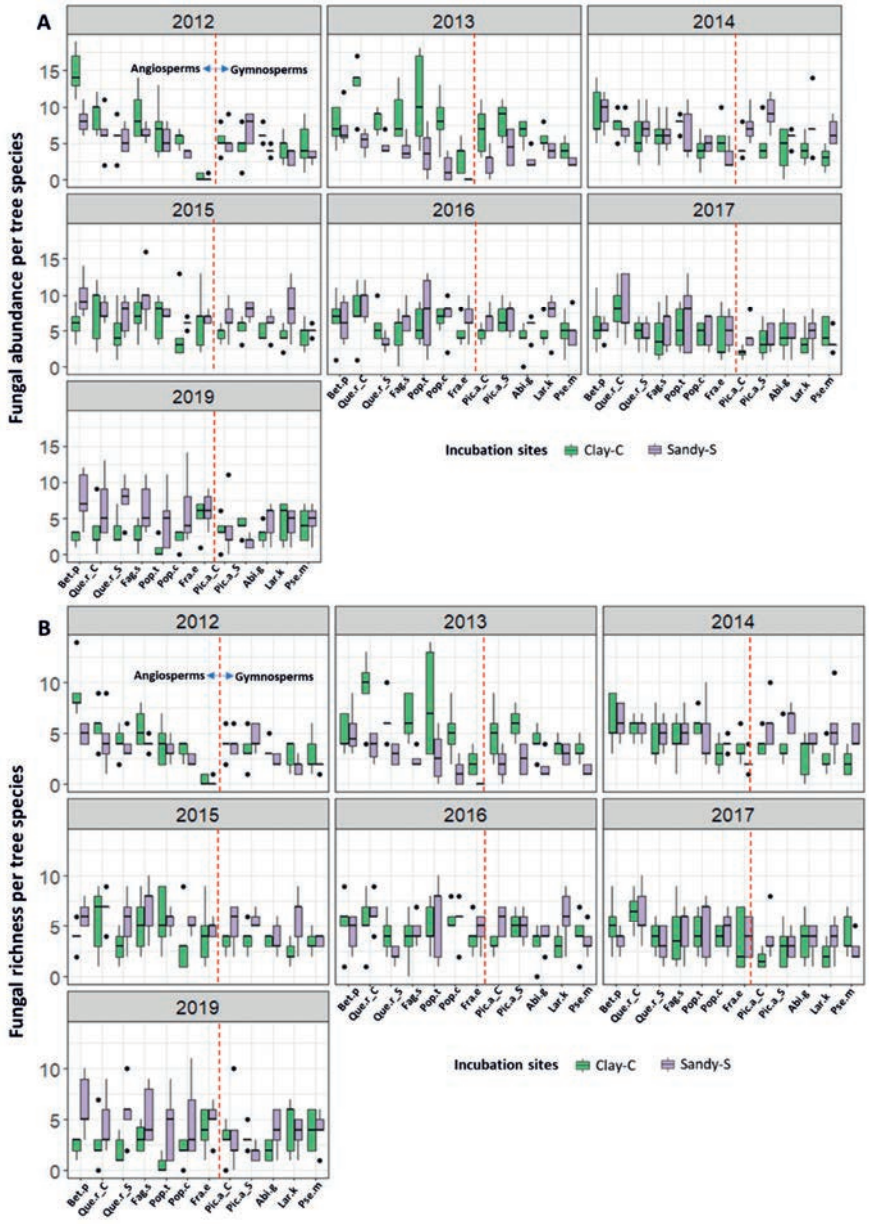


Fig. S3.2. Mean fungal abundance (A) and richness (B) variation amongst different tree species incubated in two different forest sites.

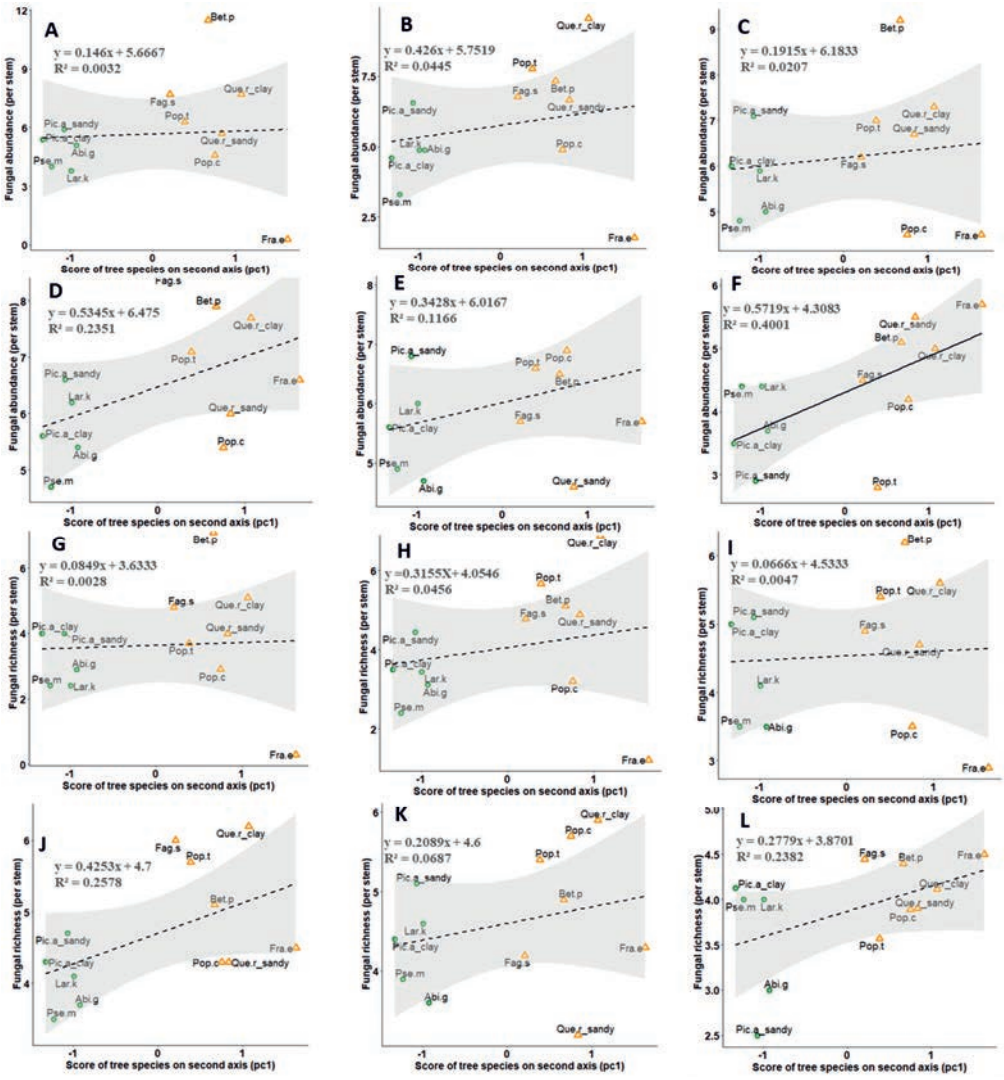


Fig. S3.3. Relation between tree species scores on the first PCA Axis (Fig. 2) and fungal abundance and richness in year 2012 (A & G), 2013 (B & H), 2014 (C & I), 2015 (D & J), 2016 (E & K) and 2019 (F & L). Ten tree species were included, with two species (*Quercus robur* and *Picea abies* coming from two different growing sites with sandy and clay). Data of gymnosperm species are shown as dark green circles (n = 5) and angiosperm species are shown as orange triangles (n = 7). Dash regression line indicates a nonsignificant relationship between fungal abundance/richness and pc1, while solid regression line indicates a significant relationship.

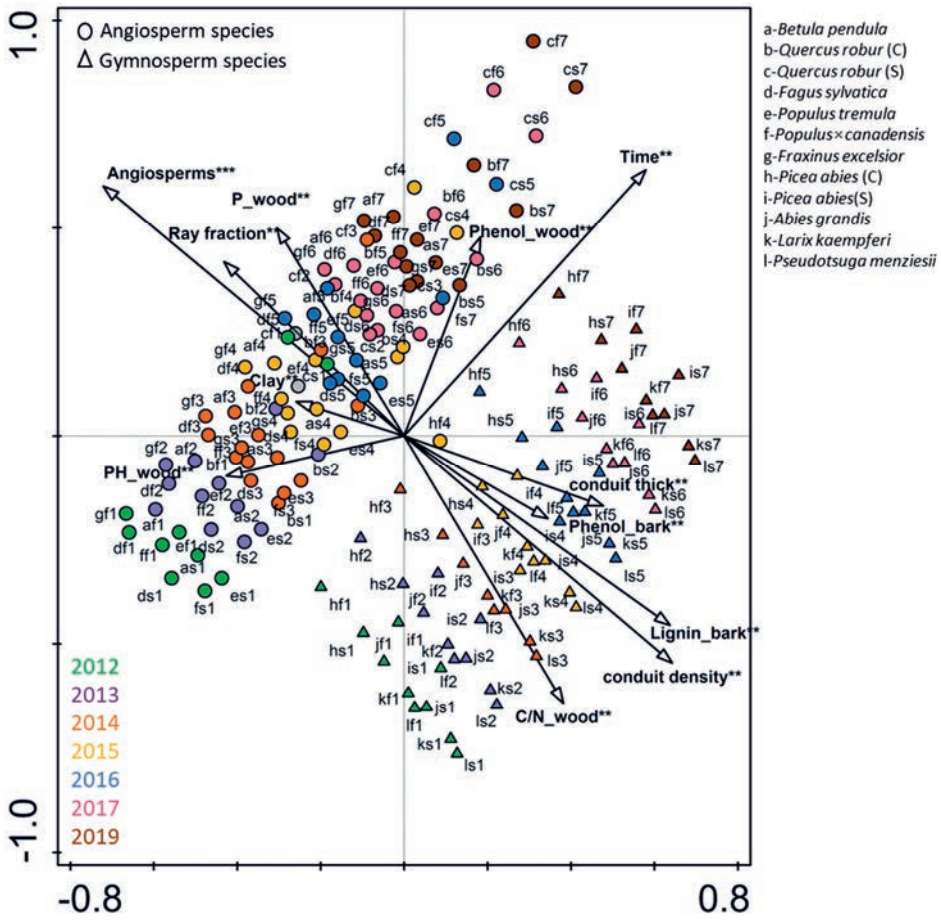


Fig. S3.4. Canonical Correspondence Analysis (CCA) ordination diagram showing the relations of fungal species to decay time (2012-2019), forest sites (clay vs. sandy), phylogenetic group (angiosperm vs. gymnosperm species) and stem traits selected by the forward selection. The first and second axes are shown, explaining 8.3 % of the total variability in fungal community composition. Asterisks denote environmental variables with significant effects in constraining community composition (*p<0.05; ** p<0.01; ***p<0.001).

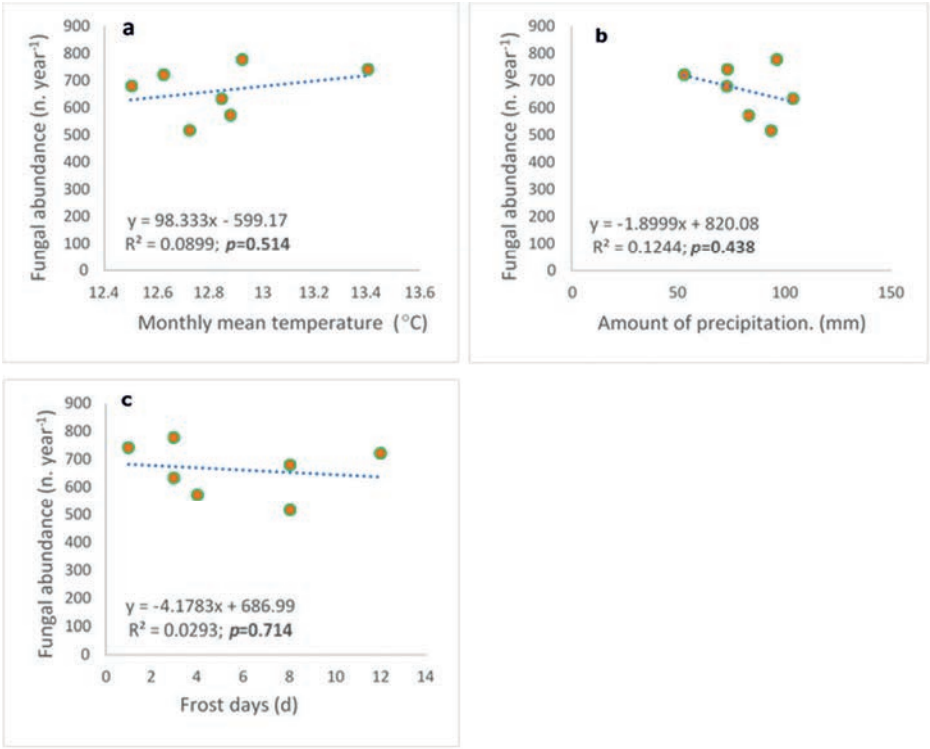


Fig. S3.5. Relationships between climate variables and fungal abundance. Each dots indicate different survey years, i.e., 2012, 2013, 2014, 2015, 2016, 2017 and 2019. a) relationship between mean temperature (°C) and fungal abundance; b) relationship between amount of precipitation (mm) and fungal abundance; c) relationship between forest days (d) and fungal abundance, frosts are defined as falls in air temperature to below zero in the evening and at night with a temperature above zero during the daytime. Four months climate data was used in this study, i.e., from August to November, climate data was collected from https://www.knmi.nl/nederland-nu/klimatologie/daggegevens.

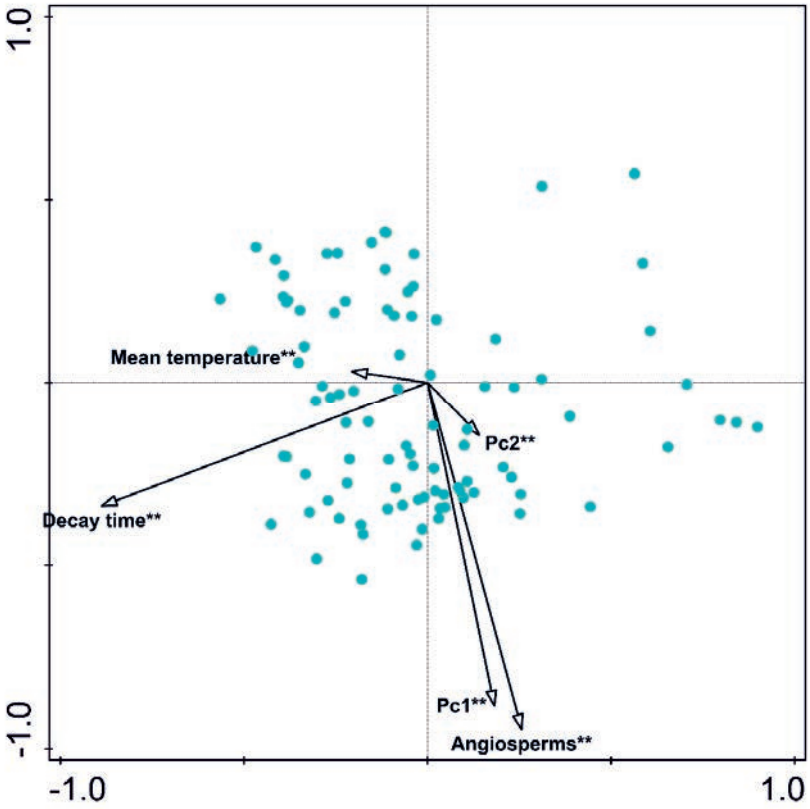


Fig. S3.6. Canonical Correspondence Analysis (CCA) ordination diagram showing how fungal composition in dead wood in the Schovenhorst forest site is significantly affected by decay time (2012-2019), phylogenetic group (Gymnosperms vs. Angiosperms), stem traits (i.e., species scores on pc1, pc2, Fig. 2) and climate variables (from August to November, mean temperature-°C, amount of precipitation-mm, frost days-d). The first and second axes are shown, explaining 12.59% of the total variability in fungal community composition. Asterisks denote environmental variables with significant effects in constraining community composition (*p<0.05; ** p<0.01; ***p<0.001). Among the three climate variables, only mean temperature showed significant effect on fungal community composition, only accounting for 2.7% of total variation and lower than that of "decay time", which account for 6.1% of total variation.

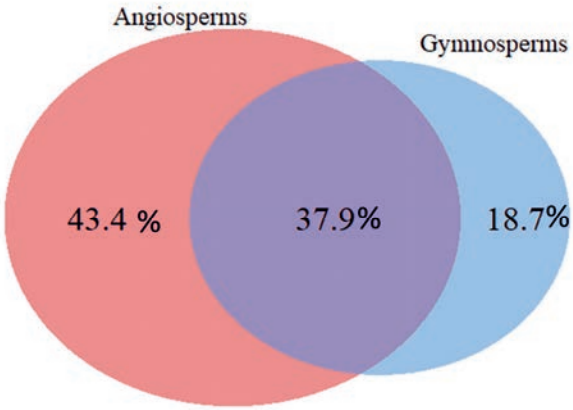


Fig. S3.7. Venn diagram with all 255 fungal species observed on dead wood of 10 temperate tree species, displaying the percentage of shared and unique fungi of angiosperm species and gymnosperm species.

CHAPTER 4

Stem traits affect fungal diversity and community composition in different stem compartments across 14 temperate tree species

Shanshan Yang, Lourens Poorter, Ute Sass-Klaassen, Eiko Kuramae, J. Hans C. Cornelissen, Marcio Leite, Ohana de Assis Costa, Frank J. Sterck

Submitted for publication

ABSTRACT

The quantity and quality of dead wood is important for forest biodiversity, such as wood inhabiting fungi. We evaluated how fungal richness and composition varied across stem compartments and 14 common temperate gymnosperm and angiosperm tree species, and how this was driven by stem traits. Fresh logs were incubated in a common garden experiment in a forest site in the Netherlands. After one and four years of decay, the fungal composition of bark, outer wood, and inner wood was assessed using Internal Transcribe Spacer amplicon sequencing. We found that fungal richness and Shannon diversity differed significantly amongst tree species and stem compartments; bark samples held significantly higher fungal diversity than inner wood and outer wood. Most samples were dominated by saprotrophic fungi, and brown-rot increased over succession. Gymnosperm and angiosperm species tended to hold different fungal communities, and distinct fungi were found in inner wood compared to outer wood and bark. Overall, stem traits associated with accessibility (i.e., conduit diameter, conduit fraction, and conduit wall thickness), food source of fungi (i.e., nutrients and lignin), and physical defence (i.e., wood and bark density, bark punch resistance, and bark thickness) were important factors regulating fungal community composition in different compartments of decaying stems. However, the role of stem traits changed during decay: traits associated with accessibility were more important during the first year of decay, whereas lignin/cellulose became most important after four years of decay. Hence, stem trait differences across tree species and their stem compartments have significant afterlife effects in regulating fungal diversity and composition, and contribute to explain the role of dead wood in forest biodiversity.

KEYWORDS

Fungal diversity; fungal composition; stem traits; dead wood; stem compartments

4.1 | INTRODUCTION

4.1.1 | The importance of dead wood

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 becomes slowly available when stems decompose (Yatskov et al. 2003). Moreover, dead wood provides a wide range of niches for diverse forest dwelling species (Moll et al. 2018, Vogel et al. 2020) and therefore plays a vital role in forest biodiversity conservation.

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 are divided into three main functional groups; white-rot Basidiomycetes, brown-rot Basidiomycetes and soft-rot Ascomycetes, that all degrade cellulose and hemicellulose while white-rot fungi can also degrade recalcitrant lignin (Blanchette 2000). White-rot fungi use extracellular lignocellulolytic enzymes for degradation, brown-rot fungi use hydrogen peroxide and iron ions (Koenigs 1974), and soft-rot fungi degrade the wood through the excretion of cellulase (Schmidt 2006). Other functional fungal groups such as ectomycorrhiza, lichens and pathogenic fungi are also associated with dead wood. Stems of different tree species can support different fungal communities. These fungi have their own strategies to compete for limited resources such as nutrients, water, oxygen and space, and degrade the wood matrix in different ways. The complex interaction of different groups of fungi, with some of the groups being generalists and others specialist and some being active in the initial decomposition and others in more advanced decomposition stages makes that dead wood largely contributes to 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) as forest biodiversity is positively related to 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, Lee et al. 2020) and the relevance of various environmental drivers for decomposition (Krah et al. 2018, Müller et al. 2020). However, up to now, it remains unclear which stem traits determine the fungal communities and how important these traits are in contributing to the diversity and composition of wood-inhabiting fungal communities.

4.1.2 | Determinants of fungal diversity and composition

Stem physical and chemical traits determine the accessibility and substrate quality of dead wood for different fungi, and therefore fungal diversity and composition (Fukasawa et al. 2009, Rajala et al. 2012, Baldrian et al. 2016). For example, angiosperm tree species differ from gymnosperm species in anatomical structure and lignin composition and therefore in fungal community composition (Cornwell et al. 2009, Kahl et al. 2017, Yang et al. 2021). 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 quality (i.e., wood moisture content) are commonly studied 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 amongst stem compartments, (i.e., bark, sapwood and inner wood) despite the fact that they can strongly differ in wood anatomical and chemical traits (Yang et al. 2021). These studies often included only 1-2 tree species (Kubartová et al. 2012, van der Wal et al. 2016) or yielded inconsistent results (Leonhardt et al. 2019). 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 six years of decay (Leonhardt et al. 2019). Such inconsistent findings may be attributed to different target tree species and decay stages, and these studies do not include changes in fungal infestation during decay for different stem compartments (including sapwood, heartwood and bark tissues). Therefore, how stem traits affect fungal infestation during decay processes in different stem compartments across a broad range of species remains unclear. Such a knowledge gap emphasizes the need for further studies disentangling dead wood fungal diversity and composition in relation to substrate characteristics in more detail. First of all, including more and anatomically contrasting tree species is necessary to assess all potential substrate related factors affecting fungal diversity and composition. Moreover, by using next generation sequencing of phylogenetic markers it is possible to assess the complete fungal community inhabiting different stem compartments (e.g., comparison among inner wood, sapwood and bark).

4.1.3 | Research question and hypotheses

In this study, we used phylogenetic marker Internal Transcribe Spacer (ITS) region amplicon next generation sequencing to evaluate the fungal communities in different stem compartments (inner wood, sapwood, and bark) of logs from 14 common gymnosperm and angiosperm tree species from temperate forest in Northwestern Europe. We included ring-porous and diffuse-porous angiosperm species and included heartwood and non-heartwood forming to cover a broad range of wood qualities (Table 4.1). Fresh logs were incubated in a common garden experiment in the same forest site in the Netherlands, thus assuring that they were exposed to similar environmental conditions and were exposed to the same fungal community. All tree logs were 100 cm long and had the same diameter (c. 25 ± 3 cm), thus assuring a similar exposed surface area and substrate quantity.

Our main question is how fungal richness, diversity, and fungal community composition varies amongst stem compartments and tree species, and how is this driven by stem traits? We hypothesize that i) bark supports a richer and more diverse fungal community than inner and outer wood because it is most exposed to fungal colonization, ii) outer (sap)wood is more palatable and more accessible than the inner wood, whereas inner wood - in case of heartwood forming species - requires fungal communities with specialists to degrade extractives and iii) fungal community composition differs amongst host tree species and stem compartments because physical-chemical traits differ among species and stem compartments.

4.2 | MATHERIALS AND METHODS

4.2.1 | Study site

A common garden experiment was carried out in a typical 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).

4.2.2 | Experimental design

We take advantage of the LOGLIFE project, in which freshly cut stems of temperate tree species have been left to decompose in a common garden experiment. Ten tree species were incubated in February, 2012, and four additional tree species were incubated in February, 2015. In total, six angiosperm species and eight gymnosperm species were incubated (see tree species list in Table 4.1). Most species were extracted from the local forest Schovenhorst, but 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 the harvested trees (Yang et al. 2021). One-meter length stem segments of equal diameter (25 ± 3 cm) were selected to reduce the confounding effects of stem size on fungal colonization. Before incubation, five individual trees were harvested 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.

4.2.3 | Sample preparation

Samples for fungal DNA extraction. After one and four years of decay (i.e., marked as T1 and T4, respectively), one log of a tree species was extracted from each plot, in total four logs per tree species were extracted at each harvest time (Fig. 4.1). Each harvested tree log was cut into two equal parts, one part (c. 50 cm) was returned to its original location for later investigation whereas the other part was used for fungal analysis. It was vertically divided into two parts (each is one quarter of the whole 1 m-length tree log) and disks from the middle of the ¼ tree log were extracted (Fig. 4.1). Sawdust samples of different stem compartments (heartwood/inner wood, sapwood/outer wood and bark (only available for T1) were collected using an electric drill. Sawdust samples were only collected from the inner parts to avoid contamination. Stem consists of wood and bark, and some species convert the inward part of the functional sapwood into heartwood and some species are non-heartwood forming species; to avoid confusion, hereafter we used the terms of inner wood, outer wood, and bark to indicate the three stem compartments. To obtain a representative sample 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. 4.1), and stored in a freezer at -20°C for further molecular analysis.

Before extracting the DNA, the sawdust samples were further ground into fine powder with a Retsch MM400 ball mill (Retsch, Haan, Germany). Specifically, sawdust samples and a metal ball (20 mm) were firstly put into a stainless steel beaker (50 ml), the sawdust filled 30% - 40% of the beaker; then we closed the beaker and put it in the liquid nitrogen until frozen; after that, we fixed it on the machine to grind the sawdust for 3 minutes at 30 Hz; finally, sample powder was carefully transferred from the beaker and stored at -20 °C. 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 and water between samples to prevent sample contamination.

Samples for physical-chemical trait measurement. To test how stem traits affect fungal communities 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 Table S4.1. 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 a MM400 ball mill (Retsch, Haan, Germany) and used for measurements of pH, C/N, C/P, N/P ratios, lignin concentration, nitrogen (N) and phosphorus (P) concentrations. Sampling and measurements were described in detail in Yang et al. (2021).

4.2.4 | DNA extraction, amplification, and sequencing

DNA was isolated from 0.10 g - 0.25 g fresh weight of ground samples using the PowerSoil DNA Isolation Kit according to the manufacturer's instructions (MO BIO Laboratories, Inc.). Standard manufacturer protocols were followed with some modifications: after adding solution C1 (causing cell lysis), samples were incubated at 60 °C for 30 minutes; to complete homogenization and cell lysis, tissuelyser was used to shake the samples for 5 minutes, then invert the sample holder, then shaken for another 5 minutes. After adding solution C6 (releasing DNA from the spin filter), samples were incubated at 30 °C for 15 minutes. The extracted DNA quality was determined using a NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA) according to the 260/280 nm and 260/230 nm absorbance ratios. All DNA was stored at -80°C.

For the PCR, we used the primer pair ITS1F (5' - CTTGGTCATTTAGAGGAAGTAA-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 reactions with Qiagen HotStar Taq master mix (Qiagen Laboratories Inc.) under 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. Success of amplification of each reaction was verified on a 2 % agarose gel. Each amplicon was quantified with the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies). The library was then generated by

pooling the same quantity (ng) of each amplicon. Cleaning-up of the pool (or library) was done with sparQ PureMag Beads (from Quantabio). The library was quantified using Kapa the Illumina GA with a Revised Primers-SYBR Fast Universal kit (Kapa Biosystems). Average fragment size was determined using a LabChip GX (PerkinElmer) instrument. Before sequencing, 15% of Phix control library was added to the amplicon pool (loaded at a final concentration of 9pM) to improve the unbalanced base composition in the flowcell. Subsequently, sequencing was performed with the MiSeq Reagent Kit v2 500 cycles in an Illumina MiSeq System (Illumina Inc.).

4.2.5 | Bioinformatics

We followed the standard ITS pipeline (https://benjjneb.github.io/dada2/ITS_workflow.html) with a modification: in the "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). In brief, using the "filterAndTrim" function, fungal reads with higher than two expected errors (maxEE=2) and shorter than the length of 50 base-pairs (minLen=50) were discarded. Then, amplicon sequence variants (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 chimera 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 the 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.

Based on the bioinformatic tool FUNGuild (Nguyen et al. 2016), all detected fungal communities (ASVs) were grouped into different guilds according to their trophic modes: 1) "saprotroph", which was further divided into "**white-rot**", "**brown-rot**", "**soft-rot**" and "**undefined saprotroph**"; 2) "**pathotroph**", including all "animal pathogen", "plant pathogen", "Lichen Parasite" and "Fungal Parasite"; 3) "**symbiotroph**", including all "Endophyte", "Ectomycorrhizal", "Lichenized" and "Epiphyte"; 4) "**multiple trophic modes**", i.e., all ASVs that potentially change their trophic modes during life cycles. Notably, classification with confidence ranking of "highly probable" or "probable" were selected, while the confidence ranking of "possible" and "NA" was grouped into "**undefined**".

4.2.6 | Statistical analyses

In this study, we focus on how multiple stem traits affect fungal richness, diversity, and community composition of different stem compartments (inner wood, outer wood, and bark) across the 14 tree species. To address the research aim, multiple 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 index 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 index) differs between tree species and stem compartments, two-way ANOVAs were conducted with the alpha diversity indices as dependent variables followed by Tukey's HSD post hoc test. The normality of the residuals was checked with the Shapiro test and Q-Q plots

Centered-log ratio (CLR) transformation is a promising approach to deal with compositional data, which converts 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 zeroes, 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 are associated with different physical-chemical traits of the three stem compartments, principal component analysis (PCA) was conducted with the fungal observed richness and Shannon diversity index of different stem compartments as supplementary variables that did not influence the ordination. Then Pearson correlations were conducted to show the relationships between fungal alpha diversity and log-transfromed stem traits, as well as the first two axes extracted from the PCA of each stem compartment. Additional PCAs were performed to visualize how fungal community composition differ across stem compartments of different tree species. To test how stem traits regulate fungal community composition in the three main stem compartments across different tree species at two decay periods (T1 and T4), constrained linear ordination analyses (redundancy analyses, RDAs) were conducted in CANOCO 5.0 using a Monte Carlo permutation test (MCPT, number of permutations 4999). These explanatory effects were evaluated in MCPT with a forward step-wise procedure and only the variables with significant effects were included.

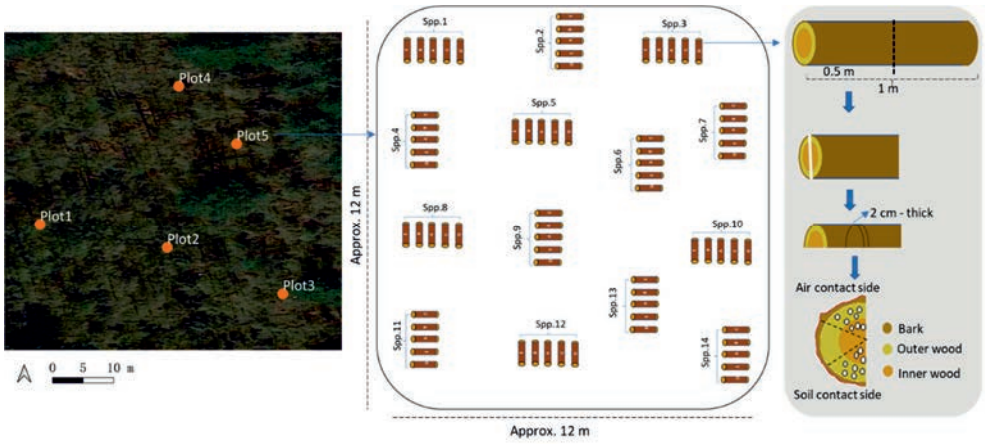


Fig. 4.1 Experimental design of the decomposition experiment in Schovenhorst. Five trees per species (N=14) were incubated in five plots per site. Each plot measured approximately 12 by 12 m, with a minimum distance of 20 m between plots. Within each plot, five one-meter length tree logs (A-E) belonging to the same individual tree were left to decompose. Sampling for DNA extraction is shown in right panel and was described in the Materials and Methods.

Table 4.1 Basic information of the 14 studied tree species with their name, abbreviation, major phylogenetic group they belong to, collection site, and wood structure.

Species	Abbre-viation	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	No	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

4.3 | RESULTS

4.3.1 | Alpha diversity and its determining factors

A total of 1466 (T1 - after one year decay) and 814 (T4 - after four years decay) fungal amplicon sequence variants (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 one year of decay, fungal richness and Shannon diversity index values (H) significantly differed among tree species and stem compartments; bark samples had a significantly higher fungal richness (54.5 ASVs) and Shannon diversity index (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. 4.2, Fig. S4.1a,). Bark samples of *Fraxinus excelsior* had the highest fungal richness (137 ASVs) and Shannon diversity index (H=3.1), while the outer wood of *Chamaecyparis lawsoniana* had the lowest fungal richness and Shannon diversity index (5.8 ASVs, 0.4 H). After four years (T4) fungal richness still significantly differed among tree species and stem compartments (Fig. 4.2b, $P<0.05$). Notably, the observed significant differences among stem compartments were mainly determined by the presence of bark samples; differences among stem compartments were less obvious or disappeared when bark samples were removed from the analyses (Fig. S4.2).

Pearson correlations were used to show the associations between physical-chemical traits of inner wood, outer wood and bark, and the fungal richness and Shannon diversity index (Table 4.2). For inner wood, fungal richness and Shannon diversity index correlated negatively with PC1 scores. Fungal alpha diversity correlated positively with carbon and lignin concentrations, but negatively with N and P concentrations and conduit diameter at T4 (Table 4.2, Fig. 4.3a). 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 and C/P ratio at T4 (Table 4.2, Fig. 4.3b). For bark, fungal richness and Shannon diversity index values tended to be lower for species with high lignin and cellulose concentrations after one year of bark decay (Table 4.2, Fig. 4.3c). These findings were supported and better visualized by principal component analyses shown in Fig. S4.3.

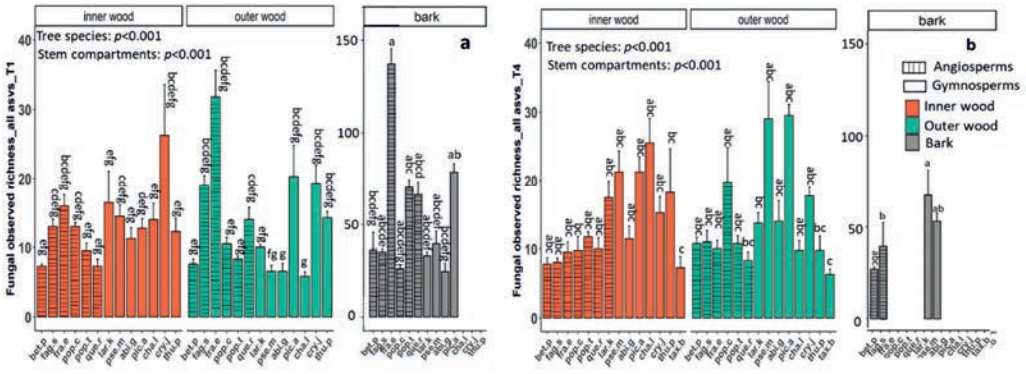


Fig. 4.2 Richness of the fungal communities. Bar plots illustrate variation in fungal ASV richness among different stem compartments of 14 tree species after (a) one year (T1) and (b) four years (T4) of decay for inner wood (orange), outer wood (green), and bark (dark grey, mind different scales) of 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; (Results of two-way ANOVA with two factors (Tree species & stem compartments) are shown in the upper left. Pairs of bars that do not share similar letters are significantly different (Tukey's HSD: $P<0.05$).

Table 4.2 Pearson correlations between fungal alpha diversity and traits of each stem compartment (inner wood, outer wood, and bark). ASV indicates fungal richness and H indicates Shannon Diversity after one (T1) or four (T4) years of stem decay.

Traits	**Inner wood**				**Outer wood**				**Bark**	
	ASV T1	ASV T4	H T1	H T4	ASV T1	ASV T4	H T1	H T4	ASV T1	H T1
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 forming	*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: values in bold show significant correlations (P<0.05), values with an underline show strong correlation (P<0.001), and values in Italic show correlations with 0.05<P<0.1. Traits and fungal alpha diversity were log-transformed. PC1 and PC2 are the scores of first two PCA axes obtained from Fig. S4.3.

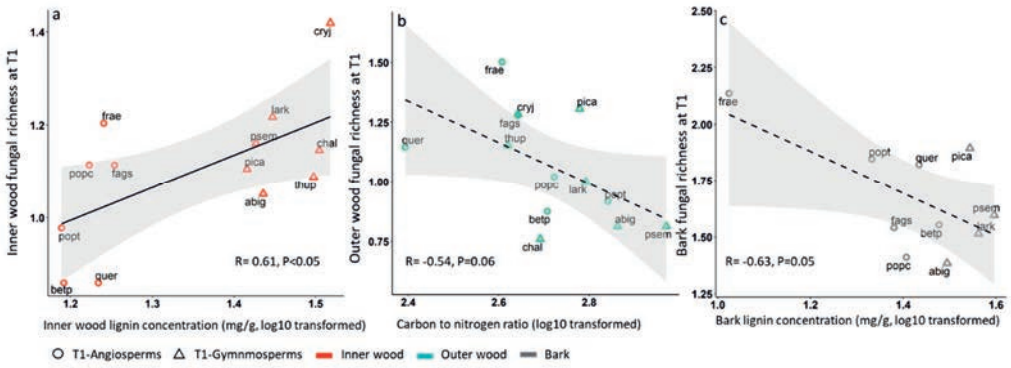


Fig. 4.3 Relationship between initial stem traits and mean fungal richness after one year (T1) of stem decay in a) inner wood (orange), b) outer wood (blue), and c) bark (grey). Data of gymnosperm species are shown as triangles and angiosperm species are shown as circles. The traits shown here are the most strongly correlated to fungal richness (Table 4.2). Solid lines indicate significant correlation, whereas dashed lines indicate marginally significant correlations (P<0.1). 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 & b, whereas only 10 tree species were available to collect bark samples and shown in panel c.

4.3.2 | Fungal community composition

Among the 1466 (814) ASVs detected at T1 and T4 (number between parentheses), 555 (391) ASVs were assigned to specific ecotypes (described in the method section). A total of 259 (232) ASVs belonged to saprotrophic fungi consisting of 80 (93) white-rot fungi, 8 (35) brown-rot fungi, 7 (5) soft-rot fungi, and 164 (97) undefined saprotrophic fungi. Additionally, 117 (50) fungi belonged to pathotrophs, 42 (33) to symbiotrophic fungi, and 137 (77) fungi had multiple trophic modes. The relative abundance of these fungal ecotypes differed among tree species and decay time (i.e., T1 & T4), but no clear pattern was found among stem compartments in terms of fungal ecotypes (Fig. 4.4); after one year of decay, the mean relative abundance of pathotrophs was high in angiosperm species (22.2%), while gymnosperm species were dominated by white-rot fungi (54.0%). Pathotrophs tended to disappear over time (from 14.1% at T1 to 2.5% at T4) while brown-rot fungi increased over time (from 0.1% at T1 to 11.0% at T4). Overall, most samples were dominated by saprotrophic fungi, especially for white-rot

fungi, and the abundance of brown-rot fungi increased over succession. Similar results were found when we used the data with Centered-log ratio (CLR) transformation, shown in Fig. S4.4. We found that most samples were dominated by saprotrophic fungi.

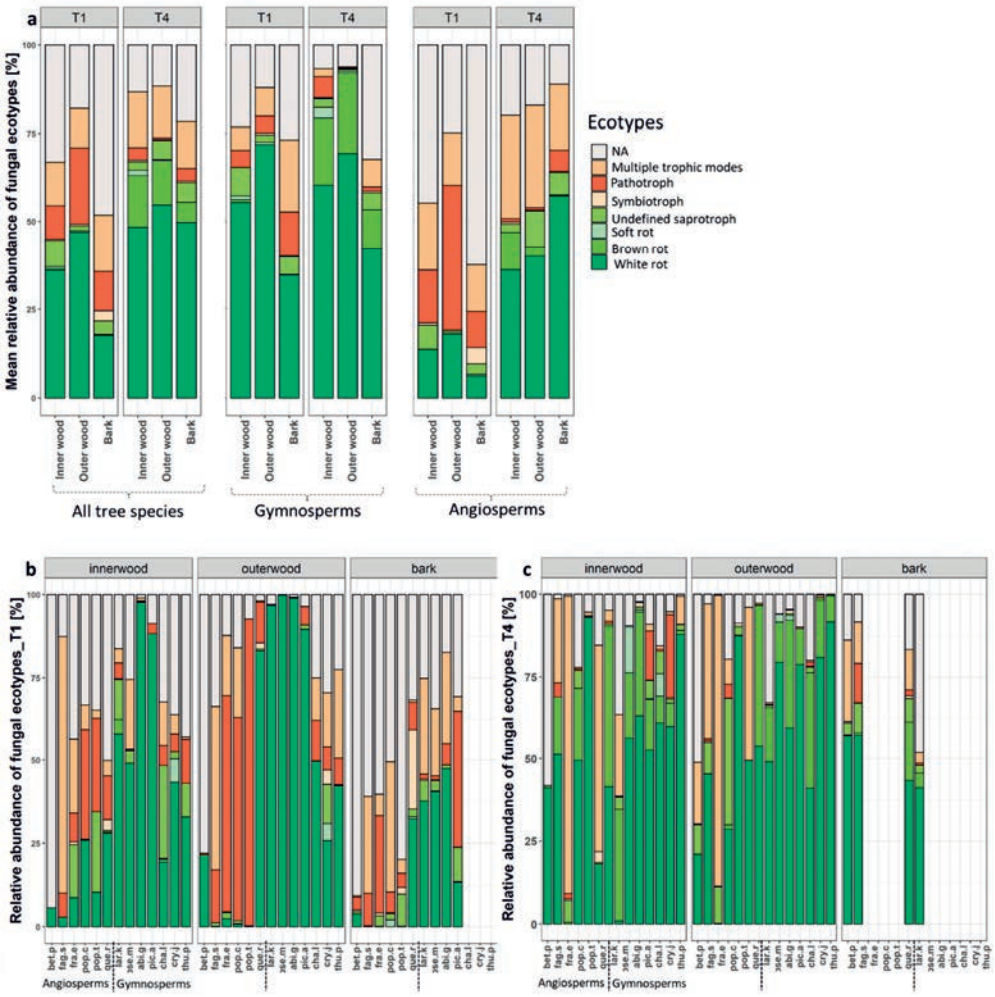


Fig. 4.4 Mean relative abundance of different fungal ecotypes (fungi with multiple trophic modes, pathotrophs, symbiotrophs, undefined saprotrophs, soft-rot, brown-rot, white-rot). The abundance is given for three stem compartments (inner wood, outer wood, and bark) of 14 temperate tree species after (a) one year (T1) and (b) four years (T4) of decay. For tree species abbreviations see Table 4.1.

Principal component analyses showed that fungal composition differed among the three main stem compartments (inner wood, outer wood, and bark) and across tree species. 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) (Fig. 4.5a-b). After one year of decay, the first PCA axis ran mostly from the outermost stem part (bark) to the innermost stem part (inner wood). Inner wood tended to hold distinct fungal communities from those in outer wood and bark at T1 (Fig. 4.5a), but such differences disappeared at T4 (Fig. 4.5b). Gymnosperm and angiosperm species tended to hold different fungal communities since they were split by the first axis at T1 (Fig. 4.5a) and by the second axis at T4 (Fig. 4.5b), which ran from gymnosperm species (shown as triangles) to angiosperm species (shown as circles). 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. 4.5c, whereas no convergent succession pattern was found for angiosperm species but fungal communities shifted along the second PCA axis over time (Fig. 4.5d). A principal component analysis that combined all tree species, stem compartments, and different decay time is provided in Fig. S4.5.

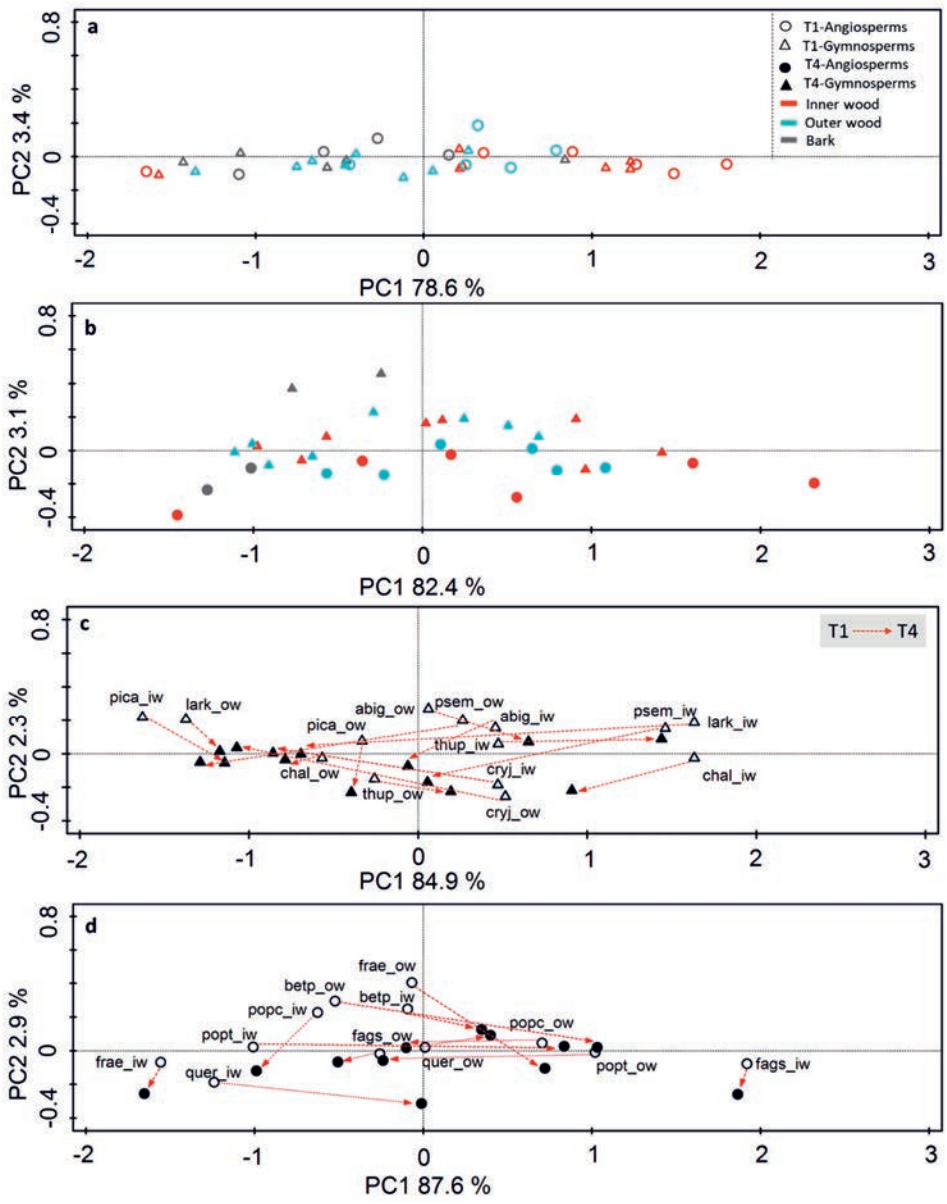


Fig. 4.5 Principal component analyses of fungal community composition across different stem compartments of angiosperm species (circles) and gymnosperm species (triangles) at (a) T1 (open symbols) and (b) T4 (filled symbols). Different colours indicate different stem compartments; inner wood (orange), 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).

4.3.3 | Factors affecting 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 associated with accessibility (i.e., conduit diameter, fraction, and wall thickness), food source of fungi (i.e., N, P, C, and lignin), and physical defence (i.e., wood and bark density, bark punch resistance, and bark thickness) were important factors regulating fungal community composition in decaying stems (Table 4.3, Fig. S4.5). Specifically, tree major taxa significantly affected fungal composition of all stem compartments in both decay periods (except outer wood at T4). The importance of traits for wood samples shifted over time; traits associated with accessibility (i.e., conduits) were important at T1, while lignin/cellulose became the most important driver of fungal community composition at T4. Nitrogen was a more important driver of variation in fungal community composition than P, since N had a significant effect on all three stem compartments, whereas P only showed a significant effect on bark fungal communities. After one year, traits could better explain variation in fungal composition of the bark (30.2%) than fungal composition of the inner wood (11.8%) or outer wood (25.4%), and the same applied to T4. Traits associated with nutrients and physical defence significantly affected bark fungal communities.

Table 4.3 Results of a redundancy analysis (RDA), showing the relationship between fungal community composition and stem traits based on a forward selection procedure. Significance was assessed with 999 permutations. RDAs were conducted for two times (T1 & T4) and three stem compartments (inner wood, outer wood, and bark) separately. The percentage explained variation is shown.

Factors	**Inner wood**		**Outer wood**		**Bark**
	T1	**T4**	**T1**	**T4**	**T1**
Tree major taxa	5.4	2.7	7.7	-	5.2
Conduit fraction	2.7	-	-	-	.
Conduit diameter	3.1	-	4.0	-	.
Conduit wall thickness	-	2.6	-	-	.
Ray fraction	3.2	-	5.3	-	.
N	-	4.5	2.6	3.7	3.9
P	-	-	-	-	4.0
C	2.8	-	4.7	-	3.3
Lignin/cellulose	-	4.8	-	5.1	7.1
Phenols	-	3.7	-	-	-
pH	-	-	5.2	3.2	-
Wood density	-	-	4.0	-	.
Bark density	.	.	.	.	4.1
Bark punch resistance	.	.	.	.	4.0
Bark thickness	.	.	.	.	3.8

4.4 | DISCUSSION

We evaluated how fungal diversity and community composition vary amongst stem compartments and tree species, and how this is driven by stem traits. Tree species and stem compartments differed in stem accessibility, nutrient levels, and physical-chemical defence (Fig. S4.3) which relates to differences in fungal diversity and community composition (Fig. 4.3, Table 4.2 & 4.3) (Leonhardt et al. 2019, Lee et al. 2020). Across tree species, fungal richness in the inner wood increased with lignin concentration, which may because of the differences between lignin characteristics in Gymnosperms and Angiosperms. Fungal composition differed strongly between gymnosperm and angiosperm species, and was especially in T1 initially driven by traits related to accessibility of wood and bark defence, and later by resource quality (the lignin/cellulose ratio).

4.4.1 | Fungal alpha diversity varies across tree species and compartments, and is regulated by different stem traits.

We hypothesized that fungal richness and Shannon diversity vary amongst stem compartments and tree species. As expected, bark had a higher fungal diversity than wood (Fig. 4.2), likely because it is the outermost part of a tree and more exposed to fungal colonization. Additionally, bark has a highly complex heterogeneous structure consisting of periderm, cortex and the (secondary) phloem (Srivastava 1964, Corder 1973, 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 (i.e., N & P) compared to wood tissues (Table 2.2, Chapter 2), thus providing more food sources to microorganisms and therefore resulting in a higher fungal species richness (Weedon et al. 2009, Bebber et al. 2011, Kazartsev et al. 2018). Additionally, a higher diversity of fungal species can be brought to bark through a diversity of bark beetles (Stokland et al. 2012). Fungal richness was similar for inner wood and outer wood especially in T4. Similar fungal richness of inner wood and outer wood was also found after a 6 years decay experiment in Germany with 13 tree species (Leonhardt et al. 2019). Sapwood has higher nutrient levels, than inner wood and heartwood-forming species have. Moreover inner wood is less permeable and hence accessible and is chemically protected with extractives (Noll et al. 2016, Yang et al. 2021). Yet, the 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. Moreover, wood moisture

content, which in the living tree and hence in the initial decay stage is generally lower in the heartwood of gymnosperm species than in the inner and heartwood of angiosperm species (Tsoumis 1991), might explain differences in fungi diversity between species.

Variation in fungal alpha diversity can be explained by different stem traits, but such effect can vary between stem compartments and change with decay time. Remarkably, fungal richness and Shannon diversity index in inner wood were found to be higher in heartwood forming species than non-heartwood forming species (Table 4.2). Interestingly, tree species with decreased accessibility (i.e., conduit diameter) had higher fungal diversity in inner wood. The reason could be that gymnosperm species have narrow tracheids compared to the large vessels in angiosperm species, but their higher amount of lignin can provide more food resources for fungi. Outer wood fungal richness was positively correlated with phosphorus concentration at T1 and positively correlated with C/N ratio at T4 (Table 4.2, Fig. 4.3, Fig. S4.3), and bark fungal richness at T1 tended to increase with higher nitrogen concentrations but decreased with cellulose concentrations. Our findings indicated that the main food sources for fungi inhabiting in sapwood and bark changed over time, with N and P serving as the main food source at a early decay stage whereas carbon sources become more important at a later decay stage. Macronutrients such as N and P, and carbon compounds as available from lignin and cellulose are important for fungal growth and reproduction (Lee et al. 2020). Fungi have different food sources and may specialize for cytoplasm, i.e., living cells, as source of nutrients or cell walls as source of carbon (Stokland et al. 2012). Cytoplasm is rich in proteins, lipids and starch, and represents a high-quality food source that is easy to digest for e.g., blue stain fungi. Cell walls are rich in lignin and cellulose and provide an abundant carbon source for fungi that are specialized in degrading cellulose (i.e., brown-rot fungi), or both cellulose and lignin (i.e., white-rot fungi). In the early decay stage, fungi living on soluble sugars, starches, lipids and proteins (e.g., blue stain fungi) invade the wood mainly through the ray parenchyma cells that transport various assimilates from the inner bark to the sapwood (Schweingruber and Börner 2018). When wood decay proceeds, white-rot fungi become more important and attack the cell walls (Tura 2016) to break down lignin and cellulose, which are important carbon sources. Another study suggests that this switch towards dead wood acting as a critical carbon source may already occur two years after decay: samples from *Pinus massoniana* had higher C/N ratio and lignin concentration, and therefore a higher fungal richness than samples from *Schima superba* (Purahong et al. 2017).

During the first year of decay, wide conduits and parenchyma are thought to facilitate fungal access to wood (Cornwell et al. 2008, Zanne et al. 2015). Yet, in our study in the first

year, and especially the fourth year, conduit diameter and ray fraction were negatively related to fungal richness in the inner wood (Table 4.2). Probably this is a 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 but one (*Quercus robur*) lack heartwood. As heartwood forming species have higher fungal diversity (Table 4.2), this may explain our counterintuitive result.

In sum, fungal alpha diversity varied across tree species and stem compartments, and such variation can be explained by different stem traits. Fungal diversity patterns are explained by tree chemical traits related to resource quality, and not by anatomical traits related to accessibility. At the initial decay stage high nutrient concentrations in sapwood and low cellulose concentrations in bark increase fungal diversity, whereas the presence of heartwood in the inner wood increases fungal diversity especially in later decay stages.

4.4.2 | 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. 4.4). Angiosperm species were initially dominated by pathotrophs, possibly because these pathotrophic fungi primarily occur in living trees with wounds. In angiosperm species, these pathotrophs may stay active during early decay because angiosperm species have a higher proportion of ray parenchyma (Fig. 2.3 in Chapter 2), 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) (Campbell 1985, Anthony et al. 2017). The possible reason that gymnosperm species, in contrast, were dominated by saprotrophs at T1 is that most gymnosperm species have an effective defence system with resin ducts that inhibit the colonization of fungal communities (Franceschi et al. 2005, Stokland et al. 2012). Moreover, the endophytic and pathogenic fungi in freshly cut stems of gymnosperm species may be rapidly replaced by saprotrophic fungi. This interpretation is supported by a former study, in which *Larix* tree stems were dominated by endophytic and pathogenic fungi at the time of tree cutting, but the abundance of these fungi were largely diminished after a year of decay (van der Wal et al. 2016). We found that most samples were dominated by saprotrophic fungi, especially for white-rot fungi (Fig. 4.4). White-rot fungi can decompose the recalcitrant lignin

(Blanchette 2000), thus facilitating the establishment of brown-rot fungi which can only degrade cellulose and hemicellulose. This agrees with our observation that the brown-rot fungi increased in abundance over time, which is consistent with results from an earlier study too (van der Wal et al. 2013).

We hypothesized that tree species and stem compartments vary in their physical-chemical traits, leading to different substrates and different fungal community composition. In accordance with the findings of a fungal fruiting body survey on a similar set of species (Chapter 3), we found that gymnosperm and angiosperm species held different fungal communities in different stem compartments in an early decay stage (T1 in Fig. 4.5a & b). Inner wood differed in fungal community compared to outer wood and bark (Fig. 4.5a). Other studies also showed that heartwood and sapwood of *Larix* and *Quercus* trees had distinct fungal communities after one year of decay (van der Wal et al. 2016). Heartwood is characterized by high concentrations of extractives which can only be degraded by fungal specialists (Scheffer 1966, Stokland et al. 2012), which results in distinct fungal communities between inner and outer wood in heartwood forming species. Such differences between inner wood and outer wood became weaker however or even disappeared over time (Fig. 4.5b) (van der Wal et al. 2016, Leonhardt et al. 2019). Perhaps, a combination of exhaustion of the sugar and protein resources and creation of a more homogeneous substrate due to the enzyme activity of fungal decomposers (Witkamp 1966) may lead to a more homogeneous fungal community composition especially in the outer wood, but also in inner wood and bark over time. Moreover, there were clear successional shifts in fungal species composition, but in different directions for tree species in the two major taxa (Fig. 4.5); fungal communities in gymnosperm species tended to converge from T1 to T4 (Fig. 4.5c), while in angiosperm species shifted along the second PCA axis over time (Fig. 4.5d). Similarly, in the fungal fruiting body study of the thesis (Chapter 3), we found distinct fungal communities between two tree major groups, which converged in later stages of decay.

In conclusion, there was a clear successional shift in functional group composition 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 species showed different fungal successional trajectories.

4.4.3 | Stem traits have significant afterlife effects on modulating fungal community composition

We found that stem traits vary across tree species and stem compartments, and have significant afterlife effects on modulating dead wood fungal communities (Table 4.3; Fig. S4.3). The RDA showed that tree major taxa and stem traits associated with accessibility (i.e., conduit diameter, fraction, and wall thickness) (Cornwell et al. 2008, Zanne et al. 2015, Lee et al. 2020), physical defence (i.e., wood and bark density, bark punch resistance, and bark thickness) (Purahong et al. 2016, McDonald et al. 2017), nutrients (Lee et al. 2019), and carbon food sources (e.g., C, N, lignin) (Rajala et al. 2010, 2012, Hoppe et al. 2016) were important factors regulating fungal community composition in decaying stems. Remarkably, nitrogen concentration showed a significant effect on the fungal community composition in different stem compartments at both decay periods (T1 and T4), indicating its important role on fungal communities' assembly. Other studies in a natural, beech-dominated, forest also found that nitrogen was the key driver driving fungal community composition in deadwood (Baldrian et al. 2016, Purahong et al. 2016). Nitrogen is an important resource for fungi living on nutrients and sugars (e.g., blue stain fungi) (Stokland et al. 2012). Nitrogen is also a major constituent of the proteins that play an important role in the synthesis and activities of degrading enzymes (Baldrian et al. 2016); increased nitrogen content can significantly affect fungal community composition and increase the activities of hemi-cellulase and cellulase, which may therefore facilitate the decay rate of dead wood (Van Der Wal et al. 2007). Gymnosperm species and angiosperm species differed strikingly in their trait values (Chapter 2) and therefore also in their fungal composition for stem compartments (Kubartová et al. 2012) and decay stages (cf. Chapter 3), even when the other trait effects were accounted for (Table 4.5). The distinct fungal communities suggest a long adaptation between major plant lineages and their fungi partners, or due to the structure and the origin of the species in the two groups; most of the gymnosperm species tested in our study are heartwood forming species and are exotic, and therefore hold distinct fungal communities compared to angiosperm species.

The effects of stem traits on fungal communities varied over succession; in the early decay stage, fungi living on nutrients and sugars enter the wood mainly through the rays and therefore traits associated with accessibility play an important role at T1. When wood decay advanced the lignin to cellulose ratio became the most important factor explaining the variation in fungal communities. Similarly, lignin concentration contributed significantly in shaping fungal community structure in *Picea* deadwood (Hoppe et al. 2016). In our study white-rot

fungi and brown-rot fungi became abundant after four years of decay (Fig. 4.4). White-rot fungi can degrade recalcitrant lignin due to their ligninolytic extracellular oxidative enzymes, meanwhile they may also degrade cellulose or leave large amounts of white-colored cellulose or hemicellulose along with the degraded lignin fragments. However, brown-rot fungi are unable to degrade lignin, they will circumvent the lignin barrier and selectively degrade the cellulose and hemicellulose by producing oxalic acid and chelators (Goodell 2003, Abdel-Hamid et al. 2013). The lignin to cellulose ratio therefore affects the relative balance between these two fungal groups, and hence modulates the fungal community composition and structure.

In sum, stem traits associated with accessibility, food source, and physical defence regulated fungal community composition and their importance shifted over succession; traits associated with accessibility (i.e., rays and conduits) were important in the early decay stage, whereas lignin/cellulose ratio became most important during the later decay stage.

4.4.4 | Conclusions

In this chapter, we used next generation amplicon sequencing to assess fungal communities in decaying stems. Compared to the fungal fruiting body survey applied in chapter 3, this method allows to capture the whole fungal community and compare the fungal communities inside the tree logs. Overall, we found that fungal alpha diversity and community composition varied across tree species, and specifically between gymnosperm and angiosperm species, and stem compartments. Such variation was significantly determined by different stem traits; bark has significantly higher fungal richness compared to wood samples, implying its important role in forest biodiversity. Stem traits associated with accessibility, resource for fungi, and physical defence were important factors regulating fungal alpha diversity and composition in decaying stems. The afterlife effects of stem traits changed over time; stem nutrients and accessibility significantly modulated fungal composition at the initial decay stage, whereas lignin promoted fungal alpha diversity and the balance between lignin and cellulose had profound effects on fungal community composition after four years of decay. When decay proceeds, stem quality will be altered by the exhaustion of easy decomposable resources and enzyme activities of multiple decomposers which strongly affects the composition of the fungi community. Therefore, to better understand fungal succession, it would be good to not only use living stem traits but also to include dead stem traits – which were not included in our approach - at each decay stage, and to evaluate the enzyme activities that reflect the activity of different fungal decomposers (cf. Noll et al. 2016). By doing so, we can gain a better understanding of how

various factors jointly determine fungal diversity and composition in dead stems, and then further test their cascading effects on dead wood decomposition rate which is related to carbon and nutrient cycling of ecosystems.

ACKNOWLEDGEMENTS

The authors thank many participants of the LOGLIFE Team in various years for help with setting up, maintenance and harvesting. The Schovenhorst estate and Staatsbosbeheer Flevoland are very hospitable and helpful with establishing and in hosting LOGLIFE throughout the experimental period. Financial support was provided by the Nora Croin Michielsen Fonds, and the Chinese Scholarship Council (no. 201706910085).

SUPPLEMENTARY MATERILS

Table S4.1 Basic information of all measured traits for inner wood, outer wood and bark of 14 tree species with their name, abbreviation, major phylogenetic group they belong to, collection site, and wood structure.

Stem compartments	Stem traits	Abbreviation	Unit
Inner wood (i.e., heartwood if present)	Conduit fraction	Con_f_iw	%
	Conduit diameter	Con_d_iw	μm^2
	Conduit density	Con_D_iw	cm^{-2}
	Conduit wall thickness	Con_t_iw	µm
	Conduit wall thick/radius	Con_t/r_iw	µm/ µm
	Ray fraction	Ray_f_iw	%
	Nitrogen concentration	N_iw	%
	Phosphorus concentration	P_iw	%
	pH	pH_iw	NA
	Phenol concentration	Phenol_iw	%
	Tannin concentration	Tannin_iw	%
	Wood density	Wd_iw	g/cm^3
	Carbon concentration	C_iw	%
	Carbon/nitrogen	C/N_iw	NA
	Carbon/phosphorus	C/P_iw	NA
	Nitrogen/phosphorus	N/P_iw	NA
	Lignin concentration	Lignin_iw	%
	Cellulose concentration	Cellulose_iw	%
	Lignin/cellulose	Lign/cellu_iw	NA
Outer wood (i.e., sapwood)	Conduit fraction	Con_f_ow	%
	Conduit diameter	Con_d_ow	μm^2
	Conduit density	Con_D_ow	cm^{-2}
	Conduit wall thickness	Con_t_ow	µm
	Conduit wall thick/radius	Con_t/r_ow	µm/ µm
	Ray fraction	Ray_f_ow	%
	Nitrogen concentration	N_ow	%
	Phosphorus concentration	P_ow	%
	pH	pH_ow	NA
	Phenol concentration	Phenol_ow	%
	Tannin concentration	Tannin_ow	%
	Wood density	Wd_ow	g/cm^3
	Carbon concentration	C_ow	%
	Carbon/nitrogen	C/N_ow	NA
	Carbon/phosphorus	C/N_ow	NA
	Nitrogen/phosphorus	N/P_ow	NA
	Lignin concentration	Lignin_ow	%
	Cellulose concentration	Cellulose_ow	%
	Lignin/cellulose	Lign/cellu_ow	NA
Bark	Nitrogen concentration	N_b	%
	Phosphorus concentration	P_b	%
	pH	pH_b	NA
	Phenols	Phenol_b	%
	Tannins concentration	Tannin_b	%
	Bark density	Bark density	g/cm^3
	Carbon concentration	C_b	%
	Carbon/nitrogen	C/N_b	NA
	Carbon/phosphorus	C/P_b	NA
	Nitrogen/phosphorus	N/P_b	NA
	Lignin concentration	Lignin_b	%
	Cellulose concentration	Cellulose_b	%
	Lignin/cellulose	Lignin/cellu_b	NA
	Bark punch resistance	Bark punch resistance	N
	Total bark thickness	Total bark thickness	cm
	Inner bark thickness	Inner bark thickness	cm
	Outer bark thickness	Outer bark thickness	cm

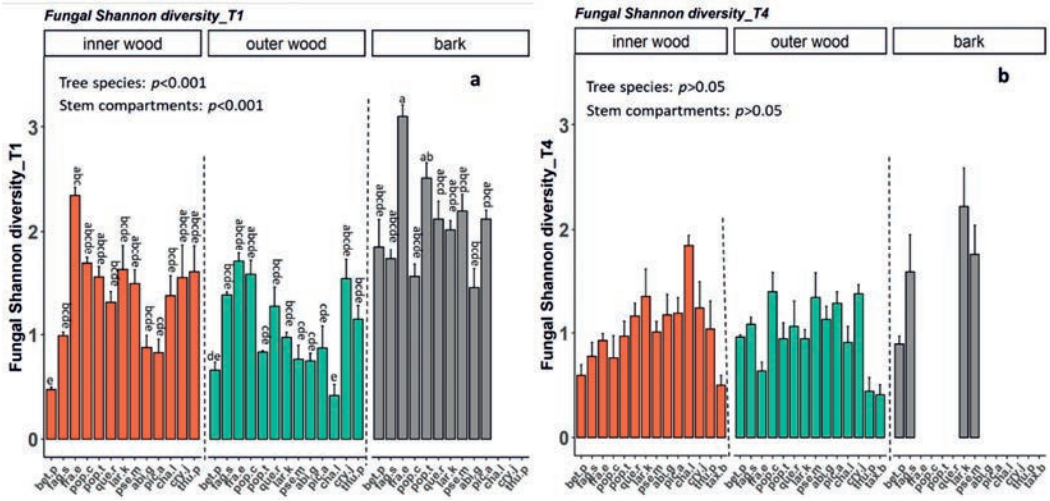


Fig. S4.1 Shannon diversity index of fungal communities. Bar plots illustrating variation in fungal Shannon diversity index values (a, b) among different stem compartments of 14 tree species at two decay periods: T1-decay for one year (a); T4-decay for four years (b). Bars show the mean of three (bark at T4) or four replicates (others) per species, the error bars correspond to standard errors of the mean. Bar colours indicate different stem compartments; inner wood (orange), outer wood (green), and bark (dark grey). Results of two-way ANOVA with two factors (Tree species & stem compartments) are shown in the upper left. Bars that do not share similar letters are significantly different among pairwise comparisons (Tukey's HSD: $P<0.05$).

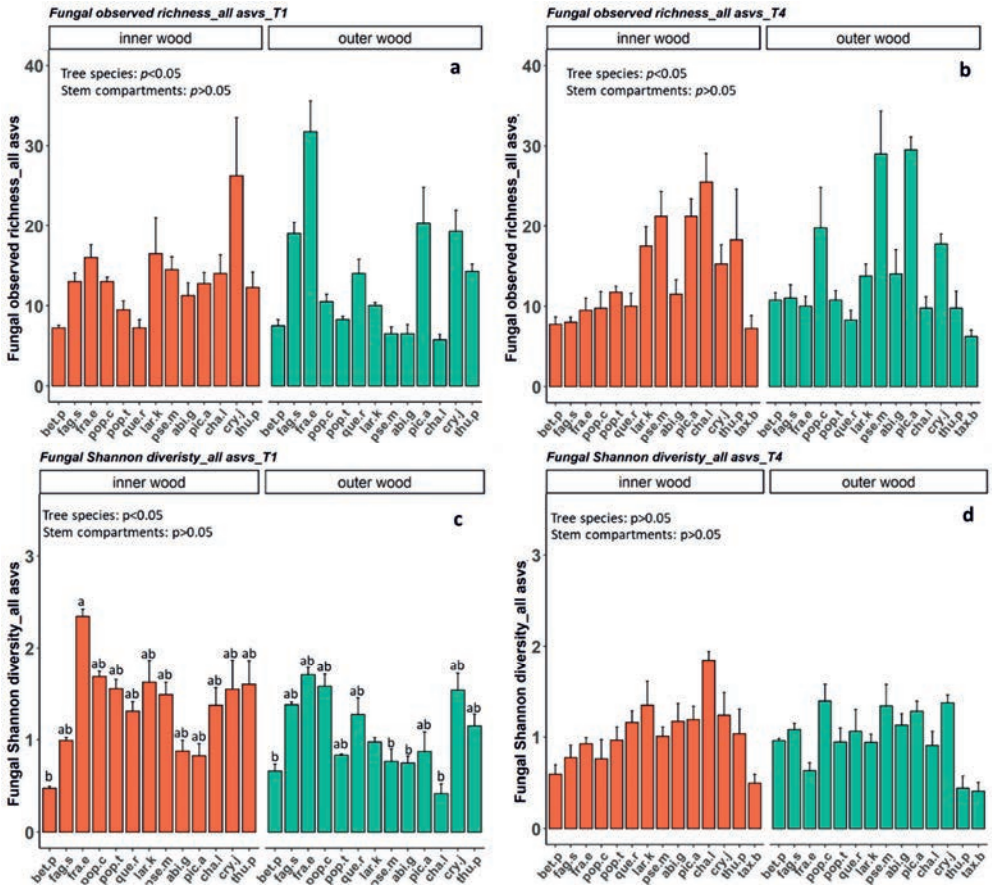


Fig. S4.2 Richness and Shannon diversity index of fungal communities. Bar plots illustrating variation in fungal richness (a, b) and Shannon diversity index values (c, d) among different stem compartments of 14 tree species at two decay periods: T1-decay for one year (a, c); T4-decay for four years (b, d). Bars show the mean of four replicates per species, the error bars correspond to standard errors of the mean. Bar colours indicate different stem compartments; inner wood (orange) and outer wood (green). Results of two-way ANOVA with two factors (Tree species & stem compartments) are shown in the upper left. Bars that do not share similar letters are significantly different among pairwise comparisons (Tukey's HSD: $P<0.05$).

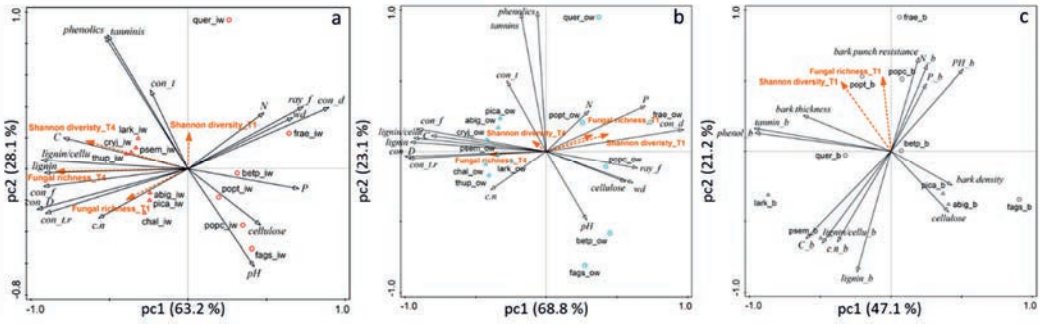


Fig. S4.3 Principal component analysis showing the associations among different physical-chemical traits of three stem compartments; inner wood (a), outer wood (b), and bark (c). Fungal richness and Shannon diversity index of different stem compartments were detected after one (T1) and four (T4) years' decay and plotted as supplementary variables. Note: given the bark fungal richness and Shannon diversity index is available for ten tree species at T1 but available for most of the tree species at T4, bark PCA was only shown for T1.

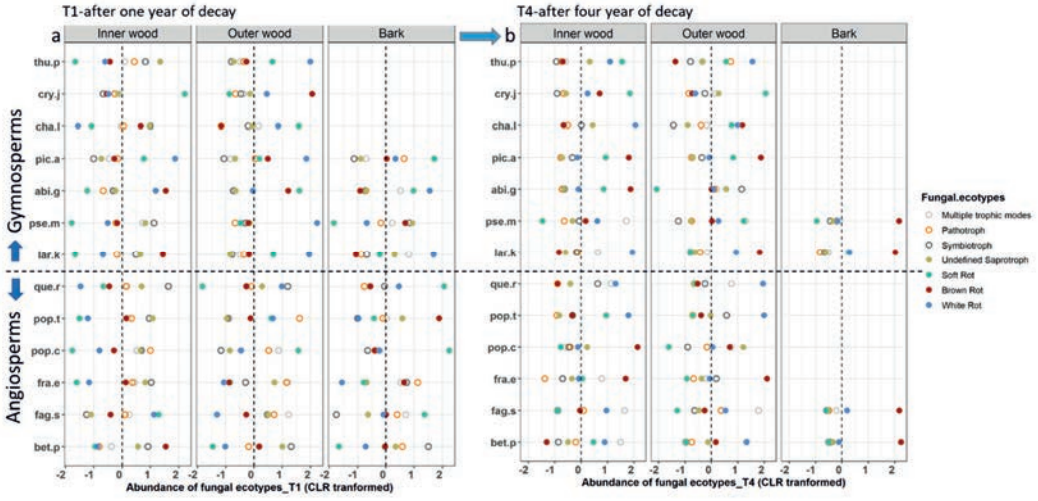


Fig. S4.4 Mean abundance of different fungal ecotypes (fungi with multiple trophic modes, pathotrophs, symbiotrophs, undefined saprotrophs, soft-rot, brown-rot, white-rot). The abundance is given for three stem compartments (inner wood, outer wood, and bark) of 14 temperate tree species after (a) one year (T1) and (b) four years (T4) of decay. For tree species abbreviations see Table 4.1.

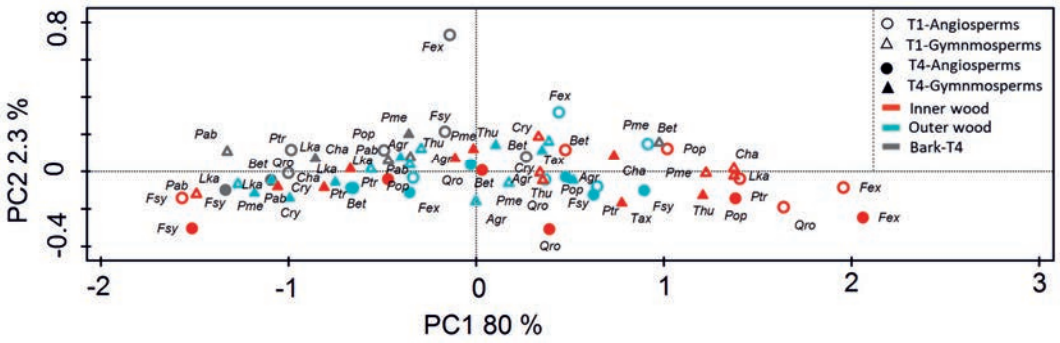


Fig. S4.5 Principal component analyses of fungal community (ASV) composition across different stem compartments of 14 tree species at two decay periods. Data of gymnosperm species are shown as triangles and angiosperm species are shown as circles. Data at T1 are shown in open symbols, while data at T4 are shown in solid symbols. Different colours indicate different stem compartments; inner wood (orange), outer wood (blue) and bark (grey).

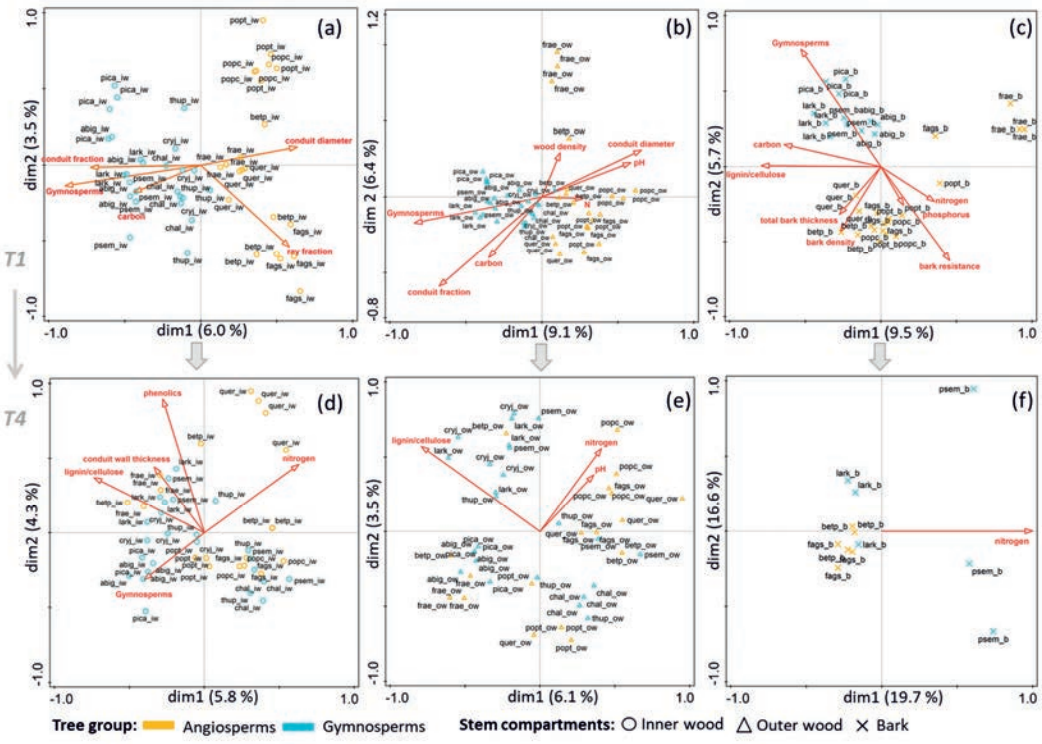


Fig. S4.5 Redundancy Analysis (RDA) showing how fungal community composition in different stem compartments at two decay periods is affected by tree major taxa (Gymnosperms vs. Angiosperms) and different physical-chemical traits. Specifically, inner wood, outer wood, and bark at T1 (after one year's decay) are shown in (a), (b), and (c) separately; inner wood , outer wood, and bark at T4 (after four years' decay) are shown in (d), (e), and (f) separately. Different colours indicate different tree groups; angiosperm species are coloured in orange while gymnosperm species are coloured in blue. Different symbols indicate different stem compartments; inner wood (circle), outer wood (triangle) and bark (cross). Only significant factors ($P < 0.05$) are shown, and the detailed statistic outputs are shown in Table 4.3.

CHAPTER 5

General discussion

5.1 | Introduction

Temperate tree species vary largely in stem traits, which facilitates species coexistence and diversity, and impacts tree- and ecosystem functioning (Villéger et al. 2008, Sterck et al. 2011) and increases the stability and adaptability of ecosystems under climate change (Yachi and Loreau 1999). Moreover, these stem traits have profound tree afterlife effects (Cornelissen et al. 2012, Zuo et al. 2016, Kahl et al. 2017). A large part of forest carbon and to a lesser extent nutrients is locked up in tree stems, and once trees die the carbon and nutrients becomes slowly available during the process of decomposition (Harmon et al. 1986, Cornwell et al. 2009). Dead wood is also an important source of biodiversity; it provides habitat and substrate for different animals and microorganisms (Moll et al. 2021). Dead wood decomposition is the outcome of complex interactions between stem traits and different decomposers (Weedon et al. 2009, Cornelissen et al. 2012), which are affected by abiotic drivers, such as temperature, moisture, light and soil conditions. Fungi are the only decomposer that can substantially decompose major polymers (i.e., cellulose, hemicellulose and lignin) of dead wood (van der Wal et al. 2013). Stem traits determine the accessibility and substrate quality of dead stems for different fungal and other decomposers, and therefore determine the wood decomposition rate (Fukasawa et al. 2009, Rajala et al. 2012, Baldrian et al. 2016). When it comes to the positive impact of deadwood on biodiversity, up to now most emphasis has been given to the quantity rather than the quality of dead wood in the forest. Wood quality refers to anatomical and chemical stem traits of tree species, which may have strong afterlife effects, by shaping the diversity and structure of wood-colonizing fungal communities, which in turn, affect wood decomposition rate and biogeochemical cycling. In this thesis, I investigated the interactions among stem traits, fungal decomposers, and environmental factors. In addition, I will show how they affect each other and jointly determine wood decomposition (see Fig. 1.2 & Fig. 5.1).

I took advantage of a long-term common garden decomposition experiment (LOGLIFE), in which logs of 14 temperate tree species have been incubated in two Dutch forests. I first quantified how tree species differ in their anatomical and chemical stem traits (*chapter 2*), then assessed how these species traits relate to the diversity and composition of fungi fruiting bodies observed on stem surfaces over 8 years of decay (*chapter 3*), and studied the fungal communities in different stem compartments (inner wood, outer wood and bark) respectively 1 and 4 year after stem incubation using ITS amplicon sequencing (*chapter 4*). Specifically, the following questions were addressed:

1) How do stem traits differ across temperate tree species and what trait trade-offs and associated plant strategies are found (*chapter 2*)?
2) How do fungal abundance, richness, and community composition as assessed by fungal fruiting body surveys depend on tree species and especially, how do fungal communities vary during the eight years of decomposition (*chapter 3*)?
3) How do fungal diversity and community composition as assessed by ITS amplicon sequencing vary amongst three different stem compartments (inner wood, outer wood, and bark) in 14 different tree species, and how is this driven by stem traits (*chapter 4*)?

In this final chapter (*chapter 5*), I firstly synthesize the main findings of previous chapters to answer my research questions. Then I integrate all components (see Fig. 1.2 & Fig. 5.1) by analysing the effects of stem traits and fungal communities on wood decomposition rates of the 14 study species. Finally, I provide suggestions for future research in forest ecosystems and provide recommendations for dead wood management.

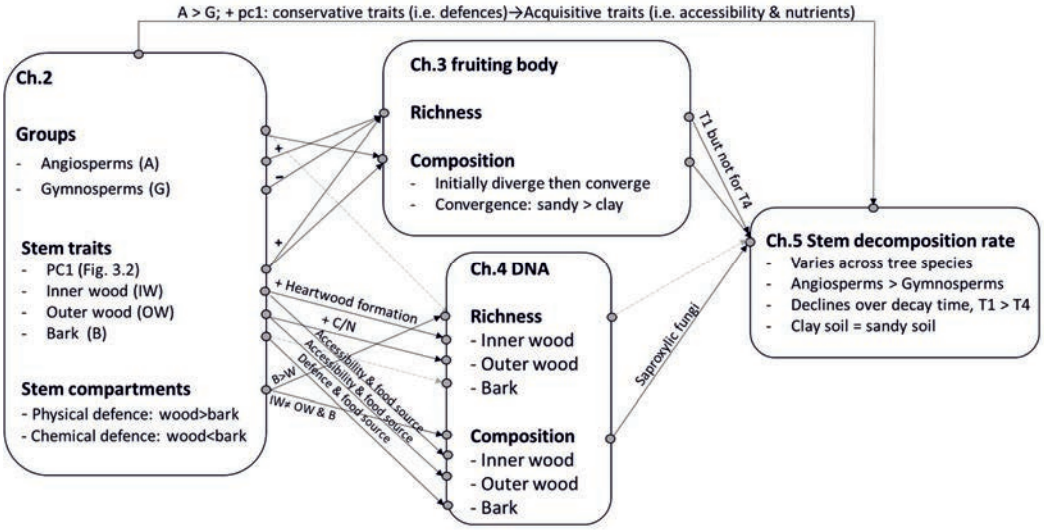


Fig. 5. 1 Conceptual diagram showing how tree species differ in their stem traits and plant strategies (chapter 2), how traits have afterlife effects by affecting the diversity and composition of fungi (chapter 3 & 4) , and how stem traits and fungi together affect wood decomposition (chapter 5). Solid lines indicate significant effects, while dashed lines indicate non-significant effects. The texts or symbols next to the arrows indicate whether the effects are positive (+) or negative (-). PC1 is obtained from Fig 3.2 with low values indicating lower conservative trait values (i.e., defences) and high values indicating acquisitive trait values (i.e., accessibility & nutrients). A: angiosperm species, G: gymnosperm species; IW: inner wood, OW: outer wood, B: bark. T1: after one year of decay, T4: after four years of decay.

5.2 | Stem traits of living trees - variation, associations, and plant strategies

In chapter 2, I tested how stem traits vary across temperate tree species and their stem compartments (inner wood, outer wood, and bark), and what trait trade-offs and plant strategies are found.

5.2.1 | Gymnosperms vs. Angiosperms

I found that most of the trait variation was explained by major taxa (38%), which could be expected from the fundamentally different wood characteristics between Gymnosperms and Angiosperms. Gymnosperms have tracheids that are narrow with low hydraulic conductance but high hydraulic safety, and they lack fibres for support and defence. Most Gymnosperms form chemically protected heartwood. In contrast, Angiosperms have vessels that can be wide and very conductive, especially in ring-porous species, and those vessels are embedded within fibres and parenchyma cells. Among temperate angiosperm trees many, especially pioneer species, lack chemically protected heartwood. For these two taxonomic groups as well as for species within those two groups, I expected that trait trade-offs determine a stem strategy spectrum running from "slow", i.e., conservative, to "fast", i.e., acquisitive, species. As expected, I found that Gymnosperms and Angiosperms occupy the ends of a slow-fast spectrum in stem traits, but also within these group, continuous trait and strategy variation was found. High hydraulic safety (e.g., small conduits, thicker conduit walls) and strong physical defence (e.g., high lignin concentration) may allow Gymnosperms to avoid freezing- and drought-induced cavitation, have persistent evergreen leaves, and occupy dry and cold, unproductive habitats. High hydraulic conductivity (e.g., large conduits) and nutrient storage (high parenchyma fraction) capacity in Angiosperms may be associated with their winter deciduous phenology and their tendency to occupy more mesic, seasonally productive habitats. It is thus no surprise that the differences between these two major taxa were at the base of explaining the largest gradients in stem trait variation across species.

5.2.2 | Stem compartments

I expected that a large trait variation would be found between different stem compartments, but that traits of wood and bark are partly coordinated because they derive from the same cambium

(Rosell et al. 2014). I found that stem traits showed distinct radial patterns from bark to innermost wood. Bark had higher concentrations of nutrients (nitrogen & phosphorus), and antifungal substances (phenols & tannins), whereas wood compartments had stronger tissues (i.e., high C/N ratio) (Fig. 5.1), indicating that bark serves as a first layer of chemical defence while wood is in general better physically defended (e.g., high lignin/cellulose ratio). Moreover, across species such reinforced bark can sometimes compensate for the weak physical strength of wood, as implied by the negative correlation between bark punch resistance and wood lignin concentration.

5.3 | Succession of fungal communities in decaying stems

In chapter 3, I show the afterlife effect of stem traits for stem-inhabiting fungi by evaluating fungal succession pattern over 8 years using fungal fruiting bodies surveys in decaying stems of 10 temperate tree species.

5.3.1 | Fungal richness depends on stem traits

I predicted that fungal richness would be higher for tree species with high nutritional quality and will initially increase over time because more nutrient sources become available during decay. At early decay stages, I observed higher fungal richness in tree species (Angiosperms and especially *Betula pendula*) with higher stem and bark concentrations of N and P. Possibly, the higher nutrient levels allow for more physiological activity and sporocarp formation (Lee et al. 2019). Moreover, stronger chemical and physical defence may impede fungal growth (Lourenço et al. 2015), as indicated by lower fungal richness found in (mainly heartwood-forming Gymnosperms) tree species with higher antifungal substance and relatively thicker cell walls (Fig. 5.1).

5.3.2 | Fungal communities vary amongst host tree species

Wood-inhabiting fungi are mainly substrate restricted (Boddy and Heilmann-Clausen 2008) and therefore I predicted that fungal species would have colonization preferences for different substrates as determined by tree species and decay time. As predicted, I found that tree species with different stem traits tend to host specific fungal communities, and especially, a clear split in fungal community composition was found between gymnosperm species and angiosperm

species (Fig. 5.1), which may be due to the strikingly difference in their stem traits as shown in section 5.2. This finding is supported by other studies that found differences in fungal communities where white-rot fungi typically colonize angiosperm species and brown-rot fungi colonized gymnosperm species (Ryvarden and Gilbertson 1993, Hatakka and Hammel 2011).

5.3.3 | Successional patterns in fungal composition

I predicted that fungal community composition would change over time and show clear succession patterns since the substrate conditions can be changed by the enzyme activities of multiple decomposers during decay (van der Wal et al. 2013, Hiscox et al. 2015). I found that fungal communities in decaying stems initially diverged and then converged in later decay stages (Fig. 5.1). This may be caused by the fact that well-dispersed generalists will firstly colonize the stems together with endophytes, and when decay proceeds, traits of decaying stems become more similar and therefore result in more similar fungal communities (Rajala et al. 2011). Such a convergent pattern in fungal communities during succession also occurs for wood-inhabiting invertebrates, which were observed for the same logs in the same forest sites as investigated in this thesis (Zuo et al. 2021). Moreover, a clear convergent succession pattern was found for fungal communities in decaying wood on sandy forest site while no obvious pattern was observed for the same species on a clay forest site. This indicates that site conditions can affect the succession patterns of fungal communities, and that succession is context-dependent.

In sum, tree species with a range in stem traits present a diversity in substrates and affect therefore the colonization by fungal communities. However, such tree-fungal interactions may change over time (Parisi et al. 2018). Compared to the long lifespan of a tree and the overall dead wood decay trajectory, succession of fungal communities occurs at relatively short time scales (within 8 years), and such a succession pattern is affected by site conditions and dynamic changes in substrate conditions during decay (Andringa et al. 2019).

5.4 | ITS amplicon sequencing detects fungal communities in decaying stem compartments

Fruiting body surveys (as applied in chapter 3, section 5.3) provide a conservative estimate of fungal communities, especially for fungal richness. To capture the whole fungal community in

decaying stems, I used advanced molecular techniques (i.e., ITS amplicon sequencing) in chapter 4 to detect microscopely invisible fungal communities within inner wood, sapwood, and bark after one and four years of decay.

5.4.1 | Alpha diversity and its determining factors

I predicted that fungal richness and alpha diversity (estimated with the Shannon diversity index) in decaying wood would vary across tree species and between stem compartments due to in stem trait differences, leading to different substrates over time (van der Wal et al. 2016, Leonhardt et al. 2019, Lee et al. 2020). As expected, I found that fungal alpha diversity varied across tree species and that bark samples had a generally higher fungal diversity (Fig. 5.1). This higher fungal diversity in bark is probably related to its higher nutritional values, highly complex, heterogeneous structure, and the fact that bark is most exposed to fungal infestation (Corder 1973, Harmon et al. 1986). Moreover, I found that at the first year of decay, outer wood and bark tended to have higher fungal richness and diversity which were associated with their larger conduit diameters and higher nutrient levels, but after four years of decay, fungal diversity was associated with higher concentrations of carbon and lignin (Table 4.2). A possible reason could be that different fungi prefer food sources that dominate different moments in time: at initial decay stage a larger conduit size may facilitate fungal access to wood while the higher nutrient concentrations can provide more food sources for early-arriving fungi (Cornwell et al. 2008, Zanne et al. 2015). When wood decay proceeds, more white and brown rot fungi occur and attack the cellulose and lignin in the cell walls. Therefore, carbon and lignin concentrations, as important components of cell wall, become important factors and serve as food sources for these groups of fungi later during decay.

5.4.2 | Fungal composition and its determining factors

In accordance with the findings of the fungal fruiting body survey (Chapter 3, see section 5.3), Gymnosperm species and angiosperm species held different fungal communities, and within these groups, these communities tended to converge when decay proceeds, especially for gymnosperm species. Moreover, distinct fungal communities were found in inner wood compared to those in outer wood and bark (Fig. 5.1) (van der Wal et al. 2017). The reason might be that heartwood forming species require fungal communities with specialists to degrade extractives, and therefore result in distinct fungal communities. When stem decay

advanced, differences in fungal communities among stem compartments became less obvious, likely because the enzyme activity of fungal decomposers may have led to a more homogeneous substrate.

Moreover, stem traits that associated with accessibility (i.e., conduit diameter, conduit fraction, and cell-wall thickness) (cf. Cornwell et al. 2009; Zanne et al. 2015), food source of fungi (i.e., N, P, C, lignin and ray fractions) (cf. Baldrian et al., 2016; Moll et al. 2021), and physical defence (i.e., wood and bark density, bark punch resistance, and bark thickness) were important factors regulating fungal community composition in decaying stems. However, the importance of these traits for fungal composition shifted over time; traits associated with accessibility were important at early decay stage (T1). Fungi living on nutrients and sugars enter the wood mainly through the rays and enable access to the inner wood for other species. With wood decay proceeded, lignin/cellulose ratio became the most important driver of fungal community composition at T4. This finding is in line with the study of Hoppe et al. (2016), who found that a high lignin concentration can contribute in shaping fungal community composition in *Picea* dead wood. The reason may be that white-rot fungi and brown-rot fungi become abundant when decay proceeds (Fig. 4.4). They mainly consume lignin and cellulose and therefore have profound effects on regulating fungal communities.

5.5 | Methodological differences between fungal fruiting body survey and ITS amplicon sequencing

The survey of fruiting bodies is a widely used method to detect wood-inhabiting fungi (Halme et al. 2012). The formation of fruiting bodies (i.e., sporocarps) requires large energy investments and can therefore indicate how active and successful fungi species are. In addition, this method is relatively low-cost and straightforward, and thus enables large scale comparative studies (Runnel et al. 2015). However, fungal fruiting body surveys are only able to capture part of the fungal communities that are in the reproductive phase and able to form sporocarps (Abrego et al. 2016, Purhonen et al. 2017). Therefore, there is an increasing debate on whether fungal fruiting body survey is a valid method to evaluate the dynamics of wood-inhabiting fungal communities, especially for assessing fungal richness compared to fungal DNA-based methods (Runnel et al. 2015, Frøslev et al. 2019, Saine et al. 2020). Molecular techniques have the potential to detect traces of all fungi, even the non-active spores. Hence, this technique may not properly indicate which fungi are actually wood decomposers. The question remains to

what extent both methods detect similar or different species. In this chapter, I address this question by comparing fungal richness and composition detected by both methods and then provide methodological recommendations for future studies.

5.5.1 | Do the two methods show similar results for fungal richness

Fungal richness of 10 tree species (Table S3.1) obtained from both methods was compared. Across the 10 tree species, fungal fruiting body-based fungal richness and fungal DNA-based fungal richness not only largely differed in scale, but were even not significantly correlated (Fig. 5.2 a & b). Fruiting body surveys may strongly underestimate species richness and are a less accurate method compared to molecular techniques.

5.5.2 | Do the two methods show similar results for fungal composition?

To answer this question, I compared the presence/absence of fungi based on fruiting bodies and amplicon sequence variants (ASVs) using a similar multivariate DCA approach. The scores of the tree species on the first DCA axis were extracted to capture fungal community composition. The fungal composition as detected by the two methods was positively correlated, especially for decomposition after 4 years (T4) (Fig. 5.2 c & d). The reason could be that there is a community turnover during succession, which resulted in fungal communities more associated with conifer species (and heartwood forming oak) and the remaining broadleaf species. This distinction was similarly detectable by both methods. Moreover, it is also likely that the ability to detect fungi by the two methods may vary over time; more spores may have germinated over time, and fungi can have better access to unprotected or even protected (heartwood) inner wood due to hyphen expansion in the wood or through cracks or disappearance of the bark and outer wood and/or the ability to break down extractives in inner wood. Hence, both methods show stronger correlation in the detected fungal composition with increasing decomposition time and advanced decay stage.

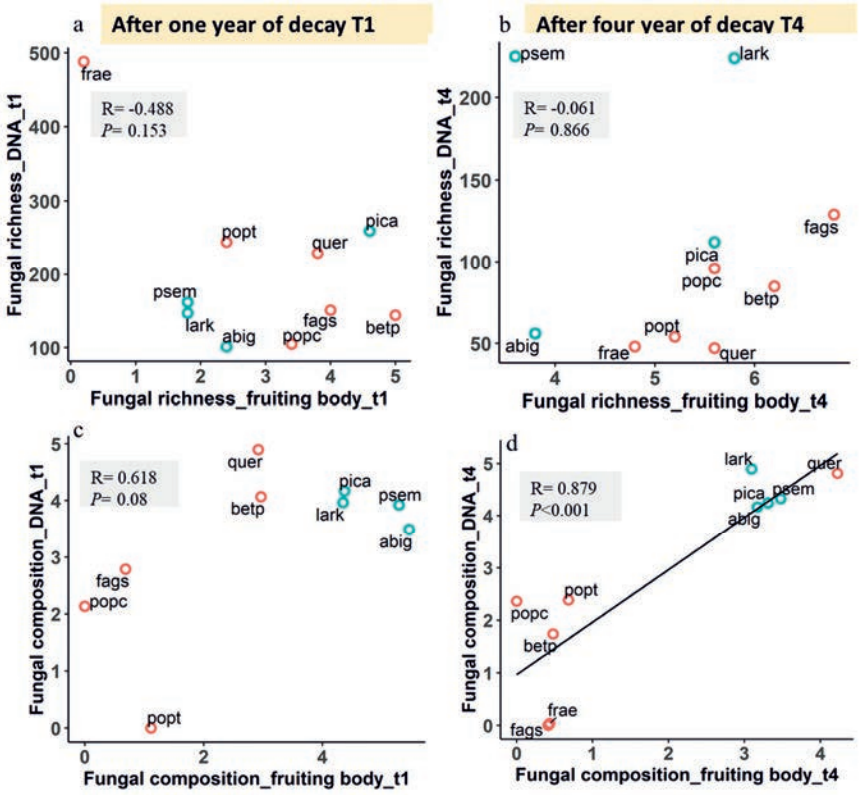


Fig. 5. 2 Pearson correlations between fungal fruiting body survey and fungal DNA-based method in terms of fungal richness (a & b) and fungal composition (c & d) on logs of 10 tree species, at two decay periods; T1: after one year of decay (a & c), T4: after four years of decay (b & d). Fungal richness_fruiting body: the number of observed fungal species in two logs per tree species; Fungal richness_DNA: the number of ASVs (i.e., amplicon sequence variants) per tree species. Fungal composition is based on the first DCA axis based on the presence-absence data. Correlation coefficients and *p*-values are shown. Solid regression lines indicate significant relations ($p<0.05$). No line is shown when there is no significant relationship ($p>0.05$). Open red dots refer to angiosperm species and green dots refer to gymnosperm species. For abbreviations of the tree species, see Table S3.1.

5.6 | How does wood decomposition vary amongst species, and to what extent can it be predicted by stem traits, fungal richness, and fungal composition?

5.6.1 | Variation in wood decomposition rate for each tree species

Wood decomposition rate for each species was calculated over a four-year period based on a negative exponential decay model (Olson 1963). The annual decay rate parameter k was obtained from $WD_t = WD_0 \exp(-kt)$, where WD_t is wood density at time t (years), and WD_0 is initial wood density at time T_{zero}. Moreover, the time to decompose 95 % ($t_{0.95}$) of the material is calculated, i.e., residence time, using the formula $t_{0.95} = 3/k$ (Harmon et al. 1986). Wood decomposition rate varied across tree species, ranging from lowest decay rates (k: 0.019 y^{-1}) and longest estimated residence time ($t_{0.95}$=159 years) for the two Gymnosperms *Taxus baccata* and *Chamaecyparis lawsoniana* to highest decay rates (0.17 y^{-1}, 0.16 y^{-1}) and shortest residence time ($t_{0.95}$=18 years, 19 years) for the two Angiosperms *Populus tremula* and *Betula pendula* (Table 5.1). Similarly, an experimental decomposition study in Germany found that decomposition rates were lowest for the Gymnosperms and fastest for Angiosperms, but for another set of species in their case: *Larix* and *Pseudotsuga* versus *Carpinus, Fagus* and *Populus*, respectively (Kahl et al. 2017). The angiosperm species included in my study had, on average, a higher decomposition rate (0.12 y^{-1}) compared to the gymnosperm species (0.06 y^{-1}), probably because Gymnosperms have higher resin content and higher proportion of recalcitrant guaiacyl units in lignin (Kahl et al. 2017). Also, the studied Angiosperm species mainly included non-heartwood forming species (except *Q. robur*), whereas most gymnosperm species (except *A. grandis*) are heartwood-forming species. The relatively slow wood decomposition rates of the studied Gymnosperms after four years could indicate that their dead stems are better for long term carbon storage in comparison to angiosperm species. Yet, the current estimates of decomposition rates and residence times were based on first four years of decay. The time is rather short in terms of the whole dead wood decay process. Moreover, the decay rate and residence time did not take into account sapwood-heartwood ratio's and associated differences in – especially initial - decomposition rates between wood compartments. It can therefore only be taken as rough estimator and needs long-term observation and further refinement through detailed analyses of dynamics in decomposition of different stem components.

Dead tree decomposition is a complex ecological process, largely regulated by a variety of abiotic and biotic drivers. Abiotic drivers such as temperature, moisture, and local site conditions (Kubartová et al. 2012, Krah et al. 2018, Müller et al. 2020). Biotic drivers are linked to stem traits (e.g., substrate quality) (Kahl et al. 2017), as well as the large community of

interacting, facilitating and competing dead wood inhabiting organisms, such as the community of bark beetles (Zuo et al. 2016) that provide access to the stem for fungal decomposers (van der Wal et al. 2013), and other organisms like bacteria (Moll et al. 2018, Kuramae et al. 2019) and mites (Moore et al. 2004). During the past decades, researchers have investigated how individual drivers affect dead tree decomposition, but how various decomposition drivers interact with each other and how these interactions contribute to wood decomposition of different tree species is still unclear (but see Kahl et al. 2017). Therefore, in this chapter, I analyze how early-stage wood decomposition rates vary across tree species, and then test and discuss below whether and how various biotic and abiotic factors determine the stem decay process (Fig. 5.1).

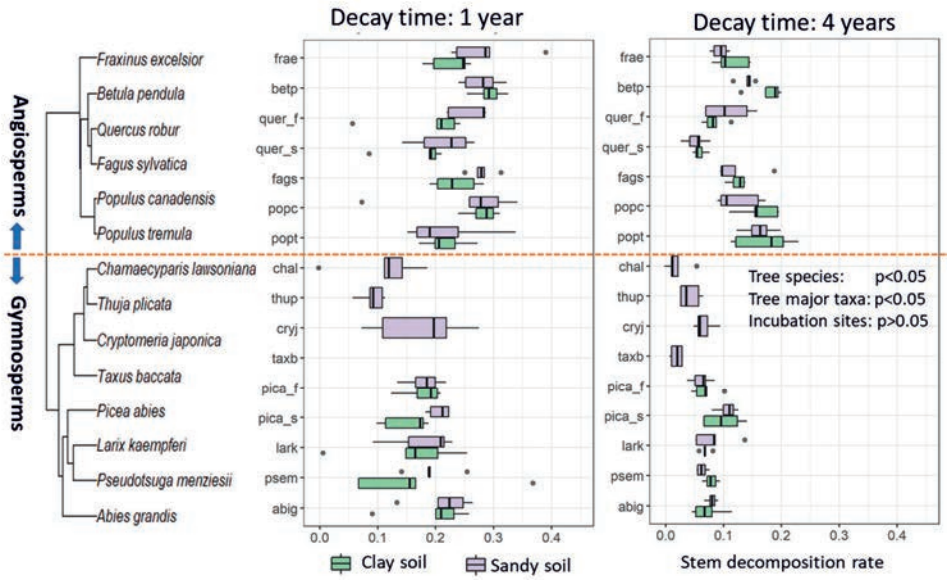


Fig. 5.3 Boxplot showing wood decomposition varies across tree species, incubation sites and decay time. Green boxes indicate decay rate of stems incubated in the clay soil site, while purples boxes indicate those in the sandy soil site. Wood decomposition rate was based on wood density loss and a negative exponential decay model, and calculated for a four-year period. ANOVAs were conducted to test if significant differences (p<0.05) in decomposition rate were found across tree species, and between two tree major taxa (Gymnosperms vs. Angiosperms), and incubation sites (clay soil vs. sandy soil).

Table 5.1 Decomposition rate and residence time ($t_{0.95}$=3/*k*) of the two major taxa (average of 8 Gymnosperms and 6 Angiosperms) and tree species (n=14; *Q. robur* and *P. abies* on respectively sandy and clay soils). *k*: the decay rate of tree species after four years of decay. The length of bars indicate the length of expected residence time of stems.

Categories	Decay rate *k*	Residence time (years)
Angiosperms	**0.121**	**28**
Gymnosperms	**0.059**	**70**
Populus tremula	0.166	18
Betula pendula	0.159	19
Populus×canadensis	0.143	21
Fagus sylvatica	0.122	25
Fraxinus excelsior	0.104	29
Picea abies-sandy	0.102	29
Quercus robur-clay	0.095	31
Abies grandis	0.076	40
Larix kaempferi	0.075	40
Pseudotsuga menziesii	0.071	42
Cryptomeria japonica	0.066	46
Picea abies-clay	0.064	47
Quercus robur - sandy	0.055	54
Thuja plicata	0.042	72
Taxus baccata	0.019	159
Chamaecyparis lawsoniana	0.019	159

5.6.2 | Which factors mainly affect early stage wood decomposition?

This thesis takes advantage of LOGLIFE project, which is a long-term common garden experiment with controlled macroclimates (e.g., temperature & precipitation) (Cornelissen et al. 2012). In this study, stems of tree species were incubated using the same experimental design in two sites with different soil types, which allows me to test the site effect. I found no significant difference in decomposition rate between the two sites with different soil types (clay vs. sandy) (Fig. 5.3), indicating that soil conditions and forest type have limited effect on stem decay rate. My study further explored the potentially determinative roles of stem traits and fungal decomposers in wood decomposition (Cornwell et al. 2008, Dossa et al. 2020). I therefore tested how stem traits, fungal richness and fungal composition individually and jointly affect the wood decomposition process in the first four years after incubation. Since saprotrophic fungi that have significant abilities to degrade organic matter are the most important wood decomposers (Moore et al. 2004), they were extracted from the entire set of detected fungi based on the molecular analysis to test their composition effects on wood

decomposition. Decomposition rate was positively associated with stem traits (Table 5.2 & 5.3, Fig. 5.4) which are associated with accessibility and permeability and negatively associated with physical protection of the wood matrix. This positive association is supported by the significant correlation between decomposition rate and PC1 (as calculated in Chapter 2), running from conservative trait values (e.g., high lignin concentration and conduit wall thickness) to acquisitive trait values (e.g., wide conduit diameter and high nutrient concentrations). This suggests that large conduit diameters together with a high nutrient level facilitate quick and easy colonization and start of decomposition of the wood matrix by fungi. Instead, a high lignin and extractive content may delay fungal colonization and hence slow down the decomposition process. This is in agreement with the result from a similar decomposition experiment with 13 temperate tree species in Germany. The later study indicated that decay rate positively correlated with fungi enzyme activities and wood nutrient concentrations (e.g., N, P), but negatively with lignin content and the presence of anti-fungal substances (e.g., phenols) (Kahl et al. 2017). Moreover, I found that the wood decay process was not only significantly regulated by the stem traits, but also the composition of saprotrophic fungi when wood decay proceeded (Table 5.3). This implies that stem traits are the main determinants for wood decomposition at the initial decay stage, but the composition of saprotrophic fungi combined with stem traits play an important role in wood decomposition at later stages.

It must be mentioned that we only measured stem traits at the beginning of the experiment, in the intact wood before incubation. Yet, as a consequence of the decomposition process, stem properties change over time, which can explain the decreasing role of those stem traits on decomposition. Moreover, the decomposition process itself may lead to convergence in stem traits across species with time (cf. Lee et al., 2020). Similarly, it is difficult to tease apart whether fungal composition drives wood decomposition, or the other way around. It is, in fact, a feedback loop where fungi change the stem substrate, causing changes in fungal community composition (Purahong et al. 2017, Lee et al. 2020). In our study, fungal diversity did not affect stem decomposition rate. However, a comparative study in Germany found both fungal diversity and beetle diversity could predict stem decomposition (Kahl et al. 2017), supposedly a larger variation in wood-inhabiting fungi may secrete wide sets of extracellular enzymes to mediate stem decay, whereas a larger variation in beetles allows for more and different access holes to stems. Interestingly, community composition of all detected fungi could not significantly predict stem decay rate (P>0.05) but community composition of saprotrophic fungi was significantly associated with decay rate (table 5.2, Fig. 5.4). The reason

could be that the fungal DNA captures different groups of fungi, and only specific groups (i.e., saprotrophic fungi) decompose the stems.

Table 5.2 Pairwise Pearson correlations between different factors and wood decomposition rate of different tree species incubated at the sandy Schovenhorst forest site. These factors are stem traits, as indicated by PC1 & 2, the scores of first two PCA axis of stem traits (Fig. 2.4); PC1 indicates a spectrum of species ranging from conservative trait values to acquisitive trait values, and PC2 from traits with high pH value to strong chemical defence (N=14). Fungal richness was based on fungal fruiting body survey (N=10), all ASVs (amplicon sequence variants) detected by a molecular technique, and saprotrophic fungi separately (N=14). Fungal community composition was described by the first two DCA axes of fungal composition. FB: fungal fruiting body, DNA: ITS amplicon sequencing. Pearson correlation coefficients and *P* values are shown. Significant correlations are shown in bold.

Factors	After one year of decay		After four years of decay	
	Coefficient (r)	*P* value	Coefficient (r)	*P* value
Stem traits				
PC1	**0.81**	**<0.001**	**0.61**	**<0.05**
PC2	-0.23	0.45	-0.42	0.14
Fungal richness				
Fruiting body	**0.69**	**<0.05**	0.45	0.19
All DNA	0.51	0.07	0.01	0.98
Saprotrophic DNA	0.18	0.56	-0.18	0.54
Fungal composition				
Fruiting body_dca1	-0.65	0.06	**-0.79**	**<0.01**
Fruiting body_dca2	**0.69**	**<0.05**	-0.43	0.21
All DNA_dca1	-0.54	0.06	-0.36	0.21
All DNA_dca2	0.30	0.32	0.37	0.19
Saprotrophic DNA_dca1	-0.04	0.91	**-0.53**	**0.05**
Saprotrophic DNA_dca2	**0.62**	**<0.05**	**-0.53**	**0.05**

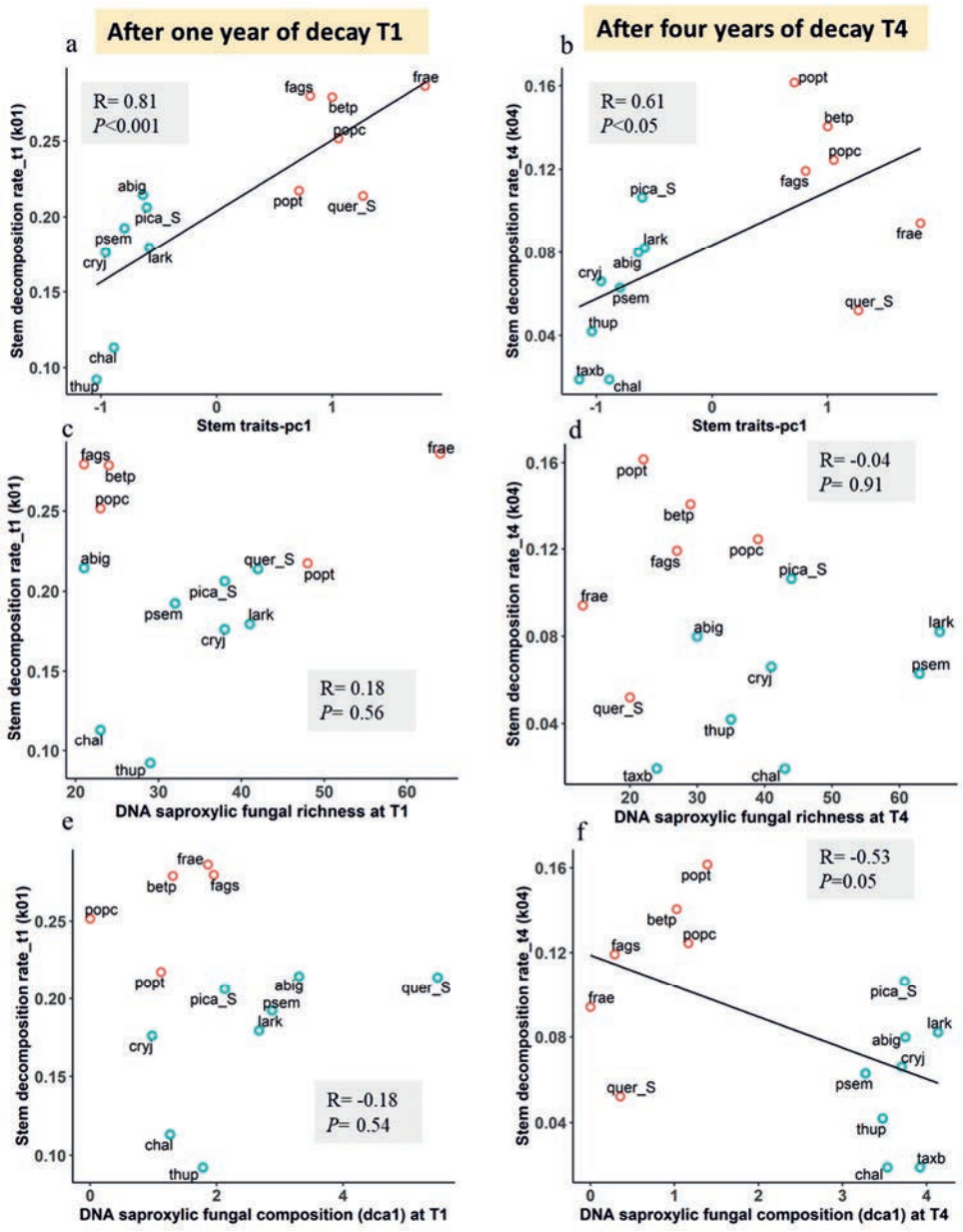


Fig. 5.4 Relationships between wood decomposition rate of 14 tree species and stem trait values (a, b), fungal richness (c, d) and fungal composition (e, f), after 1 year (T1, left column) and 4 years of decay (T4, right column). Factors of stem traits are indicated by the scores of first PCA axis of stem traits, showing species ranging from conservative to acquisitive trait values. Saprotrophic richness is based on ASVs (amplicon sequence variants), and saprotrophic fungal composition are indicated by the first DCA axis of fungal composition. Pearson correlation coefficients and *P* values are shown. Solid lines indicates significant regressions ($p<0.05$), no correlation line is shown if there no significant correlation

are found (p>0.05). Open red dots refer to angiosperm species and green dots refer to gymnosperm species;. for abbreviations of different species, see table 2.1.

Table 5.3 Summary of forward multiple regression analysis showing the effect of multiple factors on wood decomposition rate of 14 tree species. The factors used in this analysis are: stem traits, as indicated by PC1 & PC2 (Fig. 2.4) (the scores of first two PCA axis of stem traits, PC1 shows species from conservation traits to acquisitive traits, and PC2 from traits with high pH value to strong chemical defence); richness of saprotrophic fungi as detected by a molecular technique; saprotrophic fungal composition, as indicated by the first two DCA axes of saprotrophic fungal composition. Standardized regression coefficients, t values and significance levels (P) are shown. The adjusted r^2 of the model at T1 is 0.62 and the model at T4 is 0.77.

Summary of multiple regression				
Decay time	**Significant factors**	**Standardized regression coefficient**	***t* value**	***P* value**
T1	PC1	0.81	4.5	<0.001
T4	PC1	2.11	4.2	<0.01
	DCA1	1.35	2.7	<0.05
	DCA2	-0.69	-4.8	<0.001

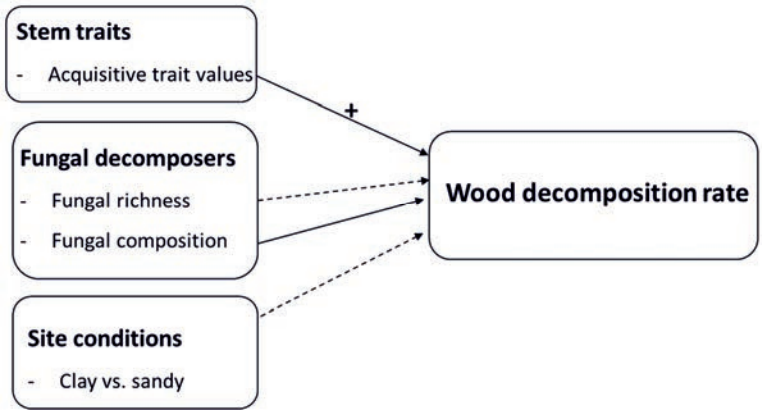


Fig. 5.5 Diagram summarizing the effects of multiple drivers on wood decomposition rate of temperate tree species. Solid lines indicate significant effects, while dashed lines indicate no significant effects. "+" shows positive effect.

5.7 | Recommendations for further research

Based on the results of this thesis, I provide the following methodological and conceptual recommendations for future research.

- *Fungal communities in decaying wood: what methods to apply?* Fungal fruiting body surveys can predict the most active fungi and fungal community compositions in decaying stems. However, results from fungal fruiting body surveys can be biased by limited survey time (i.e., once in the peak fructification time). Fungal fruiting body surveys do not provide information on fungal diversity and fungi composition inside the stem. A more comprehensive assessment of wood-inhabiting fungi (and other microorganisms) can be achieved by using molecular techniques. Applying molecular techniques on different stem compartments moreover allows to understand the relationship between stem characteristics and fungi richness and composition and - if focussing on wood-decomposing fungi - with wood decomposition. Therefore, I recommend that future studies on dead wood related to biodiversity and decomposition should integrate fungal fruiting body surveys conducted at multiple times with fungal DNA-based methods to obtain a comprehensive fungal community in dead wood.

- *Comparative common garden experiments.* The common garden experiment used in this study allows to test how stem quality and decomposers affect wood decomposition by excluding the confounding effects of environmental, i.e., site related variation. However, this experiment is only conducted in two temperate forests, but with large differences in soil and forest type. Nevertheless, no differences were observed in decomposition rates, fungal richness or fungal composition, implying that species effects overrule environmental effects. Yet, incubation in a more natural setting, i.e., tree species in a mixed forest where they have been growing could provide more insight in relevance of the local fungal community on biodiversity and decomposition. Climate gradients are widely thought to be the predominant control on the activity of decomposer organisms and hence decomposition rates (Weedon et al. 2009), but were excluded in our experiment. Therefore, I recommend more – and methodological harmonised - common garden experiments to be conducted in different climate zones and in

different forest settings (i.e., planted *vs* natural) or start global meta-analyses based on existing dead wood experiments to increase the possibility for generalization, and predictability of driving factors for dead-wood related biodiversity and decomposition.

- *Wider range of species.* Our generalizations are based on many (20) stem traits of important temperate tree species, but with only eight and six species for gymnosperm species and angiosperm species, respectively, and a limited number of heartwood forming angiosperm species (only *Quercus robur*) and non-heartwood forming gymnosperm species (only *Abies grandis*). Hence, it would be good to test our findings for a wider range of tree species with contrasting stem traits.

- *Quantifying stem traits in different decay stages.* Stem traits significantly affect and regulate fungal communities and decomposition rate, especially in the early stage of decay. When decay proceeds, stem quality will be altered by the physical and enzyme activities of multiple decomposers, so trait modifications may play a role over the entire stem decay process. Therefore, I recommend not only to quantify the stem traits measured for living trees, but also in logs in later decay stages.

- *Quantifying traits from inner bark and outer bark.* Bark is the first protective layer and includes inner and outer bark are derived from different cambial layers and fulfil different functions: inner bark for transport, storage, and photosynthesis, whereas outer bark is mainly for protection (Rosell et al. 2014). Therefore, I recommend to measure the traits, such as bark thickness, nutrients, and anti-fungal substances, for inner bark and outer bark separately for future studies.

- *Assessing the complete decomposer community.* The decay process of dead stems does not only depend on the stem quality and fungal decomposers, but also on the composition and activity of other members of the decomposer community, such as bark beetles and wood-boring insects, which act as ecosystem engineers for a range of other invertebrate colonizers. The invertebrates have been investigated in LOGLIFE (Zuo et al. 2016), which shows that bark beetles are important in facilitating access of other arthropods. Moreover, wood-inhabiting bacteria also play important roles in wood decomposition as they can degrade cellulose and

hemicellulose, and contribute to lignin degradation by chemical modification and render resources available to other decomposers (Johnston et al. 2016, Moll et al. 2018). It would be interesting to test how stem traits, fungal decomposers, and other decomposer communities (i.e., invertebrates, bacteria) interact and jointly affect wood decomposition.

- *Decay patterns within the stems.* In this study I compared the wood decay rate of different tree species, but how to efficiently quantify the decay rate and patterns in different stem compartments deserves more attention. Traditionally, wood density and mass loss is measured gravimetrically based on wood samples of various size, which at the same time defines the resolution of mass-loss measurements. Therefore, a new technique, i.e., X-ray CT scanning that can make high-resolution scans of wood (Van den Bulcke et al. 2009) can be a valuable tool and can be applied in future studies. This tool can assess not only the differences in decomposition rates across different tree species, but also the decomposition patterns in specific wood compartments (e.g., heartwood and sapwood). With this tool, we can test how stem traits and decomposers of different stem compartments affect decay rate of a specific wood compartments, which allows a better understanding of wood decay patterns and allows to test the most important drivers for the wood decay process in different parts of the stem.

5.8 | Recommendations for forest management

Based on the results of this thesis, I provide the following recommendations for management.

- *Increase the amount and diversity of dead wood.* This study has shown that dead wood diversity can promote fungal diversity. Specifically, promoting the amount and a wide array of dead wood from different tree species that cover a range of tree economic strategies (from conservative to acquisitive) and being present in various decay stages will increase the diversity of fungi (this study), invertebrates (Zuo et al. 2016) and other organisms (e.g., birds) (Kroll et al. 2012), thereby forest biodiversity. Foresters could consider increasing the variation in stem traits across co-existing

species in their forests, and allow for dead wood accumulation and decomposition of these species in their forests.

- *Cycling versus storage.* Forest managers can maintain dead wood in the forest according to different management aims. On poor sandy soils, the development of a humus layer may be important for nutrient, and water retention. In this case fast species, such as angiosperm *Populus tremula*, *Betula pendula*, and *Populus×canadensis* are preferred, not only for the quality of their leaf litter (as currently being done, Hommel et al. 2007), but also for the quality of their stem litter. On intermediate to high quality soils, more emphasis could be given to carbon retention, by using tree species with low decay rate, such as gymnosperm *Chamaecyparis lawsoniana*, *Taxus baccata* and *Thuja plicata*. Those species have longer lifespan (i.e., residence time), ranging from 72 to 159 years (Table 5.1).

5.9 | Conclusions

Stem trait variations have important implications for species distribution, and afterlife effects on the diversity and composition of deadwood-inhabiting fungi during the first four years of decomposition. Stem traits that increase accessibility, nutritional quality, and reduce physical defence drive fungal community composition in decaying stems. Wood decomposition rate in the first four years varies 9-fold across tree species and is mainly determined by stem traits and fungal community composition, but not by fungal richness. Decomposition rate decreased with "slow", conservative trait values (e.g., lignin, conduit wall thickness) and increased with "fast" acquisitive trait values (e.g., conduit diameter, nutrients). We conclude that stem traits and plant strategies differ among stem compartments (i.e., inner wood, outer wood, and bark) of different temperate tree species, and these traits have profound afterlife effects by interacting with fungal decomposers, and jointly affect wood decomposition and contribute to forest biodiversity.

REFERENCES

Abarenkov, K., R. Henrik Nilsson, K. H. Larsson, I. J. Alexander, U. Eberhardt, S. Erland, K. Høiland, R. Kjøller, E. Larsson, and T. Pennanen. 2010. The UNITE database for molecular identification of fungi–recent updates and future perspectives. New Phytologist **186**: 281–285..

Abdel-Hamid, A. M., J. O. Solbiati, and I. K. O. Cann. 2013. Insights into Lignin Degradation and its Potential Industrial Applications. Advances in Applied Microbiology **82**:1-28.

Abrego, N., P. Halme, J. Purhonen, and O. Ovaskainen. 2016. Fruit body based inventories in wood-inhabiting fungi: Should we replicate in space or time? Fungal Ecology **20**:225-232.

Adler, A., T. Verwijst, and P. Aronsson. 2005. Estimation and relevance of bark proportion in a willow stand. Biomass and Bioenergy **29**:102-113.

Andringa, J. I., J. Zuo, M. P. Berg, R. Klein, J. van't Veer, R. de Geus, M. de Beaumont, L. Goudzwaard, J. van Hal, and R. Broekman. 2019. Combining tree species and decay stages to increase invertebrate diversity in dead wood. Forest Ecology and Management **441**:80-88.

Anthony, M. A., S. D. Frey, and K. A. Stinson. 2017. Fungal community homogenization, shift in dominant trophic guild, and appearance of novel taxa with biotic invasion. Ecosphere **8 (9):** e01951.

Arnolds, E. J. M., and A. van den Berg. 2013. Beknopte standaardlijst van Nederlandse paddenstoelen 2013. Nederlandse Mycologische Vereniging.

Arnstadt, T., B. Hoppe, T. Kahl, H. Kellner, D. Krüger, J. Bauhus, and M. Hofrichter. 2016. Dynamics of fungal community composition, decomposition and resulting deadwood properties in logs of Fagus sylvatica, *Picea abies* and *Pinus sylvestris*. Forest Ecology and Management **382**:129-142.

Baber, K., P. Otto, T. Kahl, M. M. Gossner, C. Wirth, A. Gminder, and C. Bässler. 2016. Disentangling the effects of forest-stand type and dead-wood origin of the early successional stage on the diversity of wood-inhabiting fungi. Forest Ecology and Management **377**:161-169.

Baldrian, P. 2008. Chapter 2 Enzymes of saprotrophic basidiomycetes. British Mycological Society Symposia Series. Academic Press **28**:19-41.

Baldrian, P., P. Zrůstová, V. Tláskal, A. Davidová, V. Merhautová, and T. Vrška. 2016. Fungi associated with decomposing deadwood in a natural beech-dominated forest. Fungal Ecology **23**:109-122.

Bantle, A., W. Borken, R. H. Ellerbrock, E.-D. Schulze, W. W. Weisser, and E. Matzner. 2014. Quantity and quality of dissolved organic carbon released from coarse woody debris of different tree species in the early phase of decomposition. Forest Ecology and Management **329**:287-294.

Baraloto, C., C. E. Timothy Paine, S. Patino, D. Bonal, B. Hérault, and J. Chave. 2010. Functional trait variation and sampling strategies in species-rich plant communities. Functional Ecology **24**:208-216.

Barge, E. G., D. R. Leopold, K. G. Peay, G. Newcombe, and P. E. Busby. 2019. Differentiating spatial from environmental effects on foliar fungal communities of *Populus trichocarpa*. Journal of Biogeography **46**:2001-2011.

Barnett, J., and G. Jeronimidis. 2009. Wood quality and its biological basis. London, UK: Blackwell.

Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, B. Dai, F. Scheipl, and G. Grothendieck. 2011. Package 'lme4'. Linear mixed-effects models using S4 classes. R package version **1**.

Bauhus, J., K. Puettmann, and C. Messier. 2009. Silviculture for old-growth attributes. Forest Ecology and Management **258**:525-537.

Bebber, D. P., S. C. Watkinson, L. Boddy, and P. R. Darrah. 2011. Simulated nitrogen deposition affects wood decomposition by cord-forming fungi. Oecologia **167**:1177-1184.

Biedermann, P. H., and F. E. Vega. 2020. Ecology and evolution of insect–fungus mutualisms. Annual review of Entomology **65**:431-455.

Blackman, C. J., T. J. Brodribb, and G. J. Jordan. 2010. Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. New Phytologist **188**:1113-1123.

Blanchette, R. A. 2000. A review of microbial deterioration found in archaeological wood from different environments. International Biodeterioration and Biodegradation **46**:189-204.

Boddy, L. 1994. Latent decay fungi: the hidden foe? Arboricultural Journal **18**:113-135.

Boddy, L., U. Büntgen, S. Egli, A. C. Gange, E. Heegaard, P. M. Kirk, A. Mohammad, and H. Kauserud. 2014. Climate variation effects on fungal fruiting. Fungal Ecology **10**:20-33.

Boddy, L., and J. Heilmann-Clausen. 2008. Basidiomycete community development in temperate angiosperm wood. British Mycological Society Symposia Series. Academic Press **28**: 211-237.

Bonan, G. B. 2008. Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. Science **320**:1444-1449.

Brant, A. N., and H. Y. Chen. 2015. Patterns and mechanisms of nutrient resorption in plants. Critical Reviews in Plant Sciences **34**:471-486.

Buée, M., J.-P. Maurice, B. Zeller, S. Andrianarisoa, J. Ranger, R. Courtecuisse, B. Marçais, and F. Le Tacon. 2011. Influence of tree species on richness and diversity of epigeous fungal communities in a French temperate forest stand. Fungal Ecology **4**:22-31.

Buee, M., M. Reich, C. Murat, E. Morin, R. Nilsson, S. Uroz, and F. Martin. 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytologist **184**:449-456.

Cáceres, M. D., and P. Legendre. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology **90**:3566-3574.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods **13**:581-583.

Campbell, R. N. 1985. Longevity of Olpidium brassicae in air-dry soil and the persistence of the lettuce big-vein agent. Canadian Journal of Botany **63**:2288-2289.

Chang, C., R. S. P. van Logtestijn, L. Goudzwaard, J. van Hal, J. Zuo, M. Hefting, U. Sass-Klaassen, S. Yang, F. J. Sterck, L. Poorter, and J. H. C. Cornelissen. 2020. Methodology matters for comparing coarse wood and bark decay rates across tree species. Methods in Ecology and Evolution **11**:828-838.

Chave, J., D. Coomes, S. Jansen, S. L. Lewis, N. G. Swenson, and A. E. Zanne. 2009. Towards a worldwide wood economics spectrum. Ecology Letters **12**:351-366.

Christensen, M., K. Hahn, E. P. Mountford, P. Odor, T. Standovár, D. Rozenbergar, J. Diaci, S. Wijdeven, P. Meyer, and S. Winter. 2005. Dead wood in European beech (*Fagus sylvatica*) forest reserves. Forest Ecology and Management **210**:267-282.

Cochard, H., F. Froux, S. Mayr, and C. Coutand. 2004. Xylem wall collapse in water-stressed pine needles. Plant Physiology **134**:401-408.

Corder, S. P. 1973. Introducing applied linguistics. Harmondsworth, England: Penguin.

Cornelissen, J. H. C., H. M. Quested, R. S. P. Van Logtestijn, N. Pérez-Harguindeguy, D. Gwynn-Jones, S. Díaz, T. V. Callaghan, M. C. Press, and R. Aerts. 2006. Foliar pH as a new plant trait: can it explain variation in foliar chemistry and carbon cycling processes among subarctic plant species and types? Oecologia **147**:315-326.

Cornelissen, J. H. C., U. Sass-Klaassen, L. Poorter, K. van Geffen, R. S. P. van Logtestijn, J. van Hal, L. Goudzwaard, F. J. Sterck, R. K. W. M. Klaassen, G. T. Freschet, A. van der Wal, H. Eshuis, J. Zuo, W. de Boer, T. Lamers, M. Weemstra, V. Cretin, R. Martin, J. d. Ouden, M. P. Berg, R. Aerts, G. M. J. Mohren, and M. M. Hefting. 2012. Controls on Coarse Wood Decay in Temperate Tree Species: Birth of the LOGLIFE Experiment. AMBIO **41**:231-245.

Cornwell, W. K., J. H. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, and N. Pérez-Harguindeguy. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters **11**:1065-1071.

Cornwell, W. K., J. H. C. Cornelissen, S. D. Allison, J. Bauhus, P. Eggleton, C. M. Preston, F. Scarff, J. T. Weedon, C. Wirth, and A. E. Zanne. 2009. Plant traits and wood fates across the globe: rotted, burned, or consumed? Global Change Biology **15**:2431-2449.

Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M. Kleyer, C. Wirth, I. Colin Prentice, E. Garnier, G. Bönisch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles, J. Dickie, A. N. Gillison, A. E. Zanne, J. Chave, S. Joseph Wright, S. N. Sheremet Ev, H. Jactel, C. Baraloto, B. Cerabolini, S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S. Joswig, A. Günther, V. Falczuk, N. Rüger, M. D. Mahecha, and L. D. Gorné. 2016. The global spectrum of plant form and function. Nature **529**:167-171.

Diaz, S., J. Hodgson, K. Thompson, M. Cabido, J. H. Cornelissen, A. Jalili, G. Montserrat-Martí, J. Grime, F. Zarrinkamar, and Y. Asri. 2004. The plant traits that drive ecosystems: evidence from three continents. Journal of Vegetation Science **15**:295-304.

Domec, J. C., M. L. Pruyn, and B. L. Gartner. 2005. Axial and radial profiles in conductivities, water storage and native embolism in trunks of young and old-growth ponderosa pine trees. Plant, Cell & Environment **28**:1103-1113.

Dossa, G. G., E. Paudel, D. Schaefer, J.-L. Zhang, K.-F. Cao, J.-C. Xu, and R. D. Harrison. 2020. Quantifying the factors affecting wood decomposition across a tropical forest disturbance gradient. Forest Ecology and Management **468**:118166.

Dossa, G. G., D. Schaefer, J. L. Zhang, J. P. Tao, K. F. Cao, R. T. Corlett, A. B. Cunningham, J. C. Xu, J. H. Cornelissen, and R. D. Harrison. 2018. The cover uncovered: bark control over wood decomposition. Journal of Ecology **106**:2147-2160.

Douglas Bates, M., B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw **67**:1-48.

Dufrêne, M., and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs **67**:345-366.

Epron, D., M. Bahn, D. Derrien, F. A. Lattanzi, J. Pumpanen, A. Gessler, P. Högberg, P. Maillard, M. Dannoura, and D. Gérant. 2012. Pulse-labelling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects. Tree Physiology **32**:776-798.

Europe, F. 2020. State of Europe's forests 2020. Forest Europe: Bratislava, Slovakia.

Evert, R. F. 2006. Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development. Hoboken, NJ: John Wiley & Sons, Inc.

Fajardo, A., C. Martínez-Pérez, M. A. Cervantes-Alcayde, and M. E. Olson. 2020. Stem length, not climate, controls vessel diameter in two trees species across a sharp precipitation gradient. New Phytologist **225**:2347-2355.

Feild, T. S., and N. C. Arens. 2007. The ecophysiology of early angiosperms. Plant, Cell & Environment **30**:291-309.

Fengel, D., and G. Wegener. 2011. Wood: chemistry, ultrastructure, reactions. Berlin: Walter de Gruyter.

Fichtner, F., F. F. Barbier, M. G. Annunziata, R. Feil, J. J. Olas, B. Mueller-Roeber, M. Stitt, C. A. Beveridge, and J. E. Lunn. 2021. Regulation of shoot branching in Arabidopsis by trehalose 6-phosphate. New Phytologist **229**:2135-2151.

Frøslev, T. G., R. Kjøller, H. H. Bruun, R. Ejrnæs, A. J. Hansen, T. Læssøe, and J. Heilmann-Clausen. 2019. Man against machine: Do fungal fruitbodies and eDNA give similar biodiversity assessments across broad environmental gradients? Biological Conservation **233**:201-212.

Franceschi, V. R., P. Krokene, E. Christiansen, and T. Krekling. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytologist **167**:353-376.

Frank, A. C., and A. M. Pirttilä. 2018. Endophytes of forest trees: biology and applications. Springer International Publishing.

Freschet, G. T., J. H. C. Cornelissen, R. S. P. van Logtestijn, and R. Aerts. 2010. Evidence of the 'plant economics spectrum' in a subarctic flora. Journal of Ecology **98**:362-373.

Fuhr, M., M. Schubert, C. Stührk, F. Schwarze, and H. Herrmann. 2013. Penetration capacity of the wood-decay fungus Physisporinus vitreus. Complex Adaptive Systems Modeling **1**:1-15.

Fukami, T., I. A. Dickie, J. Paula Wilkie, B. C. Paulus, D. Park, A. Roberts, P. K. Buchanan, and R. B. Allen. 2010. Assembly history dictates ecosystem functioning: Evidence from wood decomposer communities. Ecology Letters **13**:675-684.

Fukasawa, Y., T. Osono, and H. Takeda. 2009. Dynamics of physicochemical properties and occurrence of fungal fruit bodies during decomposition of coarse woody debris of *Fagus crenata*. Journal of Forest Research **14**:20.

Gómez-Brandón, M., M. Probst, J. A. Siles, U. Peintner, T. Bardelli, M. Egli, H. Insam, and J. Ascher-Jenull. 2020. Fungal communities and their association with nitrogen-fixing bacteria affect early decomposition of Norway spruce deadwood. Scientific Reports **10**:1-11.

Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology **2**:113-118.

Gleason, S. M., M. Westoby, S. Jansen, B. Choat, U. G. Hacke, R. B. Pratt, R. Bhaskar, T. J. Brodribb, S. J. Bucci, and K. F. Cao. 2016. Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. New Phytologist **209**:123-136.

Gloor, G. B., J. M. Macklaim, V. Pawlowsky-Glahn, and J. J. Egozcue. 2017. Microbiome datasets are compositional: and this is not optional. Frontiers in Microbiology **8**:2224.

Goodell, B. 2003. Brown-rot fungal degradation of wood: our evolving view. Goodell, B., Nicolas, D.D., Schultz, T.P., Eds. Wood Deterioration and Preservation. ACS Symposium Series. vol. **845**: 97–118. American Chemical Society, Washington, DC.

Gossner, M. M., B. Wende, S. Levick, P. Schall, A. Floren, K. E. Linsenmair, I. Steffan-Dewenter, E.-D. Schulze, and W. W. Weisser. 2016. Deadwood enrichment in European forests–Which tree species should be used to promote saproxylic beetle diversity? Biological Conservation **201**:92-102.

Grime, J., K. Thompson, R. Hunt, J. Hodgson, J. Cornelissen, I. Rorison, G. Hendry, T. Ashenden, A. Askew, and S. Band. 1997. Integrated screening validates primary axes of specialisation in plants. Oikos:259-281.

Hacke, U. G., and J. S. Sperry. 2001. Functional and ecological xylem anatomy. Perspectives in Plant Ecology, Evolution and Systematics **4**:97-115.

Halme, P., J. Heilmann-Clausen, T. Rämä, T. Kosonen, and P. Kunttu. 2012. Monitoring fungal biodiversity–towards an integrated approach. Fungal Ecology **5**:750-758.

Harmon, M. E., J. F. Franklin, F. J. Swanson, P. Sollins, S. V. Gregory, J. D. Lattin, N. H. Anderson, S. P. Cline, N. G. Aumen, and J. R. Sedell. 1986. Ecology of coarse woody debris in temperate ecosystems. Advances in Ecological Research **15**: 133-302.

Hatakka, A., and K. E. Hammel. 2011. Fungal biodegradation of lignocelluloses. 319-340 Industrial applications. Springer.

Hayslett, M., J. Juzwik, and B. Moltzan. 2008. Three *Colopterus* beetle species carry the oak wilt fungus to fresh wounds on red oak in Missouri. Plant Disease **92**:270-275.

Heilman, P. E., and R. F. Stettler. 1986. Nutritional concerns in selection of black cottonwood and hybrid clones for short rotation. Canadian Journal of Forest Research **16**:860-863.

Heilmann-Clausen, J., and L. Boddy. 2005. Inhibition and stimulation effects in communities of wood decay fungi: exudates from colonized wood influence growth by other species. Microbial Ecology **49**:399-406.

Heilmann-Clausen, J., and M. Christensen. 2004. Does size matter?: on the importance of various dead wood fractions for fungal diversity in Danish beech forests. Forest Ecology and Management **201**:105-117.

Hillis, W. E. 1971. Distribution, properties and formation of some wood extractives. Wood Science and Technology **5**:272-289.

Hillis, W. E. 2012. Heartwood and tree exudates. Springer Science & Business Media **4**.

Hiscox, J., M. Savoury, C. T. Müller, B. D. Lindahl, H. J. Rogers, and L. Boddy. 2015. Priority effects during fungal community establishment in beech wood. The ISME Journal **9**: 2246-2260.

Hoffman, G. E., and E. E. Schadt. 2016. variancePartition: Interpreting drivers of variation in complex gene expression studies. BMC Bioinformatics **17**:17-22.

Hommel, P. W., R. de Waal, B. Muys, J. Den Ouden, and T. Spek. 2007. Terug naar het lindewoud: strooiselkwaliteit als basis voor ecologisch bosbeheer. KNVV Uitgeverij, Zeist, NL.

Hoppe, B., W. Purahong, T. Wubet, T. Kahl, J. Bauhus, T. Arnstadt, M. Hofrichter, F. Buscot, and D. Krüger. 2016. Linking molecular deadwood-inhabiting fungal diversity and community dynamics to ecosystem functions and processes in Central European forests. Fungal Diversity **77**:367-379.

Hudgins, J. W., E. Christiansen, and V. R. Franceschi. 2004. Induction of anatomically based defense responses in stems of diverse conifers by methyl jasmonate: a phylogenetic perspective. Tree Physiology **24**:251-264.

Huston, M. A., and D. L. DeAngelis. 1994. Competition and coexistence: the effects of resource transport and supply rates. The American Naturalist **144**:954-977.

Jensen, K. H., J. Liesche, T. Bohr, and A. Schulz. 2012. Universality of phloem transport in seed plants. Plant, Cell & Environment **35**:1065-1076.

Johnston, S. R., L. Boddy, and A. J. Weightman. 2016. Bacteria in decomposing wood and their interactions with wood-decay fungi. FEMS Microbiology Ecology **92**.

Jupa, R., L. Plavcová, V. Gloser, and S. Jansen. 2016. Linking xylem water storage with anatomical parameters in five temperate tree species. Tree Physiology **36**:756-769.

Juutilainen, K., P. Halme, H. Kotiranta, and M. Mönkkönen. 2011. Size matters in studies of dead wood and wood-inhabiting fungi. Fungal Ecology **4**:342-349.

Juutilainen, K., M. Mönkkönen, H. Kotiranta, and P. Halme. 2017. Resource use of wood-inhabiting fungi in different boreal forest types. Fungal Ecology **27**:96-106.

Kahl, T., T. Arnstadt, K. Baber, C. Bässler, J. Bauhus, W. Borken, F. Buscot, A. Floren, C. Heibl, D. Hessenmöller, M. Hofrichter, B. Hoppe, H. Kellner, D. Krüger, K. E. Linsenmair, E. Matzner, P. Otto, W. Purahong, C. Seilwinder, E. D. Schulze, B. Wende, W. W. Weisser, and M. M. Gossner. 2017. Wood decay rates of 13 temperate tree species in relation to wood properties, enzyme activities and organismic diversities. Forest Ecology and Management **391**:86-95.

Kazartsev, I., E. Shorohova, E. Kapitsa, and H. Kushnevskaya. 2018. Decaying *Picea abies log* bark hosts diverse fungal communities. Fungal Ecology **33**:1-12.

Kergunteuil, A., P. Descombes, G. Glauser, L. Pellissier, and S. Rasmann. 2018. Plant physical and chemical defence variation along elevation gradients: a functional trait-based approach. Oecologia **187**:561-571.

Kielak, A. M., T. R. Scheublin, L. W. Mendes, J. A. van Veen, and E. E. Kuramae. 2016. Bacterial community succession in pine-wood decomposition. Frontiers in Microbiology **7**:1-12.

Kirk, T. K., and R. L. Farrell. 1987. Enzymatic" combustion": the microbial degradation of lignin. Annual Reviews in Microbiology **41**:465-501.

Koenigs, J. W. 1974. Hydrogen peroxide and iron: a proposed system for decomposition of wood by brown-rot basidiomycetes. Wood and Fiber Science **6**:66-80.

Kohout, P., M. Charvátová, M. Štursová, T. Mašínová, M. Tomšovský, and P. Baldrian. 2018. Clearcutting alters decomposition processes and initiates complex restructuring of fungal communities in soil and tree roots. The ISME journal **12**:692-703.

Krah, F. S., S. Seibold, R. Brandl, P. Baldrian, J. Müller, and C. Bässler. 2018. Independent effects of host and environment on the diversity of wood-inhabiting fungi. Journal of Ecology **106**:1428-1442.

Kroll, A. J., S. D. Duke, M. E. Hane, J. R. Johnson, M. Rochelle, M. G. Betts, and E. B. Arnett. 2012. Landscape composition influences avian colonization of experimentally created snags. Biological Conservation **152**:145-151.

Kropp, R. K. 1982. Responses of five holothurian species to attacks by a predatory gastropod, *Tonna perdix*. Pacific Science **32**: 445-452

Kubartová, A., E. Ottosson, A. Dahlberg, and J. Stenlid. 2012. Patterns of fungal communities among and within decaying logs, revealed by 454 sequencing. Molecular Ecology **21**:4514-4532.

Küffer, N., F. Gillet, B. Senn-Irlet, D. Job, and M. Aragno. 2008. Ecological determinants of fungal diversity on dead wood in European forests. Fungal Diversity **30**: 83-95.

Kuramae, E. E., M. F. A. Leite, A. K. A. Suleiman, C. M. Gough, B. T. Castillo, L. Faller, R. B. Franklin, and J. Syring. 2019. Wood decay characteristics and interspecific interactions control bacterial community succession in *Populus grandidentata* (Bigtooth Aspen). Frontiers in Microbiology **10**:1-14.

Kwaśna, H., A. Mazur, R. Kuźmiński, R. Jaszczak, M. Turski, J. Behnke-Borowczyk, K. Adamowicz, and P. Łakomy. 2017. Abundance and diversity of wood-decay fungi in managed and unmanaged stands in a Scots pine forest in Western Poland. Forest Ecology and Management **400**:438-446.

Lambers, H., and H. Poorter. 2004. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Advances in Ecological Research **34**:283-362.

Lambevska, A., K. Rusevska, and M. Karadelev. 2013. New data on the taxonomy, distribution and ecology of the genus *Peniophora Cooke* (Basidiomycota, Fungi) in the Republic of Macedonia. Macedonian Journal of Ecology and Environment **15**:69-79.

Lamlom, S. H., and R. A. Savidge. 2003. A reassessment of carbon content in wood: variation within and between 41 North American species. Biomass and Bioenergy **25**:381-388.

Lamounier, K. C., L. C. S. Cunha, S. A. L. de Morais, F. J. T. de Aquino, R. Chang, E. A. do Nascimento, M. G. M. de Souza, C. H. G. Martins, and W. R. Cunha. 2012. Chemical Analysis and Study of Phenolics, Antioxidant Activity, and Antibacterial Effect of the Wood and Bark of *Maclura tinctoria* (L.) D. Don ex Steud. Evidence-Based Complementary and Alternative Medicine :1-7.

Lassauce, A., Y. Paillet, H. Jactel, and C. Bouget. 2011. Deadwood as a surrogate for forest biodiversity: meta-analysis of correlations between deadwood volume and species richness of saproxylic organisms. Ecological Indicators **11**:1027-1039.

Lee, M. R., B. Oberle, W. Olivas, D. F. Young, and A. E. Zanne. 2020. Wood construction more strongly shapes deadwood microbial communities than spatial location over 5 years of decay. Environmental Microbiology **22**:4702-4717.

Lee, M. R., J. R. Powell, B. Oberle, W. K. Cornwell, M. Lyons, J. L. Rigg, and A. E. Zanne. 2019. Good neighbors aplenty: fungal endophytes rarely exhibit competitive exclusion patterns across a span of woody habitats. Ecology **100**: e02790.

Leonhardt, S., B. Hoppe, E. Stengel, L. Noll, J. Moll, C. Bässler, A. Dahl, F. Buscot, M. Hofrichter, and H. Kellner. 2019. Molecular fungal community and its decomposition activity in sapwood and heartwood of 13 temperate European tree species. PLoS ONE **14**:1-21.

Lindahl, B. D., and R. D. Finlay. 2006. Activities of chitinolytic enzymes during primary and secondary colonization of wood by basidiomycetous fungi. New Phytologist **169**:389-397.

Lindenmayer, D. B., and W. F. Laurance. 2017. The ecology, distribution, conservation and management of large old trees. Biological Reviews **92**:1434-1458.

Lombardi, F., B. Lasserre, R. Tognetti, and M. Marchetti. 2008. Deadwood in relation to stand management and forest type in Central Apennines (Molise, Italy). Ecosystems **11**:882-894.

Loram-Lourenço, L., F. d. S. Farnese, L. F. d. Sousa, R. D. F. B. Alves, M. C. P. d. Andrade, S. E. d. S. Almeida, L. M. d. F. Moura, A. C. Costa, F. G. Silva, J. Galmés, H. Cochard, A. C. Franco, and P. E. Menezes-Silva. 2020. A Structure Shaped by Fire, but Also Water: Ecological Consequences of the Variability in Bark Properties Across 31 Species From the Brazilian Cerrado. Frontiers in Plant Science **10**: 1718.

Lourenço, A., D. M. Neiva, J. Gominho, A. V. Marques, and H. Pereira. 2015. Characterization of lignin in heartwood, sapwood and bark from Tectona grandis using Py–GC–MS/FID. Wood science and technology **49**:159-175.

Lusk, C. 2001. Leaf life spans of some conifers of the temperate forests of South America. Revista Chilena De Historia Natural **74**: 711-718

Müller, J., M. Ulyshen, S. Seibold, M. Cadotte, A. Chao, C. Bässler, S. Vogel, J. Hagge, I. Weiß, P. Baldrian, V. Tláskal, and S. Thorn. 2020. Primary determinants of communities in deadwood vary among taxa but are regionally consistent. Oikos **129**:1579-1588.

Martin, A., G. Dimke, M. Doraisami, and S. Thomas. 2020. Carbon fractions in the world's dead wood. Nature Communications **12**: 1-9.

McDonald, L., M. van Woudenberg, B. Dorin, A. M. Adcock, R. T. McMullin, and K. Cottenie. 2017. The effects of bark quality on corticolous lichen community composition in urban parks of southern Ontario. Botany **95**:1141-1149.

McMurdie, P. J., and S. Holmes. 2013. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE **8**.

Meyer, P., and M. Schmidt. 2011. Accumulation of dead wood in abandoned beech (*Fagus sylvatica* L.) forests in northwestern Germany. Forest Ecology and Management **261**:342-352.

Moll, J., A. Heintz-Buschart, C. Bässler, M. Hofrichter, H. Kellner, F. Buscot, and B. Hoppe. 2021. Amplicon Sequencing-Based Bipartite Network Analysis Confirms a High Degree of Specialization and Modularity for Fungi and Prokaryotes in Deadwood. Msphere **6**:e00856-20.

Moll, J., H. Kellner, S. Leonhardt, E. Stengel, A. Dahl, C. Bässler, F. Buscot, M. Hofrichter, and B. Hoppe. 2018. Bacteria inhabiting deadwood of 13 tree species are heterogeneously distributed between sapwood and heartwood. Environmental Microbiology **20**:2912-3744.

Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. de Ruiter, Q. Dong, A. Hastings, N. C. Johnson, K. S. McCann, K. Melville, and P. J. Morin. 2004. Detritus, trophic dynamics and biodiversity. Ecology Letters **7**:584-600.

Morais, M. C., and H. Pereira. 2012. Variation of extractives content in heartwood and sapwood of Eucalyptus globulus trees. Wood Science and Technology **46**:709-719.

Morris, H., L. Plavcová, P. Cvecko, E. Fichtler, M. A. F. Gillingham, H. I. Martínez-Cabrera, D. J. McGlinn, E. Wheeler, J. Zheng, and K. Ziemińska. 2016. A global analysis of parenchyma tissue fractions in secondary xylem of seed plants. New Phytologist **209**:1553-1565.

Mueller, R. C., F. S. Paula, B. S. Mirza, J. L. Rodrigues, K. Nüsslein, and B. J. Bohannan. 2014. Links between plant and fungal communities across a deforestation chronosequence in the Amazon rainforest. The ISME journal **8**:1548-1550.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta **27**:31-36.

Navarro Hoyos, M., F. Sánchez-Patán, R. Murillo Masis, P. J. Martín-Álvarez, W. Zamora Ramirez, M. J. Monagas, and B. Bartolomé. 2015. Phenolic assesment of *Uncaria tomentosa* L.(Cat's Claw): Leaves, stem, bark and wood extracts. Molecules **20**:22703-22717.

Nguyen, N. H., Z. Song, S. T. Bates, S. Branco, L. Tedersoo, J. Menke, J. S. Schilling, and P. G. Kennedy. 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology **20**:241-248.

Noll, L., S. Leonhardt, T. Arnstadt, B. Hoppe, C. Poll, E. Matzner, M. Hofrichter, and H. Kellner. 2016. Fungal biomass and extracellular enzyme activities in coarse woody debris of 13 tree species in the early phase of decomposition. Forest Ecology and Management **378**:181-192.

Oberle, B., M. R. Lee, J. A. Myers, O. L. Osazuwa, M. J. Spasojevic, M. L. Walton, D. F. Young, and A. E. Zanne. 2019. Accurate forest projections require long-term wood decay experiments because plant trait effects change through time. Global change biology **26**: 864-875.

Olson, J. S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology **44**:322-331.

Olson, M., J. A. Rosell, C. Martínez-Pérez, C. León-Gómez, A. Fajardo, S. Isnard, M. A. Cervantes-Alcayde, A. Echeverría, V. A. Figueroa-Abundiz, and A. Segovia-Rivas. 2020. Xylem vessel-diameter–shoot-length scaling: ecological significance of porosity types and other traits. Ecological Monographs **90**: e01410.

Olson, M. E., and J. A. Rosell. 2013. Vessel diameter–stem diameter scaling across woody angiosperms and the ecological causes of xylem vessel diameter variation. New Phytologist **197**:1204-1213.

Ottosson, E., A. Kubartová, M. Edman, M. Jönsson, A. Lindhe, J. Stenlid, and A. Dahlberg. 2015. Diverse ecological roles within fungal communities in decomposing logs of *Picea abies*. FEMS Microbiology Ecology **91**.

Ovaskainen, O., D. Schigel, H. Ali-Kovero, P. Auvinen, L. Paulin, B. Nordén, and J. Nordén. 2013. Combining high-throughput sequencing with fruit body surveys reveals contrasting life-history strategies in fungi. The ISME journal **7**:1696-1709.

Palarea-Albaladejo, J., and J. A. Martín-Fernández. 2015. ZCompositions - R package for multivariate imputation of left-censored data under a compositional approach. Chemometrics and Intelligent Laboratory Systems **143**:85-96.

Parfitt, D., J. Hunt, D. Dockrell, H. J. Rogers, and L. Boddy. 2010. Do all trees carry the seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in sapwood of a wide range of angiosperm trees. Fungal Ecology **3**:338-346.

Parisi, F., S. Pioli, F. Lombardi, G. Fravolini, M. Marchetti, and R. Tognetti. 2018. Linking deadwood traits with saproxylic invertebrates and fungi in European forests-a review. iForest-Biogeosciences and Forestry **11**:423.

Peay, K. G., M. Garbelotto, and T. D. Bruns. 2010. Evidence of dispersal limitation in soil microorganisms: Isolation reduces species richness on mycorrhizal tree islands. Ecology **91**:3631-3640.

Pietsch, K. A., K. Ogle, J. H. Cornelissen, W. K. Cornwell, G. Bönisch, J. M. Craine, B. G. Jackson, J. Kattge, D. A. Peltzer, and J. Penuelas. 2014. Global relationship of wood and leaf litter decomposability: the role of functional traits within and across plant organs. Global Ecology and Biogeography **23**: 1046–1057.

Pittermann, J., J. S. Sperry, U. G. Hacke, J. K. Wheeler, and E. H. Sikkema. 2006a. Inter-tracheid pitting and the hydraulic efficiency of conifer wood: the role of tracheid allometry and cavitation protection. American Journal of Botany **93**:1265-1273.

Pittermann, J., J. S. Sperry, J. K. Wheeler, U. G. Hacke, and E. H. Sikkema. 2006b. Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. Plant, Cell & Environment **29**:1618-1628.

Poorter, H., and R. Villar. 1997. The fate of acquired carbon in plants: chemical composition and construction costs. Plant Resource Allocation. Academic Press, New York:39-72.

Poorter, L., L. Bongers, and F. Bongers. 2006. Architecture of 54 moist-forest tree species: traits, trade-offs, and functional groups. Ecology **87**:1289-9170.

Poorter, L., C. V. Castilho, J. Schietti, R. S. Oliveira, and F. R. C. Costa. 2018. Can traits predict individual growth performance? A test in a hyperdiverse tropical forest. New Phytologist **219**:109-121.

Poorter, L., I. McDonald, A. Alarcón, E. Fichtler, J. C. Licona, M. Peña-Claros, F. Sterck, Z. Villegas, and U. Sass-Klaassen. 2010. The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. New Phytologist **185**:481-492.

Purahong, W., T. Arnstadt, T. Kahl, J. Bauhus, H. Kellner, M. Hofrichter, D. Krüger, F. Buscot, and B. Hoppe. 2016. Are correlations between deadwood fungal community structure, wood physico-chemical properties and lignin-modifying enzymes stable across different geographical regions? Fungal Ecology **22**:98-105.

Purahong, W., K. A. Pietsch, G. Lentendu, R. Schöps, H. Bruelheide, C. Wirth, F. Buscot, and T. Wubet. 2017. Characterization of unexplored deadwood mycobiome in highly diverse subtropical forests using culture-independent molecular technique. Frontiers in Microbiology **8**:574.

Purahong, W., T. Wubet, G. Lentendu, B. Hoppe, K. Jariyavidyanont, T. Arnstadt, K. Baber, P. Otto, H. Kellner, and M. Hofrichter. 2018. Determinants of deadwood-inhabiting fungal communities in temperate forests: molecular evidence from a large scale deadwood decomposition experiment. Frontiers in Microbiology **9**:2120.

Purhonen, J., S. Huhtinen, H. Kotiranta, J. S. Kotiaho, and P. Halme. 2017. Detailed information on fruiting phenology provides new insights on wood-inhabiting fungal detection. Fungal Ecology **27**:175-177.

Rühling, Å., and G. Tyler. 1991. Effects of simulated nitrogen deposition to the forest floor on the macrofungal flora of a beech forest. AMBIO:261-263.

Rajala, T., M. Peltoniemi, J. Hantula, R. Mäkipää, and T. Pennanen. 2011. RNA reveals a succession of active fungi during the decay of Norway spruce logs. Fungal Ecology **4**:437-448.

Rajala, T., M. Peltoniemi, T. Pennanen, and R. Mäkipää. 2010. Relationship between wood-inhabiting fungi determined by molecular analysis (denaturing gradient gel electrophoresis) and quality of decaying logs. Canadian Journal of Forest Research **40**:2384-2397.

Rajala, T., M. Peltoniemi, T. Pennanen, and R. Mäkipää. 2012. Fungal community dynamics in relation to substrate quality of decaying Norway spruce (*Picea abies* [L.] Karst.) logs in boreal forests. FEMS Microbiology Ecology **81**:494-505.

Rajala, T., T. Tuomivirta, T. Pennanen, and R. Mäkipää. 2015. Habitat models of wood-inhabiting fungi along a decay gradient of Norway spruce logs. Fungal Ecology **18**:48-55.

Rayner, A. D., and L. Boddy. 1988. Fungal decomposition of wood. Its biology and ecology. John Wiley & Sons Ltd.

Reich, P. B. 2014. The world-wide 'fast-slow' plant economics spectrum: A traits manifesto. Journal of Ecology **102**:275-301.

Reich, P. B., M. B. Walters, and D. S. Ellsworth. 1997. From tropics to tundra: global convergence in plant functioning. Proceedings of the National Academy of Sciences **94**:13730-13734.

Reiterer, A., I. Burgert, G. Sinn, and S. Tschegg. 2002. The radial reinforcement of the wood structure and its implication on mechanical and fracture mechanical properties—a comparison between two tree species. Journal of materials science **37**:935-940.

Renvall, P., and T. Niemelä. 1993. Ocotea usambarensis and its fungal decayers in natural stands. Bulletin du Jardin botanique national de Belgique/Bulletin van de Nationale Plantentuin van Belgie **62**:403-414.

Rosas, T., L. Galiano, R. Ogaya, J. Peñuelas, and J. Martínez-Vilalta. 2013. Dynamics of non-structural carbohydrates in three Mediterranean woody species following long-term experimental drought. Frontiers in Plant Science **4**:400.

Rosell, J. A., S. Gleason, R. Méndez-Alonzo, Y. Chang, and M. Westoby. 2014. Bark functional ecology: evidence for tradeoffs, functional coordination, and environment producing bark diversity. New Phytologist **201**:486-497.

Rosell, J. A., M. E. Olson, T. Anfodillo, and N. Martínez-Méndez. 2017. Exploring the bark thickness–stem diameter relationship: clues from lianas, successive cambia, monocots and gymnosperms. New Phytologist **215**:569-581.

Rosenzweig, M. L., and Z. Abramsky. 1993. How are diversity and productivity related? Pages 52-65 *in* R. E. Ricklefs and D. Schulter, editors. Species diversity in ecological communities: historical and geographical perspectives. University of Chicago Press, Chicago, Illinois, USA..

Rungwattana, K., and P. Hietz. 2018. Radial variation of wood functional traits reflect size-related adaptations of tree mechanics and hydraulics. Functional Ecology **32**:260-272.

Runnel, K., H. Tamm, and A. Lõhmus. 2015. Surveying wood-inhabiting fungi: Most molecularly detected polypore species form fruit-bodies within short distances. Fungal Ecology **18**:93-99.

Ruokolainen, A., E. Shorohova, R. Penttilä, V. Kotkova, and H. Kushnevskaya. 2018. A continuum of dead wood with various habitat elements maintains the diversity of wood-inhabiting fungi in an old-growth boreal forest. European Journal of Forest Research **137**:707-718.

Ryvarden, L., and R. Gilbertson. 1993. European Polypores, Synopsis Fungorum **6**:168-171. Fungiflora, Oslo.

Ryvarden, L., and I. Melo. 2017. Poroid fungi of Europe. Synopsis Fungorum **37**: 1-430. Fungiflora. Oslo.

Saine, S., O. Ovaskainen, P. Somervuo, and N. Abrego. 2020. Data collected by fruit body- and DNA-based survey methods yield consistent species-to-species association networks in wood-inhabiting fungal communities. Oikos **129**:1833-1843.

Scheffer, T. C. 1966. Natural resistance of wood to microbial deterioration. Annual Review of Phytopathology **4**:147-168.

Schelhaas, M. J., G. J. Nabuurs, and A. Schuck. 2003. Natural disturbances in the European forests in the 19th and 20th centuries. Global Change Biology **9**:1620-1633.

Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, and B. Schmid. 2012. Fiji: an open-source platform for biological-image analysis. Nature Methods **9**:676-682.

Schmidt, O. 2006. Wood and tree fungi. Springer, Berlin, Germany.

Schwarze, F. W. M. R., S. Fink, and G. Deflorio. 2003. Resistance of parenchyma cells in wood to degradation by brown rot fungi. Mycological Progress **2**:267-274.

Schweingruber, F. H., and A. Börner. 2018. The plant stem: a microscopic aspect. Springer Nature.

Seibold, S., C. Bässler, R. Brandl, B. Büche, A. Szallies, S. Thorn, M. D. Ulyshen, and J. Müller. 2016. Microclimate and habitat heterogeneity as the major drivers of beetle diversity in dead wood. Journal of Applied Ecology **53**:934-943.

Sinsabaugh, R. L., R. Antibus, A. Linkins, C. McClaugherty, L. Rayburn, D. Repert, and T. Weiland. 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology **74**:1586-1593.

Sisk-Hackworth, L., and S. T. Kelley. 2020. An application of compositional data analysis to multiomic time-series data. NAR Genomics and Bioinformatics **2**:1-8.

Sperry, J. S., U. G. Hacke, and J. Pittermann. 2006. Size and function in conifer tracheids and angiosperm vessels. American Journal of Botany **93**:1490-1500.

Spicer, R., and N. M. Holbrook. 2007. Parenchyma cell respiration and survival in secondary xylem: does metabolic activity decline with cell age? Plant, Cell & Environment **30**:934-943.

Srivastava, L. M. 1964. Anatomy, chemistry, and physiology of bark. International review of forestry research **1**:203–277.

Steppe, K., F. Sterck, and A. Deslauriers. 2015. Diel growth dynamics in tree stems: linking anatomy and ecophysiology. Trends in Plant Science **20**:335-343.

Sterck, F., L. Markesteijn, F. Schieving, and L. Poorter. 2011. Functional traits determine trade-offs and niches in a tropical forest community. Proceedings of the National Academy of Sciences **108**:20627-20632.

Sterck, F. J., F. Schieving, A. Lemmens, and T. L. Pons. 2005. Performance of trees in forest canopies: explorations with a bottom-up functional–structural plant growth model. New Phytologist **166**:827-843.

Sterck, F. J., R. Zweifel, U. Sass-Klaassen, and Q. Chowdhury. 2008. Persisting soil drought reduces leaf specific conductivity in Scots pine (*Pinus sylvestris*) and pubescent oak (*Quercus pubescens*). Tree Physiology **28**:529-536.

Stokland, J. N., J. Siitonen, and B. G. Jonsson. 2012. Biodiversity in Dead Wood. Cambridge University Press, Cambridge

Strid, Y. M. E. 2012. Bark beetles facilitate the establishment of wood decay fungi. Acta Universitatis Agriculturae Sueciae **72**: 46 (PhD dissertation, Univ. Uppsala).

Sun, S., X. Zeng, D. Zhang, and S. Guo. 2015. Diverse fungi associated with partial irregular heartwood of Dalbergia odorifera. Scientific Reports **5**:1-7.

Swenson, N. G., and B. J. Enquist. 2007. Ecological and evolutionary determinants of a key plant functional trait: wood density and its community-wide variation across latitude and elevation. American Journal of Botany **94**:451-459.

Team, R. c. 2019. R: A Language and Environment for Statistical Computing (Version 3.5. 2, R Foundation for Statistical Computing, Vienna, Austria, 2018).

ter Braak, C. J. F., and P. Smilauer. 2012. Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer Power, Ithaca, NY.

Thorn, S., S. Seibold, A. B. Leverkus, T. Michler, J. Müller, R. F. Noss, N. Stork, S. Vogel, and D. B. Lindenmayer. 2020. The living dead: acknowledging life after tree death to stop forest degradation. Frontiers in Ecology and the Environment **18**:505-512.

Tsoumis, G. 1991. Science and technology of wood: structure, properties, utilization. Van Nostrand Reinhold New York.

Tudor, D., S. C. Robinson, and P. A. Cooper. 2013. The influence of pH on pigment formation by lignicolous fungi. International Biodeterioration & Biodegradation **80**:22-28.

Tura, D. 2016. Wood-Inhabiting Fungi: Applied Aspects Wood-Inhabiting Fungi: Applied Aspects. Fungi: Applications and Management Strategies:245-292.

Tyree, M. T., and M. Zimmermann. 2002. Hydraulic architecture of whole plants and plant performance. Pages 175-214 Xylem structure and the ascent of sap. Springer, Berlin, Heidelberg: 175-214.

Vítková, L., R. Bače, P. Kjučukov, and M. Svoboda. 2018. Deadwood management in Central European forests: Key considerations for practical implementation. Forest Ecology and Management **429**:394-405.

Van den Bulcke, J., M. Boone, J. Van Acker, M. Stevens, and L. Van Hoorebeke. 2009. X-ray tomography as a tool for detailed anatomical analysis. Annals of Forest Science **66**:1-12.

Van Der Wal, A., W. De Boer, W. Smant, and J. A. Van Veen. 2007. Initial decay of woody fragments in soil is influenced by size, vertical position, nitrogen availability and soil origin. Plant and Soil **301**:189-201.

van der Wal, A., T. D. Geydan, T. W. Kuyper, and W. De Boer. 2013. A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. FEMS Microbiology Reviews **37**:477-494.

van der Wal, A., P. Klein Gunnewiek, M. de Hollander, and W. de Boer. 2017. Fungal diversity and potential tree pathogens in decaying logs and stumps. Forest Ecology and Management **406**:266-273.

van der Wal, A., P. J. A. Klein Gunnewiek, J. H. C. Cornelissen, T. W. Crowther, and W. de Boer. 2016. Patterns of natural fungal community assembly during initial decay of coniferous and broadleaf tree logs. Ecosphere **7**:e01393.

Venugopal, P., K. Junninen, R. Linnakoski, M. Edman, and J. Kouki. 2016. Climate and wood quality have decayer-specific effects on fungal wood decomposition. Forest Ecology and Management **360**:341-351.

Vile, D., B. Shipley, and E. Garnier. 2006. Ecosystem productivity can be predicted from potential relative growth rate and species abundance. Ecology Letters **9**:1061-1067.

Villéger, S., N. W. Mason, and D. Mouillot. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecology **89**:2290-2301.

Violle, C., M. L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! Oikos **116**:882-892.

Vleminckx, J., C. Fortunel, O. Valverde-Barrantes, C. E. Timothy Paine, J. Engel, P. Petronelli, A. K. Dourdain, J. Guevara, S. Béroujon, and C. Baraloto. 2021. Resolving whole-plant economics from leaf, stem and root traits of 1467 Amazonian tree species. Oikos:1-16.

Vogel, S., M. M. Gossner, U. Mergner, J. Müller, and S. Thorn. 2020. Optimizing enrichment of deadwood for biodiversity by varying sun exposure and tree species: an experimental approach. Journal of Applied Ecology **57**:2075-2085.

Wallander, H. 1995. A new hypothesis to explain allocation of dry matter between mycorrhizal fungi and pine seedlings in relation to nutrient supply. Nutrient Uptake and Cycling in Forest Ecosystems. Springer, Dordrecht: 243-248

Weedon, J. T., W. K. Cornwell, J. H. C. Cornelissen, A. E. Zanne, C. Wirth, and D. A. Coomes. 2009. Global meta-analysis of wood decomposition rates: a role for trait variation among tree species? Ecology Letters **12**:45-56.

Wheeler, J. C., and C. Sneden. 1989. Abundance ratios as a function of metallicity. Annual review of astronomy and astrophysics **27**:279-349.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications **18**:315-322.

Wijdeven, M. A., J. Willemsen, and F. Rutjes. 2010. The 3文ydroxypiperidine Skeleton: Key Element in Natural Product Synthesis (Eur. J. Org. Chem. 15/2010). European Journal of Organic Chemistry **2010**:2819-2819.

Witkamp, M. 1966. Decomposition of Leaf Litter in Relation to Environment, Microflora, and Microbial Respiration. Ecology **47**:194-201.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, and M. Diemer. 2004. The worldwide leaf economics spectrum. Nature **428**:1476-4687.

Yachi, S., and M. Loreau. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proceedings of the National Academy of Sciences **96**:1463-1468.

Yang, S., F. J. Sterck, U. Sass-Klaassen, J. H. C. Cornelissen, R. S. P. van Logtestijn, M. Hefting, L. Goudzwaard, J. Zuo, and L. Poorter. 2021. Stem trait spectra underpin multiple functions of temperate gymnosperm and angiosperm tree species. Preprint, Research Square.

Yatskov, M., M. E. Harmon, and O. N. Krankina. 2003. A chronosequence of wood decomposition in the boreal forests of Russia. Canadian Journal of Forest Research **33**:1211-1226.

Zanne, A. E., B. Oberle, K. M. Dunham, A. M. Milo, M. L. Walton, and D. F. Young. 2015. A deteriorating state of affairs: How endogenous and exogenous factors determine plant decay rates. Journal of Ecology **103**:1421-1431.

Zanne, A. E., M. Westoby, D. S. Falster, D. D. Ackerly, S. R. Loarie, S. E. J. Arnold, and D. A. Coomes. 2010. Angiosperm wood structure: global patterns in vessel anatomy and their relation to wood density and potential conductivity. American Journal of Botany **97**:207-215.

Zhang, L., Y. Chen, G. Hao, K. Ma, F. Bongers, and F. J. Sterck. 2020. Conifer and broadleaved trees differ in branch allometry but maintain similar functional balances. Tree Physiology **40**:511-519..

Zheng, J., and H. I. Martínez-Cabrera. 2013. Wood anatomical correlates with theoretical conductivity and wood density across China: evolutionary evidence of the functional differentiation of axial and radial parenchyma. Annals of Botany **112**:927-935.

Zobel, B. J., and J. P. Van Buijtenen. 2012. Wood variation: its causes and control. Timell TE 1989 Springer-Verlag Heidelberg.

Zuo, J., M. P. Berg, R. Klein, J. Nusselder, G. Neurink, O. Decker, M. M. Hefting, U. Sass‐Klaassen, R. S. Logtestijn, and L. Goudzwaard. 2016. Faunal community consequence of interspecific bark trait dissimilarity in early‐stage decomposing logs. Functional Ecology **30**:1957-1966.

Zuo, J., M. P. Berg, J. van Hal, R. S. van Logtestijn, L. Goudzwaard, M. M. Hefting, L. Poorter, F. J. Sterck, and J. H. Cornelissen. 2021. Fauna community convergence during decomposition of deadwood across tree species and forests. Ecosystems **24**:926-938.

Zuo, J., M. M. Hefting, M. P. Berg, R. S. P. van Logtestijn, J. van Hal, L. Goudzwaard, J.-C. Liu, U. Sass-Klaassen, F. J. Sterck, and L. Poorter. 2018. Is there a tree economics spectrum of decomposability? Soil Biology and Biochemistry **119**:135-717.

R

SUMMARY

Summary

Forests play a key role for terrestrial biodiversity. Trees are large and long-living organisms that define forest structure and form a key element in nutrient and carbon cycling. Tree species differ in ecological strategies and related functional traits, which may allow them to partition resources and coexist. It is well know that tree species differ in stem traits, but the afterlife effects of such traits for the diversity of decomposers (e.g., fungi, insects) within dead stem logs and their dynamics with ongoing decay are poorly known. To fill this knowledge gap, I studied the effects of stem traits on the infestation, composition and diversity of fungi during different phases of decay. I benefited from an existent long-term common garden decomposition experiment, called "LOGLIFE". I showed how stem traits and related stem functions co-vary across species, and how these traits affect afterlife effects on the succession of diversity and composition of wood and bark inhabiting fungi over time, and the implications for wood decomposition. In this study, I included eight conifer and six broadleaf tree species in order to cover a wide variety of stem traits and thus explore the afterlife implications of such variation.

In **chapter 2** I investigated how stem traits vary across wood and bark of 14 temperate tree species, and what trait trade-offs and plant strategies are found. Stem trait variation was largely explained by major taxa and stem compartments, i.e., inner, outer wood and bark. A continuous plant strategy gradient was found across and within taxa, running from hydraulic safe Gymnosperms to conductive Angiosperms. Gymnosperms strongly converged in their trait strategies because of their uniform tracheids, whereas Angiosperms strongly diverged because of different vessel arrangement and tissue types. Bark had higher concentrations of nutrients and phenolics, whereas wood had stronger physical defence. This indicates that stem compartments fulfill different strategies; bark serves as storage organ and a first physical and chemical defence layer, while wood is physically well defended by having stronger tissues.

In **chapter 3** I investigated the effects of stem traits for the succession of fungi fruiting bodies over 8 years of decay. Fungal communities diverged early in the decay process because tree species largely differed in substrate conditions. However, these fungal communities converged later, likely because this substrate became more similar with ongoing decay. Dead wood quality, as determined by species stem traits and decay stage, is therefore an important driver of fungal diversity and fungi community composition. These results imply that forest with larger

variation in stem traits and stem logs in different phases of decay will promote fungal and other microbial diversity and, thereby, forest biodiversity.

In **chapter 4**, Internal Transcribe Spacer (ITS) region amplicon next generation sequencing was used to assess the effects of stem traits on the entire fungal community within different stem compartments (inner wood, sapwood and bark) after one and four years of decay. Bark contained higher fungal diversity than wood. Fungal community composition differed between inner wood versus outer wood and bark. Stem traits regulate therefore the fungal composition via their effects on compartment accessibility, fungal nutrition, and physical or chemical defence against fungi. Traits associated with accessibility were important in the initial decay stage whereas traits related to nutrition (e.g., lignin/cellulose ratio) became most important after four years of decay. Hence, stem trait differences across tree species and their stem compartments have significant afterlife effects in regulating fungal diversity and composition, and contribute to forest biodiversity.

In **chapter 5**, I integrated the results from chapters 2, 3 and 4 by assessing how stem traits and fungal communities related to differences in stem decomposition rate amongst tree species. The molecular technique is able to detect microscopely invisible fungal communities within the decaying logs and provided therefore a better approach than fungal fruiting body surveys to assess fungal diversity while fungal fruiting body surveys can better indicate the most active fungi that form fruiting bodies when inactive mycelia hidden inside the wood. Wood decomposition rate varied markedly across tree species (from 0.019 to 0.166). It was mainly determined by stem traits and composition of saprotrophic fungal community, but not by fungal diversity. Early in decay, stem traits are the strongest determinants of decomposition rate whereas later in decay the fungal composition becomes more important.

I conclude that stem traits from an important component of tree strategies, with potentially strong effects on species performance, coexistence, and ecosystem functioning. Moreover, these stem traits have profound afterlife effects by interacting with fungal decomposers. Hence, stem traits and their fungal decomposers affect stem decomposition and carbon and nutrient cycling, and contribute to forest biodiversity.

AFFILIATION OF CO-AUTHORS

Affiliations of co-authors

Lourens Poorter, Frank J. Sterck, Ute Sass-Klaassen, Leo Goudzwaard
Forest Ecology and Forest Management Group, Wageningen University and Research, Wageningen, the Netherlands

J. Hans C. Cornelissen, Richard S.P. van Logtestijn, Chenghui Chang
Systems Ecology, Department of Ecological Science, VU University (Vrije Universiteit) Amsterdam, Amsterdam, the Netherlands

Mariet Hefting
Landscape Ecology, Institute of Environmental Biology, Utrecht University, Utrecht, the Netherlands

Eiko Kuramae, Marcio Leite, Ohana de Assis Costa
Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, the Netherlands

Juul Limpens
Plant Ecology and Nature Conservation Group, Wageningen University and Research, Wageningen, the Netherlands

Juan Zuo
Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, China

Emiel Brouwer
B-WARE Research Centre, Radboud University, Nijmegen, the Netherlands

Mirjam T. Veerkamp, Bart van den Berg
Pelikaanweg 54, 3985 RZ Werkhoven, the Netherlands

Nico Dam, Marjo Dam
Hooischelf 13, 6581 SL Malden, the Netherlands

ACKNOWLEDGEMENT

Acknowledgements

Finally, nailed it!!!

During my four years' PhD journey, challenges and difficulties just like rugged mountains standing along the way. Luckily I have met so many amazing people, those who like the stars, glow in the dark, guide me, take care of me, and support me to pursue my dreams! Because of you, I started the journey of PhD, and because of you, this journey is much more fun than I expected. Here I'd like to express my sincere gratitude to all of you, who have always been there with me.

First of all, I want to thank the best supervision team. Lourens, you are the most smart person I have ever seen. I still remember the first time that I searched for your information online, what attracted me most is not only your long list of outstanding scientific papers, but also your cheerful smiles. Then I hypothesized that you are a smart, easy-going and optimistic person that can bring me wisdom of both study and life. Not surprisingly, my hypothesis was confirmed. You are a great responsible supervisor, when I encountered difficulties, you always helped me to find good solutions; when I got lost and lagged behind, you helped me to do re-planning and nicely "push" me to meet the deadlines; when I doubted about myself, you always encouraged me with kind words and brought me confidence; when I stressed too much, you told me a wise sentence you had heard - "90 % of what you are worried about now will never happen", which is so true and do make me feel relieved..... you are also a good cooker, excellent ping-pang ball player.....your various identities made you become a respectable and amiable tutor.

Thank you Frank, you are the first supervisor that I met when arrived Wageningen, I appreciate a lot for your warm welcome and invitation for the nice Italian food. You offered me a lot of help especially at the early stage of my PhD, when lacking funding brought me a lot of difficulties to proceed my research. Thank you and Mirjam offered me free accommodations in the expensive Amsterdam when I was doing lab work in VU Amsterdam. And thank you for helping me to build the collaboration with the Kuramae group in NIOO, and to apply fund to support my molecular experiment. I also appreciate that you generously shared your life experience and showed your passion for life, which always inspired me to rethink my life. I am

happy that you like the Chinese food, especially the Hot-Pot, which my hometown is famous for. I enjoyed a lot for the several nice dinners with you, we tried Amsterdam Kokohili restaurant, Sichuan Hot-Pot, Thai Hot-Pot..... these are all my precious memories.

Thank you Ute, I admire a lot for your passion for work and science. You gave me a lot of valuable suggestions about courses and life. I appreciate a lot for your help with applying for funds to attend an international course, which enabled me to broaden my view of science and to get to know many nice researchers. You are a very hard-working and admirable woman, you set me an example of being a good teacher! I am so lucky to have many nice talks with you, we discussed not only about research, but also about society and wisdom of life, which brought me a lot of inspirations and thoughts. You are a caring and warm-hearted person, you can easily catch my struggles and tiredness, and kindly reminded me to take a break, recharge and move on. You are the one who relived my anxious about turning to age 30 in the coming June, as you have showed me that being mature and confident, being enthusiastic for life, 30+ is something that should be looked forward to.

Thank you, wonderful LOGLIFE team and all related co-authors (Agata, Chenghui, Eiko, Emiel, Frank, George, Hans, Juan, Jurgen, Juul, Leo, Lourens, Marcio, Mariet, Marjo, Mattias, Mirjam, Nico, Ohana, René, Richard, Ute and other related researchers and students). Thank you Hans Cornelissen for establishing the LOGLIFE project, which has offered me the great opportunity to explore the world of dead wood. I also thank you for the valuable inputs on my proposal and manuscripts, you are a very nice person based on my experience and the compliments from my VU friends, the most frequent sentence I heard from them is “Hans is the best teacher I have ever seen.....”. Thank you Richard Logtesteijn for offering me help with lab work, you are a funny person and I enjoyed a lot chatting with you. Thank you Juan Zuo, you are a very kind sister who gave me a lot of suggestions for research, careers and life. Thank you Chenghui Chang for your support in various aspects. I cherish a lot for the 10-year’s friendship and sincerely appreciate your continuous encouragement and help. Thank you Leo Goudzwaard for your help with fieldwork, you are a very knowledgeable, responsible and kind person. I gained way much more than just collecting research data during fieldwork; you taught me how to identify tree species, birds, and find good walnuts in the forests. I also enjoyed a lot the dinner and nice talks with you and Paula, you both are very nice persons that I will remember forever.

I am so happy that I joined the most wonderful group - Forest Ecology and Forest Management group in WUR, where I met all the lovely FEMily members. I love the interesting conversations, relaxed and happy moments with you during coffee breaks, lunch breaks, international dinners, and FEM excursions. Therefore I'd like to thank you Ambra, Aldicir, Alejandra, Arildo, Barbara, Bo Zhou, Carlos and Fabianne, Carolina, Catarina, Daisy, Danaë, Danju, Douglas, Ellen, Etienne, Francisca, Frank, Frans, Frits, Gert-Jan, Jan, Jazz, Jente, Johan, Joke, José, Juan Ignacio, Kathelyn, Koen, Lan, Laura, Louis, Lourens, Leo, Lu, Maaike, Madelon, Marielos, Marco, Marleen, Marlene, Martin, Masha and Yasmani, Mathieu, Meike, Misaki, Nathalia, Nicola, Paul, Pauline, Peter, Pieter, Qi, Rens, Rodrigo, Richard, Sophie, Surya, Sylvana, Tomas, Tomonari, Ursula, Ute, Xiaohan, Xuqing, Yanjun. Thank you my dear office mates, Richard Sikkema for your kind and beautiful sunflowers-gift and all the interesting trip stories. Thank you Barbara for your open-mined patience in explaining "famous" FEM jokes and for being my best language teacher, you are so charmosa and you helped me to "nailed it", thank you! Hi my old officemate Federico. Thank you for all the nicknames you created for me; "dangerous girl", and "Shanshanshanshan! (Symphony No. 5)", which made me became "famous" and integrated into the group quickly. Thank you for playing the nice noisy drum, which largely practiced my patience and improved my artistic taste, except for all your "mean" words for me, you are still a nice person, I have to say that I am happy to have a friend like you. Thank you Alan & Indira, Laura, Linar, Diana and Jazz, I cherish the happy moments with you during those Hot-Pot parties. Thank you Ursula, the most cute Mexican girl, we had millions of nice dinners these years, you are a sincere and kind girl, thank you for your caring, generous and the open-hearted talks. Thank you Lan for your kind support, I appreciate a lot for your company and well prepared dinners for me when I did lab work in Amsterdam, that's is really sweet. A special thanks to my paranymph Sophie, a lovely girl always with big smiles and bring people happiness. Thank you my paranymph Qi (plus Jingjing, and Jiajia, what a smart strategy - invited one paranymph and got two for free), thank you Qi for being my big brother, therefore I can always get free nice dinners these years; thank you Jingjing for your caring and support for all aspects of my life; thank you Jiajia for your pure eyes and innocent smiles, which can always make me feel the beauty of the world, I am super happy and lucky to be one of your "family" members ..... Many thanks to all PE&RC staff, especially thank you Claudius van de Vijver and Lennart Suselbeek for your efforts and time investing on those great courses and training programme, thank you for your quick and useful feedbacks whenever I had a PhD-related questions.

Many thanks to everyone that I met in the Netherlands, Anna, Azkia, Biyao, Bin Tuo, Chen Zhang, Chengcheng, Chenxuan, Chunfeng, Erlinda, Feng Xue, Frank van Langevelde, Fred, Guangyuan, Han Wang, Hao Chen, Hongyu, Ignas, Jiao Zhou, Jianli, Jingjing, Jin Gan, Jin Wang, Jiyao, Kaiyi, Kevin, Judith, Ke Chen & Jiyao, Liang Zheng, Li Kang, Li Lin, Lili, Lubin, Marc, Meijun, Meixiu, Mengting, Mengying, Min Xu, Minjie, Ning Ding, Panpan & Xiaoma, Qi Zheng, Qijun & Jie sister & Lele, Qinghua & Yingying, Qing Zhan, Qiong Wu, Shenglai, Shudong, Saori, Shuang Song, Shuo Zhang, Shuwen, Taojun, Tian Tao, Wenjie, Wenhao, Wenyu, Xilong, Xiaqu, Xianfeng, Xiang Wang, Xiaohan, Xiaoxiao, Xiangyu, Xiaolin, Xiaomei & Yale, Xin Li, Xinfang, Xin Wang & Lei Zheng, Xu Chen, Yang Hu, Yaobin, Yifan, Yiran, Yu Peng, Yuanli, Yue Zhao, Yuting, Yueling, Yongran, Zezhong, Zhezeng, Zhihong, Zhijun, Zulin Mei, etc. Life is not that hard at my final stage of PhD because of your company, warmth, and encouragement. Thank you my "emergency" assistant Zhikang, hard-working Hui Jin, romantic Fangfang, and sunny Manqi. Here are three special angles that I have to mention and express my sincere gratitude, Nana – a self-disciplined beautiful sports lady, a kind and heart-warmed friend, Yanjie – a naughty, helpful, smart and free cutie, Yanjun – my "twin" sister, we came with the same flight four years ago, and submitted our thesis at the same day, your warm company, our tons of laughs, and occasionally "fights" make our friendship deeper. We spent most of the weekends together (before Yanjie became Dr. Yanjie) and had many beautiful and unforgettable moments, you brought me a lot of happiness, cheers, warmth, and support, I cannot finish my PhD thesis without your firm support and trust, I love you girls. Oh, there is another guy - Lei Deng, the only one who can compete with Qi in Wageningen in terms of cooking skills, please keep fighting for the No.1 chef, and as a nice friend, I am quite willing to be a referee. I also want to thank my special friends Dengxiao, Huan Cheng, Lina, Mengdi & Xin Li, Mao Ye, Yutong, and all our Benshuo brothers and sisters, Baoru, Mei Li, Peipei, Shufen, Jie Chen, Wei Luo, Shibin, Wenbin, Xing Huang, Lin Zhou, Cheng Zhang, Song Guo, Yi Liu, I cherish the 6-year's classmateship and ten years friendship, I appreciate all the warmth and happiness you offered me.

Thank you my best enlightenment teacher – Tingchun Xiong, you taught me to study hard and play hard, your humorous and optimistic personality influenced my whole life. Without your help, I may not be able to walk out of our small village and explore a bigger world. I also want to thank my Bachelor & Master supervisor – Tingxing Hu, who guided me to explore the beautiful world of science, and taught me to dream passionately. Your encouragement and selfless dedication supported me to start the PhD journey. Thank you the whole members of

“Army” Hu (Cao He, Chenghao, Dexiang, Gang Chen, Guangliang, Hong Chen, Hongling, Jie Luo, Jiulong, Lihua, Liping, Qian Wang, Ruyi, Wei Ding, Wei He, Weiwei, Xiaomei, Xin Zhou, Xiuyan, Xue jiang, Yong Peng, Yuhua.....), your kind support made my bachelor and master journey so joyful and unforgettable. There must be many other people who helped me during the challenging PhD journey, here I’d like to express my sincere gratitude and wishes to all of you.

Last but most important, thank you my dear mom & dad, my grandparents, my big family members, you are my warm harbour, no matter how far I go, you will always be there for me. Your deep love are my key motivation to move forward. I also want to thank myself, tasted quite a lot tears and difficulties but never give up. Thank you my dear grandpa, Anjun Yang, an odd old man that I deeply love, thank you for bringing me an enjoyable childhood with a lot of love and novel hand-make toys. Thank you for all the fantastic homemade bedtime stories that I will never be bored of, thank you for your mental support helping me to accomplish the thesis, thank you for teaching me to cherish every one you love now before it’s too late..... I love you and I miss you. 我亲爱的家人，感谢你们给予的无尽的爱，勇气与自由，我爱你们!

BIOGRPAPHY

Short biography

Shanshan Yang was born on 22 June, 1992 in Chengdu, China. She had a beautiful and unforgettable childhood at a charming little village-Daxing, Pujiang. Growing up at a place surrounded by trees, rivers and mountains built her interests in nature, which is an important motivation for Shanshan to start the Bachelor of Forestry in Sichuan Agriculture University (Yaan campus, Sichuan) in 2011. For her bachelor thesis, she studied the impact of decomposing *Cinnamomum septentrionale* leaf litter on the growth and physiological characteristics of three crops (corn, cucumber, and cowpea). From 2014, she continued her master's research on Forest Silviculture in the same university (Chengdu campus, Sichuan). She carried out the research on testing the allelopathy effect of *Cinnamomum septentrionale* leaf litter on maize growth and the relieving effect of fertilization. Since 2017, Shanshan was supported by a PhD scholarship from the Chinese Scholarship Council and started her PhD research at Forest Ecology and Forest Management Group, Wageningen University and Research (Wageningen, the Netherlands). Shanshan's research was based on a long-term common garden dead wood experiment - LOGLIFE, she mainly aimed at investigating how stem traits and plant strategies differ among stem compartments (i.e., inner wood, out wood, and bark) of different temperate tree species, and how these stem traits interact with fungal decomposers affecting stem decomposition and add to forest biodiversity. Now Shanshan has finished her PhD thesis and is ready to continue the scientific journey with passion.

Journal publications

Yang SS, Limpens J, Streck FJ, Sass-Klaassen U, Cornelissen, JHC, Hefting M, van Logtestijn R, Goudzwaard L, Dam N, Dam M, Veerkamp M, den Berg B, Brouwer E, Chang CH, Poorter L. Dead wood diversity promotes fungal diversity. Oikos, 2021. Doi: 10.1111/oik.08388.

Chang CH, van Logtestijn RSP, Goudzwaard L, van Hal J, Zuo J, Hefting M, Sass-Klaassen U, **Yang SS**, Sterck FJ, Poorter L, Cornelissen, JHC. Methodology matters for comparing coarse wood and bark decay rates across tree species. Methods in Ecology and Evolution, 2020. 11(7):828-838.

Yang SS, et al. Stem trait spectra underpin multiple functions of temperate tree species. Under review.

Yang SS, et al. Stem traits, compartments, and tree species affect fungal communities on decaying wood. Under review.

PE&RC Training and Education Statement

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)
- Dead wood is a source of life: stem traits, fungal communities, and stem decomposition of temperate tree species

Writing of project proposal (4.5 ECTS)
- Dead wood is a source of life: stem traits, fungal communities, and stem decomposition of temperate tree species

Post-graduate courses (9.3 ECTS)
- Structural Equation Modelling (SEM); WUR (2018)
- International course on wood anatomy & tree-ring ecology; Swiss Federal Institute for Forest, Snow and Landscape Research; WSL (2019)
- Linear models; WUR (2019)
- Introduction to R for statistical analysis; WUR (2019)
- Generalized linear models; WUR (2019)
- Mixed linear models; WUR (2019)
- Forest management across Europe, towards climate-smart strategies; PE&RC (2021)

Deficiency, refresh, brush-up courses (12.5 ECTS)
- Basic statistics; WUR (2017)
- Forest ecology and forest management; WUR (2018)
- Ecology methods; WUR (2018)

Laboratory training and working visits (11.4 ECTS)
- Physical-chemical traits measurement of wood and bark; Vrije Universiteit Amsterdam (2018-2019)
- Wood decomposition rate, CT scan analysis; Ghent University (2019)
- Sample preparation for fungal DNA analysis; Vrije Universiteit Amsterdam (2019)
- Fungal DNA extraction-decayed wood and bark; Netherlands Institute of Ecology (NIOO-KNAW) (2019)

Competence strengthening / skills courses (2.2. ECTS)
- Brain friendly working and writing; WUR (2019)
- Reviewing a scientific manuscript; WUR (2020)
- Scientific writing; WUR (2021)

Scientific integrity/ethics in science activities (0.3 ECTS)
- Scientific integrity; WUR (2020)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)
- PE&RC First years weekend (2017)
- PE&RC Day (2019)

Discussion groups / local seminars or scientific meetings (7 ECTS)
- Symposium frontiers in resilience research (2017)
- Journal club; FEM (2017-2020)
- LOGLIFE annual meeting (2018, 2020)
- R user discussion group (2019)
- Introduction to LaTeX lunch workshop (2019)
- R club; FEM (2020)
- The 4th dimension in earth sciences; guest lecture tree story: what we can learn about climate and forest history from the rings in trees (2021)

International symposia, workshops and conferences (5.4 ECTS)

- BES: Festival of Ecology; online; the United Kingdom (2020)
- Netherlands Annual Ecology Meeting; online; the Netherlands (2021)

Supervision of BSc/MSc students (8 ECTS)

- Mass loss and decay of two tree species in a decomposition experiment
- CT-scanning for assessing wood decomposition
- Wood anatomy in relation to decay rate of 4 conifer species
- Density, permeability and anatomical structures of decayed wood
- Dead wood decomposition rate (LOGLIFE)
- Decomposition patterns of 10 temperate wood species

Colophon

The research described in this thesis was financially supported by China Scholarship Council, under Grant Agreement Number (201706910085) and the Nora Croin Michielsen Fonds (2018).

Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Cover illustration:
Jeroen Helmer

Printed:
ProefschriftMaken