Norwegian University of Life Sciences
Faculty of Environmental Sciences and
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Philosophiae Doctor (PhD)
Thesis 2017:88

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Vedlevende insekter påvirker kolonisering og suksesjon av sopp i død ved

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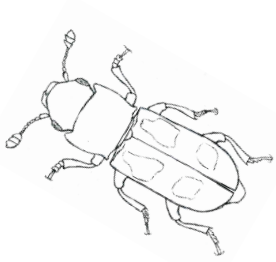

Oh, and lastly; thanks to the insects and the fungi! For all their hard work which keeps our ecosystems going, and for all their fascinating diversity and awesomeness.

Rannveig M. Jacobsen, Ås, September 2017

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Paper I

Birkemoe, T., Jacobsen, R. M., Sverdrup-Thygeson, A. & Biedermann, P. H. W. (In prep.) Insect-fungus interactions in dead wood systems. In Ulyshen, M. (editor) *The diversity, ecology and conservation of saproxylic insects*. Springer.

Paper II

Jacobsen, R. M., Kauserud, H., Sverdrup-Thygeson, A., Bjorbækmo, M. M. & Birkemoe, T. (2017). Wood-inhabiting insects can function as targeted vectors for decomposer fungi. Fungal Ecology, 29: 76-84. DOI: 10.1016/j.funeco.2017.06.006. CC BY-NC-ND 4.0

Paper III

Jacobsen, R. M., Sverdrup-Thygeson, A., Kauserud, H. & Birkemoe, T. Revealing hidden insect-fungus interactions in detritivore networks. *Submitted.*

Paper IV

Jacobsen, R. M., Sverdrup-Thygeson, A., Kauserud, H., Mundra, S. & Birkemoe, T. Exclusion of invertebrates influences saprotrophic fungal community and wood decay rate in an experimental field study. *Submitted.*

Paper V

Jacobsen, R. M., Birkemoe, T. & Sverdrup-Thygeson, A. (2015). Priority effects of early successional insects influence late successional fungi in dead wood. Ecology and Evolution, 5 (21): 4896-4905. DOI: 10.1002/ece3.1751. CC BY 4.0

Sammendrag

Insekter og sopp utgjør en stor andel av alle arter på landjorda, og leverer viktige økosystemtjenester som pollinering, karbonlagring og nedbrytning. Sopp er spesielt viktig for nedbrytning av plantemateriale, siden de kan produsere enzymer som effektivt bryter ned cellulose og lignin. Dette gjør sopp til de viktigste nedbryterne av død ved, hvilket gir grunnlag for en næringskjede med stort mangfold av vedlevende arter. Arter som lever i død ved utgjør rundt 25% av alle arter tilknyttet skog i de nordiske landene, og flertallet av de vedlevende artene er insekter og sopp.

Død ved er et levested med begrenset varighet, siden de vedlevende artene nødvendigvis vil bryte ned habitatet over tid. Dette gjør spredning spesielt viktig for vedlevende arter. Vedlevende insekter sprer seg hovedsakelig ved å fly, og de kan bruke syn og lukt til å finne passende substrat. Man antar at vedlevende sopp i hovedsak sprer sporene sine med vinden, med unntak av noen få arter som lever i mutualistiske forhold med treveps, barkbiller eller ambrosiabiller. Men ved en gjennomgang av relevant litteratur (**artikkel I**) fant vi flere studier som indikerte at spredning med insekter kan være viktig for vedlevende sopp generelt. Mange vedlevende insekter blir tiltrukket av og besøker fruktlegemer av vedlevende sopp, og disse insektene kan bære med seg intakte soppsporer i tarmene eller på hudskjelettet. Dermed kan vedlevende insekter kanskje fungere som en alternativ spredningsmåte for sopp, som i motsetning til vindspredning er målrettet mot egnede substrat. I denne avhandlingen undersøker vi hvorvidt spredning med insekter påvirker kolonisering og suksesjon av sopp i død ved.

I **artikkel II** samlet vi vedlevende insekter fra ferske ospestokker i felt, og brukte DNA metabarcoding og elektronmikroskopi til å undersøke soppmateriale på disse insektene. Mange ulike arter vedlevende insekter viste seg å inneholde sopp-DNA, deriblant DNA som matchet vedlevende sopp som silkekjuke (*Trametes versicolor),* knuskkjuke *(Fomes fomentarius)* og rødrandkjuke *(Fomitopsis pinicola).* Elektronmikroskopi viste at iallfall noe av dette soppmaterialet kom fra sporer på insektenes hudskjelett. I **artikkel III** analyserte vi disse dataene videre som interaksjonsnett basert på antall individuelle insekter med DNA fra ulike arter sopp. Strukturen på nettverkene varierte avhengig av hvilken funksjonell gruppe med sopp som ble analysert. For eksempel var nettverket mellom vedlevende insekter og vedlevende sopp mer spesialisert enn nettverket med plantepatogene sopp eller sopp forsøksvis klassifisert som insektsymbionter. Vedlevende sopp og insekter utviste omtrent samme grad av spesialisering som tidligere studier har beregnet for nettverk basert på frøspredning med dyr. Dette er i tråd

med vår hypotese om at vedlevende insekter som besøker sporulerende sopp deretter sprer sporene til nye substrat, hvilket resulterer i et moderat spesialisert interaksjonsnettverk.

I **artikkel IV** og **V** studerte vi kortsiktige og langsiktige effekter av insekt-sopp-interaksjoner på sammensetningen av soppsamfunn. I **artikkel IV** testet vi effekten av å ekskludere invertebrater større enn 1 mm fra ferske ospestokker. Vi brukte DNA metabarcoding til å sammenligne soppsamfunnet i ospestokker med og uten invertebrater, og fant en signifikant effekt av eksklusjon av invertebrater. Ospestokkene uten invertebrater hadde en annen sammensetning av sopp i veden og høyere vedtetthet, hvilket indikerte at nedbrytningen hadde gått saktere i de to årene siden eksperimentet startet. Resultatene våre antyder at invertebrater påvirker soppens kolonisering av død ved, hvilket kan resultere i ytterlige effekter på soppsamfunnets suksesjon. I samsvar med dette fant vi i **artikkel V** at insekter i tidlig suksesjon av død ved ser ut til å påvirke soppsamfunnet senere i suksesjonen. Vi samlet insekter med vindusfeller de første fire årene etter å ha drept ospetrær, og registrerte fruktlegemer av sopp på de samme ospene tolv år senere. Det viste seg at flatkjuke (*Ganoderma applanatum*) var mye vanligere på ospene der spesifikke fungivore billearter (*Glischrochilus quadripunctatus* og *Agathidium nigripenne*) hadde vært tallrike de første årene etter trærnes død. Gult dvergbeger (*Bisporella citrina*) var derimot vanligere der det hadde vært mange ved-borende biller (hovedsakelig trebukker). Antall ved-borende biller var positivt korrelert med tap av bark fra ospene, hvilket var fordelaktig for gult dvergbeger.

Selv om også andre insekt-sopp-interaksjoner enn sporespredning kan ha hatt innvirkning på resultatene i **artikkel IV** og **V**, så viste **artikkel II** og **III** at vedlevende insekter sprer vedlevende sopp til nye substrat, hvilket dermed sannsynligvis har bidratt til effektene i **artikkel IV** og **V**. **Artikkel V** viste at insekt-sopp-interaksjoner kan ha langsiktige konsekvenser, og **artikkel IV** indikerte at disse interaksjonene også kan påvirke nedbrytning av ved. Vi oppmuntrer derfor til langtidsstudier av effektene av insekt-sopp-interaksjoner som inkluderer mål på økosystemtjenester som vednedbrytning.

Summary

Insects and fungi comprise a large proportion of all species in terrestrial habitats, and provide important ecosystem services such as pollination, carbon sequestration and decomposition. Fungi are especially important for decay of plant material, as their extensive enzymatic machinery enables them to efficiently decompose cellulose and lignin. Thus, fungi are the primary decomposers of wood, and fuel a very diverse food chain of species dependent on dead wood, i.e. saproxylic species. Saproxylic species comprise approximately 25% of all forest-dwelling species in the Nordic countries, and the most species rich eukaryotic saproxylic taxa are fungi and insects.

Dead wood is an inherently ephemeral habitat, since the saproxylic species occupying this habitat will inevitably contribute to its destruction through decomposition. This continual process of community assembly and disassembly makes dispersal especially important to saproxylic species. Saproxylic insects mostly disperse by flying, and can target suitable substrates by sight and odour. Saproxylic fungi are generally assumed to disperse by wind-borne spores, with the exception of a few species known to be dispersed by mutualistic wood wasps, bark beetles or ambrosia beetles. However, upon review of relevant literature, we found in **paper I** that several studies indicate a broader role for insect-vectored dispersal of fungal propagules. Many saproxylic insects are attracted to and visit fungal fruit bodies, and can carry viable fungal propagules internally or externally. Thus, saproxylic insects might function as an additional dispersal mode for saproxylic fungi, which unlike wind dispersal can be targeted to suitable substrates. In this thesis, we have investigated whether insect-vectored dispersal might influence community assembly and succession of fungi in dead wood.

In **paper II** we sampled saproxylic insects from recently cut aspen logs in the field, and used DNA metabarcoding and scanning electron microscopy (SEM) to investigate fungal material carried by these insects. We found that several different species of saproxylic insects carried fungal DNA, including DNA annotated as wood-decay fungi such as *Trametes versicolor, Fomes fomentarius* and *Fomitopsis pinicola*. The SEM-pictures revealed that at least some of this fungal material was carried as spores on insect exoskeletons. We analysed this data further in **paper III** as interaction networks based on fungal DNA isolated from individual insects. We found that the networks differed in structure depending on functional group of the fungi. For instance, the networks between saproxylic insects and wood-decay fungi were more specialized than networks with plant pathogenic fungi or fungi tentatively classified as insect symbionts. Interestingly, the degree of specialization between saproxylic insects and wood-decay fungi

was similar to that of animal-mediated seed dispersal networks in previous studies. We suggest that this interaction network might be based on opportunistic spore-feeding and subsequent spore dispersal by the insects, resulting in a moderate degree of specialization.

In **paper IV** and **V**, we assessed the short-term and long-term effects of insect-fungus interactions on fungal communities. In **paper IV** we experimentally excluded invertebrates larger than 1 mm from recently cut aspen logs. We used DNA metabarcoding to compare the fungal communities established in these logs after two years in the field, with those of logs that were accessible to invertebrates. We found that invertebrate exclusion significantly affected fungal community composition and resulted in higher wood density, indicating reduced wood decay rates. Thus, invertebrates seemed to influence community assembly of fungi, which might result in priority effects that affect subsequent succession of both insects and fungi. Correspondingly, in **paper V** we found that colonization history of saproxylic insects in early succession seemed to influence the fungal community in late succession. Insects were sampled with flight interception traps during the first four years after tree death, and fungal fruit bodies were registered at the same dead wood after twelve years. The polypore *Ganoderma applanatum* occurred more frequently at sites where certain species of fungivorous beetles (*Glischrochilus quadripunctatus* and *Agathidium nigripenne*) had been abundant after tree death, while the ascomycete *Bisporella citrina* occurred more frequently where wood-boring beetles (mainly cerambycids) had been abundant after tree death. Abundance of wood-boring beetles seemed to increase bark loss, which benefitted *B. citrina.*

Thus, while insect-fungus interactions other than insect-vectored propagule dispersal could have affected the results in **papers IV** and **V**, **papers II** and **III** showed that dispersal of saproxylic fungi by insects does occur and this interaction probably contributed to the effects seen in **papers IV** and **V**. **Paper V** showed that insect-fungus interactions can have long-term consequences, and **paper IV** indicated that these interactions can influence the process of wood decomposition. We therefore call for long-term studies of the effects of insect-fungus interactions that incorporate measures of ecosystem processes such as wood decay.

Synopsis

1. Introduction

Insects and fungi comprise a large proportion of the biodiversity in terrestrial habitats. Recent estimates of global species richness for terrestrial arthropods range from 2.8 to 13.7 million species (Hamilton et al. 2010; Hamilton et al. 2011; Ødegaard 2000), while estimates for fungal species richness suggest between 1.5 to 3 million species (Blackwell 2011; Hawksworth 2012). As recent predictions for total eukaryotic species richness range from 2 to 8 million species (Costello et al. 2011; Costello et al. 2013; Mora et al. 2011), it is clear that very many of Earth's eukaryotic species are arthropods and fungi.

Several species of insects and fungi are predominantly found in brown food webs based on substrates such as litter, dung and dead wood. Species that depend on dead wood (Fig. 1), i.e. saproxylic species (Speight 1989), are a major component of forest biodiversity and include many species threatened by extinction (Gärdenfors 2010; Henriksen & Hilmo 2015; Rassi et al. 2010). Saproxylic species perform an essential ecosystem function by decomposing dead wood, with wood-decay fungi as the main agent of mass loss (Boddy 2001; Kubartová et al. 2015). Fungi have developed an extensive enzymatic machinery which enables them to efficiently decompose recalcitrant components in wood such as cellulose and lignin (Boer et al. 2005; Floudas et al. 2012). Insects and other arthropods (with the exception of termites) do not seem to have a comparable direct effect on mass loss of wood, but have been shown to significantly affect decomposition rates through mechanisms such as substrate alteration, nitrogen fertilization and biotic interactions (Ulyshen & Wagner 2013; Ulyshen 2016). Due to the influence of invertebrates and fungi on decomposition of dead wood, these saproxylic species are integral to nutrient cycling in forest ecosystems (Cornwell et al. 2009; Fekete et al. 2014; Gonzalez-Polo et al. 2013).

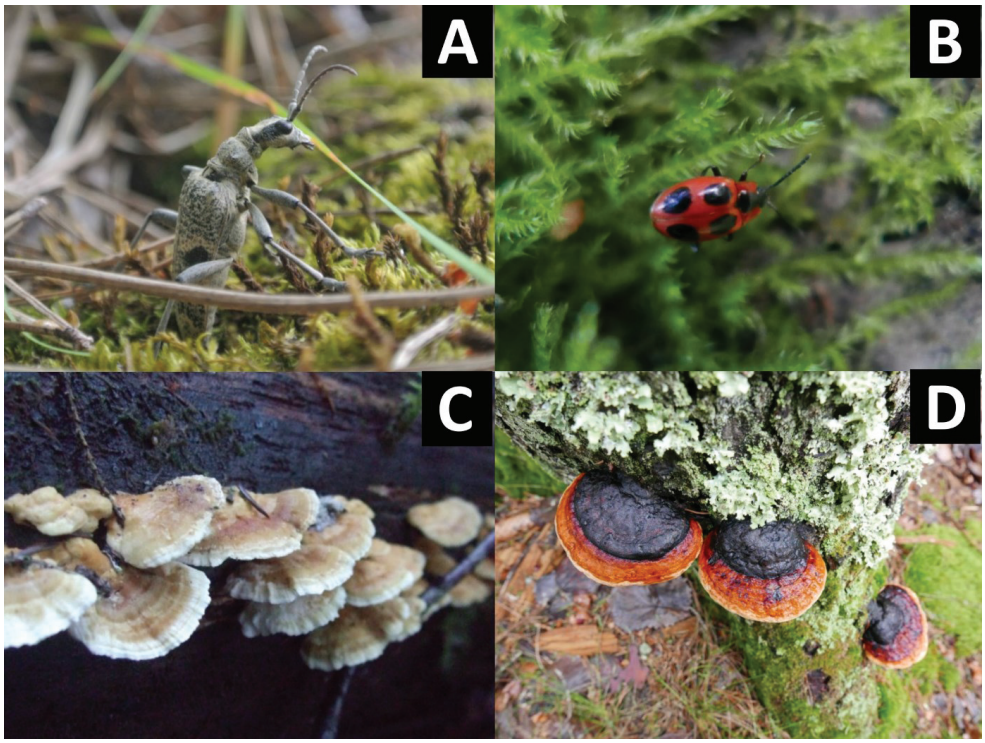

Figure 1) Examples of saproxylic insects and fungi. (A) The long-horn beetle Rhagium mordax, ovipositing on dead wood of oak (Quercus sp.). (B) The fungivorous beetle Endomychus coccineus, here on an aspen log from ***paper IV****. (C) The annual polypore Trametes ochracea, here growing on an aspen log from* ***paper V****. (D) The red belt conk (Fomitopsis pinicola), a perennial polypore seen here growing on spruce dead wood. Photos: R. M. Jacobsen.*

The decomposition process means that brown food webs are inherently ephemeral, in the sense that each habitat patch inevitably disappears due to the actions of the species inhabiting that patch. Therefore, community assembly and disassembly are continual processes that occur regularly in brown food webs (O'Neill 2016; Pechal et al. 2014; Vanschoenwinkel et al. 2010), making dispersal essential to the persistence of species that depend on these ephemeral habitats (Southwood 1977). Dispersal is a relatively stochastic process, although there is a degree of determinism due to dispersal-related traits of species. Even so, there is usually some random variation in arrival order of species during community assembly, which can affect community composition and subsequent succession if environmental filters do not override this effect (Chase 2010). By introducing an element of stochasticity to community composition, dispersal and variation in arrival order has been shown to increase beta diversity among habitat patches

(Chase 2010; Vannette & Fukami 2017). Effects of arrival order on community composition have been termed priority effects, due to studies showing that species arriving early are sometimes prioritized over species arriving late (Dickie et al. 2012; Peay et al. 2011; Shorrocks & Bingley 1994). The advantage of early arrival could stem from monopolizing resources or increasing population size, thereby gaining a competitive advantage over late arrivals. This would lead to inhibitory succession (Connell & Slatyer 1977). However, positive priority effects leading to facilitative succession have also been demonstrated. Early colonizers can for instance alter the habitat in ways that are beneficial to the late colonizer (Hughey et al. 2012; Jacobsen et al. 2015a; Ottosson et al. 2014), or early colonizers can vector propagules of the late colonizer (Hughey et al. 2012; Weslien et al. 2011). Priority effects might be especially important in habitats such as dead wood, where substrate alterations are irreversible and the habitat has a very restricted expanse. Correspondingly, several experimental studies have demonstrated a significant effect of arrival order on community composition of wood-decay fungi, which in turn affects wood decay rates (Dickie et al. 2012; Fukami et al. 2010; Hiscox et al. 2015; Hiscox et al. 2016; Leopold et al. 2017).

Despite the importance of dispersal for dead wood communities, there are relatively few studies of dispersal ecology of insects and especially fungi (Driscoll et al. 2014; Holyoak et al. 2008). For saproxylic insects, dispersal *mode* is usually evident as most species fly, while dispersal *range* has only been estimated for a few species (Haack et al. 2017; Nilssen 1984; Ranius 2006; Svensson et al. 2011) and is often inferred from correlations with habitat amount at different scales (Bergman et al. 2012; Jacobsen et al. 2015b; Ranius et al. 2011; Sverdrup-Thygeson et al. 2014). For saproxylic fungi, there are several studies of dispersal *range*, either using direct measurements such as spore-capture (Hallenberg & Kuffer 2001; Kallio 1970; Norros et al. 2012) or indirect indications such as genetic differentiation of distant populations (Franzen et al. 2007; Högberg & Stenlid 1999; Parrent et al. 2004) or lack thereof (Gosselin et al. 1999; Högberg et al. 1999; Kauserud & Schumacher 2003). However, in studies of fungal dispersal it is often only assumed that spores are dispersed by wind. Dispersal *mode* is rarely investigated.

For sessile organisms such as fungi, an essential aspect of dispersal mode is the dispersal vector. Wind is a passive vector, providing undirected, random dispersal of propagules. The small spores of saproxylic fungi can potentially be dispersed by wind over large distances (Norros et al. 2014), but spore deposition rate (Norros et al. 2012) and probability of spore establishment (Edman et al. 2004) declines rapidly with distance from the fruit body. In contrast, animals such as saproxylic insects can function as targeted dispersal vectors if they share the habitat

preferences of the fungi. Insect vectors can potentially even deliver the spores directly to optimal microhabitats such as the cambium under bark. Several authors have suggested that insects and other arthropods might contribute to fungal dispersal (Malloch & Blackwell 1992; Norros 2013; Talbot 1952; Watkinson et al. 2015), and fungal fruit bodies are frequently found growing in situations where wind dispersal would be inefficient, i.e. very close to the soil surface or beneath bark (Norros & Halme 2017; Talbot 1952). Dispersal of spores with arthropod vectors such as saproxylic insects could be complementary to wind dispersal, providing benefits such as dispersal under different environmental conditions or dispersal targeted towards suitable substrates.

The capacity for navigation (Nathan et al. 2008) is an important distinction between wind-dispersed and animal-dispersed species, which influences their population dynamics. Plants with targeted dispersal of seeds by animal vectors have been found to have higher tolerance for habitat fragmentation relative to wind-dispersed plants (Marini et al. 2012; Montoya et al. 2008; Purves & Dushoff 2005), provided the animal vector survives in the fragmented habitat (Cordeiro & Howe 2003; Galetti et al. 2006). Similarly, targeted dispersal with saproxylic insects might help saproxylic fungi persist in fragmented forests with low volumes of dead wood. Animal-dispersed and wind-dispersed plants have also been found to respond differently to edge effects and connectivity (Damschen et al. 2008). Knowledge of dispersal mode is therefore essential in conservation planning, which is highly relevant for the many saproxylic fungi threatened by extinction (Gärdenfors 2010; Henriksen & Hilmo 2015; Rassi et al. 2010).

Few studies have investigated the importance of insect-vectored dispersal for saproxylic fungi. Of course, symbiotic fungi of bark beetles, ambrosia beetles and wood wasps are dispersed by these insects (Batra 1963; Harrington 2005; Slippers et al. 2011), but these well-known mutualists only comprise a small fraction of the diversity of saproxylic insects and fungi. While there are several studies indicating that insect-vectored dispersal might be of broader importance to the saproxylic community (e.g. Lim 1977; Weslien et al. 2011; Strid et al. 2014), this field of research has until recently lacked focused effort. However, recent advancements in molecular methods seem to be fuelling an increased interest in insect-fungus interactions and their role in structuring decomposer communities (Crowther et al. 2013; Leopold et al. 2017; Strid et al. 2014; Ulyshen et al. 2016).

Objectives

In this thesis I use several different approaches to investigate the importance of insect-vectored dispersal for saproxylic fungi, with DNA metabarcoding as an important tool to identify fungi isolated from dead wood or insects. Specifically, my research questions were:

1) What is currently known regarding insect-fungus interactions in dead wood; specifically, are there indications that insect-vectored dispersal might be important to non-mutualistic saproxylic fungi? (**Paper I**)

2) Do saproxylic insects carry fungi to dead wood, and does the composition of fungi depend on the insect taxon? (**Paper II**)

3) For saproxylic insects that do carry fungi, how specialized are the interactions with different functional groups of fungi? (**Paper III**)

4) Does exclusion of insects and other invertebrates affect wood decay and the fungal community that establishes in dead wood? (**Paper IV**)

5) Does initial colonization history of saproxylic insects affect the fungal community present in the dead wood several years later? (**Paper V**)

Thus, I start by reviewing current knowledge of insect-fungus interactions, and I will focus here on the section regarding insect-vectored dispersal of fungi (**paper I**). Our own research then aims to fill the knowledge gaps regarding insect-vectored dispersal, by first taking a detailed look at whether the underlying interactions necessary for insect-vectored dispersal of saproxylic fungi are taking place (**paper II**) and how specialized these interactions are (**paper III**), then moving on to the short-term (**paper IV**) and the long-term (**paper V**) effects of insect-fungus interactions on the fungal community in dead wood.

2. Methods

*Table 1) Summarized methods for **papers I-IV**.*

	Data	Experimental units	Main analyses
Paper I	Literature review for a book chapter.	Not applicable.	Not applicable.

Paper II	Fungal DNA extracted from insect samples (same as **paper III**).	343 beetle individuals sampled from aspen logs at eight sites in two landscapes.	Binomial GLMs were used to test whether beetle taxa differed in how often they carried fungal DNA. PCA (Hellinger-transformed data) was used to explore whether composition of fungi depended on beetle taxon.
Paper III	Fungal DNA extracted from insect samples (same as **paper II**).	Networks with 17 taxa of insects (187 individuals) and 3 functional groups of fungi; 35 taxa of insect symbionts, 22 taxa of wood-decayers, 60 taxa of plant pathogens.	Degree of specialization (H_2'), modularity (QuanBiMo) and nestedness (WNODF) were analysed to assess the structure of the insect-fungus networks, and tested against null models.
Paper IV	Fungal DNA extracted from wood samples, density measures of wood core samples.	30 sites in two landscapes with four logs/treatments at each site; cage, cage control, control and ethanol-baited positive control. 60 samples per treatment were taken after two years of wood decay. In addition, 53 wood samples were taken directly after tree felling.	RDA (Hellinger-transformed data) with conditional design variables was used to investigate the effect of experimental treatment on fungal communities. Linear mixed models were used to test whether wood density and number of sequences from specific fungal species differed between treatments.
Paper V	Insects sampled with flight interception traps and registration of fungal fruit bodies.	55 sites in two landscapes, each site with an aspen high stump and log. Insects sampled in year one to four after tree death, fungi registered in year twelve.	Binomial GLMs were used to test whether abundance of fungivorous or wood-boring beetles affected occurrence of any of three species of fungi on the aspen dead wood at the sites.

2.1 Literature review (paper I)

Our topic for the book chapter (**paper I**) was "Insect-fungus interactions in dead wood systems", which we split into four main sections; section 4 "Fungivory and its effects" by T. Birkemoe, section 5 "Insect-vectored dispersal of non-mutualistic fungi" by R. M. Jacobsen, section 6 "Symbioses between insects and fungi in dead wood" by P. H. W. Biederman and section 7 "Indirect interactions" by A. Sverdrup-Thygeson. All authors also contributed to editing the entire manuscript, and to introductory and concluding sections. Literature was found with Web of Science and Google Scholar, as well as through library services in order to obtain older references and books.

2.2 Study system (papers II-V)

We chose to focus on saproxylic beetles (Coleoptera), as they are the most species-rich insect group in dead wood (Stokland et al. 2012).

Our study system was aspen (*Populus tremulae*) dead wood in managed forests in the south boreal vegetation zone (Moen 1998) in Southern Norway. The forests were dominated by spruce (*Picea abies*), with smaller proportions of pine (*Pinus sylvestris*), birch (*Betula pubescens*) and aspen.

We chose to use aspen dead wood in our studies as it is a very species rich substrate with high conservation value in boreal forests (Jonsell et al. 1998; Tikkanen et al. 2006) and wood decay progresses relatively rapidly (Angers et al. 2012; Kahl et al. 2017), making it more likely that relevant decay processes would take place during the duration of our experiments. Additionally, bark beetles are not numerically dominant in early decay stages of aspen dead wood (Sverdrup-Thygeson & Ims 2002; Sverdrup-Thygeson & Birkemoe 2009) like they frequently are in coniferous dead wood in spruce-dominated forests, thereby allowing us to investigate the effects of other insect-fungus interactions than bark beetle mutualisms.

All studies were conducted in two landscapes (Fig. 2); Losby forest holdings in Østmarka (Lat. 59.87, Long. 10.97, 150–300 m.a.s.l.) and Løvenskiold-Vækerø forest holdings in Nordmarka (Lat. 60.08, Long. 10.58, 200–500 m.a.s.l.).

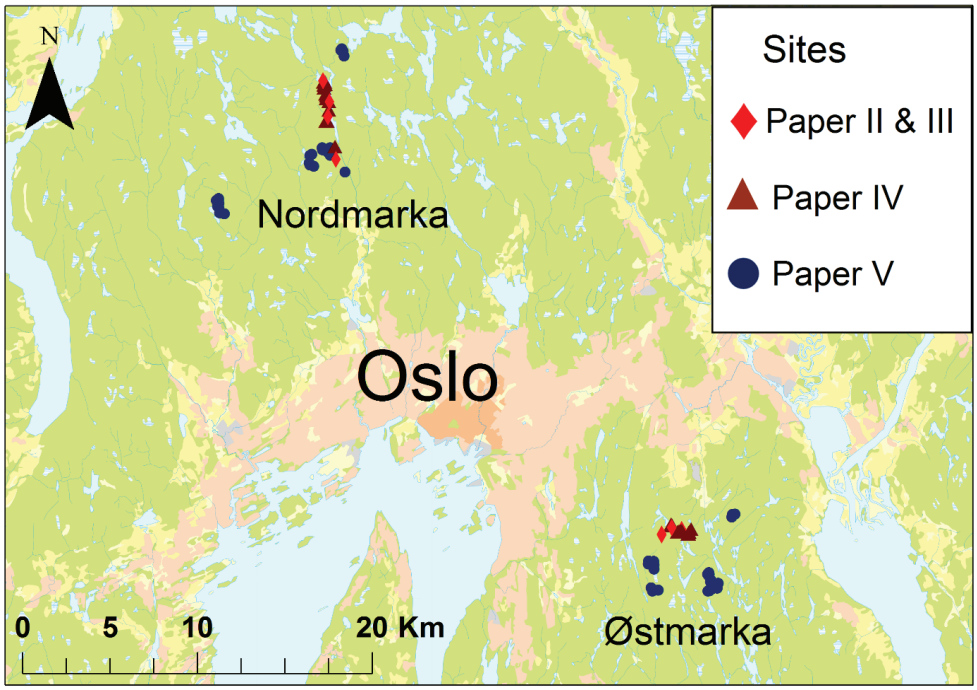

*Figure 2) Study sites for **papers II-V** in Nordmarka and Østmarka, South-East Norway.*

2.3 Dead wood

Papers II – IV

The dead wood for **papers II – IV** came from 17 aspen trees from the same stand in Ås municipality (Lat. 59.66, Long. 10.79, 92 m.a.s.l.) which were felled in March 2014 and cut into 1 meter long logs with average diameters of 27.6 cm (range 20.5 - 36.4 cm).

Fresh wood samples were taken for **paper IV** from sections between every two or three logs (Fig. 3). The wood samples were taken by drilling 10 cm into the wood after first removing the bark, at two different locations on the circumference of the section. Both the drill bit (12 mm) and knife used for removing the bark were sterilized between each sample with ethanol and fire. Wood samples were stored at -80ºC.

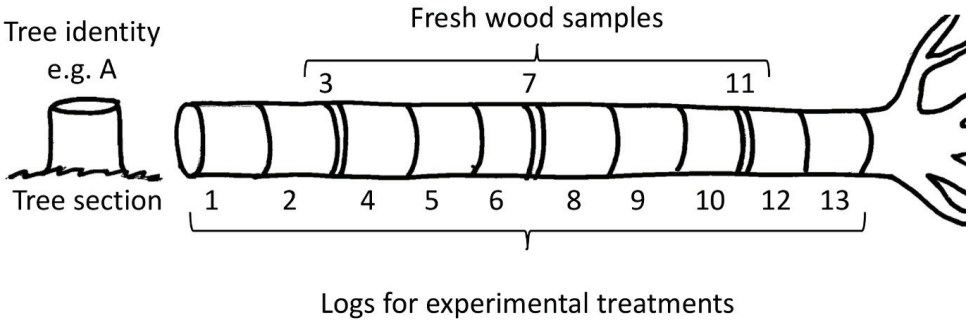

*Figure 3) An example of how a felled tree was divided into logs for **papers II-IV** and fresh wood samples for **paper IV**, and the classification of tree identity and tree section in **paper IV**.*

Paper V

The dead wood for **paper V** was created in 2001 in each landscape by cutting mature aspen trees (diameter ≥ 20 cm at breast height, i.e. 1.3 m above ground) into 4 meter tall high stumps (Fig. 4) with the fallen top half of the trees forming logs. This was done at 30 sites in each landscape, resulting in a total of 60 high stumps and logs. However, high stumps and/or logs were missing at five sites in 2013.

*Figure 4) Aspen high stump for **paper V** in Østmarka. Photo: R.M. Jacobsen.*

2.4 Study design

Papers II & III

In spring 2014, twenty-four aspen logs were distributed at four sites in each landscape with a mean distance of 1574 meters between sites within a landscape. All selected sites were in semi-shaded, mature spruce forest. Beetles were sampled during May to August in 2014 and 2015 on a total of 11 sampling occasions per site. Beetles were sampled individually with tweezers either directly from the logs or from sticky traps on the logs that had been exposed for one to two days prior to the sampling occasions. The tweezers were sterilized with ethanol and fire between handling of each beetle, and the beetles were placed in

separate Eppendorf-tubes (2 ml) and killed by freezing at – 80ºC to facilitate subsequent DNA analysis.

Initially, some of the beetle individuals were rinsed with sterilised water, which we intended to analyse separately as fungal material from the exoskeleton. However, the beetles defecated in the tubes, mixing fungal material carried externally and internally, and we therefore discontinued the rinsing treatment.

The insects were identified to species or genus in a sterile environment and using sterilized equipment by R. M. Jacobsen, and 343 beetles individuals that could be confidently identified and were saproxylic according to Dahlberg and Stokland (2004) were selected for extraction of fungal DNA.

Paper IV

Four aspen logs were placed at 15 study sites in each landscape in spring 2014. All sites were in mature, semi-shaded forest and mean distance between sites was 120 meters in Østmarka and 276 meters in Nordmarka.

The four logs at each site were assigned the following treatments; cage, control, cage control and ethanol-baited positive control. The logs were placed within a few metres of each other, except the ethanol-baited logs which were placed approximately 10 meters from the other treatments.

The cage treatment was designed to exclude insects and other invertebrates. It consisted of a fine mesh net (1 x 1 mm mesh size) suspended around the log by a scaffolding and a plastic sheet beneath the log (Fig. 5). As the plastic sheet would also prevent colonization by fungi in the soil, it was included in all treatments to avoid systematic differences.

The control treatment therefore consisted of a log on a plastic sheet.

The cage control treatment was designed to control for microclimatic effects of the cage. It was identical to the cage treatment, with the exception of four large holes of approximately 20 cm in diameter cut in the net to allow invertebrates to colonize the logs.

The ethanol-baited treatment was designed to function as a positive control, based on the presumption that evaporating ethanol would attract saproxylic insects, as documented by e.g. Allison et al. (2004) and Bouget et al. (2009). The treatment consisted of a 1 litre bottle of 96% ethanol with small holes for evaporation attached to the log throughout the summer seasons.

These four treatments were hypothesized to form a gradient, with very few invertebrates (only those smaller than 1 mm) colonizing caged logs, normal colonization of control and cage control logs, and an increased number of invertebrates (mainly saproxylic insects) colonizing ethanol-baited logs.

*Figure 5) Example of a study site in **paper IV** with cage control (furthest back), control and cage (in the front) treatments. The ethanol-baited log is not visible. Photo: R.M. Jacobsen.*

The treatments were applied for two seasons, 2014 and 2015, but cage and cage control treatments were temporarily removed during winter to allow snow to fall naturally on the logs.

Wood samples for DNA analysis were taken in November 2015 by drilling 10 cm into the wood after first removing the bark. Both the drill bit (8 mm) and knife used for removing the bark were sterilized between each sample using ethanol and fire. For each log, wood samples were taken 25 cm (end sample) and 50 cm (mid sample) from the end of the log. Each end sample and mid sample consisted of wood chips from drilling into the log at three different locations on the circumference; top and both sides.

Wood samples for density measurements were taken at the same positions as the DNA samples (25 cm and 50 cm from one end) with a core sample drill, in two replicates (top and side) pooled together for analysis. These samples were further sub-divided into the outer 5 cm (without bark)

and the inner 5 cm section of the sample. Green volume was measured by water displacement, followed by oven drying at 103°C overnight and measurement of dry mass to calculate density (dry mass divided by green volume).

Paper V

Aspen high stumps and logs were created in 2001 at 15 sites in closed canopy forest and 15 sites in open clear-cuts in each landscape, for a total of 60 sites (for more information, see Sverdrup-Thygeson and Birkemoe (2009) or Sverdrup-Thygeson and Ims (2005)). Insects were sampled with trunk window traps (40 cm x 60 cm) mounted on the high stumps from May to August in 2002 – 2005, i.e. in year one to four after the aspen trees had been killed (Fig. 6). All sampled beetles were identified to species and categorized by tree species preference and feeding guild according to the literature (Dahlberg & Stokland 2004; Hansen et al. 1908-1965; Hågvar 1999; Palm 1959; Schigel 2011). Only saproxylic species known to utilize deciduous wood in the feeding guilds fungivores and wood-boring beetles were analysed further in our study.

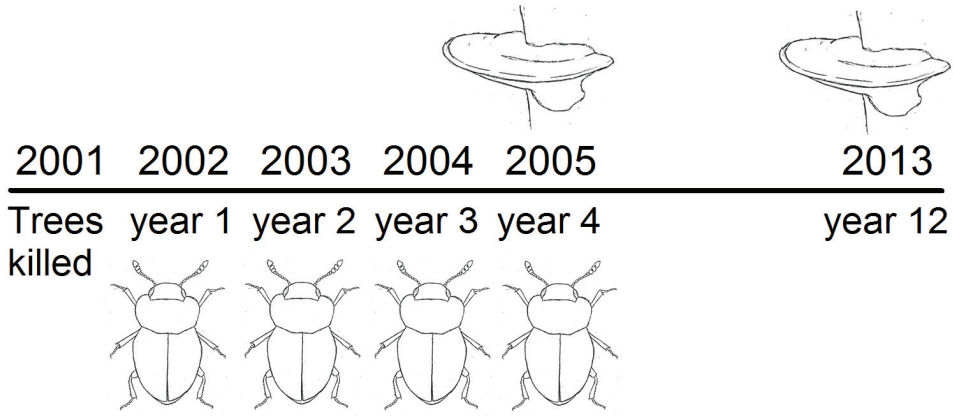

*Figure 6) Time line for **paper V** showing time of tree death, followed by four years of insect sampling and registration of fungal fruit bodies four years (only cursory) and twelve years after tree death. Illustrations: R.M. Jacobsen.*

Proportion of bark cover on the logs and high stumps was recorded in 2005, in addition to a cursory registration of easily recognisable fruit bodies. Fungi were registered by presence or absence of fruit bodies on high stumps or logs at the 55 intact sites in 2013, including both basidiomycete and ascomycete macrofungi, but only bark fungi that could be identified in the

field. Fungi were categorized according to tree species preference recorded in the literature (Ryvarden & Melo 2014). Only wood-decay fungi with a preference for deciduous wood and occurrence at 10 – 45 sites were analysed further, and of the five species fulfilling these criteria, we chose to focus on three species with contrasting biology; *Ganoderma applanatum* (saprotrophic, perennial polypore), *Phellinus tremulae* (parasitic and saprotrophic, perennial polypore) and *Bisporella citrina* (saprotrophic ascomycete with annual fruit bodies).

2.5 DNA analysis (papers II-IV)

DNA was extracted from the insect samples (**papers II – III**) and the wood samples (**paper IV**) following a modified version of the CTAB protocol (Murray & Thompson 1980). Extracted DNA from wood samples was cleaned using the E.Z.N.A.® Soil DNA kit (Omega Bio-tek, Norcross, USA) as recommended by the manufacturers. DNA from insect and wood samples, including negative controls and technical replicates, was used in a 10x dilution for amplification by polymerase chain reaction (PCR) as described in **papers II-IV**. The PCR products were cleaned using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, USA), following a modified version of the manufacturer's protocol. The cleaned amplicons were checked by gel electrophoresis and pooled according to band strength to equalize amount of DNA per sample.

The insect samples were combined in two pooled samples which were further cleaned with the ChargeSwitch® kit (Invitrogen, California, USA). DNA-concentration was measured with the Qubit® BR DNA kit (Invitrogen, California, USA) and the sample quality was confirmed by Nanodrop™ (Thermo Fisher Scientific, Madison, USA) before submission to GATC Biotech for adaptor-ligation and Illumina HiSeq Rapid Run 300bp paired-end sequencing.

The wood samples were also combined in two pooled samples, which were submitted to StarSEQ for clean-up, DNA-concentration, quality control, adaptor ligation and Illumina MiSeq 300bp paired-end sequencing.

2.6 Bioinformatics

Papers II – III

The sequence data from the insect samples was quality controlled as described in **paper II** and clustered by single-linkage clustering with maximum distance 0.015 using the SCATA pipeline (https://scata.mykopat.slu.se/). The samples were randomly subsampled to 10 000 sequences per sample and all clusters with only one sequence were removed from the dataset. The most

abundant sequence of each cluster was designated the representative sequence. Taxonomy was assigned to the representative sequences of each cluster/operational taxonomic unit (OTU) taking the top hit of a Basic Local Alignment Search Tool (BLASTn) (Altschul et al. 1990) search against the NCBI (National Center for Biotechnology Information) and UNITE (Abarenkov et al. 2010) databases. OTUs were classified into ecological guilds using FUNGuild (Nguyen et al. 2016), with the addition of the guild "insect symbionts", which was based upon available literature (references in Table S2 in **paper II**). OTUs with affinity to the class Agaricomycetes were further grouped into taxa known to decay dead wood. For statistical analysis, only OTUs represented by at least 20 sequences were included to focus on fungi more likely to be ecologically relevant and remove OTUs that had appeared due to PCR and sequencing errors (Bjørnsgaard Aas et al. 2016).

Paper IV

Sequence data from the wood samples was quality controlled as described in **paper IV** using VSEARCH v. 2.0.3 (Rognes et al. 2016), QIIME v 1.8.0 (Caporaso et al. 2010) and MOTHUR v.1.31.2 (Schloss et al. 2009), and clustered with 97% similarity threshold using VSEARCH. The most abundant sequence of each cluster/OTU was designated the representative sequence. All OTUs with less than 10 sequences were removed to minimize impact of rare OTUs stemming from sequencing and PCR errors (Nguyen et al. 2015). The representative sequences of the OTUs were subjected to BLASTn search (Altschul et al. 1990) against the quality-checked UNITE+INSD fungal ITS sequence database (released 20 November 2016), containing both identified and unidentified sequences (Kõljalg et al. 2013). OTUs with no blast hit or with similarity to plant sequences were excluded from further analysis, and the remaining OTUs were further classified into their ecological guild using FUNGuild (Nguyen et al. 2016).

2.7 Statistical analysis (papers II-V)

All analyses were conducted in R version 3.2.0 or 3.3.2 (R Core Team 2016).

Generalized linear models (GLM) with binomial distribution and logit link were used to test for effect of insect taxa (genus in **paper II** and species in **paper V**) or groups (fungivores and wood-borers in **paper V**) on presence or absence of fungal DNA (**paper II**) or fungal fruit bodies (**paper V**). The effect of wood-borer abundance on bark cover and of bark cover on presence of fungal fruit bodies was also tested by binomial GLMs (**paper V**).

Fungal community composition, i.e. composition of OTUs, was explored by principal component analysis (PCA) of Hellinger-transformed abundance (i.e. number of sequences) data (Borcard et al. 2011) in **paper II**. Effect of beetle genus (**paper II**) or experimental treatment (**paper IV**) on OTU composition was analysed by redundancy analysis with conditional design variables, and tested against permutations (999) of the data.

Linear mixed models with restricted maximum likelihood were used to test the effect of insect genus (**paper II**) or experimental treatment (**paper IV**) on number of OTUs (**paper IV**), log-transformed number of sequences from specific species of fungi (**paper IV**), density of wood core samples (**paper IV**) and arcsine-transformed (Crawley 2012) proportion of sequences from decomposer fungi (**paper II**) or wood saprotroph fungi (**paper IV**).

For **paper III**, we constructed quantitative networks based on the number of beetle individuals in which fungal OTUs annotated to specific species or genera occurred, for three functional groups of fungi; insect symbionts, wood-decayers and plant pathogens. We chose these functional groups as they were most abundant in insect samples, and we expected that they would differ in the specificity of their interactions with the saproxylic beetles. For each of these networks, we estimated the degree of specialization by the H_2' index as described in Blüthgen et al. (2006), modularity with the QuanBiMo algorithm developed by Dormann and Strauss (2014) and the weighted nestedness metric based on overlap and decreasing fill (Almeida-Neto & Ulrich 2011). We also estimated species-level specialization (Blüthgen et al. 2006). We tested the statistical significance of the metrics with two-sided tests against null models (n=1000) defined by Patefield's algorithm (Patefield 1981).

3. Results

3.1 Paper I

Are there indications that insect-vectored dispersal might be important to non-mutualistic saproxylic fungi?

Here I summarize section 5, "Insect-vectored dispersal of non-mutualistic fungi", in the book chapter (**paper I**). While we focused on the role of insects in our review, we included relevant references to studies of other invertebrates as well.

To show that insect-vectored dispersal can be important for saproxylic fungi, several aspects of this interaction should be documented; 1) Insects must come into contact with living saproxylic fungi (as opposed to the dead fruit bodies colonized by many fungivores), preferably during

sporulation. 2) Insects must be capable of carrying propagules externally or internally, in a viable state. 3) Insects must bring propagules of saproxylic fungi to dead wood. 4) The propagules must establish in the substrate and thereby affect the saproxylic fungal community. We found varying degrees of documentation for these four aspects of insect-vectored dispersal, summarized in Table 2;

1) A few studies have found that some saproxylic insects (including species without larval development in fruit bodies) are attracted to odour emission by polypores, and one study showed that odour emission increases during sporulation. Several studies have documented that saproxylic insects visit polypore fruit bodies during sporulation.

2) Several studies have found that saproxylic insects carry propagules of saproxylic fungi on their exoskeletons or in their guts, and more recently fungal DNA has been isolated from several different species of saproxylic insects. There are also a few studies documenting that fungal propagules can remain viable after passage through insect guts.

3) A few studies have shown that bark beetles bring DNA or propagules of non-mutualistic fungi to dead wood. To our knowledge, our study is the first to show that several other species of saproxylic insects bring saproxylic fungi to dead wood (**paper II**).

4) We found no studies that followed the establishment of fungal propagules brought to dead wood by insects. However, a few studies have experimentally excluded invertebrates from recently cut logs and assessed the effect on establishment of the fungal community in the dead wood. Strid et al. (2014) combined this experimental approach with data on fungi vectored by bark beetles in the same areas, which indicated that propagule dispersal by bark beetles influenced the composition of the fungal communities. We used a similar approach in **paper IV** and found indication that insect-vectored dispersal of fungi influence the fungal community in a system where bark beetles do not dominate.

*Table 2) Literature that supports the hypothesis that invertebrates are important dispersal agents for non-mutualistic saproxylic fungi, summarized from **paper I**, section 5.*

Relevant finding	References
Species-specific polypore odours attract saproxylic invertebrates (including species without larval development in fruit bodies)	(Fäldt et al. 1999; Johansson et al. 2006; Jonsell & Nordlander 1995)

Polypore odour emission increases during sporulation	(Fäldt et al. 1999)
Saproxylic invertebrates (including species without larval development in fruit bodies) visit sporulating polypores	(Hågvar 1999; Krasutskii 2006; Krasutskii 2007a; Krasutskii 2007b; Krasutskii 2010; Nikitsky & Schigel 2004; Park et al. 2014; Schigel 2011; Yamashita et al. 2015)
Invertebrates carry DNA or propagules of wood-inhabiting microfungi	Greif & Currah (2007), Strid et al. (2014)*, Jacobsen et al. (2017) (**paper II**)
Invertebrates carry DNA or propagules from saproxylic fungi	Persson et al. (2011)*, Strid et al. (2014)*, Castello et al. (1976)*, Harrington et al. (1981)*, Pettey and Shaw (1986)*, Tuno (1999), Lim (1977), Park et al. (2014), Talbot (1952), Jacobsen et al. (2017) (**paper II**)
Propagules of saproxylic fungi are viable after passage through invertebrate guts	Drenkhan et al. (2016), Tuno (1999), Lim (1977), Talbot (1952)
Invertebrates bring DNA or propagules of saproxylic fungi to dead wood	Persson et al. (2011)*, Strid et al. (2014)*, Castello et al. (1976)*, Harrington et al. (1981)*, Jacobsen et al. (2017) (**paper II**)
Exclusion of invertebrates affects fungal community assembly in dead wood	Müller et al. (2002)*, Strid et al. (2014)*, **paper IV**
Invertebrate colonization affects subsequent fungal community in dead wood	Weslien et al. (2011), Jacobsen et al. (2015a) (**paper V**)

* References concerning bark beetles (Scolytinae) interacting with non-mutualistic fungi.

3.2 Paper II

Do saproxylic insects carry fungi to dead wood?

We obtained fungal DNA from 187 of the 343 beetle individuals that had been selected for DNA-analysis. In total, 1069 fungal OTUs (1,714,063 sequences) represented by at least 20 sequences each were isolated from the beetles, of which 23% of the OTUs and 34% of the

sequences were classified as decomposer fungi. The beetle genera *Glischrochilus, Rhizophagus, Xylita* and *Epuraea* frequently carried fungal DNA in general (Table 3), while the genus *Endomychus* frequently carried DNA from wood-decay fungi (p-value <0.001 in binomial GLM, excluding its host fungus *Chondrostereum purpureum,* which was the only macrofungus visibly fruiting on the logs during insect sampling). Scanning electron microscopy showed that at least some of the fungal material was carried as spores on the beetle exoskeletons.

Table 3) Generalized linear model (GLM, binomial distribution and logit link) with presence or absence of fungal DNA as response and insect genus (no. of individuals ≥ 9, genus Agathidium in the intercept), rinsing treatment and trap method as explanatory variables. Significant p-values marked in bold. N = 312.

Presence of fungal DNA	**Estimate**	**SE**	**z-value**	**p-value**
Intercept	0.23	0.42	0.55	0.584
Rinsed (Yes)	-1.21	0.36	-3.33	**0.001**
Trap (Sticky)	-0.70	0.40	-1.76	0.078
Insect genus:				
Endomychus	1.20	0.69	1.74	0.082
Epuraea	1.48	0.75	1.96	**0.050**
Glischrochilus	2.35	0.57	4.16	**<0.001**
Rhizophagus	1.36	0.60	2.26	**0.024**
Xylita	1.99	0.87	2.30	**0.021**
Fam. Staphylinidae;				
Acrulia	0.35	0.67	0.52	0.605
Anthophagus	0.34	0.59	0.58	0.562
Oxypoda	-0.91	0.54	-1.69	0.092
Quedius	0.42	0.65	0.65	0.518
Null deviance: 427.4 on 311 degrees of freedom				
Residual deviance: 301.1 on 300 degrees of freedom				

Does the composition of fungi depend on the insect taxon?

Beetle genus had a clear effect on composition of fungal OTUs in unconstrained ordination (Fig. 7), which was confirmed by its significant effect in constrained ordination where beetle genus explained 18.3% of the variance in fungal OTU composition (p-value = 0.001 from comparisons with 999 permutations of the data). The beetle genus *Glischrochilus* and to some extent *Rhizophagus* formed a cluster in unconstrained ordination (Fig. 7A) that was characterized by abundance of OTUs annotated as *Phialophora bubakii* and *Candida* spp. (Fig. 7C), while the beetle genus *Endomychus* formed another cluster (Fig. 7B) characterized by abundance of *Cladosporium cladosporioides, Fusarium merismoides* and *Chondrostereum purpureum* (Fig. 7D).

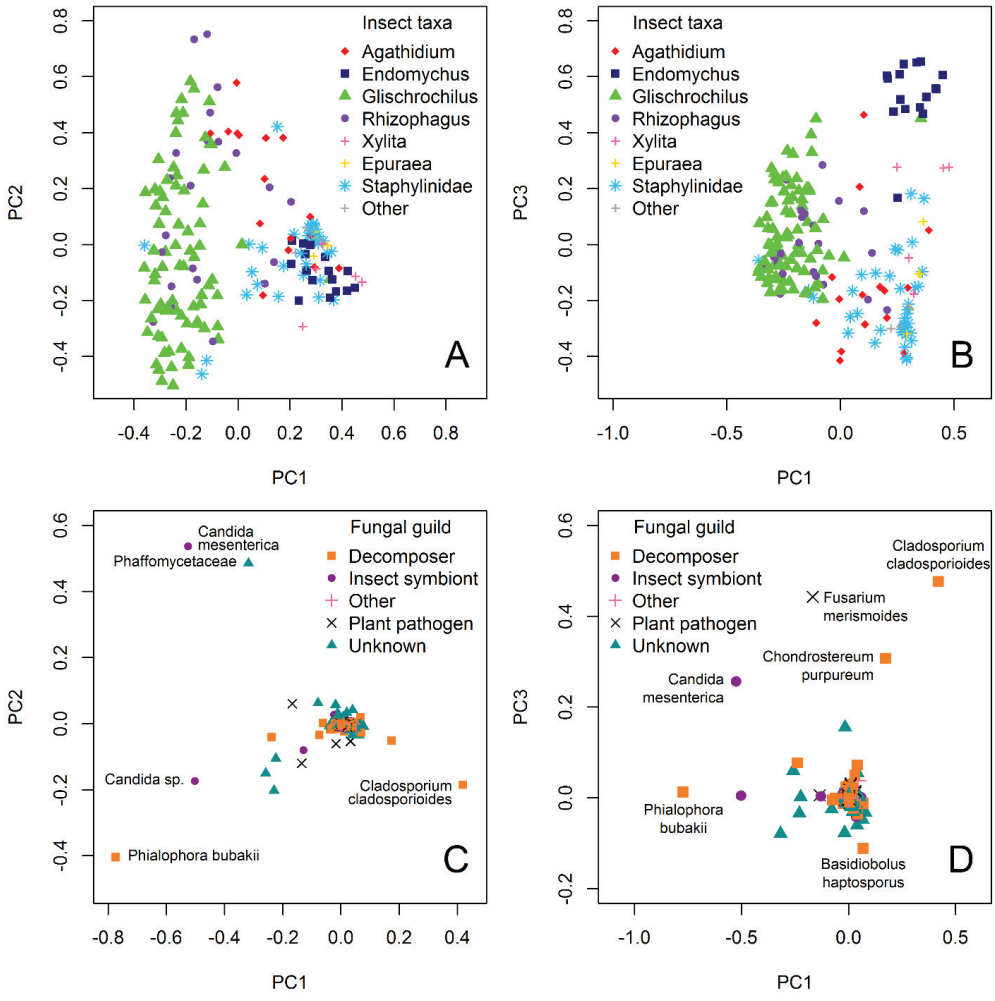

Figure 7) Principal component analysis of the composition of fungal OTUs in the insect samples (all genera included, N = 187), based on Hellinger-transformed abundance data. A and B show insect scores, with symbols representing insect taxa. C and D show OTU scores, with symbols representing fungal guild of the OTUs and the most influential OTUs labelled with matching taxon identity. A and C shows principal component axis 1 and 2, while B and D shows principal component axis 1 and 3.

3.3 Paper III

How specialized are the interactions between saproxylic insects and different functional groups of fungi?

Based on the fungal DNA isolated from 187 beetle individuals (**paper I**), we constructed quantitative networks between the 17 beetle taxa (species or genera) and three functional groups of fungi; 35 taxa (OTUs annotated to species or genera) of insect symbionts, 22 taxa of wood-decayers in the class Agaricomycetes and 60 taxa of plant pathogens. All three networks were significantly more specialized and less nested than the null models, and the networks with insect symbionts and wood-decayers were also significantly more modular (Fig. 8).

The network between saproxylic beetles and wood-decay fungi was most specialized (H_2' = 0.21). One of the wood-decay fungi, *C. purpureum,* was visibly fruiting on all logs during insect sampling and could thereby have occurred in all samples indiscriminately. We therefore constructed a network for wood-decay fungi without *C. purpureum*, which increased degree of specialization further (H_2' = 0.29). Without *C. purpureum*, the network between saproxylic beetles and wood-decay fungi was organised in six modules (Fig. 9).

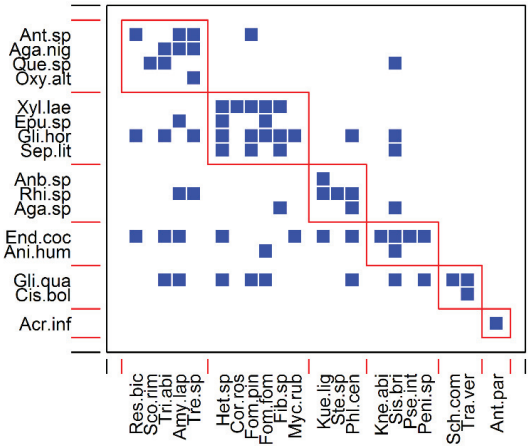

Figure 9) Modules in the network between wood-inhabiting beetles and wood-decay fungi with C. purpureum excluded, as organised by the QuanBiMo algorithm (Dormann & Strauss 2014). Lines demarcate modules, squares indicate interactions between insects and fungi.

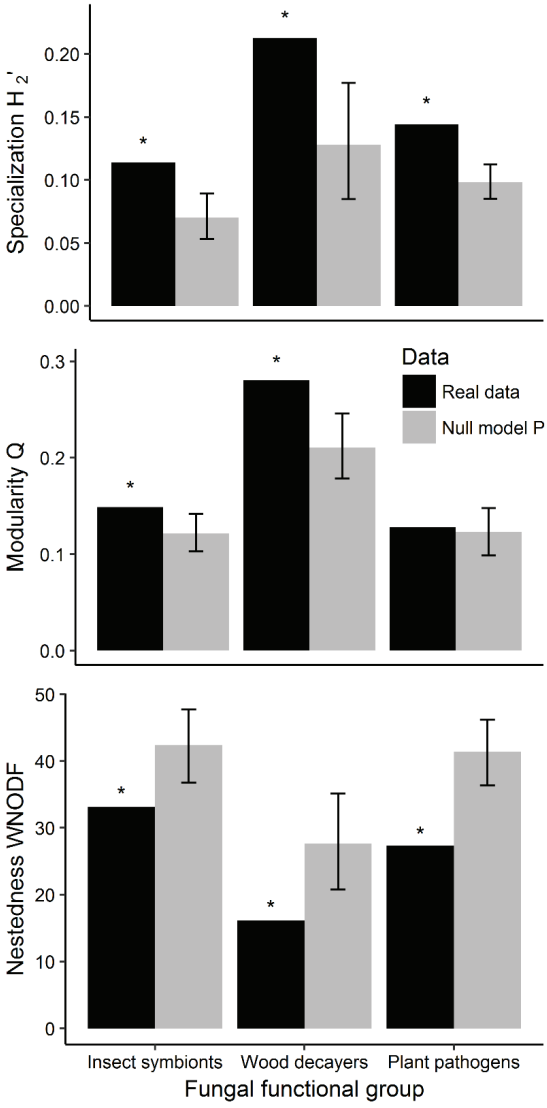

Figure 8) Network specialization (H_2' ranges from 0 for least specialized to 1 for most specialized and reflects tendency for species to prefer certain interactions irrespective of partner abundance), modularity (Q ranges from 0 for least modular to 1 for most modular and reflects tendency for interactions to be sorted into compartments) and weighted nestedness (WNODF ranges from 0 for least nested to 100 for most nested and reflects tendency for abundant species to be involved in most interactions) for networks between wood-inhabiting beetles and the fungal functional groups insect symbionts, wood-decayers and plant pathogens. Black bars represent the original networks, while grey bars represent networks randomized with constant marginal sums according to null model P (Patefield 1981) with 95% confidence intervals (CI). Asterisks (*) above the black bars signify significant (P-value < 0.05) differences between the original and the randomized networks.

3.4 Paper IV

Does exclusion of insects and other invertebrates affect wood decay and the fungal community that establishes in dead wood?

We isolated 1737 fungal OTUs (18,455,289 sequences) from the wood samples (n = 292), of which 570 OTUs (11,768,009 sequences) were classified as wood-decayers (including mixed guilds such as wood saprotroph/plant pathogen). Fungal community composition, in terms of abundance of fungal OTUs, did differ significantly between experimental treatments (Fig. 10A, RDA1 p-value = 0.001 and RDA2 p-value = 0.010 based on 999 permutations). The treatments formed a gradient from caged logs to ethanol-baited logs, with cage control and control logs being intermediate to these contrasting treatments. This corresponded with our hypothesized gradient of invertebrate colonization of the logs.

The first ordination axis, RDA1, formed a gradient where high values signified abundance of fungal OTUs annotated as *Trametes ochracea* and *T. versicolor,* while low values signified abundance of fungal OTUs annotated as *C. purpureum*, among others (Fig. 10B). Linear mixed models confirmed that, in comparison with caged logs, *T. ochracea* was more abundant in ethanol-baited logs (p-value = 0.006) and *T. versicolor* was more abundant in both ethanol-baited logs (p-value = 0.001) and cage control logs (p-value = 0.028). Abundance of *C. purpureum* did not differ significantly between treatments, but was higher in the mid section of logs relative to the end section (p-value = 0.008).

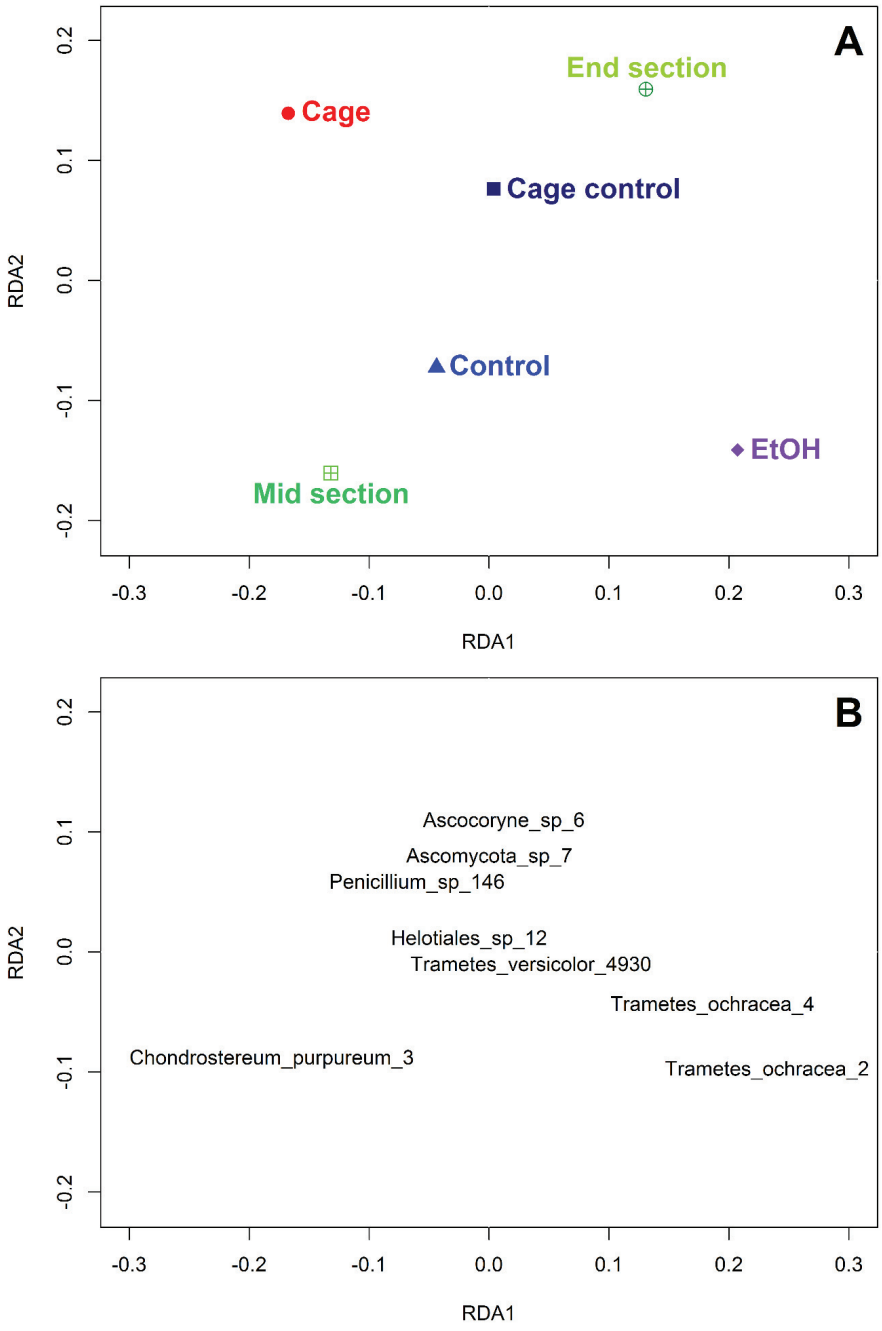

Figure 10) Ordination of treatment wood samples by redundancy analysis of Hellinger-transformed abundance of fungal OTUs, with experimental treatment and log section as constraining variables, and tree identity, tree section, log diameter, landscape and site as

conditional variables. (A) Centroids of constraining variables: Log section (end or mid) and experimental treatments; cage (for invertebrate exclusion), cage control, control and ethanol-baited positive control (EtOH). (B) A few of the most abundant fungal OTUs are plotted according to their species scores to illustrate trends in community composition along gradients.

The experimental treatments explained a relatively small, but significant proportion of the variance in fungal community composition in the treatment wood samples (adjusted $R^2 = 0.016$, p-value = 0.001 based on 999 permutations). The largest proportion of the variance was explained by the identity of the individual tree from which the logs had been cut (adjusted $R^2 = 0.158$, p-value = 0.001 based on 999 permutations). The exclusion treatment resulted in a significantly higher wood density for caged logs relative to control logs (Table 4), implying a lower rate of wood decay in caged logs.

Table 4) Linear mixed model fit by restricted maximum likelihood (REML) explaining density of wood core samples by experimental treatment (cage in the intercept), sample section (inner/outer), log section (mid/end) and log diameter as fixed effects and site, tree identity and tree section nested under tree identity as random effects.

Fixed effects	**Estimate**	**Std. error**	**t-value**	**p-value**
Intercept	*0.349*	*0.014*	*25.75*	*<0.001*
Cage control logs	-0.003	0.004	-0.81	0.418
Control logs	-0.008	0.004	-2.04	**0.041**
Ethanol-baited logs	-0.002	0.004	-0.60	0.546
Sample section (Outer)	0.015	0.002	8.63	**<0.001**
Log section (Mid)	0.002	0.002	0.98	0.328
Diameter	0.001	<0.001	2.62	**0.009**
Random effects	***Variance***	***Std. deviance***		
Site	*0*	*0*		
Tree identity (ID)	*0.001*	*0.024*		
Tree ID/Tree section	*<0.001*	*0.011*		
Residual	*<0.001*	*0.019*		
REML criterion at convergence: -2210.4				

3.5 Paper V

Does initial colonization history of saproxylic insects affect the fungal community present in the dead wood several years later?

During the first four years after tree death, 23 beetle species (961 individuals) assigned to the wood-borer guild and 56 species (3456 individuals) assigned to the fungivore guild were sampled from the sites with aspen high stumps and logs. In year 12 after tree death, 62 species of fungi were registered in the fruit body survey of the aspen high stumps and logs. The chosen study species, *G. applanatum, P. tremulae* and *B. citrina*, were present at 14, 19 and 41 sites (of 55 sites in total), respectively.

Of these three species of fungi, only the saprotrophic polypore *G. applanatum* was affected by abundance of fungivores at the sites during the first four years after tree death. Specifically, abundance of the fungivorous beetles *Glischrochilus quadripunctatus* (p-value = 0.071) and *Agathidium nigripenne* (p-value = 0.034) during the first four years after tree death increased the likelihood that fruit bodies of *G. applanatum* would be present in year twelve after tree death (Fig. 11).

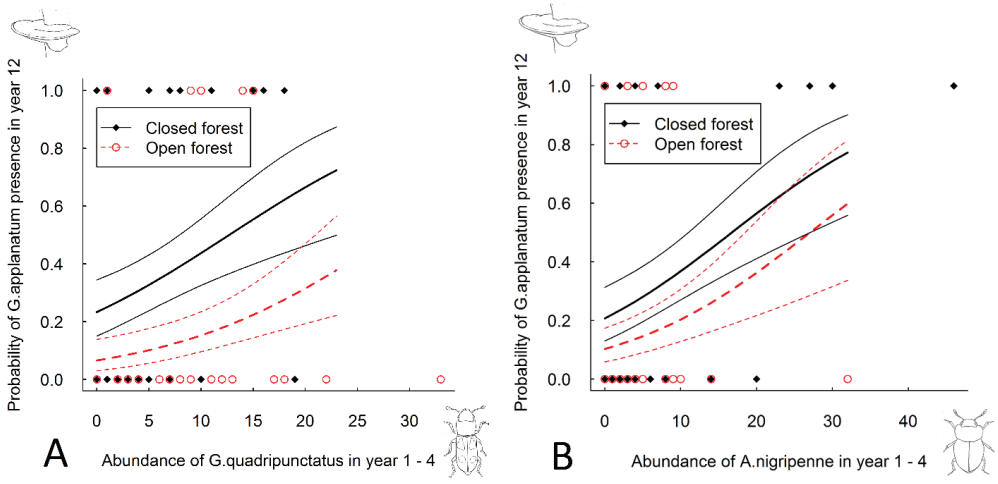

Figure 11) Observed presence of G. applanatum in year 12, with prediction lines and 95% confidence intervals based on binomial GLMs with abundance of Glischrochilus quadripunctatus (A) or Agathidium nigripenne (B) in the first four years after tree death as explanatory variable. Prediction lines only extend to 23 individuals of G. quadripunctatus and to 32 individuals of A. nigripenne. Illustrations: R.M. Jacobsen.

The saprotrophic ascomycete *B. citrina* was the only one of the three species of fungi that responded to abundance of wood-boring beetles. *B. citrina* was more likely to be present in year twelve at sites where wood-boring beetles had been abundant during the first four years after tree death (p-value = 0.042, Fig. 12A). Abundance of wood-boring beetles was negatively correlated with bark cover in year four after tree death (p-value = 0.045, Fig. 12B), and correspondingly, presence of *B. citrina* was also negatively correlated with bark cover (p-value <0.001 in binomial GLM).

The parasitic and saprotrophic polypore *P. tremulae* did not respond to abundance of either beetle guild.

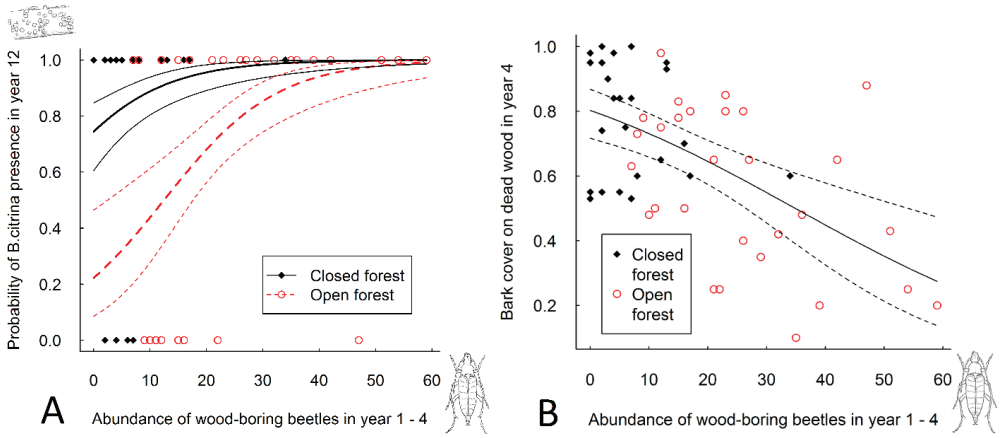

Figure 12) Observed presence of Bisporella citrina in year 12 (A) or bark cover (0 - 1, 1 = 100 % cover) remaining in year 4 after tree death (B), with prediction lines and 95% confidence intervals based on binomial GLMs (logit link) with abundance of wood-boring beetles in the first four years after tree death as explanatory variable. Illustrations: R.M. Jacobsen.

4. Discussion

Together, the papers presented here strongly suggest that insect-vectored dispersal of fungal propagules does influence the community of saproxylic fungi in dead wood. In our literature review (**paper I**) we found several previous studies demonstrating that some saproxylic insects are attracted to fungal odours, visit sporulating fruit bodies and can vector fungal propagules externally or internally in a viable state. In **paper II** we showed that several different species of saproxylic insects do bring fungal material, including spores, to recently cut logs. The insects carried a taxon-specific mix of fungi, and certain species frequently carried wood-decay fungi.

In **paper III**, we found that the dispersal of fungi in **paper II** was based on non-random interactions with the insects, with a degree of specialization that was higher for wood-decay fungi than for plant pathogenic or insect symbiont fungi. In **paper IV** we excluded insects and other invertebrates from recently cut logs during the two first years of wood decay, and showed that invertebrate exclusion resulted in a composition of saproxylic fungi that differed from the fungal community in accessible logs. A comparison with the fungi isolated from insects in **paper II** indicated that at least some of the effect was due to insect-vectored dispersal of saproxylic fungi. Finally, in **paper V**, we found that colonization history of saproxylic insects in the first four years after tree death affected the community of wood-decay fungi twelve years later. Although we cannot prove the exact cause of the correlations in **paper V**, our results from **paper I-IV** show that it could be a consequence of saproxylic insects directly influencing the fungal community through dispersal of propagules. All in all, our results strongly suggest that certain saproxylic fungi benefit from insect-vectored dispersal.

4.1 Which fungi might benefit from insect-vectored dispersal?

In **paper II** we showed that saproxylic insects carry a diversity of fungi to recently cut logs. The majority of the fungal OTUs that were classified to functional guilds were decomposers, followed by the tentative insect symbiont group and plant pathogenic fungi. The decomposer guild included wood-decay fungi, which are most likely to benefit from dispersal by saproxylic insects to dead wood. Correspondingly, the degree of specialization between insects and fungi was highest for wood-decay fungi (**paper III**). The network between saproxylic insects and wood-decay fungi (**paper III**) had a degree of specialization that was lower than that of pollination networks, but similar to that of animal-mediated seed dispersal networks (Blüthgen et al. 2007). This corresponds with our hypothesis that the network was based on opportunistic spore-feeding and subsequent dispersal by the insects, which resembles animal-mediated seed dispersal and would be expected to result in a similarly moderate degree of specialization.

Insects disperse fungi to dead wood

Since the insects in **paper II** were sampled from logs during the first and second year following tree death, we consider it most likely that the majority of fungal taxa isolated from the insect samples were brought to the logs by the insects. The SEM-pictures of fungal spores on insect exoskeletons strengthened this argument (**paper II**, Fig. 13), as did the absence of macroscopic fruit bodies on the logs, with the exception of *C. purpureum*. The wood-decay fungus *C. purpureum* was fruiting on all logs in the second year of insect sampling, and correspondingly

occurred in very many insect samples (81 insect individuals, while the second most frequent wood-decay fungus, *Sistotrema brinkmannii,* was obtained from only 17 individuals). As no other wood-decay fungi were fruiting on the logs during insect sampling, nor occurred as frequently and abundantly in the insect samples as *C. purpureum*, the other wood-decay fungi isolated from the insect samples were most likely dispersed to the logs by the insects.

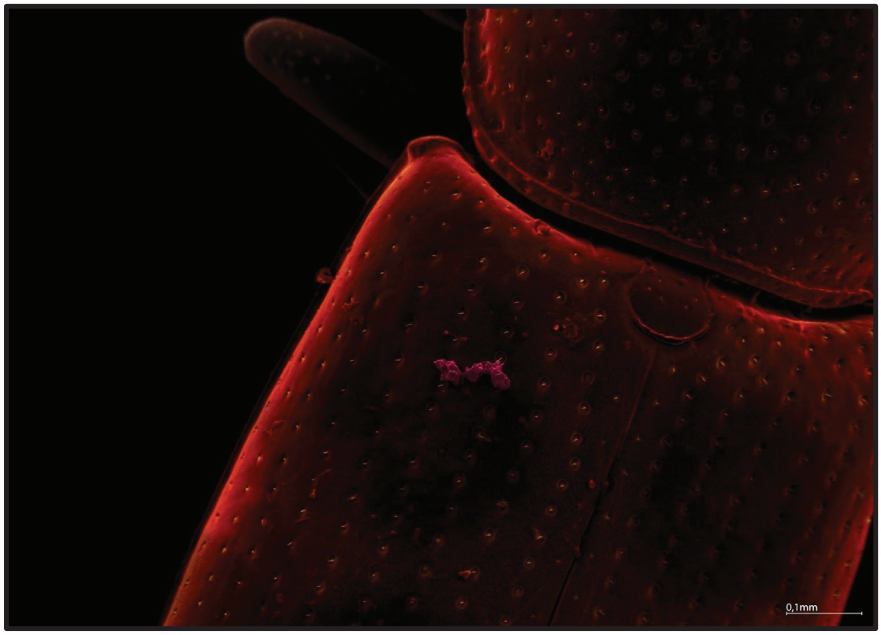

Figure 13) Picture of the elytra of a Rhizophagus beetle taken with scanning electron microscopy by R.M. Jacobsen and coloured by Egil Paulsen for an artistic exhibition. The pink colour marks a cluster of what is most likely fungal spores. For more detailed pictures, see ***paper II***.

Dispersal of tree pathogenic fungi

Although we classified *C. purpureum* as a wood-decay fungus, it is often classified as a plant pathogen (Nguyen et al. 2016) since it can colonize living trees through wounds (Boddy 2001). This is similar to the ecology of the polypore *P. tremulae,* which can colonize the heartwood of living trees parasitically and continue living as a saprotroph after tree death. As such, both *C. purpureum* and *P. tremulae* were obtained from the fresh wood samples taken directly after tree felling in **paper IV** (Table 5). Saprotrophs that can colonize living trees as plant pathogens or parasites might not benefit much from insect-vectored dispersal *after* tree death. Correspondingly, *P. tremulae* did not respond to colonization history of saproxylic insects after

tree death in **paper V**, and *C. purpureum* was not significantly affected by invertebrate exclusion from dead wood in **paper IV**. However, parasitic saprotrophs like *C. purpureum* and *P. tremulae* might benefit from dispersal by insects to wounds in trees, which is a well-known dispersal mode for tree pathogens (Cease & Juzwik 2001; Webber & Gibbs 1989). The high diversity of fungi isolated from fresh wood samples in **paper IV** and the strong effect of tree identity on fungal community composition after two years of wood decay, indicates that fungi colonizing trees prior to tree death might exert significant influence on the development of the fungal community in dead wood. The role of insect-vectored dispersal in colonization of living trees by parasitic saprotrophs or wood-decay fungi latently present in the wood (Parfitt et al. 2010; Song et al. 2017) should be researched further.

Dispersal of wood-decay fungi

Several wood-decay fungi were isolated from saproxylic insects in **paper II** (Table 5), and the fungi *T. versicolor, Fomes fomentarius* and *S. brinkmannii* were shown in **paper III** to be significantly specialized in their interactions with the insects, indicating a potential for species-specific dispersal. *T. versicolor* and *S. brinkmannii* were also obtained from the aspen wood samples in **paper IV**, while *F. fomentarius* was absent (Table 5). As a perennial polypore, *F. fomentarius* might take longer than two years after tree death to establish, or aspen might not be an ideal substrate for this species, which is most commonly observed on birch in our study area (Ryvarden & Melo 2014). *S. brinkmannii* was obtained in low abundance from the aspen wood in **paper IV** and did not show any clear response to the experimental treatments. However, in an exclusion experiment with spruce logs, *S. brinkmannii* occurred more frequently in logs accessible to insects and was also isolated from bark beetles (Strid et al. 2014). Thus, *S. brinkmannii* might benefit from insect-vectored dispersal to coniferous wood.

Some of the wood-decay fungi isolated from insects in **paper II** are known to prefer or specialize on coniferous wood, such as *Fomitopsis pinicola* (Fig. 1D)*, Trichaptum abietinum* and *Heterobasidion sp.* This is not surprising, as the insects were sampled in a spruce-dominated forest, nor is it surprising that these species did not occur in the wood samples from aspen logs in **paper IV**. Although these species might not benefit from dispersal with saproxylic species targeting deciduous wood, both *F. pinicola* and *Heterobasidion sp.* have previously been isolated from conifer-associated bark beetles (Castello et al. 1976; Harrington et al. 1981; Pettey & Shaw 1986). Correspondingly, both *F. pinicola* (Weslien et al. 2011) and *H. parviporum* (Strid et al. 2014) have been found to occur more frequently in spruce dead wood colonized by bark beetles.

The polypore *T. versicolor,* however, was abundant in aspen wood samples in **paper IV**, as was the closely related *T. ochracea* (Fig. 1C). Both these polypores were found to be significantly less abundant in caged logs from which invertebrates were excluded, in comparison with logs that were accessible to invertebrates (**paper IV**). These species were most abundant in ethanol-baited logs, indicating that they somehow benefited from the saproxylic insects that were presumably attracted by the ethanol (Allison et al. 2004; Bouget et al. 2009). Previous studies have found that early arrival is important for establishment of *T. versicolor* in new substrates (Dickie et al. 2012; Fukami et al. 2010; Leopold et al. 2017). *T. versicolor* might therefore benefit significantly from dispersal with insect vectors that colonize dead wood in early stages of decay, such as the nitidulid beetle *G. quadripunctatus* from which *T. versicolor* was isolated in **paper II**. We did obtain *T. versicolor* in low abundance from some of the fresh wood samples as well (Table 5), indicating that *T. versicolor* had been latently present in some of the living trees, but dispersal by saproxylic insects could still increase establishment success by providing different mating types or simply by increasing the number of propagules and thereby reducing chances of local extinction prior to establishment. Early establishment of *T. versicolor* has been found to result in priority effects that influence subsequent development of the fungal community (Dickie et al. 2012; Fukami et al. 2010; Leopold et al. 2017), and so the effect of invertebrate exclusion on assembly of fungal communities in **paper IV** might also affect subsequent succession of fungi.

*Table 5) Summarized findings from **papers II-IV** for agaricomycete wood-decay fungi isolated from five or more insect individuals in **paper II** and for the three species of fungi analysed in **paper V**. * Mean no. of sequences per sample*

Wood-decay fungi	**Vectored by insects in paper II (no. of insect individuals)**	**Significantly specialized interactions with insects in paper III**	**In living aspen trees in paper IV (*)**	**In aspen logs after two years of decay in paper IV (*)**	**Responding to experimental treatments in paper IV**
Amylocystis lapponica	Yes (7)	No	No	No	-
Bisporella citrina	No	-	No	No	-

Chondrostereum purpureum	Yes (81)	No	Yes (516)	Yes (8839)	No
Fibulorhizoctonia	Yes (5)	No	No	No	-
Fomes fomentarius	Yes (16)	Yes (11 occurrences in *G. hortensis*)	No	No	-
Fomitopsis pinicola	Yes (7)	No	No	No	-
Ganoderma applanatum	No	-	No	No	-
Peniophora	Yes (5)	No	No	No	-
Phellinus tremulae	No	-	Yes (31)	Yes (9)	Too infrequent for testing
Phlebia centrifuga	Yes (8)	No	No	No	-
Sistotrema brinkmannii	Yes (17)	Yes (9 occurrences in *E. coccineus*)	Yes (6)	Yes (15)	Too infrequent for testing
Trametes versicolor	Yes (5)	Yes (4 occurrences in *G. quadri-punctatus)*	Yes (25)	Yes (940)	Yes, less abundant in caged logs
Trametes ochracea	No	-	Yes (599)	Yes (24 425)	Yes, less abundant in caged logs
Trechispora	Yes (5)	No	No	No	-
Trichaptum abietinum	Yes (6)	No	No	No	-
Heterobasidion sp.	Yes (6)	No	No	No	-

Dispersal of the Artist's conk (*G. applanatum*)

In **paper V**, we found that fruit bodies of *G. applanatum* (Fig. 14) were more likely to be present at sites where the fungivorous beetles *G. quadripunctatus* and *A. nigripenne* had been abundant several years earlier. We suggested that this correlation might be due to dispersal of spores from *G. applanatum* by these fungivores, since both *G. quadripunctatus* and *A. nigripenne* have been found to visit sporulating polypores (Hågvar 1999; Krasutskii 2007b; Nikitsky & Schigel 2004; Schigel 2011) and previous studies indicate that *Ganoderma* species might benefit from insect-vectored spore dispersal (Lim 1977; Tuno 1999). However, we did not obtain DNA of *G. applanatum* from any of the insects in **paper II**, wherein both *G. quadripunctatus* and *A. nigripenne* were among the analysed species. When Tuno (1999) sampled adult insects from fruit bodies of *G. applanatum* in Japan, the majority of individuals were drosophilid flies of the genus *Mycodrosophila*. The guts of these flies were found to contain thousands of spores, and while spores were also found in the guts of a few beetles in genus *Scaphisoma*, none of the spores in the beetle guts were viable. The *Mycodrosophila* flies, however, excreted and dropped thousands of viable spores. In a study by Lim (1977), spores of *Ganoderma phillippi* (previously *G. pseudoferreum)* would not germinate until having passed through the guts of tipulid fly larvae, and the adult flies were found to carry thousands of spores on their exoskeletons. These findings suggest that flies might be more important dispersal vectors of *Ganoderma* spores than beetles.

*Figure 14) Fruit bodies of the Artist's conk (Ganoderma applanatum) on an aspen high stump in **paper V**. Photo: R. M. Jacobsen.*

As such, the correlations in **paper V** might not be due to dispersal of *G. applanatum* by fungivorous beetles. Another option is that the beetles *G. quadripunctatus* and *A. nigripenne,* both of which were found to frequently carry fungal DNA in **paper II**, promoted a specific fungal community in dead wood through propagule dispersal, and that *G. applanatum* benefited from this by subsequently colonizing as a successor species (Niemelä et al. 1995; Ottosson et al. 2014). However, it is also possible that *G. quadripunctatus* and *A. nigripenne* do disperse

spores of *G .applanatum* very rarely, and that our sample size in **paper II** was too low to include the few individuals carrying spores. After all, if most individuals of these species were to disperse *G. applanatum*, then the benefit of an increasing abundance of these species at sites in **paper V** should quickly reach an asymptote where the likelihood of *G. applanatum* being dispersed and establishing would not increase further. Our data in **paper V** does not show such an asymptote (Fig. 11), perhaps indicating that few individuals of *G. quadripunctatus* and *A. nigripenne* carried spores of *G. applanatum* and thus probability of insect-vectored dispersal increased with increasing abundance of these species. Relatively random and opportunistic spore-feeding by these fungivores might result in such a pattern, which is in line with the moderate degree of specialization between saproxylic insects and wood-decay fungi found in **paper III**.

4.2 Which insects are effective dispersal vectors?

In **paper II** we showed that insect taxa differ in how frequently they carry fungi, and that the composition of fungi depends on insect taxon. In line with this, insect species have been found to differ in their attraction to fungal volatiles in general or even to specific species of fungi (Fäldt et al. 1999; Johansson et al. 2006; Jonsell & Nordlander 1995). Previous studies have also showed that the effect the insect digestive system has on spore viability depends on the insect species (Kadowaki et al. 2011; Lilleskov & Bruns 2005; Lim 1977; Tuno 1999), ranging from an increase in germination rate (Lim 1977) to destruction of most spores (Kadowaki et al. 2011). Thus, the effectiveness of potential spore vectors varies between insect species, and also seems to depend on traits of the fungus such as thickness of spore walls (Nuss 1982) and pigmentation of spores (Kobayashi et al. 2017). This is similar to animal-mediated seed dispersal, for which trait-dependent and species-specific dispersal effectiveness has been extensively studied and presented in a framework explaining seed dispersal effectiveness of different animal vectors (Schupp et al. 2010). To encourage focused research on insect-vectored (or invertebrate-vectored) spore dispersal, we suggested a similar framework for spore dispersal effectiveness in **paper I** (Fig. 15). We have tried to discern the factors that might vary between potential insect vectors and affect the spore dispersal effectiveness of a particular insect-fungus interaction. While traits of the fungus are not presented explicitly in the framework, they are included implicitly as they will influence variables such as digestion effects, attraction to fruit bodies and which habitat will allow establishment of dispersed spores. Most variables presented in the spore dispersal effectiveness framework are inadequately studied however, and there is no insect-fungus interaction for which we have knowledge of enough variables to estimate

spore dispersal effectiveness. Hopefully, our work to systematize the knowledge of insect-vectored spore dispersal can inspire future research to remedy this.

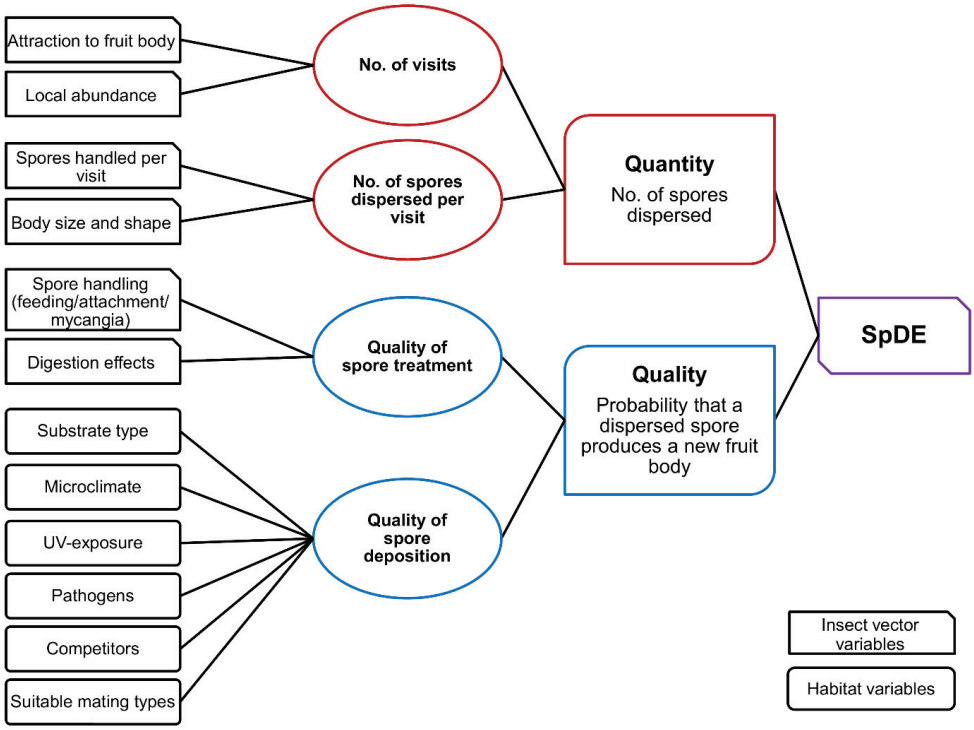

Figure 15) A theoretical framework for studies of spore dispersal effectiveness (SpDE) of insect or invertebrate vectors of fungal spores (or other propagules). The list of variables is not exhaustive. The figure is adapted from the seed dispersal effectiveness framework described in Schupp et al. (2010).

Beetles frequently carrying fungal DNA

The insect taxa that frequently contained fungal DNA in **paper II** were *G. quadripunctatus* (Fig. 16), *G. hortensis, Epuraea sp., Rhizophagus sp., Xylita laevigata* and *Endomychus coccineus* (Fig. 1B) (Table 6). Scanning electron microscopy showed that at least some of this fungal DNA probably stemmed from spores on insect exoskeletons (Fig. 13). Furthermore, *G. quadripunctatus, G. hortensis, X. laevigata* and *E. coccineus* frequently contained DNA of

wood-decay fungi (Table 6). The two nitidulid beetles, *G. quadripunctatus* and *G. hortensis,* have often been found to visit sporulating fruit bodies (Table 6). Jonsell and Norlander (1995) found that *G. hortensis* exhibited a significant attraction to fruit bodies of *F. fomentarius,* and DNA of *F. fomentarius* was indeed isolated from eleven individuals of *G. hortensis* in **paper II**. This probably contributed to the significant specialization estimated for both *F. fomentarius* and *G. hortensis* in the network between insects and wood-decay fungi in **paper III**. *E. coccineus* was also found to be significantly specialized in its interaction with wood-decay fungi in **paper III**, which could of course be due to its host fungus *C. purpureum* being isolated from all but one individual of *E. coccineus*. The wood-decay fungus *S. brinkmannii* might also have been a contributing factor, as it was isolated from nine of sixteen *E. coccineus* individuals. It is also worth noting that fourteen of sixteen *E. coccineus* individuals carried fungal DNA annotated as *Entoloma sp.,* a mushroom genus with mainly saprotrophic and some mycorrhizal species (Nguyen et al. 2016). Thus, although larvae of *E. coccineus* develop on fruit bodies of *C. purpureum*, adult *E. coccineus* seem to have a wider fungivorous diet and might be effective dispersal vectors for a range of saprotrophic fungi.

*Figure 16) The beetle Glischrochilus quadripunctatus on an aspen log from **paper IV**.* Photo: R.M. Jacobsen.

*Table 6) Summarized findings from **papers II-III** for the insect taxa analysed in these papers (ind. = individuals), and previous studies documenting visits to polypore fruit bodies by these taxa. Values that were significant in tests in the papers are marked in bold – note that some taxa presented separately here were combined in analyses in **paper II**. *Agaricomycete wood-decay fungi, excluding C. purpureum which was fruiting on the logs during insect sampling.*

Insect taxa	Ind. analysed in paper II	Ind. (%) with fungal DNA in paper II	Ind. (%) with DNA from wood-decay fungi* in paper II	Significantly specialized interactions with wood-decay fungi in paper III	Visits polypore fruit bodies
Agathidium nigripenne	22	11 (50%)	2 (10%)	No	1, 2

Agathidium sp.	14	5 (36%)	3 (21%)	No	2, 3, 4
Endomychus coccineus	20	**16 (80%)**	**14 (70%)**	**Yes**	5
Epuraea sp.	13	**6 (46%)**	2 (15%)	No	1, 2, 4, 5, 6, 7, 8
Glischrochilus hortensis	34	**31 (91%)**	**18 (53%)**	**Yes**	1, 2, 3, 4, 5
Glischrochilus quadripunctatus	55	**48 (87%)**	**13 (24%)**	No	1, 5, 7
Rhizophagus sp.	31	**23 (74%)**	8 (26%)	No	1, 2, 3, 5, 6
Xylita laevigata	9	**6 (67%)**	**4 (44%)**	No	6
Staphylinids;					
Acrulia inflata	13	6 (46%)	1 (8%)	No	2, 5, 8
Anthobium sp.	6	5 (83%)	1 (17%)	No	-
Anthophagus sp.	23	9 (39%)	3 (13%)	No	-
Oxypoda alternans	59	8 (14%)	2 (3%)	No	1, 2, 5, 9
Quedius sp.	19	7 (37%)	2 (11%)	No	1, 6, 9

1) Hågvar (1999); 2) Nikitsky & Schigel (2004); 3) Kaila et al. (1994); 4) Krasutskii (2007a); 5) Schigel (2011); 6) Hågvar & Økland (1997); 7) Krasutskii (2007b); 8) Krasutskii (2010); 9) Kochetova et al. (2011).

Bark beetles as vectors for non-mutualistic fungi

Bark beetles were not a dominant taxon in our study system, and correspondingly we were only able to sample one individual in **paper II** (*Trypodendron domesticum,* an ambrosia beetle which naturally carried an abundance of sequences from its mutualist fungus, *Phialophoropsis ferruginea*). Several previous studies have, however, shown that bark beetles might vector non-mutualistic fungi (**paper I**). For instance, bark beetles (mainly *Dendroctonus* species) have often been found to carry fungal propagules of *F. pinicola* while in flight (Castello et al. 1976; Harrington et al. 1981; Pettey & Shaw 1986), or even post-flight while in their egg-laying galleries (Castello et al. 1976; Harrington et al. 1981). Persson et al. (2011) isolated DNA of *F. fomitopsis* from bark beetles (*Pityogenes chalcographus* and *Crypturgus* sp.) and their galleries, but not from the surrounding wood, clearly suggesting that *F. pinicola* colonized the dead wood

from the bark beetle galleries. Correspondingly, Weslien et al. (2011) found a positive correlation between colonization of spruce stumps by the bark beetle *Hylurgops palliatus* and presence of *F. pinicola* fruit bodies, which might have been caused by propagule dispersal. Strid et al. (2014) excluded invertebrates from recently cut spruce logs and found strong indications that wood-decay fungi dispersed by bark beetles (*Ips typographus, Pityogenes chalcographus* and *Crypturgus sp.*) influenced the fungal community. Thus, several species of bark beetles might function as dispersal vectors for wood-decay fungi.

4.3 Management implications

Sessile organisms like plants or fungi respond differently to edge effects, connectivity and fragmentation depending on whether they are dispersed by wind or animal vectors, as demonstrated for plants (Damschen et al. 2008; Montoya et al. 2008). While saproxylic fungi are usually assumed to be wind-dispersed, our studies show that saproxylic insects might contribute to dispersal of several species of fungi (**papers I-V**). Targeted insect-vectored dispersal might allow fungi to persist in forests with low volumes of dead wood or in fragmented landscapes. Of the wood-decay fungi isolated from insects in **paper II** (Table 5), *F. pinicola, Heterobasidion sp.* and *F. fomentarius* do seem to have a high tolerance for habitat fragmentation (Nordén et al. 2013), and populations of *F. pinicola, Heterobasidion sp*. and *T. abietinum* have been shown to have high genetic diversity and little geographic differentiation (Högberg et al. 1999; Kauserud & Schumacher 2003; Stenlid et al. 1994), indicating effective spore dispersal. Our results suggest that targeted dispersal by insect vectors might have contributed to this effective spore dispersal and high fragmentation tolerance.

However, the benefit of animal-mediated dispersal in fragmented landscapes depends on the fragmentation tolerance of the animal vector (Cramer et al. 2007). Saproxylic insects differ in their response to habitat fragmentation or connectivity, presumably due to differences in substrate requirements or dispersal capacity (Brunet & Isacsson 2009; Buse et al. 2016; Schiegg 2000; Sverdrup-Thygeson et al. 2017). For instance, substrate generalists seem to have higher tolerance of habitat fragmentation than specialists (Schiegg 2000; Sverdrup-Thygeson et al. 2017). Although generalist saproxylic insects might thereby disperse fungal propagules across fragmented landscapes, the dispersal will probably be less targeted with respect to habitat demands of specialist fungi. Furthermore, as wood-decay fungi represent a low trophic level, their fragmentation tolerance might be higher than that of specialized fungivores or higher trophic levels such as parasitoids (Komonen et al. 2000). Thus, specialized insects providing

targeted dispersal for specialized fungi might go extinct in a fragmented habitat prior to the fungi. This might result in a slow extirpation of the fungi from the habitat, if insect-vectored dispersal was important to its persistence. It is therefore important to gain knowledge of insect-fungus interactions and their importance, to be able to make informed management decisions and increase the probability of success for conservation efforts, for instance by reintroducing important insect vectors after habitat restoration.

4.5 What other insect-fungus interactions can influence the fungal community in dead wood?

In **paper I** we reviewed insect-fungus interactions in general, which includes a variety of mechanisms by which insects can influence the fungal community in dead wood. Insects can affect fungi directly by insect-vectored propagule dispersal or by fungivory, and they can affect fungi indirectly by substrate alterations. The consequences of these interactions might be species-specific. For instance, while fungivory is usually negative to the fungus, Crowther et al. (2011) demonstrated that nematode grazing stimulated growth of the fungus *Hypholoma fasciculare*, allowing it to outcompete fungi that would normally be superior competitors. Preferential grazing by fungivores can alter the competitive hierarchy of saprotrophic fungi by stimulating inferior competitors or reducing competitive ability of dominant species, depending on the preferences of the fungivore (A'Bear et al. 2014).

Insect tunnelling can facilitate spread of fungi in wood

Substrate alterations by insects can also have species-specific effects. Saproxylic insects can alter their substrate by tunnelling, and different species create tunnels of differing widths and in different layers of the wood. Tunnelling can influence the fungal community by promoting fragmentation of the substrate, by altering aeration and moisture conditions or by increasing accessibility to the wood (Ulyshen 2016). For instance, Leach et al. (1937) found that tunnels created by cerambycids in the genus *Monochamus* accelerated spread of the fungus *Phanerochaete gigantea* (previously *Phlebiopsis/Phlebia gigantea*) in the heartwood of dead pine. Since *P. gigantea* could not be isolated from any *Monochamus* individuals, it would seem that the tunnels themselves promoted spread of the fungus. However, tunnels by large buprestid beetle larvae did not facilitate spread of *P. gigantea.* Buprestid larvae did not expel frass from their tunnels like the cerambycid larvae did, and so the frass-packed tunnels did not seem as beneficial to the fungus as the open tunnels of cerambycids.

Fungi are affected by bark loss caused by insect tunnelling

Many saproxylic insects mainly create tunnels in the cambium between the bark and the wood, which can cause bark loss. Weslien et al. (2011) found a negative correlation between the number of *Monochamus sutor* emergence holes in spruce high stumps and the percentage of remaining bark, and a positive correlation between the percentage of remaining bark and number of *F. pinicola* fruit bodies. Thus, tunnelling by *M. sutor* caused bark loss that negatively affected *F. pinicola*. In **paper V**, we found that bark cover was lower for aspen high stumps and logs at sites where wood-boring beetles (mainly cerambycids) had been abundant after tree death. However, in contrast with *F. pinicola*, presence of fruit bodies of the fungus *B. citrina* was negatively correlated with bark cover, and therefore positively correlated with abundance of wood-borers. Thus, while both **paper V** and the study by Weslien et al. (2011) showed that tunnelling by wood-boring beetles increased bark loss, the fungus species exhibited contrasting responses due to different habitat preferences.

4.6 How important is insect-vectored dispersal to the fungal community?

Due to the range of possible insect-fungus interactions (**paper I**) it is difficult to quantify the effect of insect-vectored propagule dispersal per se. While the effect of saproxylic insects and other wood-inhabiting invertebrates can be tested by experimental exclusion, as in **paper IV**, this method does not discern between different insect-fungus interactions that can influence the fungal community in dead wood. Short of following the fate of single propagules from fungal fruit bodies via insect vectors to their establishment at new substrates, it is difficult to devise a method testing the effect of only insect-vectored dispersal in the field. However, comparing fungal communities established with and without insects, and considering the differences between these communities in light of fungi isolated from potential insect vectors, should allow sound inferences as to the importance of insect-vectored dispersal for the fungal community.

Experimental exclusion of invertebrates from dead wood

Presently, there are three published studies that experimentally exclude invertebrates from dead wood to study the effects on the fungal community (Müller et al. 2002; Strid et al. 2014; Ulyshen et al. 2016), in addition to **paper IV**. Müller et al. (2002) mainly studied the effects of colonization of spruce logs by the bark beetles *H. palliatus* and *T. lineatum.* Strid et al. (2014) focused on the effects of the bark beetles that were numerically dominant in their spruce logs

(mainly *P. chalcographus* and *I. typographus*), while termites were the dominant invertebrates in pine logs in the study by Ulyshen et al. (2016). In contrast, based on previous studies of saproxylic communities in aspen dead wood (Sverdrup-Thygeson & Ims 2002; Sverdrup-Thygeson & Birkemoe 2009), we do not consider any single taxon of invertebrates to be dominatingly influential in the aspen logs in **paper IV**. Notably, the three previous studies all use coniferous wood, while our study is the first to test the effect of invertebrate exclusion on deciduous wood.

Despite the differences between the study systems, all four studies found that invertebrate exclusion significantly altered the fungal communities in the dead wood. For instance, Müller et al. (2002) found that the fungi *Antrodia serialis, P. gigantea* and *Trichoderma sp.* were more frequently present in logs colonized by the ambrosia beetle *T. lineatum*. Strid et al. (2014) found that among others *P. gigantea, Trichoderma pleuroticola, Stereum sanguinolentum* and *S. brinkmannii* were significantly more frequent in logs colonized by bark beetles relative to logs from which bark beetles had been excluded, and all these species except *P. gigantea* were also isolated from bark beetle samples. Fungal OTUs annotated as *Stereum sp., Trichoderma sp.* and *S. brinkmannii* were also isolated from saproxylic insects in **paper II**. In **paper IV**, the most notable effect of invertebrate exclusion on fungal community composition was the reduced abundance of the wood-decay fungi *T. versicolor* and *T. ochracea.* These fungi were also found to increase in abundance in ethanol-baited logs. Since *T. versicolor* was isolated from saproxylic insects sampled from aspen logs in **paper II**, and previous studies have shown that several saproxylic insects are attracted to ethanol (Allison et al. 2004; Bouget et al. 2009), the increased abundance of *T. versicolor* and *T. ochracea* in ethanol-baited logs was likely caused by a positive effect of saproxylic insects on establishment of these fungi (as discussed previously in section 4.1).

What is causing the effect of invertebrate exclusion?

While it is not unlikely that the cage used for invertebrate exclusion might have altered the microclimatic conditions and thus influenced fungal community composition, we included a cage control treatment in **paper IV** to control for this effect. The cage control treatment differed from the cage treatment along the main gradient of variation in fungal community composition explained by the experimental treatments (Fig. 10), showing that the differences between the treatments along this gradient were not due to the cage per se. The studies by Strid et al. (2014) and Ulyshen et al. (2016) lacked similar cage control treatments, although they made additional observations to justify the argument that presence or absence of invertebrates was the main

effect of exclusion. Müller et al. (2002) circumvented this problem by caging all logs and opening half the cages during the flight period of their study species.

In the study by Strid et al. (2014) and **paper IV**, the change in fungal community composition was considered in light of fungal DNA isolated from saproxylic insects, which indicated that insect-vectored dispersal of fungi affected the fungal community in the logs. However, substrate alteration or fungivory by invertebrates might also have contributed to the effect of invertebrate exclusion. In **paper IV**, we did not see any indications of insect entry holes or tunnels, suggesting that there had been little substrate alteration. Strid et al. (2014) tested the effect of artificial holes drilled into the dead wood, mimicking beetle tunnels, and found that this had little impact on the fungal community. However, fungivory might have affected the fungal communities in accessible logs in both studies, although it is unclear to what degree fungivores can influence fungal growth within dead wood (Crowther et al. 2011). Previous studies have tested the effect of soil invertebrates on fungal growth and competition in soil micro- or mesocosmoses (reviewed in A'Bear et al. 2014). To our knowledge, there are no studies of the effect of saproxylic fungivores or xylomycetophages (species feeding on fungus-infested wood) on fungal establishment, growth or competitive ability within dead wood. Future studies should attempt to test whether fungivory by saproxylic insects affects fungal communities within dead wood.

How important are the effects of invertebrate exclusion?

It is difficult to quantify the magnitude of the effect of invertebrates on the fungal community in exclusion studies, and indeed no such estimate is presented in the three previous studies (Müller et al. 2002; Strid et al. 2014; Ulyshen et al. 2016). In **paper IV**, we do quantify the proportion of variance in the fungal communities that is explained solely by the experimental treatments (i.e. invertebrate exclusion and our three control treatments), which is small relative to the proportion explained by tree identity (i.e. which tree individual each log was cut from). However, the experiment only covered two years of decay after tree death, and exclusion of invertebrates would probably have had an even stronger effect in a long-term experiment. Furthermore, the difference in community composition between treatments documented in our study might increase during succession due to priority effects favouring early arrivals (Dickie et al. 2012; Fukami et al. 2010; Leopold et al. 2017), which might subsequently facilitate or inhibit successor species (Niemelä et al. 1995; Ottosson et al. 2014). Hopefully, we will be able to assess this effect in future studies.

Effect of invertebrate exclusion on wood decay

In **paper IV** we also measured wood density as an indication of wood decay, and found that wood density of caged logs was significantly higher than control logs. This indicates that the rate of wood decay had been decreased by the experimental exclusion of invertebrates. Similarly, in the study by Müller et al. (2002), logs in permanently closed cages had lost less dry weight than logs in periodically opened cages. The reduced rate of wood decay is unlikely to be due to the cage per se, as the cage control treatment in **paper IV** did not have an effect on wood density similar to the cage treatment, and mesh nets have rather been found to increase rate of decomposition in a previous study (Stoklosa et al. 2016). The apparent decrease in wood decay rate for caged logs is therefore more likely be due to either lack of direct effects of the insects on wood decay (Ulyshen 2016), or indirect effects such as the change in fungal community composition in absence of insects, or a combination of both. Previous studies have found that saproxylic insects other than termites have relatively little direct impact on mass loss of wood (Ulyshen & Wagner 2013; Ulyshen 2016), and so the indirect effect through the fungal community might be of greater importance. In either case, this shows that not only do insect-fungus interactions structure the dead wood community, they significantly influence the ecosystem process of wood decay, which is integral to the functioning of forest ecosystems (Cornwell et al. 2009; Fekete et al. 2014; Gonzalez-Polo et al. 2013).

Figure 17) Dead wood decayed by brown rot and white rot fungi. Photo: R.M. Jacobsen.

4.7 Conclusions and future perspectives

Our studies (**papers I-V**) show that insect-vectored dispersal does influence the fungal communities in dead wood, thus underlining the need for further research into the importance of this interaction for specific species of fungi, the saproxylic fungal community and its function in the forest ecosystem. Saproxylic fungi and insects perform an essential ecosystem service by decomposing dead wood (Fig. 17), and their interactions can influence the rate of

decomposition (**paper IV**, Müller et al. 2002, A'Bear et al. 2014). Decomposition of dead wood is integral to carbon and nitrogen cycles in forests (Fekete et al. 2014; Gonzalez-Polo et al. 2013; Rinne et al. 2017), and recent studies have suggested that the influence of fungal communities on wood decay must be taken into account in models of CO_2 emissions (Bradford et al. 2014; van der Wal et al. 2015). Dead wood is also a biodiversity hot spot in forests, hosting a rich community of saproxylic species (Stokland et al. 2012). In order to conserve the diversity of saproxylic species and the services they provide, we need to understand their interactions and interdependency.

Further research

To increase our understanding of the influence of invertebrates on fungal communities demonstrated in **papers IV-V** and previous studies (e.g. Müller et al. 2002; Strid et al. 2014; Ulyshen et al. 2016; Weslien et al. 2011), future studies must somehow separate effects stemming from different insect-fungus interactions. Experimental "inoculation" of dead wood with insects, i.e. caging logs and introducing specific species or functional guilds of insects, could be a possibility, although single species or guilds could still interact with fungi in multiple ways. It would also be logistically difficult to incorporate insect-vectored propagule dispersal in this experimental set-up, since insects would then have to be kept in captivity for very short periods to avoid propagules being excreted or dropped prior to inoculation. However, such an experiment could still provide interesting information.

An ideal, but maybe not feasible method to study insect-vectored propagule dispersal would be to use some sort of label for fungal propagules from a specific fruit body, and then only allow propagules to be dispersed by controlled exposure to certain insects. If the fate of these propagules could thereafter be followed by tracking the labels, the results could be very informative. However, I doubt that this is currently possible in practice.

The potential of insect species to act as spore vectors, i.e. their spore dispersal effectiveness, should also be assessed further. Several of the variables in the spore dispersal effectiveness framework (Fig. 15) could easily be studied by conventional methods, such as sampling individual insects and using microscopy to assess number of spores carried externally and internally. Spore viability should be assessed by culturing in the lab, and comparing germination rates of spores dropped or excreted by insects with those of spores sampled directly from fruit bodies, as in Lim (1977). These methods could be used to assess spore dispersal by several insect species sampled from dead wood, representing different taxa and functional

guilds, and could potentially reveal interesting systematic differences in quantity of spores dispersed and digestion effects.

To fully understand the implications of insect-fungus interactions for the ecosystem, we need long-term studies of the effect of invertebrate exclusion on the fungal community, incorporating measures of ecosystem processes such rate of decomposition. Including a treatment with fungus exclusion, perhaps through sterilisation of substrates or application of fungicides, might clarify the relative importance of direct and indirect effects of invertebrates on rate of decomposition. Comparing the effect of invertebrate exclusion on decomposer communities in substrates such as dead wood, litter and dung would also be very interesting.

5. Conferences and outreach contributions

Jacobsen, Rannveig M; Birkemoe, Tone; Sverdrup-Thygeson, Anne; Kauserud, Håvard. 2017. *The potential of insects to disperse fungi to dead wood.* Talk at the Norwegian Ecological Society meeting, Oslo, Norway.

Jacobsen, Rannveig M; Birkemoe, Tone; Sverdrup-Thygeson, Anne; Kauserud, Håvard; Botnen, Synnøve Smebye. 2016. *The potential of insects to act as spore vectors.* Talk at the XXV International Congress of Entomology, Florida, USA.

Jacobsen, Rannveig M; Birkemoe, Tone; Sverdrup-Thygeson, Anne. 2015. *Beetles in early succession influence the fungal community in late succession of dead wood.* Talk at The Royal Entomological Society International Symposium & National Science Meeting Ento'15, Dublin, Ireland.

Jacobsen, Rannveig M; Sverdrup-Thygeson, Anne; Birkemoe, Tone. 2014. *Do early colonizing beetles on dead aspen affect fungi present 10 years later?* Talk at the 8th Symposium on the Conservation of Saproxylic Beetles, Basel, Switzerland.

Jacobsen, Rannveig Margrete; Sverdrup-Thygeson, Anne; Birkemoe, Tone. 2014. *Do early colonizing beetles on dead aspen affect fungi present 10 years later?* Talk at the Nordic Society OIKOS conference, Stockholm, Sweden.

Jacobsen, Rannveig M. 2014. *Samarbeider skogens ryddehjelp?* Presentation in the science outreach competition Forsker Grand Prix, Oslo. http://www.fgposlo.no/arkiv/deltagere-2013/rannveig-m-jacobsen/

Insect ecology blog: http://blogg.nmbu.no/insektokologene/

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PAPER I

Insect-fungus interactions in dead wood systems

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Abstract

Insects and fungi are the most abundant eukaryotic organisms in dead wood. Insect-fungus interactions span a wide gradient of specificity from indirect interactions through shared habitats, to direct interactions based on nutrition, dispersal, detoxification or protection, including facultative and obligate mutualisms. In this review, we bring together old and new knowledge on these topics.

For insects, fungal tissue has higher nutritional value than wood. Adding fungi to the diet of wood-feeding insects may reduce the time needed for larval development in comparison with pure wood diets. Fungivory has been demonstrated to affect growth and competitive ability of wood decay fungi in soil, though the effect on fungal communities within wood is unclear. Substrate alteration by insect tunneling and comminution can also affect the growth and occurrence of fungi.

Exchange of dispersal and nutrition is the basis for obligate insect-fungus mutualisms. Adaptations to these mutualisms seem to have evolved rapidly, and for some insects there has been a feedback between the evolution of fungus-farming and sociality. Several recent studies indicate that insect-vectored dispersal might be an important complement to wind dispersal also for non-mutualistic saproxylic fungi, potentially providing targeted dispersal to suitable substrates. We propose a theoretical framework for insect-vectored spore dispersal effectiveness.

Insect-fungus interactions are an essential component of forest ecosystems, influencing species richness, wood decay and nutrient cycling. Several aspects of insect-fungus interactions are unknown and require further study, but increased use of molecular methods such as DNA analysis seems fuel a renewed interest in this field of research.

Contents

1. Introduction

Insects and fungi are the most abundant eukaryotic organism groups in dead wood. The high species diversity and the old evolutionary history dating back to Early Ordovician for the insects (Misof et al. 2014) and at least late Silurian for the fungi (Sherwood-Pike and Gray 1985; Misof et al. 2014) are indicative of a long history of cohabitation, which likely resulted in reciprocal adaptations and intricate interactions. Based on our current knowledge, the main interactions between fungi and insects can be grouped into four functional relationships: *1) Nutrition*. This includes insects feeding on fungi and fungi feeding on insects. The fungi provide insects with some essential nutrients and wood-degrading enzymes. In insect-fungus mutualisms, fungi may be provisioned with new substrate or 'fertilized' in different ways by the insects. The insects may also be fed upon by pathogenic fungi, fungal parasites or can be immobilized or killed by ectomycorrhizal fungi (Klironomos and Hart 2001). *2) Dispersal*. Insects disperse fungi in passive ways or in highly specialized transmission organs. *3) Detoxification*. Fungi degrade tree defenses that would be toxic to insects. *4) Protection*. Insects protect fungi by farming as known from leaf-cutter ants, termites and several ambrosia beetles. Fungi may also protect insects by reinforcement of nest-wall structures (Schlick-Steiner et al. 2008) or fighting microbial pathogens (Florez et al. 2015), although this is less studied. All four functional interactions can be based on loose relationships, or the interaction can be tight such as facultative or obligate mutualism. In the dead wood system, *indirect interactions* also occur as both fungi and insects modify the dead wood, changing the habitat for the other group.

Many reviews have covered insect-fungus interactions, including several of the functions mentioned above (Wheeler and Blackwell 1984; Wilding et al. 1989; Vega and Blacwell 2005; Shaw 1992; Boddy and Jones 2008). Surprisingly, despite insects and fungi being among the most species rich taxa in dead wood ecosystems, no review has focused on their interactions in the dead wood environment. The comprehensive book "Biodiversity in dead wood" (Stokland et al. 2012) only includes the most common interactions in addition to an overall description of fungivores. Thus, the aim of this book chapter is to address this knowledge gap and summarize the available knowledge on insect-fungus interactions in the dead wood system. Interactions with insect pathogens, insect gut symbionts and pathogens of living plants are left out. Due to the close proximity of dead wood to the soil ecosystem, well-known examples of interactions from this system are included.

2. Fungi in dead wood

Here we will provide a short introduction to the ecology of saproxylic fungi in dead wood (**Fig. 1**). For more detailed information on these fungi, we refer to the many excellent reviews and books on this topic, e.g. Rayner and Boddy (1988), Boddy (2001), Boddy and Jones (2008) or Stokland et al. (2012).

Most of the saproxylic fungi known to cause significant mass loss during wood decay belong to the white-rot and brown-rot fungus groups in the phylum Basidiomycota, which predominately degrade cellulose and lignin or only cellulose, respectively (Boddy 2001; Kubartová et al. 2015). The soft-rot ascomycete fungi are also common in dead wood and predominantly contribute to cellulose degradation, but to a much lesser extent than the basidiomycetes (Boddy 2001; van der Wal et al. 2015). High-throughput DNA-sequencing analyses have recently shown that there are higher numbers of ascomycete fungus species in dead wood, but that the basidiomycetes seem to occupy larger volumes of wood (Kubartová et al. 2015; Ottosson et al. 2015; Strid et al. 2014; van der Wal et al. 2015). Basidiomycetes have a much more complex enzymatic machinery (Floudas et al. 2012) and thus dominate over ascomycetes, especially during intermediate and late stages of wood decay (Ottosson 2013; Rajala et al. 2015).

Prior to the development of molecular methods such as high-throughput sequencing, fungal communities in dead wood were recorded by fruit body surveys. Therefore, studies of saproxylic fungi have usually focused on species with macroscopic fruit bodies, mainly of the polyphyletic group called polypores or bracket fungi (Basidiomycota, e.g. Gilbertson and Ryvarden (1986)) Molecular methods have shown that although fruit body surveys do not capture the entire fungal community in dead wood, they reflect the most abundant species that dominate the substrate (Ovaskainen et al. 2013). The discrepancy between the methods explains why species richness of fungi seems to peak at intermediate stages of decay in fruit body surveys (Jönsson et al. 2008; Lindblad 1998), while species richness continues to increase with wood decay according to molecular analyses (Kubartova et al. 2012; Ovaskainen et al. 2013; Rajala et al. 2015). In advanced decay stages, dominant basidiomycete species such as polypores are replaced by a large number of species with inconspicuous fruit bodies (Kubartova et al. 2012) and soil fungi (e.g. mycorrhiza) that colonize the dead wood (Makipaa et al. 2017; Rajala et al. 2012).

The succession of fungus species during wood decay is linked to their abilities to overcome tree defenses, enzymatically degrade wood and compete with other fungi (Rayner and Boddy 1988). Put simply, several plant-pathogenic (e.g. blue-stain fungi) and soft-rot ascomycete fungi (e.g. *Chaetomium spp., Ceratocystis spp.*) typically dominate in dying trees and early stages of decay, as they are well adapted to overcome tree defenses by metabolizing specialized toxic plant compounds such as terpenes and phenolics (Krokene 2015). These fungi grow relatively quickly through the tracheids and plant vessels, but have relatively poor cellulolytic and no ligninolytic capabilities. They consume the cell contents, leaving the structural components of the cell walls more or less intact (Nilsson 1976; Rösch and Liese 1971). Also, they are poor competitors and are thus replaced by the 'true' wood-decaying fungi, i.e. white- or brown-rot basidiomycetes. These species grow through the wood relatively slowly by substantial degradation of the recalcitrant lignocellulosic plant cell-wall structure (Rayner and Boddy 1988). Molecular methods have also revealed that fungi with a variety of other ecological roles are present in dead wood (Ottosson et al. 2015). While fungi known to be wood-decaying are most abundant, endophytic, plant- and entomopathogenic, mycoparasitic, mycorrhizal and lichenized species have also been isolated from dead wood (Ottosson et al. 2015). Some wood-decay fungi can switch between different modes, colonizing living trees as plant-pathogens and switching to a saprotrophic mode as the tree dies (Boddy 2001). Furthermore, many species of saprotrophic fungi have been found to be latently present as endophytes in the wood of the living tree, presumably waiting for the breakdown of the tree defensive system due to weakness or death of the tree (Chapela and Boddy 1988; Parfitt et al. 2010).

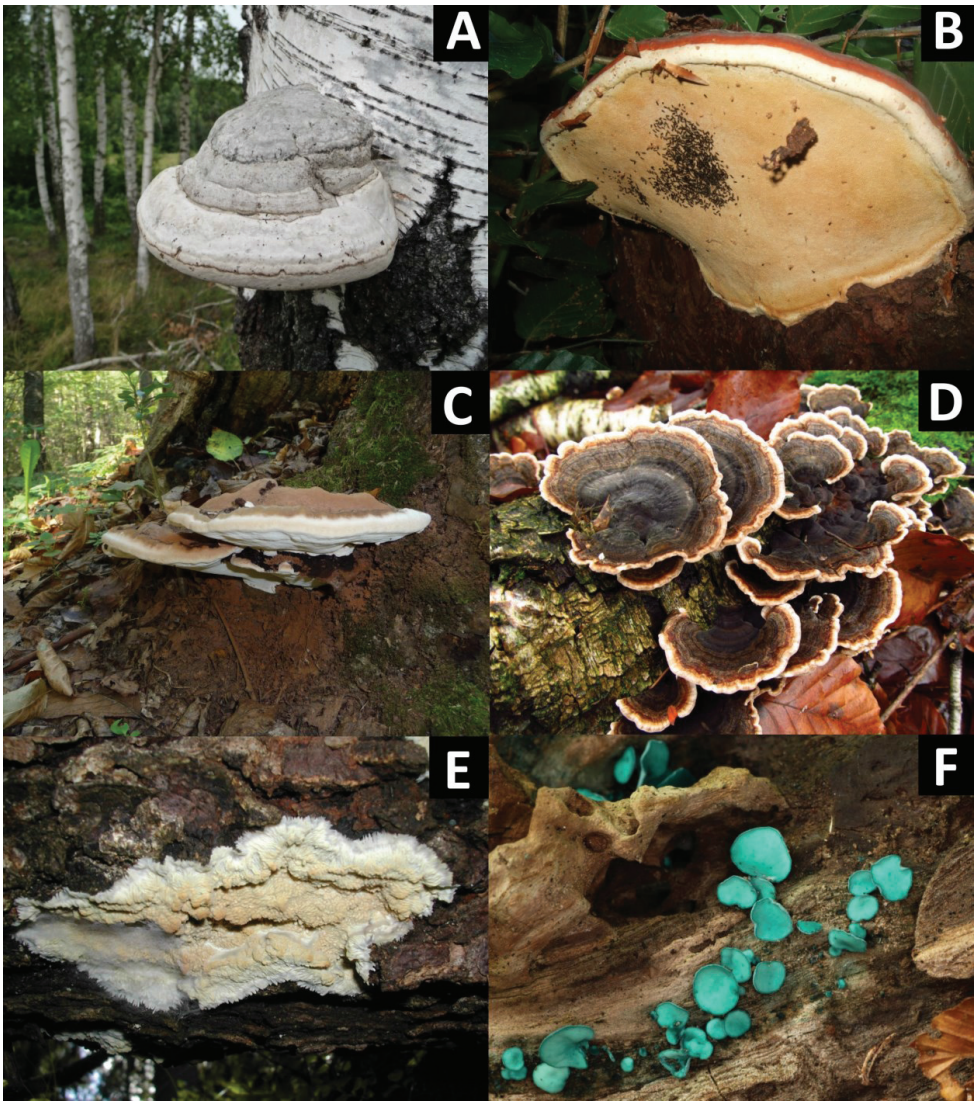

Fig. 1. Examples of saproxylic basidiomycetes (A-E) and ascomycetes (F). (A) The tinder fungus *Fomes fomentarius*. Photo by George Chernilevsky - Own work, Public Domain. (B) The red belt conk *Fomitopsis pinicola,* here with a gathering of beetles on its spore-producing hymenium. Photo by R. M. Jacobsen. (C) The artist's conk *Ganoderma applanatum*, with its copius production of spores clearly visible as brown powder beneath the fruit body. Photo by George Chernilevsky - Own work, Public Domain. (D) The turkey tail *Trametes versicolor*. Photo by Hans-Martin Scheibner - Own work, CC BY-SA 3.0. (E) The resupinate fruit body of *Phlebia centrifuga,* a polypore mainly found in old-growth forest. Photo by A. Sverdrup-

Thygeson. (F) The green elfcup *Chlorociboria aeruginascens,* an ascomycete whose hyphae can dye the wood green. Photo by H. Krisp - Own work, CC BY 3.0.

3. Fungi as providers of nutrition, detoxification and protection for insects

Fungal mycelium contains many times more nitrogen and phosphate relative to carbon in comparison with undecayed wood (Swift and Boddy 1984). Decayed wood, being a mixture of both substances, have ratios of intermediate values (Boddy and Jones 2008). Insect tissue also contains much higher concentrations of nitrogen and phosphorous than the wood itself; undecayed pine wood had 1500–2000 and 500–900 times less concentrated N and P than the cerambycid and buprestid beetles feeding on it (Filipiak and Weiner 2014). Thus, based on these nutrient contents alone, adding fungi to the diet should be highly favorable for saproxylic insects. This might be part of the reason that two subterranean termites, known to break down lignocellulose by use of their gut symbionts, still prefer sawdust infected by fungi to un-infected sawdust when given the choice (Cornelius et al. 2002) .

Most insects lack key enzymes for sterol biosynthesis (Clark and Block 1959). Plant sterols are rare, but other sterols, like the fungal ergosterol, can help with biosynthesis of juvenile hormone and thus insect development. Essential elements such as K, Na, Mg, Zn and Cu are also scarce in dead wood and can limit larval growth (Filipiak and Weiner 2017). Similar to N and P, the concentrations of these elements have been found to increase with wood decay and are likely to be transferred from the surroundings by fungi (Filipiak et al. 2016; Filipiak and Weiner 2014). Recent research has shown that the fungal communities in dead wood and soil do indeed interact closely, moving nutrients between the substrates (Makipaa et al. 2017). Thus, from the insect point of view, adding fungi to the diet reduces the quantity of food needed and provides essential elements for growth. Many wood-feeding insects, like lower termites, longhorn beetles and bark beetles, engage in facultative associations with filamentous fungi. They develop perfectly without fungi, but profit when certain fungi are present in the surroundings of the nest or within the ingested wood substrate (Becker and Kerner-Gang 1963; Geib et al. 2008; Six 2012; Klepzig et al. 2009).

Fungi not only serve as biomass with potential nutritional value, but are also active catalytic agents with diverse metabolic capabilities. Many wood-feeding insects carry one or a few species of yeasts in their digestive tracts (Vega and Dowd 2005; Suh et al. 2005). Although there are few studies of the functions of these yeasts, they seem to help the insects with degradation of the lignocellulosic plant biomass (Tanahashi et al. 2010; Vega and Dowd 2005;

Urbina et al. 2013) and probably with detoxification of toxic plant chemistry (Dowd 1992). Filamentous fungi growing within the wood may also benefit insects through their liberation of wood-degrading and detoxifying enzymes, especially if these enzymes remain active in the insect gut and thereby augment or extend the digestive capabilities of the consumer (Martin 1983). This facilitation of enzymatic degradation and detoxification is of primary importance in the bark beetle and wood-wasp mutualisms with fungi (see **section 6**), but very likely also plays a role in many non-mutualistic insect-fungus interactions. However, recent studies of beetle genomes (Cerambycidae: *Anoplophora glabripennis,* Buprestidae: *Agrilus planipennis,* Scolytinae: *Dendroctonus ponderosae*) have revealed that some wood-boring insects are not dependent on associating with fungi (or bacteria) to degrade wood, as they have acquired many plant-degrading and detoxifying enzyme families such as P450 or GST horizontally from microbes (Keeling et al. 2013; McKenna et al. 2016).

A final and almost unstudied role is the protective function that fungi may have for insects in wood, such as by outcompeting antagonistic organisms (e.g. fungal entomopathogens) (Castrillo et al. 2016), including the production of antibiotics (Florez et al. 2015). The use of fungi in ant nest construction as known for Old World *Lasius* ants (Formicinae) of the subgenera *Dendrolasius* and *Chthonolasius* (Schlick-Steiner et al. 2008; Seifert 2006) can also be seen as an example of fungi physically protecting insects.

4. Fungivory and its effects

4.1. Dead wood fungivores

Species of most insect orders living in dead wood are known to feed on fungi, although species of Diptera and Coleoptera dominate. The dipterans include the highly numerous fungus gnats (Sciaroidea: Bolitophilidae, Diadocidiidae, Ditomyiidae, Keroplatidae and Mycetophilidae), gall midges (Cecidomyiidae) and species of flat-footed flies, *Agathomyia* spp. (Platypezoidea: Platypezidae) (Halme et al. 2013; Økland 1996; Økland 1995; Hanski 1989; Jakovlev 2011). Among the beetles, species of the families Ciidae, Cryptophagidae, Endomychidae, Erotylidae, Leiodidae, Melandryidae, Ptilidae, Mycophagidae, Staphylinidae and Tenebrionidae include a large number of primarily fungivorous species living in dead wood. Several so called fungus-farming insects, like ambrosia beetles (Platypodinae and Scolytinae) and ship-timber beetles (Lymexilidae) also feed more or less exclusively on mutualistic fungi cultivated within their tunnel systems in wood (see **section 6**). The termites, being an originally wood-feeding order with gut flagellate protists, diverged into a species rich group with a large variation in food items ~ 60 million years ago (Brune 2014). The subfamily Macrotermitinae evolved in

symbiosis with fungi in the genus *Termitomyces spp.,* which they cultivate on wood fragments and other lignocellulotic material in their nests (Nobre et al. 2011). These fungivorous termites, comprising relatively few species, are highly abundant and important decomposers within their distribution range in Africa and Asia (Brune 2014; Jouquet et al. 2011). In Lepidoptera, the only groups with primarily fungivorous species seems to be Oecophoridae, Tineoidea and Oinophilidae (Rawlins 1984; Lawrence and Powell 1969) living in and feeding on fruit bodies of polypores. Fungivores also occur in smaller insect orders such as Thysanoptera (thrips), but here mainly in the suborder Tubuliforma (Mound 1974). Thrips can be abundant in early stages of wood-decay in tropical forests. For an overview of insects feeding on fungi in general, see Table II, Appendix, in Wilding et al. (1989). In addition to the above mentioned insects, other invertebrates usually defined as soil fauna such as mites, collembola, isopods and nematodes can be numerous in dead wood (Zuo et al. 2014). Fungi represent an important food source to these species (Pollierer et al. 2009).

Beetles are the most well-known saproxylic invertebrate group. In Germany, approximately 52% of the saproxylic beetle species are assumed to feed on wood and/or phloem (xylophages), 18% on fungi and an additional 10% on a mixture of fungi and wood (xylomycetophages) (Koehler 2000). In this dataset, the species feeding directly on dead wood were much larger than the fungivores (mean body length of 8.3 mm vs 2.4 mm) whereas species feeding on a mixture of wood and fungi have intermediate length (5.3 mm) (**Fig. 2**). The Jarman-Bell principle based on mammalian herbivores but extended to primates, whales and fruit bats (Müller et al. 2013) states that gut capacity remains a constant fraction of body size, whereas the specific metabolic rate decrease with increasing body mass (Owen-Smith 1988). Thus, for many mammals, larger species can tolerate a lower quality diet than smaller species. It is interesting that the same pattern in body size and food quality is found for saproxylic beetles.

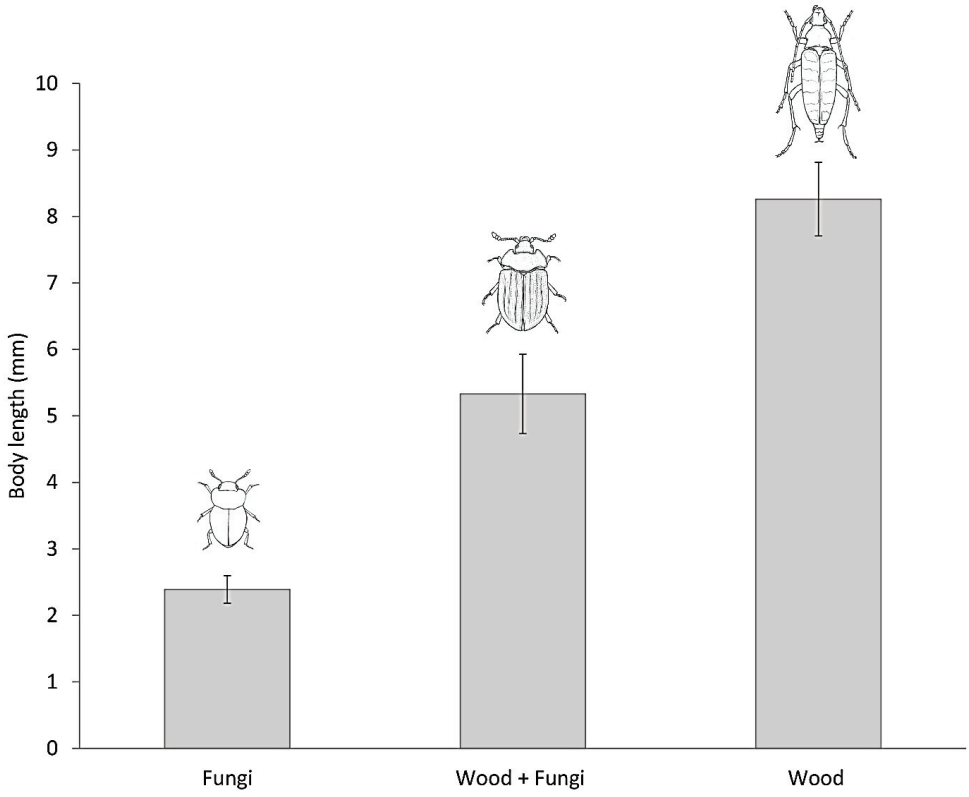

Fig. 2. Body size (mean ± 95% confidence intervals) vs diet in saproxylic beetles from Germany. Number of species is 185 (fungi), 109 (fungi and wood) and 542 (wood) (Koehler 2000). Drawings of representative species by R. M. Jacobsen.

Our knowledge of insect feeding modes in the dead wood system is limited and based on much anecdotal evidence. The relative percentage of species being recorded as fungivores and the number of species including fungi as part of their diet are likely to be higher than present estimates. Filipiak and Weiner (2014) argue that the wood-feeding beetles (xylophages) in their study are in fact fungivores or mixed wood and fungus feeders (xylomycetophages), as their wood diets are supplemented with fungi that gradually infect the decaying wood and provide essential nutritional elements. They calculate that without fungi in their diets, these wood feeders would need between 40 and 85 years in order to gain the essential nutrients needed to develop into adults. Detailed studies of stag beetles also point towards fungi as an important part of their xylophagous diet (Tanahashi et al. 2009). Fungal (and bacterial) endosymbionts

might further aid digestion in the gut (Ceja-Navarro et al. 2014; Suh et al. 2005; Brune 2014). Studies of the soil ecosystem using stable isotopes show that most litter arthropods are actually feeding on ectomycorrhiza or predating on invertebrates rather than feeding on the litter itself (Pollierer et al. 2009). Similar studies from the wood ecosystem spanning a large number of species, would be highly valuable.

4.2. Insect specialization on fungal growth forms

Fungi can be divided into filamentous fungi and yeasts (Vega and Dowd 2005). Yeasts are predominantly unicellular and reproduce asexually by budding, although several species can also produce hyphal growth and some reproduce sexually by producing ascospores. Filamentous fungi in wood on the other hand grow vegetatively as hyphae and often reproduce sexually by fruit bodies that produce fungal spores. The different forms of fungal growth represent highly different food resources for insects.

Yeasts and yeast-like fungal growth is important for insects in dead wood as many xylophagous species carry yeasts within their digestive tracts (Vega and Dowd 2005; Suh et al. 2005). Unfortunately, there is little research on the role of these gut yeasts, but they may provide the insects with enzymes for digestion and supply essential amino acids, vitamins and sterols (see **section 3**) (Tanahashi and Hawes 2016; Tanahashi et al. 2010; Suh et al. 2005). Some yeast-like fungi in the ascomycete genera *Ophiostoma* and *Ascoidea* play essential nutritional roles in the facultative and obligate mutualisms with bark, ambrosia and ship-timber beetles (see **section 6**). Yeasts have also been isolated from the guts of fungivorous beetles, where they might be nutritionally important, help with digestion of fungal polysaccharides or detoxification, or simply stem from the beetle's actual food source (Suh and Blackwell 2005; Suh et al. 2005). Several yeasts have been isolated from dead wood (Kubartova et al. 2012; Strid et al. 2014; van der Wal et al. 2015) and might therefore present a food source or supplement for saproxylic insects, but this remains to be studied.

Hyphal growth of filamentous or yeast-like fungi in more or less dense mycelium is present within and outside wood structures, and is likely to be included in the diet of many insects, even those normally identified as wood-feeding (Filipiak et al. 2016). Hyphae are a predictable resource that can be abundant in dead wood for many years. As many as 102 species of fungus gnats have been reared from larvae collected from dead wood or bark impregnated with fungi from Finland and the Russian Karelia (Jakovlev 2011). Hyphal feeders can also be found among other Diptera, Coleoptera, Thysanoptera, Collembola, Isopoda, nematodes and mites in

dead wood. Hyphae may aggregate to form linear organs known as cords (Boddy et al. 2009). These might be less palatable than looser mycelium or hyphae; whereas millipedes and isopods are known to feed on cords, smaller invertebrates such as collembola, mites and nematodes do not (Crowther et al. 2011b).

Fruit bodies are fed upon by Coleoptera, Lepidoptera and Diptera (Lawrence 1973; Rawlins 1984; Gilbertson 1984; Lawrence and Powell 1969; Komonen et al. 2004; Økland 1995; Jakovlev 2011). They can be soft and short-lived such as in many ascomycetes and some polypores, or hard and long-lived as in many perennial polypores. Both softness and longevity are likely to affect the insect's use of fungal fruit bodies. Thorn et al. (2015) found that most Ciidae, a beetle family specializing on saproxylic fruit bodies, preferred annual fruit bodies. Schigel et al. (2006) differentiate annual polypores into three groups based on their longevity (ephemeral, sturdy and hibernating), but difference in species preferences for these groups has never been formally tested. Generally, the hard and perennial polypore basidiocarps are mainly fed upon in their various stages of decay (Jonsell and Nordlander 2004).

Spores are only available during restricted time intervals. They are fed upon by a large number of opportunists (Hågvar 1999; Schigel 2011) and a few specialists. The specialists include larvae of minute beetles feeding on spores within the spore tubes of polypores (Ptilidae, Limulodidae and *Hylopsis sp.* in the Corylophidae) (Dybas 1976; Lawrence 1989). Species in the predominantly spore-feeding tribe Nanosellini (Ptiliidae: Ptiliinae) actually include the world's smallest beetles (*Scydosella musawasensis* from Nicaragua and *Vitusella fijiensis* from Fiji), which are only 0.3 mm long (Hall 1999) (**Fig. 3**). Larger fungivores have specialized on spore-feeding at the polypore surface (hymenium) (Leiodidae: *Zearagytodes maculifer*, Corylophidae: *Hylopsis sp.*)(Kadowaki et al. 2011b). Thysanoptera in the subfamily Idolothripinae are also specialized spore feeders found on dead wood in the wet tropics (Mound 1974).

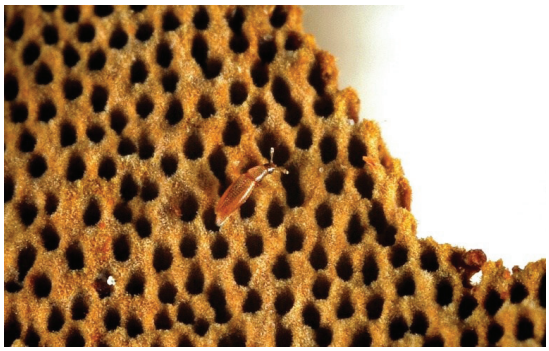

Fig. 3. Europe's smallest beetle, *Baranowskiella ehnstromi*, is only 0.5 mm long and live on spores in the pore tubes of polypore *Phellinus conchatus*. Reprinted with permission from Ole Martin ©

4.3. Effect of fungivores on fungi

Fungivory can reduce mycelium extension and spore numbers, but whether this affects fungal fitness is largely unexplored. Only a few cases of extensive damage to living fruit bodies are known. First, the two specialist beetles *Octotemnus glabriculus* and *Cis boleti* (Ciidae) may reduce the spore-producing surface (hymenium) of *Trametes versicolor* by 30-64% (Guevara et al. 2000). Second, the larvae of *Agathomyia wankowiczii* (Diptera: Platypezoidea: Platypezidae) form galls in *Ganoderma applanatum* that can cover most of the hymenium (Hanski 1989). Finally, the beetle *Cypherotylus californicus* (Erotylidae) is able to destroy soft polypores (Basidiomycetes: Polyporaceae) such as *Trametes versicolor* and *Bjerkandrea adusta* before spore production occurs (Graves 1965).

Spore feeders might also potentially decrease fungal fitness, if spores are not viable after passage through their digestive system. The digestion effects might be species-specific (**see section 5.3**). Digestion of spores from *Ganoderma cf. applanatum* by the specialist spore-feeding beetle *Zearagytodes maculifer* has been shown to reduce germination rate relative to undigested spores, suggesting a potential decrease in fungal fitness (Kadowaki et al. 2011a).

In woodland soil ecosystems, mycelium-feeding invertebrates can affect fungal growth. For instance, lab manipulations have shown that high collembola grazing intensity can cause mycelium extension of the wood decay fungi *Hypholoma fasciculare* and *Phanerochaete velutina* to decrease, while low grazing intensity can cause an increase (Crowther et al. 2012). Compensatory fungal growth at low grazing intensities has also been indicated for three soil living ascomycetes when collembola numbers were reduced by predatory mites (Hedlund and

Öhrn 2000). In a more complex system with several animal groups and as many as seven wood decay fungi, the micro- and mesofauna (nematodes and collembola) were able to increase fungal growth through stimulatory grazing, whereas the macro-fauna (isopods and millipedes) only reduced fungal growth (Crowther et al. 2011b).

Given that the size of fungal grazers seems to determine the effect on fungi, small fungivorous beetles and diptera might have similar grazing effects as nematodes and collembola and increase fungal growth at wood surfaces, while larger species will be expected to reduce fungal growth. As for fungi growing within the dead wood itself, the effect of grazing is unclear. Not surprisingly, Crowther et al. (2011a) noted that grazing isopods only reduced fungal growth outside the wood blocks. However, tunnelling beetles feeding on a mixed wood and fungal diet may have the potential to reduce or stimulate fungal growth even within dead wood. In insect-fungus mutualisms, ambrosia beetles have been shown to stimulate nutritional yeast-like 'ambrosial growth' for *Ambrosiella* fungi (Ascomycota) (**Table 1**) (Batra and Michie 1963).

Fungi are known to compete for resources, often with well-known hierarchies of inferior and dominant species (Holmer et al. 1997; Boddy 2000). Grazing by soil invertebrates has been found to influence or even reverse outcomes of competetive interactions in soil between wood-decomposing fungi. Crowther et al. (2011a) demonstrated that nematodes stimulated growth of an inferior competitor, whereas isopods restricted a dominant competitor (Crowther et al. 2011a). Grazing therefore altered the competitive hierarchy and ensured coexistense of two fungal species, which also affected wood decay rates. Thus, grazing intensity and food preferences of fungivorous invertebrates might alter fungal-mediated nutrient cycling and decomposition.

4.4. Fungal defense against fungivores

If invertebrate feeding activity reduces fungal fitness, fungi might have evolved defense mechanisms. Although there are presently few examples of reduced fitness due to fungivory, several physical and chemical defense mechanisms have been suggested. Hackman and Meinander (1979) as cited in Hanski (1989), suggest that sporulation in soil, physical protection of fruit bodies prior to sporulation, production of milky sap and toxic or repellent chemicals might defend fruit bodies against colonization of fungivores. Perennial polypores are often hard and difficult to digest which prevents invertebrate feeding prior to decay. Fruit bodies of agarics are generally short lived and small, which might ensure escape from fungivores in time and

space (Hanski 1989). Finally, compensatory growth by yeasts may be an adaptation to lower damage due to insect grazing (Vega and Dowd 2005).

Fungi produce an almost endless diversity of organic compounds not required for growth or metabolism, and many of these are known to be highly toxic to animals (Rohlfs 2015). Some of these secondary metabolites are likely to function as defense against invertebrates. Rohlfs (2015) critically reviewed the evidence for such a function and concluded that invertebrate grazing (collembola and fruit flies) on *Aspergillus* spp. might indeed increase production of fungal secondary metabolites. The production of these metabolites subsequently decreased grazing. Collembola grazing on *Aspergillus* has also been found to increase production of sexual fruit bodies, which is likely a response to escape grazers by reproduction and ensure fungal fitness as the fruit bodies are strongly chemically protected and thus remain ungrazed. Interestingly, induced chemical defenses by the fungus can be overcome by collembola when feeding in groups (Stötefeld et al. 2012), similar to gregariously feeding insect leaf-herbivores. This might explain why some fungivorous insects feed in groups. The chemistry of the induced metabolites vary, but a recent finding shows that the terpenoid compounds that function as juvenile hormones in insects are synthesized in *Aspergillus* as response to *Drosophila* grazing (Nielsen et al. 2013). Presence of this compound significantly decreased the weight of adult flies. Similar terpenoid compounds are well known from plant defenses (Toong et al. 1988). Finally, some fungi secrete chitinolytic enzymes (Klironomos and Hart 2001), making grazing a dangerous activity for insects.

4.5. Insect specialization on fungi

The specificity of interactions between insects and fungi outside the well-known, highly specific, mutualistic interactions is generally assumed to be low, or at least much lower than in plants and their associated herbivores (Hanski 1989; Hackman and Meinander 1979). Insects living inside polypores may represent a notable exception to this pattern (Paviour-Smith 1960; Orledge and Reynolds 2005; Jonsell and Nordlander 2004). Jonsell and Nordlander (2004) estimated that almost half of the beetles and moths hatching from 10 polypore species they investigated in Scandinavia were monophagous (defined as less than 20% of hatched individuals found outside the main host). A strong tendency for closely related fungi to function as hosts for the same beetles has also been found when analyzing only ciid beetles (Paviour-Smith 1960; Orledge and Reynolds 2005; Thorn et al. 2015). Despite the agreement that fungus gnats are generally highly polyphagous (Hanski 1989), recent studies have shown that phylogenetic relationship of fungi is indeed important to explain host use in these insects

(Poldmaa et al. 2016). In polypores, several species of fungus gnats can be associated with fungal species or genera. (Sevcik 2001; Jakovlev 2011; Sevcik 2003). Studies of fungus gnats associated with ascomycete fruit bodies are still scarce, but preferences also appear to occur in these interactions (Jakovlev 2011). Thus, both beetles and fungus gnats are likely to have co-evolved with and specialized on certain fungi. Defensive compounds produced by the fungi have potentially driven this process.

There are also indications that spore-feeding insects have preferences for certain species or genera of fungi. Hågvar (1999) investigated spore-feeding adult beetles on the two common polypores *Fomitopsis pinicola* and *Fomes fomentarius* in forests in Norway (**Fig. 1**). He found only a slight overlap in beetle species on the two polypores even though they sporulated within the same time period. In total, based on all literature known to us, as many as 134 species of beetles have been found to visit these two polypores, but only 27% (36) have been recorded from both (Kaila et al. 1994; Nikitsky and Schigel 2004; Schigel 2011; Hågvar and Økland 1997; Hågvar 1999; Krasutskii 2007b). Thus, even opportunistic spore-feeders (and their predators) appear to distinguish between the two polypores. Fäldt et al. (1999) found that *F. pinicola* and *F. fomentarius* emit different volatiles, and that the scents are modified during sporulation. Most likely, insects can use these signals to locate their hosts (Fäldt et al. 1999; Jonsell and Nordlander 1995).

Fungi identified by molecular methods from adult saproxylic beetles sampled from dead wood also indicate species-specific interactions and possibly feeding preferences (Jacobsen et al. submitted-a). The degree of specialization between 17 species of saproxylic beetles and 22 wood-decay agaricomycete fungi such as *F. fomitopsis* and *F. fomentarius* was similar to the specialization between seed dispersing animals and plants (Blüthgen et al. 2007). Yamashita et al. (2015) conducted the same analysis of network specialization but included beetles hatching from living and decomposing polypores, which resulted in a higher degree of specialization that was close to that of pollinator networks. These two network studies clearly indicate that spore-feeding beetles and beetles living within fungal fruit bodies exhibit feeding preferences. As expected, the specificity was highest when beetles with larval development within fruit bodies were included.

Preferences for hyphae of different fungi need to be determined by experimental work in the lab. At present hardly any studies have been carried out in the dead wood system. *Xestobium rufovillsum* thrives in wood with eight different species of fungi (Fisher 1941, 1940) which

might indicate polyphagy in this species. However, this effect might also be caused by indirect effects, such as an ability to use wood decomposed by a wide range of fungi (see **section 7**). Fungivorous soil invertebrates feeding on hyphae are regarded as generalist feeders although the mesofauna (mites and collembola) appears more specialized than the macrofauna (earthworms, diplopods, slugs and snails) (Maraun et al. 2003) and species-specific preferences do occur (Newell 1984; Tordoff et al. 2008; Crowther and A'Bear 2012; Jørgensen et al. 2003).

In the obligate mutualisms between insects and fungi, specificity for certain partners is usually high. In the best studied bark and ambrosia beetles every beetle species is associated with one or two fungus species, which serve as their primary food source (Harrington 2005; Beaver 1989; Francke-Grosmann 1967; Mayers et al. 2015). Although host switches do occur over evolutionary time scales, there is co-evolution between the beetles and the fungi (Farrell et al. 2001). It is not known what unique co-adaptations occur in specific partnerships, but it has been shown that switches between highly related *Ambrosiella* fungal mutualists (Ascomycota) between two sister species of *Xylosandrus* ambrosia beetles (Scolytinae) resulted in significant fitness losses compared to the native partnerships (Kaneko and Takagi 1966). Also, *Dendroctonus* bark beetles failed to incorporate non-native strains of their *Entomocorticium* mutualist (Basidiomycota) into their mycetangia. These findings indicate beetle adaption to particular genotypes of mutualistic fungi (Bracewell and Six 2015). Thus, Scolytinae-fungus partnerships are maintained by the selectivity of mycetangia and at least partly also by characteristic fungal volatiles that can be highly attractive to the beetles (Biedermann and Kaltenpoth 2014). The secondary fungal flora that is found in bark and ambrosia beetle nests is highly variable and depends mostly on the tree substrate, other organisms in the vicinity of the nest and environmental conditions (Beaver 1989).

4.6. Insect species richness differ between fungus species

As discussed above, several insects have been found to specialize on specific species of fungi. Are certain species or traits of fungi more frequently preferred by fungivores, thus hosting a higher species richness of associated insects?

In obligate mutualisms of ambrosia and ship-timber beetles every fungus species is associated with a single beetle species (Harrington 2005; Beaver 1989). Some fungi involved in facultative mutualisms of bark beetles can be found associated with different beetles species, but no single fungus dominates in these interactions (Kirisits 2004; Six 2012)(see **section 6.2**).

Fungal fruit bodies are discrete units from which insects can be collected or hatched, and therefore insect communities associated with fungi are best known from these structures. The lack of beetles visiting or feeding on 82 out of 198 investigated polypore species in Finland indicates that some species are inferior as insect hosts (Schigel 2012). Whether this difference relates to toxicity, nutrient content or fungal structural characteristics is unknown. Many of the avoided polypores were common species.

Rather than looking at species, Thorn et al. (2015) focused on polypore traits and insect species richness in southern Germany. They hypothesized that ciid species richness would increase with increasing fruit body size, niche diversity (fungal growth form), durability (annual < perennial), abundance and decreasing phylogenetic isolation of the host fungus. These traits have previously been found to affect species richness in herbivore-plant and parasite-host systems. Their hypotheses were generally confirmed, with the exception that species with annual fruit bodies had higher ciid species richness than the perennial species. *Trametes versicolor* **(Fig. 1.D**) had the overall highest species richness of ciids (16) and was also the most common fungus species in the area.

Despite an obviously skewed sampling effort towards common species, the importance of fruit body abundance for harboring a high diversity of insects has been noted by several authors. Yamashita et al. (2015) hatched 82 beetle species from polypores in tropical Malaysia: 53 (65%) hatched from *Ganoderma* which made up 61% of the total fungal biomass and 19 (23%) from *Phellinus* which made up 17% of the biomass. Many insect species have also been hatched from common polypores such as *Fomes fomentarius* in Norway (36 species) (Thunes et al. 2000), *Fomitopsis pinicola* in Fennoscandia (139 species) (Komonen et al. 2004) and *Polyporus squamosus* in Germany (264 species) (Gilbertson 1984). The most common polypores also harbored the highest number of insects in the Czech and Slovak Republics (Sevcik 2003).

5. Insect-vectored dispersal of non-mutualistic fungi

Saproxylic fungi are a diverse group and their dispersal ecology might be equally diverse, although for many species it is poorly known (Watkinson et al. 2015). In general, saproxylic fungi are assumed to disperse primarily by air-borne spores (Ingold and Hudson 1993; Junninen and Komonen 2011; Norros et al. 2012), although some species can also reach their substrate by hyphal cords in the soil (Boddy et al. 2009; Coates and Rayner 1985). A few species of

fungi are known to be dispersed by bark beetles, ambrosia beetles, ship-timber beetles or wood wasps (Batra 1963; Harrington 2005), in mutualisms further discussed in **section 6.3**. More generally, the role of invertebrates in fungal dispersal might be underestimated, as has been suggested several times (Talbot 1952; Harrington 2005; Malloch and Blackwell 1992; Norros 2013; Watkinson et al. 2015).

In order to disperse fungi to dead wood, an insect would first have to get in contact with propagules of a saproxylic fungus and then transport the propagules internally or externally in a manner that leaves an adequate percentage viable. These propagules should be disposed at a suitable substrate and in a suitable microclimate for the fungus, which is likely when insect and fungus habitat preferences match. Many insects may fulfill some or all of these criteria, thus functioning as dispersal vectors with different effectiveness.

5.1. Insect-vectored dispersal of polypores

The ideal insect vector for saproxylic fungi would be a species that visits sporulating fruit bodies and subsequently seeks out dead wood of a type suitable to the fungus. Several saproxylic insects seem to be attracted to fungal odors (Jonsell and Nordlander 1995; Johansson et al. 2006). Fäldt et al. (1999) showed that odor emission from fruit bodies increases during sporulation, which they suggested could be an adaptation to attract insect spore vectors. Sporulating fruit bodies do attract a large number of insect visitors, most of which are saproxylic (Hågvar 1999; Schigel 2011; Nikitsky and Schigel 2004; Krasutskii 2007a; Krasutskii 2007b; Krasutskii 2010; Park et al. 2014; Yamashita et al. 2015; Krasutskii 2006). Interestingly, many of these insect species develop in dead wood, not in fruit bodies. For instance, the nitidulid beetles *Glischrochilus quadripunctatus* and *G. hortensis* both visit sporulating polypores (Hågvar 1999; Krasutskii 2007a; Nikitsky and Schigel 2004; Schigel 2011; Krasutskii 2007b), but their main habitat seems to be weakened or recently dead trees (Dahlberg and Stokland 2004). Nitidulid beetles have been shown to carry fungal plant pathogens to wounds on living trees (Hayslett et al. 2008; Cease and Juzwik 2001). When sampled from fresh dead wood, *G. quadripunctatus* and *G. hortensis* were found to frequently carry DNA from several different fungi, including DNA from polypores such as *Trametes versicolor* and *Fomes fomentarius* (Jacobsen et al. 2017). Jonsell and Nordlander (1995) showed that *G. hortensis* is attracted by the odor of *F. fomentarius,* explaining its frequent presence on sporulating fruit bodies of that polypore (Schigel 2011; Kaila et al. 1994; Hågvar 1999; Nikitsky and Schigel 2004). Fruit bodies of *F. fomentarius* often accumulate thick layers of spores on their upper side, where many insect visitors can be found, especially during the

night (Hågvar 1999). This accumulation of spores might increase the odor and attractiveness of the fruit bodies to insect visitors, and as such it might be an adaptation to insect-vectored dispersal.

Several other saproxylic beetles sampled from fresh dead wood, such as *Endomychus coccineus* (Endomychidae) (**Fig. 4A**), *Xylita laevigata* (Melandryidae) and *Rhizophagus spp.* (Monotomidae), have also been found to frequently carry fungal DNA from a diversity of fungal taxa, including several polypores and other wood-inhabiting species (Jacobsen et al. 2017). There are a few polypore taxa involved in dispersal mutualisms with wood wasps and ambrosia beetles (see **section 6**), but bark and ambrosia beetles have also been found to carry propagules of fungi they are generally not thought to depend on such as *Fomitopsis pinicola, Trichaptum abietinum, Heterobasidion annosum, Stereum sanguinolentum* and *Cryptoporus volvatus* (Castello et al. 1976; Harrington et al. 1981; Pettey and Shaw 1986; Strid et al. 2014; Six 2012). Several of these species have also been isolated from beetle galleries without being present in the surrounding wood, suggesting that the bark beetle galleries provided their point of entry into the wood (Persson et al. 2011).

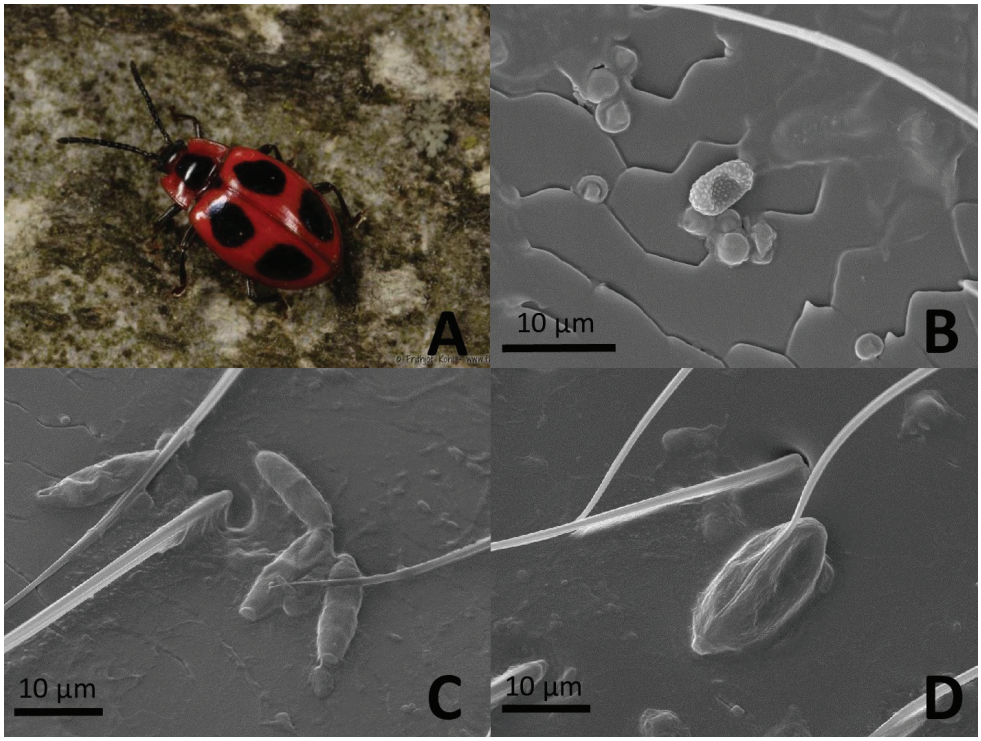

Fig. 4. Scanning electron microscope pictures (B, C, D) of what is most likely fungal spores on the exoskeleton of a fungivorous beetle, *Endomychus coccineus* (A). Photo by (c) Frithjof Kohl, reprinted with permission. The beetles were sampled from freshly dead aspen wood and stored at -80ºC prior to scanning (Jacobsen et al. 2017). (B) A few of the larger spores with coarse outer structure were found on the beetles, while the smaller spores were found in large numbers and might be yeast cells covered by biofilm. (C) The fusiform shape and the horizontal cross-walls of these spores is typical of mold fungi in the genera *Cladosporium* and *Cladophialophora* (Marie Davey, pers. com.). (D) Several of these large spores were found on one beetle.

5.2. Insect-vectored dispersal of wood-inhabiting microfungi

Although basidiomycete polypores may be the most important fungal taxa when it comes to mass loss during wood decay (Boddy 2001; Kubartová et al. 2015), molecular analyses have shown that there is a diversity of yeasts and ascomycete taxa present in dead wood (Ottosson et al. 2015; van der Wal et al. 2015; Strid et al. 2014). The role of these taxa in the dead wood is poorly known (Ottosson et al. 2015; van der Wal et al. 2015). They might contribute to wood decay directly or depend on prior decomposition by cellulolytic or lignolytic fungi (Rayner and Boddy 1988; Ottosson et al. 2015; Rajala et al. 2011), and have even been suggested to produce a synergistic effect on wood decay together with basidiomycetes (Blanchette and Shaw 1978). Several of the yeasts and filamentous ascomycete taxa isolated from wood have also been isolated from saproxylic insects (Greif and Currah 2007; Strid et al. 2014; Six 2003; Jacobsen et al. 2017), indicating that these fungi might be dispersed by insects. It has also been shown that phoretic mites on bark beetles function as vectors for certain microfungi (Blackwell et al. 1986; Hofstetter and Moser 2014). Dispersal by insects has been suggested previously for species like *Oidiodendron spp.* and *Myxotrichum spp.* that produce a peculiar spore-containing structure called a reticuloperidium (**Fig. 5**) (Greif and Currah 2003). Spores in a reticuloperidium are contained within a network of rigid and thick-walled hyphae, often with hooked or barbed appendages. Greif and Currah (2003) showed that (i) these reticuloperidia easily attach to hairs on the exoskeleton of insects and (ii) when the insects groom themselves, the reticuloperidia are torn apart and the spores are released. While the significance of this has not been tested in the field, these are intriguing observations.

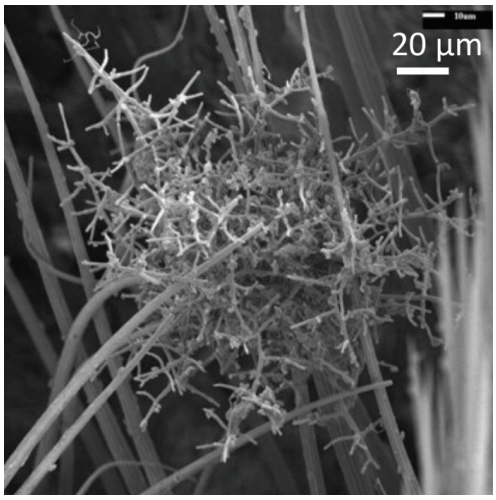

Fig. 5. Scanning electron microscope picture of the reticuloperidium of *Myxotrichum deflexum* attached to hairs of the fly *Neobellieria bullata*. Reprinted with permission from Greif and Currah (2003).

5.3. Viability of spores after insect-vectored dispersal

Several studies show that fungal spores can survive transport both on insect exoskeletons and within insect guts (Lilleskov and Bruns 2005; Tuno 1999; Lim 1977; Drenkhan et al. 2016). For instance, *Mycodrosophila* flies that visited sporulating fruit bodies of *Ganoderma applanatum* excreted and dropped up to several hundred thousand viable spores (Tuno 1999). Basidiospores of *Ganoderma* species have double spore walls which might be an adaptation to dispersal by insect vectors, whereas their small proteospores are probably better suited for wind dispersal (Nuss 1982). Lim (1977) found that the basidiospores of *Ganoderma philippii* would only germinate after passage through the gut of tipulid fly larvae. Digestion by the fly larvae seemed to reduce the spore wall thickness without damaging the spore content, which appeared to benefit germination. The emerging adult flies subsequently came into contact with the spores previously excreted by the larvae and thousands of spores attached to their exoskeletons. Thus, *G. philippii* seems to be adapted to dispersal by tipulid flies, and the flies might benefit from dispersing the fungus that their larvae feed on. However, studies of another *Ganoderma* species found that passage through the gut of a specialist spore-feeding beetle reduced germination rate (Kadowaki et al. 2011a). Digestion by this beetle species apparently reduces the originally thick spore walls to the extent that the spores burst open. Thus, whether passage through insect

guts is beneficial or detrimental to spore germination seems to depend on traits of both the fungus and the insect.

5.4. Effects of potential insect vectors on the fungal community in dead wood

Although several studies show that insect-vectored dispersal of saproxylic fungi is a distinct possibility, it is difficult to estimate the significance of this dispersal mode for the fungal community. However, there are a few published field studies that provide indications of the importance of insect-vectored dispersal for wood-inhabiting fungi, including both long-term observational studies (Weslien et al. 2011; Jacobsen et al. 2015) and short-term experimental studies (Jacobsen et al. Submitted-b; Müller et al. 2002; Strid et al. 2014).

In a field study by Weslien et al. (2011), fruit bodies of the polypore *F. pinicola* were found to occur more often on dead wood that had previously been colonized by the bark beetle *Hylurgops palliatus.* The authors suggested that spore dispersal by *H. palliatus* might have caused this correlation, as its flight period coincided with the spring sporulation of *F. pinicola*. Jacobsen et al. (2015) showed that an abundance of the nitidulid beetle *G. quadripunctatus* or the leiodid beetle *Agathidium nigripenne* during the first years after tree death increased the probability that fruit bodies of the polypore *Ganoderma applanatum* were found on the dead trees several years later. They suggested spore dispersal as the most likely mechanism to cause these patterns, especially in light of previous knowledge of insect-vectored spore dispersal for *Ganoderma* species (Tuno 1999; Lim 1977).

To experimentally assess the effect of insects on community assembly of wood-inhabiting fungi, Müller et al. (2002), Strid et al. (2014) and Jacobsen et al. (Submitted-b) used net cages with mesh sizes of 1 mm or less to exclude invertebrates from recently felled logs. Müller et al. (2002) put spruce logs in cages and opened half of the cages for three weeks in May during the flight periods of their study species, resulting in colonization of these logs mainly by the bark beetles *H. palliatus* and *Trypodendron lineatum*. Logs colonized by *H. palliatus* tended to have a higher diversity of fungi, while logs colonized by *T. lineatum* had a higher frequency of the fungi *Trichoderma sp., Antrodia serialis* and *Phlebia gigantea*. In theory, the ambrosia beetle *T. lineatum* would not be expected to vector wood-decaying polypores that might be detrimental for its nutritional fungal mutualists. However, it was not clear whether the effect of the beetles was due to dispersal of propagules or some other interaction. For instance, beetle entry holes and tunnels can increase access to the wood for the fungi even if the beetles do not bring fungal propagules to the wood (Leach et al. 1937).

To separate the effect of bark beetle tunneling and the effect of potential propagule dispersal, Strid et al. (2014) combined the exclusion experiment with drilled holes in spruce logs to mimic bark beetle tunnels. These artificial holes had a much weaker effect on the fungal community than exclusion of invertebrates. The exclusion treatment contrasted caged logs with uncaged logs, and there was also a positive control consisting of logs baited with bark beetle pheromones. Molecular analyses were used to analyze the fungal community in wood samples from the different treatments, and in samples of bark beetles. The fungal community of the bark beetle samples was most similar to that of the pheromone-baited logs, whose fungal community in turn was more similar to that of uncaged logs than caged logs. Furthermore, several fungal taxa that were isolated from the bark beetles were significantly more frequent in uncaged logs, e.g. *Stereum sanguinolentum, Bjerkandera adusta* and *Sistotrema brinkmannii* (Strid et al. 2014). These results show that bark beetles have a significant effect on the fungal community in dead wood, and that part of this effect probably stems from propagule dispersal of non-mutualistic fungi.

Jacobsen et al. (Submitted-b) excluded invertebrates from aspen logs, thereby studying the influence of saproxylic insects in a community where bark beetles were not numerically dominant. Jacobsen et al. (Submitted-b) included ethanol-baited logs as positive control and a control for microclimatic effects of the cage, which was lacking in the previous exclusion experiments. The experimental treatments were postulated to form a gradient from low invertebrate colonization in caged logs, intermediate/normal in cage control and control logs, to increased colonization of ethanol-baited logs. This gradient was reflected in the fungal community composition, especially in abundance of certain fungal taxa in the logs, such as the polypores *Trametes versicolor* and *T. ochracea* that had low abundance in caged logs and high abundance in ethanol-baited logs. As *T. versicolor* had been isolated from saproxylic insects such as *G. quadripunctatus*, sampled in the same time and place as the exclusion study (Jacobsen et al. 2017), it is likely that insect-vectored propagule dispersal contributed to the effect of invertebrate exclusion on the fungal community (Jacobsen et al. Submitted-b).

5.5. Adaptations to insect-vectored dispersal

If insect-vectored dispersal can increase the fitness of wood-inhabiting fungi, this might have resulted in adaptations to this dispersal mode. The spore-containing reticuloperidium of certain ascomycete taxa mentioned previously might be such an adaptation (**section 5.2**). Spores with appendages like spikes or hooks have been found to attach easily to invertebrate exoskeletons (Lilleskov and Bruns 2005) and might be adaptive for external dispersal by invertebrate vectors

(Halbwachs and Bâssler 2015). Likewise, the sticky spores produced by blue-stain fungi (i.e. the ascomycete genera *Ophiostoma, Leptographium, Ceratocystis, Grosmannia, Ceratocystiopsis*) easily adhere to their bark beetle vectors and facilitate external dispersal (Harrington 2005). The thick spore walls of *Ganoderma* species seem to be adaptive for internal dispersal by insect vectors (Lim 1977; Nuss 1982). Finally, fungi in mutualistic associations with insects (**section 6**) typically grow in a yeast-like form (= 'ambrosial growth') to get picked up in the spore carrying organs (mycangia) of the adult wood wasps, bark, ambrosia or ship-timber beetles (Francke-Grosmann 1967; Six 2003). There might also be less obvious adaptations in the chemical composition of spores, such as lack of defensive compounds, but the chemical defense of most fungi is poorly known (see **section 4.4**). Fruit body morphology might also facilitate spore dispersal, for instance by resulting in the accumulation of thick spore layers on top of the fruit body that attract insect visitors (Hågvar 1999). This is especially characteristic for sporulating fruit bodies of *F. fomentarius* and *G. applanatum*.

The insects on their side might not have adaptations specifically for dispersal of fungi if their interaction stems from opportunistic spore-feeding. While spore feeders might benefit from increasing the prevalence of fruit bodies in their habitat, such an indirect selection pressure might not result in adaptation. However, if the larval development benefits from presence of fungi dispersed by the adult insects, the selection pressure will be stronger. This is the basis of the evolution of obligate insect-fungus mutualisms, which has led to the development of the selective fungus-bearing pockets in insect exoskeletons called mycangia in several insect groups (see **section 6**). Mycangial structures have been found in a range of insects not known to engage in mutualisms with fungi, although their function as organs for dispersal of fungi is often inferred and not demonstrated (Grebennikov and Leschen 2010). Females of several species of saproxylic stag beetle (Lucanidae) have mycangia that they use to vertically transmit *Scheffersomyces* yeast species (Ascomycota) to their offspring during oviposition (Tanahashi et al. 2010). These yeasts are also transferred to the wood, but their main function is probably xylose fermentation in the guts of developing larvae, and they therefore seem to be primarily endosymbionts (Tanahashi and Hawes 2016). Unfortunately, most of the presumably non-mutualistic insect species with mycangium-like structures remain understudied.

5.6. Implications of insect-vectored dispersal

To summarize, several studies support the hypothesis that insects can be important dispersal agents also for non-mutualistic saproxylic fungi, but the effect of insect-vectored dispersal is

difficult to quantify. Animal-mediated seed dispersal, which has many similarities with insect-vectored spore dispersal, can be assessed with a framework that shows how different aspects of the animal vector contributes to seed dispersal effectiveness (SDE) (Schupp et al. 2010) In **Fig. 6**, we propose a similar framework for studies of spore dispersal effectiveness (SpDE), which might help structure and focus future research efforts.

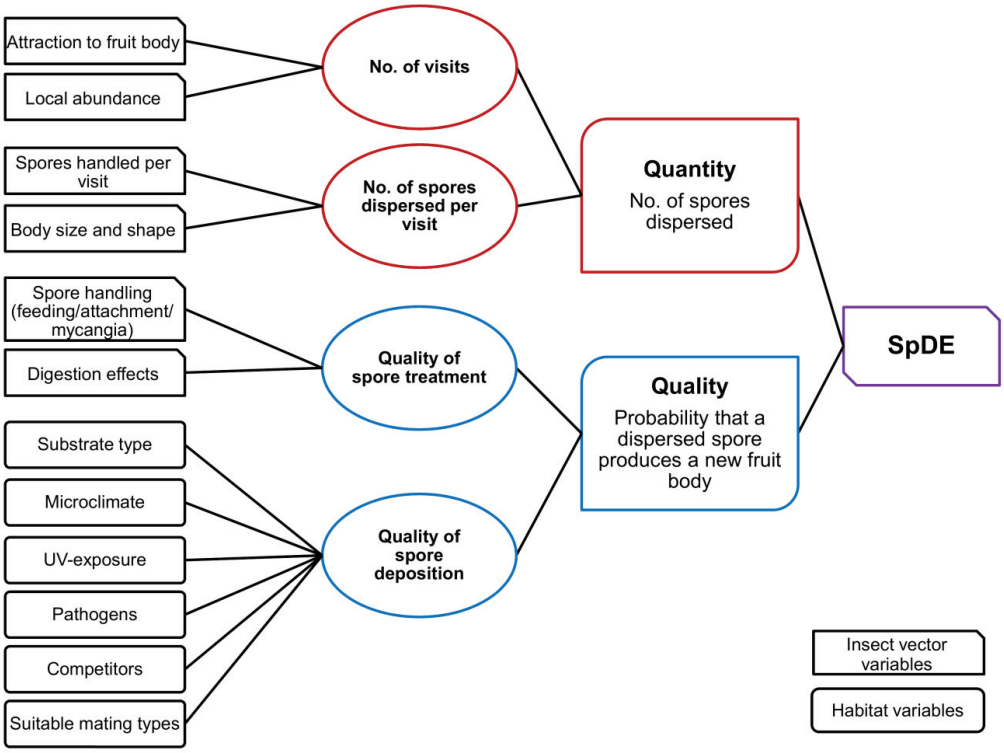

Fig. 6. A theoretical framework for studies of spore dispersal effectiveness (SpDE) of insect or invertebrate vectors of fungal spores (or other propagules). The list of variables is not exhaustive. The figure is adapted from the seed dispersal effectiveness framework described in Schupp et al. (2010).

In any case, insect-vectored dispersal does not have to replace wind dispersal to be of importance to saproxylic fungi. Insect-vectored dispersal could be a complementary form of dispersal that is especially important under certain circumstances. The most obvious difference from wind dispersal is that insect-vectored dispersal has the potential to be targeted towards the preferred substrate of the fungi, while wind dispersal is completely random. Studies comparing wind-dispersed and animal-dispersed plants have shown that animal-mediated seed

dispersal can increase tolerance to habitat fragmentation (Montoya et al. 2008; Marini et al. 2012), as long as the animal vector is present in the fragments (Cramer et al. 2007). Similarly, targeted dispersal by insects might help certain fungi persist in fragmented forests with low volumes of dead wood.

6. Symbioses between insects and fungi in dead wood

All animals live in symbiotic associations – from antagonism to mutualism – with microorganism that play an important role for pathogenicity and host nutrition (Whitman et al. 1998; Zilber-Rosenberg and Rosenberg 2008). Insects living in wood are no exception – mutualistic bacteria and fungi are of essential importance for many saproxylic insects as they help degrade plant defensive compounds, digest recalcitrant plant polymers or synthesise and assimilate essential nutrients (Dowd 1992; Klepzig et al. 2009; Riley et al. 2016). These microorganisms are usually carried within the intestinal tracts of the insects ('gut symbionts'), but there are also a few cases where symbionts are cultivated externally (henceforth termed 'ectosymbionts'). Many wood-feeders in the beetle families Cerambycidae, Passalidae, Scarabaeidae, Tenebrionidae, Lucanidae and Elateridae seem to rely on gut symbionts, in particular yeasts, but the exact functions of these gut symbionts remain understudied and are reviewed elsewhere (Davis 2014; Vega and Dowd 2005; Tanahashi and Hawes 2016). Here we focus on the insects that engage in facultative or obligate ectosymbioses with fungi, including all taxa that grow yeast-like or filamentous fungi in their tunnels within wood, i.e. the bark and ambrosia beetles in the Curculionidae, the ship-timber beetles (Lymexilidae) and the hymenopteran wood wasps (Siricidae) (Six 2013; Six 2012; Thompson et al. 2014) (**Fig. 7**). Currently, there is a lot of progress made on understanding the ecology and evolution of these ectosymbioses, which we aim to summarize in the following sections.

6.1. Characteristics of wood favoring insect-fungus mutualisms

Mutualisms between species require environmental stability and often evolve between animals and microbes in poor/restricting habitats due to benefits of division of labour (Boucher et al. 1982; Bourke 2011). Both characteristics are fulfilled in living and dead wood for insects and fungi: First, insects and fungi occur in close vicinity within wood, often for several insect/fungus generations due to the relative stability of wood as a habitat. Second, both insects and fungi have many complementary characteristics and can therefore benefit each other in various ways (Six 2012; Beaver 1989; Mueller et al. 2005; Vega and Blacwell 2005). The

primary benefit insects can provide to fungi is probably the targeted dispersal (relative to wind dispersal) of spores into new or inaccessible habitats such as the cambium or xylem of trees (see **section 5**). Insects with advanced fungiculture that actively farm their fungal crops also provide nutrients and protection to the fungus (**Table 1**). The insects, on the other hand, may profit directly by feeding on the fungus (acquiring mostly amino acids and sterols), or indirectly through fungal detoxification of defensive plant compounds (phenolics, terpenoids) and degradation of plant polymers (lignin, cellulose, pectin) (see also **section 3**). Insects may also make use of fungal volatiles to attract conspecifics or mating partners, or they can use antibiotics produced by fungi to protect themselves against pathogens or fungal competitors (**Table 1**).

6.2. Facultative mutualisms between insects and ectosymbiotic fungi

Even if some of insects profit from a wood diet supplemented with fungi (see **section 3 & 4.1**) and the fungi benefit from dispersal (see **section 5**), selection might not be strong enough to develop co-adaptations for protecting and facilitating a mutualistic partnership (Martin 1992). Partnering with another species involves costs of dependency. Therefore, the partnership can only be stable if fitness interests of partners are aligned and the association is protected against 'cheaters' in either species that do not reciprocate benefits provided by the partner (Bourke 2011; Boucher et al. 1982; Doebeli and Knowlton 1998; Bronstein 2015). Maintaining close contact and reciprocation between species is often difficult in ephemeral habitats like dead wood, where species-specific interactions can easily be broken up by invasion of non-mutualistic fungi (conspecific 'cheaters' or heterospecifics) (Biedermann and Rohlfs 2017). This is probably the reason why obligate insect-fungus mutualisms (see below) have only evolved in wood-boring insects that colonize living or recently dead wood. This habitat is free of other interfering fungi and so a partnership can be established reliably throughout the development period of the insects and be maintained over generations by vertical transmission of fungal spores between the insects.

6.3. Evolutionary origin of the obligate mutualisms between insects and ectosymbiotic fungi

Three saproxylic insect groups have evolved obligate farming mutualisms with fungi: Some bark and ambrosia beetles (Curculionidae; at least eleven independent origins, ~3500 species), ship-timber beetles (Lymexylidae; one independent origin, ~50 species) and wood wasps (Xiphydriidae, Anaxyelidae, Siricidae; one independent origin, ~270 species) (**Table 1, Fig. 7**). These mutualisms evolved between 17 and 110 million years ago during periods of global

warming (Jordal and Cognato 2012; Farrell et al. 2001) and have resulted in adaptive radiations, especially in many lineages of the scolytine ambrosia beetles and in particular in the tropics (see **Fig. 8**) (Jordal et al. 2001; Hulcr and Stelinski 2017). A fourth group of so called moisture ants (Formicinae; one to two independent origins) uses fungi to build their 'carton nests' in decayed wood (Schlick-Steiner et al. 2008).

The insect taxa involved in obligate farming mutualisms with fungi are usually among the first colonizers of freshly dead wood, which contains only a few microorganisms (e.g. plant endosymbionts) and high concentrations of plant-defensive terpenoids and phenolics (Six 2012; Beaver 1989; Krokene 2015). These insects bore tunnel systems within the xylem and inoculate the tunnel walls with vertically transmitted fungi that the female parents carry in mycangia (Francke-Grosmann 1967). Mycangia thereby secure transmission of the species-specific fungus from the natal nest to the new nest/gallery. While adult bark and ambrosia beetle tunnel in the wood themselves, inoculate the fungus and lay eggs there, the other two taxa only deposit eggs coated with fungal spores on (ship-timber beetles) or under (wood wasps) the bark and their larvae tunnel and inoculate the fungus themselves. The fungi fulfil various functions for the different insect taxa (**Table 1**). For bark beetles and ambrosia beetles they help in overcoming tree-defences and are of nutritional importance (Six 2012). Nutrition is probably also the major role of fungi for the understudied ship-timber beetles (Francke-Grosmann 1967). In both beetle groups this function is reflected by the mutualistic fungi forming so called 'ambrosial growth,' which is nutrient rich asexual fruiting structures (= thickened 'conidia' or yeast-like growth) that are usually only formed in the presence of the beetles and can form thick layers on the walls of tunnels (**Fig.7C**) (Neger 1909; Francke-Grosmann 1967). These fungi evolved from phytopathogenous ophiostomatoid (Ascomycota) fungi (Harrington 2005). The symbiotic fungi of wood wasps, on the other hand, are originally basidiomycete wood-degraders that do not form thick ambrosial layers on tunnels and whose hyphae are apparently quite nutrient poor (Thompson et al. 2013). Instead they serve as an 'external rumen' for the insects by excreting enzymes into the wood that digest lignocellulosic compounds, which are then ingested by the growing larvae (Thompson et al. 2014; Kukor and Martin 1983).

Two theories have been proposed for the evolutionary transitions from a purely plant-based diet to obligate fungus mutualisms (Mueller et al. 2005). (1) In the 'transmission first' model, a fungus makes use of an insect as a vector and then begins to supply extra nutrients (sterols, amino acids) to increase insect reproduction, which directly benefits its dispersal. Insects co-

adapt by developing fungus-specific mycangia and specialised farming behaviours until they finally rely on the fungus as a food source. (2) In the 'consumption first' model, an insect supplements its plant diet with fungi and then begins to vector the fungus as it is nutritionally profitable. Later the fungus co-adapts to the insect traits. Both models are equally tenable for all three insect groups. Given that wood-boring insects typically vector a lot of fungal spores (even ones specialized for wind dispersal; e.g. (Seibold et al. submitted; Jacobsen et al. 2017) (see **section 5**), the transmission first model may have occurred in the oldest associations between Platypodinae – *Raffaelea* fungi and Scolytoplatypodini – *Ambrosiella* fungi, for example. As these fungal lineages evolved to be nutritionally ideal for the beetles, they could have been acquired by the Xyleborini several million years later via the consumption first model (**Table 1**).

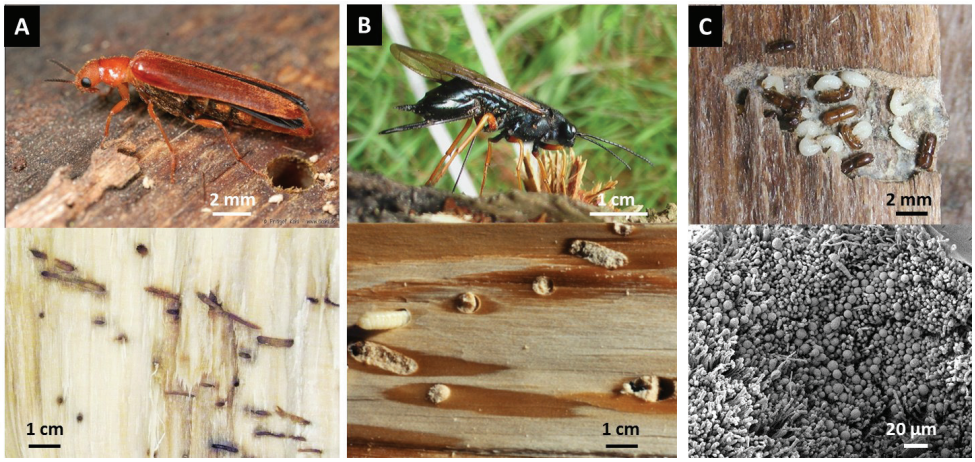

Fig. 7. Overview of the three known insect groups that engage in obligate ectosymbiotic mutualisms with fungi within wood. **A. Ship-timber beetles (Lymexilidae).** An adult female of the Palearctic *Hylocoetes dermestoides* after emergence from its tunnel (© Frithjof Kohl) and tunnels of larvae in fir wood (*Abies alba)* below (© P. Biedermann). **B. Wood wasps (Siricidae).** Adult female of the Holarctic sawfly *Sirex noctilio* on pine (*Pinus radiate)* bark (© Michaellbbecker, CC BY-SA 3.0) and a larva and tunnels below (©Vicky Klasmer, CC BY-NC 3.0 US) **C. Ambrosia beetles (Scolytinae).** Brood chamber with multiple larvae and adult females of the globally distributed facultatively eusocial fruit-tree pinhole borer *Xyleborinus saxesenii* in beech (*Fagus sylvatica)* (© P. Biedermann) and SEM picture of nutritional 'ambrosial growth' of *Raffaelea sulphurea*, the ascomycete fungus mutualist of this

species (SEM with 200× magnification made by the Elektronenmikroskopisches Zentrum Jena; © P. Biedermann).

6.4. Evolutionary consequences of obligate mutualism for insects and fungi

Mutualism with fungi allowed the insects to flourish in freshly dead wood – a nitrogen poor, carbon rich and highly toxic environment that is unsuitable for most organisms. The insects and fungi exchange transport and nutrition, so the most striking adaptations to this mutualism are the spore-carrying mycangia of the insects and the highly nutritional ambrosial growth or increased enzyme production in the fungi. Both traits probably evolved relatively rapidly, which is indicated by their repeated independent origins in various fungal and insect lineages (Six 2012; Hulcr and Stelinski 2017). In the same genera of ambrosia beetles, for example, mycangia can differ between sexes or occur in different body regions, which indicates rapid and independent origins and probably also losses due to parasitism by other fungi (Farrell et al. 2001). Ambrosial growth also evolved several times in unrelated lineages of fungi (Harrington 2005; Hulcr and Stelinski 2017).

Another consequence of the mutualism with fungi was the selection for social farming behaviours in some bark beetles and probably all ambrosia beetles (Kirkendall et al. 2015). Subsociality (i.e. both parents stay within the nest and care for their brood throughout their life, but there are no workers or caste system) is an ancestral trait for both Scolytinae and Platypodinae, so it had been present already before the origins of the mutualism with fungi (Jordal et al. 2011). Interestingly, during the evolution of the mutualism, many brood-tending behaviours apparently got modified to fungus-tending behaviours (Biedermann and Taborsky 2011). The resulting prolonged maintenance of fungus gardens due to parental care allowed multiple generations to develop within one nest (Kirkendall et al. 2015; Biedermann 2012). This was followed by evolution of division of labour between adults and their offspring in fungus-farming tasks like weeding, tending and fertilizing (= 'advanced fungiculture', **Table 1**), which apparently increased fungal yields further. This positive feedback between mutualism and social evolution (Biedermann and Rohlfs 2017) finally led to the emergence of eusociality (i.e. a society with a queen and workers in a caste system) in at least one ambrosia beetle and intermediate social structures like communal breeding and facultative eusociality in many others (Biedermann and Taborsky 2011). The eusocial ambrosia beetle colonizes living trees without killing them, which indicates that social evolution in these beetle-fungus mutualisms can only progress towards eusociality in very stable habitats (Kirkendall et al. 2015). Nevertheless, social behaviour is not a requirement for obligate mutualisms to evolve. Larvae

of ship-timber beetles and wood wasps live solitarily within their tunnels and lack active care of their fungal cultures (= 'primitive fungiculture'; **Table 1**).

The consequences of the mutualism for the fungal partners is the least studied part of the relationship. Two changes are apparent: (i) Most fungal associates became asexual during the evolution of the mutualism with the insects. Sexuality is probably selected against by the insect because it may increase conflict within fungal cultures and break up advantageous co-adaptations between insects and fungi (Mueller et al. 2005). (ii) There has been a strong selection pressure on the fungi for being or providing 'good food' for the insects, probably reflected by increased content of nitrogen, vitamins, amino acids, sterols and lipids, or by increased production of wood-degrading enzymes. Studies comparing nutrient content or enzyme production of mutualistic fungi with other wood-inhabiting fungi are lacking, but investigations of the *Termitomyces* fungi that are mutualists of certain termites have shown that they are the most palatable and nutrient rich fungi currently known (Mueller et al. 2005).

Table 1 Overview of major obligate ectosymbioses between insects and fungi within dead wood. Not displayed are five additional scolytine ambrosia beetle lineages that are hardly studied (Premnobiini, Hyorrhynchini, Bothrosternini, Scolytodes, Camptocerus; see Kirkendall et al. (2015).

	Bark beetles (Scolytinae: Curculionidae)		**Ambrosia beetles** (Platypodinae and Scolytinae: Curculionidae)				**Ship-timber beetles**	**Wood wasps**	**Moisture ants**
Insect hosts	Ipini (e.g. *Ips*)	Tomicini (e.g. *Dendroctonus*, *Tomicus*)	Platypodinae (e.g. *Platypus, Austro-platypus, Crossotarsus*)	Scolytoplatypodini (e.g. *Scolyto-platypus*)	Xyloterini (e.g. *Xyloterinus, Trypo-dendron*)	Xyleborini (e.g. *Xylosandrus, Xyleborus, Anisandrus, Xyleborinus, Ambrosio-domus*)	Lymexylidae (e.g. *Atractocerus, Elateroides*)	Xiphydriidae, Anaxyelidae, Siricidae	Formicinae (*Dendro-lasius, Chthono-lasius*)
Primary mutualistic fungi									
Ascomycete taxa	*Ophiostoma, Grosmannia, Ceratocystio-psis, Ceratocystis*, *Endoconidio-phora*	*Ophiostoma, Grosmannia, Ceratocystio-psis, Ceratocystis, Ogataea*	*Raffaelea*	*Ambrosiella, Phialo-phoropsis*	*Phialo-phoropsis*	*Ambrosiella, Raffaelea*, *Fusarium*	*Alloascoidea, Ascoidea*	*Daldinia*, *Entonaema*	Undescribed Ascomycetes
Basidio-mycete taxa	-	*Entomocorti-cium, Phlebiopsis*	-	-	-	*Flavodon*	-	*Cerrena, Stereum*, *Amylo-stereum*	
Age of symbiosis (Mya)[1]	?	?	90-110	28-46	32-51	17-25	?	?	25-44
Mode of feeding[2]									
Adults	Pm	Pm	M?	M?	M?	M	No food	No food	Omnivorous
Larvae	Pm	Pm	Xm	Xm	Xm	M, Xm[3]	Xm	Xm	Omnivorous

	Bark beetles (Scolytinae: Curculionidae)		**Ambrosia beetles** (Platypodinae and Scolytinae: Curculionidae)				**Ship-timber beetles**	**Wood wasps**	**Moisture ants**
Insect hosts	Ipini (e.g. *Ips*)	Tomicini (e.g. *Dendroctonus*, *Tomicus*)	Platypodinae (e.g. *Platypus, Austroplatypus, Crossotarsus*)	Scolytoplatypodini (e.g. *Scolyto-platypus*)	Xyloterini (e.g. *Xyloterinus, Trypodendron*)	Xyleborini (e.g. *Xylosandrus, Xyleborus, Anisandrus, Xyleborinus, Ambrosiodomus*)	Lymexylidae (e.g. *Atractocerus, Elateroides*)	Xiphydriidae, Anaxyelidae, Siricidae	Formicinae (*Dendrolasius, Chthonolasius*)
Type of fungiculture									
Mode of nesting	Phloem tunnels	Phloem tunnels	Xylem tunnels	Xylem tunnels	Xylem tunnels	Xylem (phloem) tunnels and chambers	Xylem (phloem) tunnels	Xylem tunnels	Carton nests
Mode of fungi-culture[4]	Primitive	Primitive (Advanced)[5]	Advanced	Advanced	Advanced	Advanced	Primitive	Primitive	Primitive
Nutritional role of fungal mutualists[6]									
Nutrition (amino acids, sterols)	?	+	++	++	++	++	++	+	-
Fungus acquired enzymes[7]	?	?	?	?	?	+[3]	?	++	-
Detoxification of phenolics and terpenoids[6]									
Detoxifi-cation of tree defenses[8]	++	++	?	?	?	?	?	+	-
Protective role of fungal mutualists[6]									
Protection against other fungi[9]	?	?	?	?	?	+	?	?	?
Reinforce-ment of nest structure	-	-	-	-	-	-	-	-	++

[1] Age of origin of fungiculture (Jordal and Cognato 2012; Jordal et al. 2011).

[2] Distinctions originating from the scolytine beetle literature [e.g. 20]: M (mycetophagy = eating fungal mycelium, fruit bodies or specific fungal structures), Pm (phloeomycethophagy = eating phloem and fungal biomass), Xm (xylomycetophagy = eating xylem and fungal biomass).

[3] Only in larvae of the genus *Xyleborinus* and probably *Xylosandrus* and *Ambrosiodomus* (De Fine Licht and Biedermann 2012; Biedermann 2012; Kasson et al. 2016).

[4] Primitive fungiculture is defined by only dispersal and seeding of fungi; advanced fungiculture additionally involves the active care of fungal crops (cf. Mueller et al. (2005)).

[5] Possibly advanced fungiculture in some *Dendroctonus* spp.

[6] '++' - very important role, '+' - role present, '?' - not investigated

[7] Digestion of recalcitrant plant polymers by fungus acquired enzymes that are active in the insect gut or fecal exudates (Kukor and Martin 1983; Martin 1979, 1992; De Fine Licht and Biedermann 2012; Thompson et al. 2014).

[8] Terpenoids, phenolics (Dowd 1992; Krokene 2015).

[9] Protection against antagonistic fungal competitors or pathogens, either by association with competitive fungal mutualists (e.g. *Flavodon ambrosius* with *Ambrosiodomus* spp. (Kasson et al. 2016)) or production of antibiotics by fungal mutualists (Florez et al. 2015; Nakashima et al. 1972).

7. Indirect interactions

Insect-fungus interactions can take many different forms, spanning from tight-knit mutualistic relationships, via specialized or opportunistic direct interactions, to a range of indirect interactions including modification of a common habitat. Indirect interactions go both ways: Fungi can improve habitat conditions for saproxylic insects in general by killing trees, by softening the wood and making it more accessible, or by emitting volatiles that insects use as semiochemicals. Likewise, insects can change the physico-chemical properties of dead wood by their comminution and tunneling, or through nitrogen enrichment e.g. by N_2 fixing gut symbionts (Ulyshen 2015), thus improving the conditions for exploitation by fungi.

Several studies have documented that fungal community composition and the entire insect community - not only fungivores - often correlate in dead wood. Fungal fruit bodies (e.g. of polypores) are known to shelter a variety of insects and arthropods. Kaila et al. (1994) investigated the beetle community in dead birches (*Betula* sp.) in Finland, and found that distinct beetle assemblages seemed to be associated with different polypore species. Similarly, Abrahamsson et al. (2008) found that the root rot fungi *Heterobasidion* spp. affected the

assemblage of saproxylic beetles in high-stumps of Norway spruce (*Picea abies*), probably by disfavoring certain beetle species. Jonsell et al. (2005) found that occurrence of *Fomitopsis pinicola* or *Trichaptum abietinum* had a stronger influence on species composition of saproxylic beetles in high stumps of spruce (*P. abies*) than important characteristics of dead wood such as diameter, height or bark cover. Several other studies have also found similar correlations between the species composition of fungi and insects in dead wood (Gibb et al. 2006; Johansson et al. 2007; Persiani et al. 2010; Jacobs and Work 2012), without identifying the underlying cause. These correlations could be due to a number of direct and/or indirect effects of both the insects and the fungi.

7.1. Fungi change wood characteristics relevant for the non-fungivorous insect community

Fungi create habitats also for saproxylic insects that are not fungivorous. For instance, many saprotrophic fungi can colonize living trees as parasites and directly cause or at least expedite tree death (Boddy 2001), thus creating habitats for the entire community of saproxylic beetles.

The presence of fungi and the related abundance of fungivores will also affect the abundance of saproxylic predatory insects, which in turn may affect the entire insect community within the dead wood. According to Persiani et al. (2010), the predatory beetles, together with necrotrophic fungal parasites, may control the structure and dynamics of fungal and beetle communities in dead wood through top-down mechanisms. However, the previously mentioned fact that fungi can move nutrients into the wood (section **3**) and thus affect the cohabiting insect communities, might cascade all the way up the food web to the predators in decaying wood.

The combined effect of nutrient enrichment and structural breakdown by fungi can affect the development time of the insect larval stage. For instance, in the case of the death watch beetle *Xestobium rufovillosum*, larval development has been shown to be much faster in wood already decayed by fungi (Fisher 1941, 1940). It is not known whether this is a result of direct or indirect interactions.

Non-fungivorous insects also profit due to the breakdown of the physical cellular structure of wood and the detoxification of phenolics and terpenoids. One example is the creation of cavity-bearing trees (**Fig. 9**). The activity of heart-rot fungi, specialized in decaying the dead heartwood of mature living trees, is the first step in the creation of cavities in living or dead trees. The breakdown of polymers makes the heartwood softer and facilitates further

excavations by insects or woodpeckers (Jusino et al. 2016). The nests of woodpeckers may later be inhabited by other birds or insects, and the frass, feces and dead animals that build up in the cavity is turned into a nutrient-rich wood mold, which is an important habitat for a diverse community of saproxylic insects (Sverdrup-Thygeson et al. 2010; Ranius and Jansson 2000). Cavity-bearing trees often contain a high proportion of endangered saproxylic species, as a large number of insects are specialized to these rare microhabitats (Siitonen and Ranius 2015). In warmer areas, like tropical forest or savanna woodland, termites are the key agents creating cavities in trees (N'Dri et al. 2011; Werner and Prior 2007). Carpenter ants (*Camponotus* sp.) also excavate the interior of wood and wooden structures, and prefer wood softened by moisture and fungal rot (Chen et al. 2002; Hansen and Akre 1985; Birkemoe 2002)

Other indirect effects can include chemical communication and orientation in insects. Ethanol from fermentation of sugar-rich sap can function as an orientational cue for insects during the colonization of recently dead trees (Stokland et al. 2012; Allison et al. 2004). Also, several volatiles of fungal origin have been found to be specific to wood infected by certain fungi. These may act as potential semiochemicals for wood-inhabiting insects and may mediate specific interactions between fungi and insects (Leather et al. 2014).

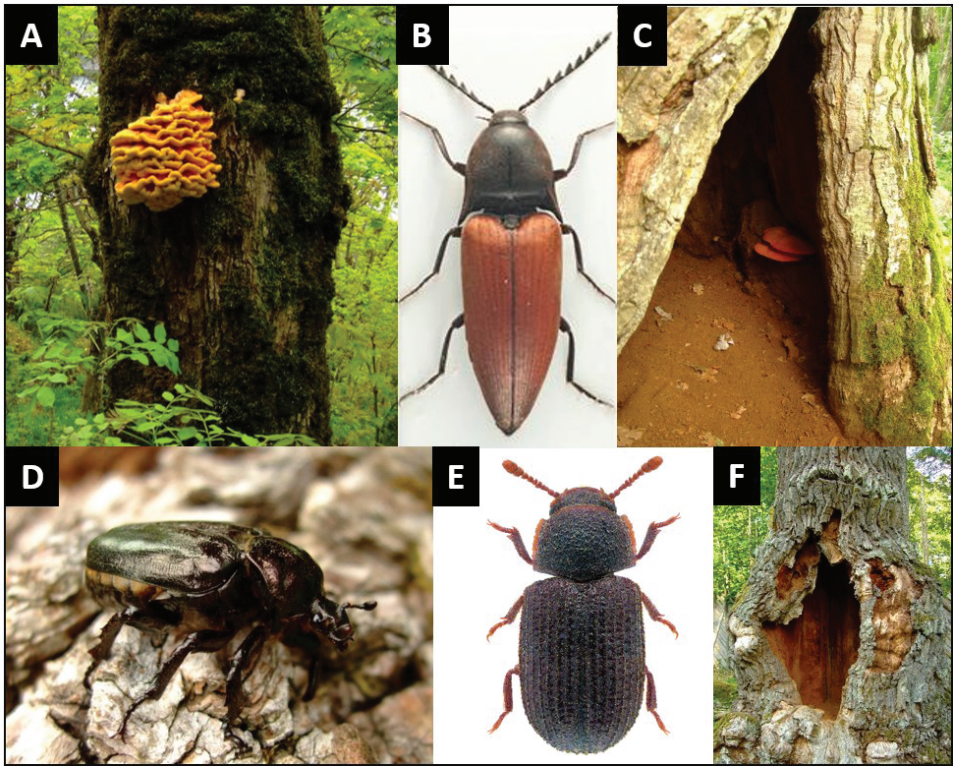

Fig. 9. Interactions between fungi and insects are important in creating the species rich and endangered communities in tree cavites. **(A)** Old oak with fruit body of chicken-of-the-woods, *Laetiporus sulphureus*, a brown heart rot polypore in hardwoods and an important agent in softening the interior of the tree. Photo: Anne Sverdrup-Thygeson. **(B)** The beetle *Ampedus hjorti* listed as Vulnerable on the IUCN Red List of threatened species. The species develops in wood mold in cavities in trunks and stumps of old oaks (*Quercus spp.)* in Europe. Photo Arnstein Staverløkk, Norsk institutt for naturforskning, CC BY 3.0. **(C)** Old oak with fruit body of the beefsteak fungus, *Fistulina hepatica*, a fungus found in many parts of the world. The species also causes brown heart rot and facilitates the colonization of the interior of e.g. old oaks by a range of insects. Photo: Anne Sverdrup-Thygeson. **(D)** The hermit beetle, *Osmoderma eremita*, a large scarab entirely dependent upon veteran trees as it inhabits decaying heartwood, listed as Near Threatened on the IUCN Red List of threatened species. Photo: Anne Sverdrup-Thygeson. **(E)** *Eledona agricola,* an uncommon tenebrionid beetle living in fruit bodies of *L. sulphureus* in Europe. Photo: Udo Schmidt (CC BY-SA 2.0) **(F)** Old oak with a large opening into a cavity filled with nutritious wood mold. Photo: Anne Sverdrup-Thygeson.

7.2. Insects change wood characteristics relevant for fungal community

Saproxylic insects can act as ecosystem engineers and modify the physical properties of the wood through their boring and tunneling activities. Wood often has a low surface area to volume ratio relative to other plant material, and the inaccessibility of the inner parts of a log may limit the availability of nutrients required by the fungal community (Cornwell et al. 2009). Through fragmentation and comminution of dead wood, insects reduce the particle size and increase the surface to volume ratio. This makes the woody tissue more susceptible to enzymatic activity (Walker and Wilson 1991). Tunneling by wood-boring insects can increase access for fungi to the interior of the wood and improve aeration of the wood, which can result in greatly increased rates of decomposition (Dighton 2003).

By consuming the cambium, subcortical early-successional insects remove the layer that attaches the bark to the woody surface, and the bark will be more prone to falling off. This type of insect-mediated ecosystem engineering has been demonstrated for both Norway spruce (*P. abies*) (Weslien et al. 2011) and aspen (*Populus tremulae*) dead wood systems (Jacobsen et al. 2015), with variable effects on the fungal community. Bark loss exposes the woody surface to wind-dispersed spores, which may increase the chances of fungal colonization. At the same time, removal of bark also leaves the wood exposed to sun and wind, which will reduce the moisture content in the wood (Ulyshen et al. 2016). These effects will therefore facilitate some species while inhibiting others, and might shift competitive relationships within the fungal community.

Insects may also change the nutrient content of wood through their relationship with nitrogen-fixing bacteria. Evidence for nitrogen fixation in the gut has been found in several groups of insects, like termites, bark beetles of the genus *Dendroctonus*, the scarabs *Osmoderma eremita* and *Cetonia aurataeformis*, the stag beetle *Dorcus rectus* and finally, in the guts of a wood-eating cockroach, *Cryptocercus punctulatus* (for references, see Ulyshen (2015)). How this affects fungal communities in dead wood is not known in detail, but it has been shown that the addition of larval frass from the cerambycid *Monochamus scutellatus scutellatus* to mineral soils led to a significant increase in microbial activity (Cobb et al. 2010).

8. Concluding remarks

In this review, we have aimed at presenting an overview of insect-fungus interactions in dead wood. We have covered direct interactions from tight mutualism to more opportunistic associations, as well as indirect interactions. When relevant we have drawn parallels to other

systems, mainly the soil ecosystem which is tightly interwoven with the dead wood system. The main interactions are summarized in **Fig. 10**. In these concluding remarks, we summarize our findings in the context of evolutionary adaptations and importance for forest ecosystems.

8.1. Evolution and adaptations

Adaptations between insects and fungi are best known from the mutualistic interactions between for instance bark beetles and fungi. There are competing theories as to how these interactions evolved, but dispersal of fungal propagules was probably a driving force. Fitness benefits from the interaction led to the evolution of adaptations such as pockets for transferring fungi ('mycangia') in insect exoskeletons and increased nutrient content in the fungi. The mutualism eventually became obligate as the fungi turned asexual, and fungus-farming has been a driver for evolution of eusociality in ambrosia beetles. Adaptations to non-mutualistic insect-fungus interactions are far less studied. Potential adaptations have been documented, such as thick spore walls allowing passage through insect guts, mycangia-like structures in insect exoskeletons and fungal propagule structures that may increase chances of insect-vectored dispersal (reticuloperidia, spikes or hooks on spores). However, further studies are required to verify the function of these structures. Fungal adaptations to invertebrate grazing might include physical or chemical defenses, or compensatory growth. Compensatory growth as a response to grazing has been demonstrated in soil as well as in mutualistic relations in dead wood. Fungi produce an almost endless diversity of organic compounds not required for growth and metabolism. Some of these secondary metabolites, that are highly toxic to animals, are likely to function as defense against invertebrates. Induced chemical defense in response to grazing by collembola and fruit flies on *Aspergillus* has recently been observed in the laboratory. Most likely, chemical protection is important in a wide range of interactions in dead wood, but this is currently an understudied field.

8.2. Specialization and biodiversity

The obligate mutualists represent the most specialized and co-dependent interactions in dead wood, but there are also species taking part in facultative mutualisms. For instance, longhorn beetles can benefit from fungi in their larval substrate, but can also develop in substrate without fungi. Fungivores have been considered generalists, but species developing in polypore fruit bodies seem to be rather specialized. Interaction networks between insect developing in polypores and their fungal hosts has been shown to exhibit a similar degree of specialization as pollinator-plant networks. In contrast, network specialization between saproxylic beetles and wood-decaying fungi isolated from these beetles was found to be more similar to that of animal-

mediated seed dispersal networks, indicating moderately specialized interactions perhaps involving opportunistic spore-feeding.

Many of the same host characteristics that influence species richness in herbivore-plant and parasitoid-host systems are important for beetle communities in polypores; abundant, large fruit bodies with diverse growth forms host the highest species number. Fungal species with these characteristics are particularly important for insect biodiversity in dead wood. Indirect interactions might also drive insect diversity; the activity of heart-rot fungi is the first step in the creation of tree cavities. Over time, cavity-bearing trees are associated with a particularly diverse community of saproxylic insects.

Grazing by fungivores can in some cases facilitate co-existence of inferior and superior fungal competitors, at least in the soil system. Thus, fungivory might increase species richness of fungi, although this requires further study. The effect of insect-vectored spore dispersal on species richness of fungi is also unknown, but it might result in significant priority effects for the vectored fungi. On a larger scale, this effect might represent an additional element of stochasticity in dead wood colonization, which could contribute to the high beta-diversity between dead wood objects and thus the high biodiversity in forest ecosystems.

8.3. Nutrient flow and decomposition

Fungi break down complex plant substances and transport essential elements from the surroundings to the wood. Fungivores, and subsequently their predators, assimilate these nutrients. To what extent wood-feeders (xylophages) get their nutrients directly from the wood, or whether fungi (or bacteria) are involved through endosymbiosis or enzymatic digestion is a matter of great interest. Recent research suggests that many wood-feeders do depend on fungi (and bacteria) to gain enough nutrients during the larval stage, although some beetles are capable of producing their own wood degradation enzymes.

Community composition of fungi can have significant effects on dead wood decomposition rates. As insects may affect fungal community composition through dispersal of propagules or grazing, they may indirectly affect decomposition rates. These causal relationships have been demonstrated in soil ecosystems, but remain to be tested in dead wood. Insects dispersing fungal propagules, especially in early succession, might have particularly strong effects on the fungal community, as several studies have found that assembly history is tightly linked with fungal community composition and rate of decomposition. Experimentally excluding insects

from fresh dead wood significantly affects the fungal community, and at least part of this effect most likely stems from absence of insect-vectored fungi.

To conclude, insect-fungus interactions in dead wood are highly diverse and form an essential component of forest ecosystems. It is likely that there are hitherto unknown evolutionary adaptations to these interactions among both insects and fungi. At present, our knowledge of insect-fungus interactions is highly fragmented, but novel methodology such as DNA analysis presents new research opportunities that are already producing interesting results. Increased knowledge of insect-fungus interactions in decomposition of dead wood is a necessary in order to conserve the diversity of species and functions involved in this ancient and essential process.

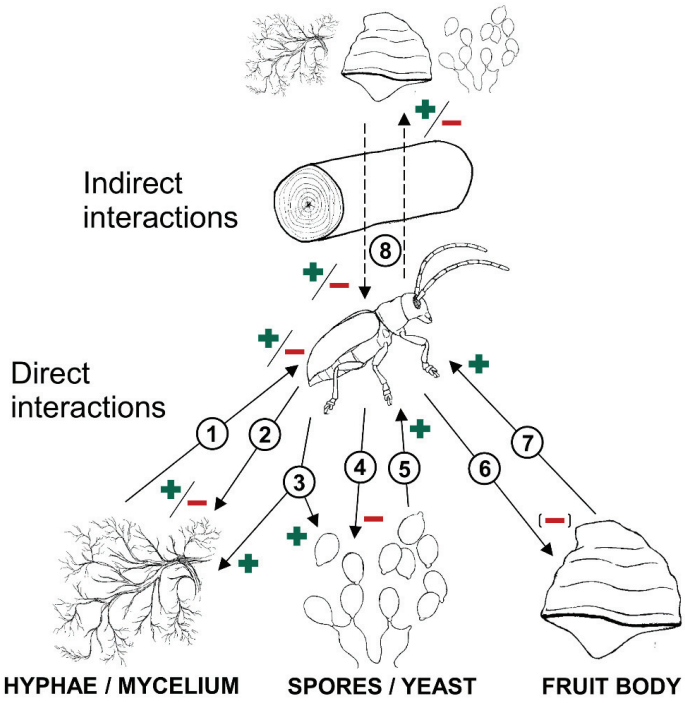

Fig. 10. An overview of insect-fungus interactions and their effects discussed in this review. (1) Some insects feed directly on fungal mycelium and obviously benefit from this. Fungal mycelium in dead wood can also benefit insects by detoxifying tree defenses, enzymatically degrading the wood or even by producing antibiotics that protect the insects against pathogens. Fungi may also protect ants by stabilizing nest structure. Other fungi, however, can feed on insects, immobilizing and killing them with mycelium and toxins. (2) Many insects feed on

fungal mycelium and hyphae and the effect on the fungus is usually negative, but the effect of this grazing can be stimulatory in some cases. Furthermore, other insects such as fungus-farming ambrosia beetles provide protection for fungal growth. (3) Insects can disperse fungal propagules such as spores, hyphae or yeast cells, with positive effects for the dispersed fungus. This is well-known from mutualistic insect-fungus associations, but there is mounting evidence that such interactions might also be important for non-mutualistic species. (4) Spores and yeast cells fed on by insects can be destroyed during digestion. (5) Spores or yeast cells can benefit insects as a main food source for fungivores or as additional nutrients for opportunists or generalists. (6) Insects feeding on fruit bodies can have a negative effect on the fungus. However, often the fruit bodies are already dead and the feeding therefore has little effect on the fungus. (7) Insects feeding on fruit bodies benefit nutritionally, and fruit bodies can also provide shelter and protection for insects. (8) Both insects and fungi can affect each other indirectly through their effects on their shared habitat. These indirect effects can be positive or negative. Drawing by R. M. Jacobsen.

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PAPER II

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Wood-inhabiting insects can function as targeted vectors for decomposer fungi

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ABSTRACT

Most wood-inhabiting fungi are assumed to be dispersed primarily by wind, with the exception of a few species involved in mutualistic relationships with insects. In this study we tested whether several species of wood-inhabiting insects can function as dispersal vectors for non-mutualistic fungi, which would indicate that wood-inhabiting fungi can benefit from targeted animal-mediated dispersal. We sampled wood-inhabiting beetles (Coleoptera) from freshly felled wood experimentally added to forests and used DNA metabarcoding to investigate the fungal DNA carried by these insects. Staphylinid beetles rarely contained fungal DNA, while *Endomychus coccineus*, *Glischrochilus hortensis* and *Glischrochilus quadripunctatus* frequently carried fungal DNA with a composition specific to the insect taxon. A large proportion of the obtained fungal sequences (34%) represented decomposer fungi, including well-known wood-decay fungi such as *Fomitopsis pinicola*, *Fomes fomentarius*, *Trichaptum abietinum* and *Trametes versicolor*. Scanning electron microscopy further showed that some of the fungal material was carried as spores or yeast cells on the insect exoskeletons. Our results suggest that insect-vectored dispersal is of broader importance to wood-inhabiting fungi than previously assumed.

1. Introduction

Dispersal is an integral aspect of community ecology and population dynamics. It is a key component of community assembly (Myers and Harms, 2009; Chase, 2010) and influences the response of species to disturbances such as fragmentation (Johst et al., 2002; Cordeiro and Howe, 2003; Montoya et al., 2008) and climate change (Brooker et al., 2007; Engler et al., 2009). Given the rapid, human-driven habitat changes presently occurring on a global scale (Cardinale et al., 2012; Haddad et al., 2015), it is crucial to understand how species disperse in order to conserve both biodiversity and ecosystem functions.

For sessile terrestrial organisms such as plants or fungi, the propagule vector is highly important for dispersal efficiency. The most important abiotic vector is wind, with water playing a minor role, whereas a multitude of different animals can serve as biotic vectors (Watkinson et al., 2015). While wind dispersal is generally considered a random process, animal-mediated dispersal can be targeted towards suitable habitats. This fundamental difference between wind- and animal-mediated dispersal results in important ecological differences between species employing these different dispersal modes. For instance, animal-dispersed plant species seem to tolerate habitat fragmentation better than wind-dispersed species (Purves and Dushoff, 2005; Montoya et al., 2008; Marini et al., 2012), as long as their dispersal agents are present in habitat fragments (Cordeiro and Howe, 2003; Galetti et al., 2006; Cramer et al., 2007).

Seed dispersal has been extensively studied for both wind-dispersed and animal-dispersed plants (Nathan and Muller-Landau, 2000; Nathan et al., 2002; Wang and Smith, 2002; Schupp et al., 2010), but studies of fungal dispersal are less

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exhaustive, partly due to the high diversity of fungal species and their variable ecology (Watkinson et al., 2015). Many fungi are wind-dispersed (Ingold, 1953; Piepenbring et al., 1998; Halbwachs and Bässler, 2015), but there are also several examples of animal-mediated dispersal (Blackwell, 1994; Johnson, 1996; Piepenbring et al., 1998; Halbwachs and Bässler, 2015). Animal-vectored dispersal of fungi is an understudied field where many interactions probably remain to be discovered (Malloch and Blackwell, 1992). Although some interactions between fungi and animal vectors are co-dependent mutualisms (Batra, 1963; Slippers et al., 2011), there is a continuum of insect-fungus interactions of differing specificity and opportunism which may lead to dispersal of fungal propagules (Talbot, 1952; Wilding et al., 1989; Blackwell, 1994; Tuno, 1998; Greif and Currah, 2007).

Fungi living in dead wood perform an essential ecosystem service by decomposing woody material and constitute a major component of forest biodiversity, including many species threatened by extinction (Gärdenfors, 2010; Rassi et al., 2010; Henriksen and Hilmo, 2015). Wood-inhabiting fungi are generally assumed to be dispersed by wind (Junninen and Komonen, 2011; Norros, 2013), with the exception of fungi associated with bark beetles, ambrosia beetles, termites or wood wasps (Batra, 1963; Martin, 1992; Harrington, 2005). However, these mutualistic species only represent a small fraction of the great diversity of arthropods and fungi that inhabit and decompose dead wood (Tikkanen et al., 2006; Stokland et al., 2012), and there are indications that animal-mediated spore dispersal of wood-decay fungi could be more widespread than previously assumed. For instance, several wood-inhabiting beetles visit fruit bodies of wood-inhabiting fungi during sporulation (Hågvar, 1999; Krasutskii, 2007b, 2010; Schigel, 2011), presumably feeding on spores, and these species could disperse spores of wood-inhabiting fungi in much the same way as invertebrates that feed on spores of soil fungi contribute to their dispersal (Rantalainen et al., 2004; Lilleskov and Bruns, 2005; Seres et al., 2007; Halbwachs et al., 2015). The few studies that have tested the effect of wood-inhabiting insects on the establishment of fungi in dead wood did find significant differences between the fungal communities that established with and without insects, but these studies all focused on bark beetles (Müller et al., 2002; Persson et al., 2011; Strid et al., 2014).

In the current study we investigate whether insect-vectored dispersal could be of broader importance to the fungal community in dead wood, potentially involving several species of insects and fungi. Studies of fungal dispersal have previously been restricted due to the difficulty of identifying spores, but the rapid development of molecular methods has presented new possibilities in this field of research. In this paper we use metabarcoding of fungal DNA from a broad range of wood-inhabiting beetle species to ask the following questions:

1. Do wood-inhabiting beetles not involved in obligate insect-fungus mutualisms frequently bring fungi to dead wood?
2. If so, what kind of fungi do the beetles carry? Specifically, do the beetles bring wood-decay fungi to newly available dead wood largely uncolonized by fungi?
3. Is the composition of fungal taxa specific to the beetle taxon?

2. Materials and methods

2.1. Sample sites

In March 2014, 17 aspen (*Populus tremula*) trees from the same stand in Ås municipality in Norway (Lat. 59.66, Long. 10.79, 92 m a.s.l.) were felled and cut into 1 m long logs with 20.5–36.4 cm diameter. The trees were felled shortly prior to insect sampling, since our intention was to study fungal dispersal to new, uncolonized habitat.

The logs were transported to two landscapes: Losby forest holdings in Østmarka (Lat. 55.98, Long.10.68, 150–300 m a.s.l.) and Løvenskiold-Vækerø (LV) forest holdings in Nordmarka (Lat. 54.49, Long. 21.24, 200–500 m a.s.l.). Both landscapes are within the southern boreal vegetation zone (Moen, 1998) and consist of forest dominated by spruce (*Picea abies*), with pine (*Pinus sylvestris*), birch (*Betula pubescens*) and aspen as subdominants. The forest holdings were managed as production forests within the regulations of the PEFC (the Programme for the Endorsement of Forest Certification schemes, Norway, http://pefcnorway.org/). Twenty-four logs were divided between four sites in each landscape with a mean distance of 1574 m between sites within a landscape and with an average of six logs per site. All selected sites were in semi-shaded, mature spruce forest.

No fungal fruit bodies were apparent on the logs during the first season (2014). In the second season (2015), fruit bodies of *Chondrostereum purpureum* appeared on all logs. No other macrofungi fruit bodies were apparent on the logs during the two seasons of field work, and thus the logs could be considered largely uncolonized habitat for wood-inhabiting fungi that might be vectored by the insects to the logs.

2.2. Insect sampling

Insects, specifically beetles (Coleoptera), were sampled from the aspen logs at each site during May to August in 2014 and 2015. To avoid contamination among samples the insects were sampled individually with tweezers either from sticky traps or directly from the logs. The tweezers were sterilized with ethanol and a gas burner between handling of each insect.

DeLaval™ fly sheets (60 × 30 cm) were used as sticky traps. At each site, one sheet was divided between three different logs and exposed for one or 2d before insect sampling. During sampling from the sticky traps, all insects found on the logs were also sampled. In total, insects were sampled on 11 occasions from each site.

Each insect was placed in a separate Eppendorf-tube (2 ml) and killed by freezing at − 80 °C, which was also the storage temperature. Insects sampled in 2014 and during the first sampling occasion in 2015 were rinsed in sterilized water to separate fungal DNA from the inside and the outside of the insects. However, it became clear that the insects defecated in the tubes, thereby contaminating their exoskeleton and the water with gut content. We, therefore, omitted rinsing the insects for the remaining sampling occasions.

The insects were identified to species or genus using available literature in a sterile environment and using sterilized equipment. Insects that could not be confidently identified at least to genus by the first author (RMJ) were not analyzed further (<20 individuals). We selected 343 beetle individuals for DNA analysis (Table S1) and put aside an additional nine individuals of some of the most abundant species for scanning electron microscopy (SEM). These were wood-inhabiting genera or species, i.e. insects with larval development either in dead wood or in fungal fruiting bodies on dead wood (Wheeler and Blackwell, 1984; Dahlberg and Stokland, 2004).

2.3. Scanning electron microscopy

We used a scanning electron microscope (Zeiss EVO 50 EP) to investigate whether any of five individuals of *Endomychus coccineus* or four individuals of *Rhizophagus* sp. carried fungal material on

their exoskeletons. The beetle samples were desiccated from storage at −80 °C and were gold-coated directly after thawing.

2.4. DNA analysis

DNA was extracted from the insect samples following a modified version of the CTAB protocol (Murray and Thompson, 1980) and diluted 10 times before polymerase chain reaction (PCR) amplification. Negative controls were included during extraction and amplification, and nine technical replicates were included during amplification. PCR was run on an Eppendorf Thermal Cycler (VWR, Radnor, USA) in a total reaction volume of 20 μl consisting of 2 μl of 5 mM primers ITS4 (White et al., 1990) and fITS7 (Ihrmark et al., 2012) each with an incorporated 12 bp identifier tag, 2 μl 2 mM dNTPs, 0.2 μl Phusion Hot Start II High-Fidelity DNA Polymerase and 4 μl 5X Phusion HF Buffer (Thermo Fisher Scientific, Waltham, USA), 1 μl bovine serum albumin (BSA), 0.6 μl dimethyl sulfoxide (DMSO), 6.2 μl milli-Q H_2O and 4 μl 10x-dilution of DNA template. PCR was run as follows: initial denaturation at 98 °C for 30 s, then denaturation at 98 °C for 10 s, annealing at 56 °C for 30 s and elongation at 72 °C for 15 s repeated 30 times, followed by a final elongation step at 72 °C for 10 min. The PCR products were then frozen to deactivate the enzyme.

The PCR products were cleaned using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, USA). Initially we followed the manufacturer's protocol, but this resulted in remnant ethanol in the samples. These samples were still usable after evaporating the ethanol by incubation at room temperature for approximately 3 h. We modified the protocol for the remaining samples by centrifuging longer after the final run-through of wash solution.

The cleaned amplicons were checked by gel electrophoresis and given a score from one to four based on their strength, with one being strongest and four weakest. The amplicons were pooled in different proportions to equalize number of sequences per sample; 1 μl was added from amplicons whose bands were given a strength score of one, 2 μl from amplicons of score two etc. Amplicons were combined in two pooled samples.

The pooled samples were cleaned-up with the ChargeSwitch® kit (Invitrogen, California, USA), DNA-concentration was measured with the Qubit® BR DNA kit (Invitrogen, California, USA), and the sample quality was confirmed by NanoDrop™ (Thermo Fisher Scientific, Madison, USA). The samples were submitted to GATC Biotech for adaptor-ligation and Illumina HiSeq Rapid Run 300 bp paired-end sequencing.

2.5. Bioinformatics

Sequencing resulted in 24 054 248 reads, of which 11 158 131 passed quality control with the following settings on the SCATA pipeline (https://scata.mykopat.slu.se/, accessed 5th of July 2016); minimum read length 150 bp, minimum average quality score 20, minimum allowed base quality 10, barcode mismatch 0.1. The samples were randomly subsampled to 10 000 reads per sample to facilitate downstream analyses and to standardize sequencing depth for samples with variable read numbers. The subsampled dataset was clustered in SCATA by single-linkage clustering with maximum distance 0.015 (minimum alignment length for clustering 0.85, mismatch penalty 1, gap open penalty 0). All singletons were removed from the dataset. The most abundant sequence of the cluster was chosen as the representative sequence for each operational taxonomic unit (OTU). Taxonomy was assigned to the representative sequences of each OTU taking the top hit of a Basic Local Alignment Search Tool (BLASTn; Altschul et al., 1990) search against the NCBI (National Center for Biotechnology Information) and UNITE (Abarenkov et al., 2010) databases. OTUs with e-values < e^{-10} and bit-scores > 100 were annotated to species level if ITS homology was 100–98%, genus for 97.9–95%, family or order for 94.9–80%, phylum for 79.9–70% and 'Fungus' for lower homology or e-values > e^{-10} and bit-scores < 100. Taxonomy was updated according to the taxonomic database Dyntaxa (https://www.dyntaxa.se/, accessed 24th of February 2017). Identified OTUs were matched against FUNGuild (Nguyen et al., 2016) with some modifications according to literature (references in Table S2) for classification into the following guilds; decomposer, insect symbiont, animal parasite, mycoparasite, plant pathogen, lichen or mycorrhiza (Table S2). The classification 'insect symbionts' was not included in the FUNGuild database and is therefore based upon available literature (references in Table S2). Some fungal species isolated from beetle guts in previous studies were included in this classification under the presumption that these species might be endosymbionts. OTUs with affinity to the class Agaricomycetes were further grouped into taxa known to decay dead wood (Table S3).

For further statistical analysis, only OTUs represented by at least 20 reads were included. The removal of the many infrequent OTUs was done intentionally to focus on fungi more likely to be ecologically relevant. Removal of OTUs with few reads will typically also remove OTUs that have appeared due to the PCR and sequencing errors (Bjørnsgaard Aas et al., 2016).

2.6. Statistics

All analyses were conducted in R version 3.2.0 (R Core Team, 2015).

We used generalized linear mixed models (GLMM, binomial distribution and logit link, lme4 package v. 1.1–12 (Bates et al., 2014)) to analyse presence-absence data of fungal DNA in insect samples and linear mixed models for proportion data with insect genus, rinsing treatment (yes/no) and trap method (sticky trap/freely sampled) as fixed effects, site nested under landscape and month nested under year as random factors. Insignificant fixed effects or random factors explaining no variation in the data were excluded from the models, and if this resulted in models without random factors we switched to generalized linear models or just linear models. The insect genus with average response variable values was placed in the intercept of the models. Proportion data was arcsine-transformed ($\sin^{-1}\sqrt{0.01}$ x proportion of decomposer sequences) to improve model fit (Crawley, 2012), which was investigated with residual plots and proved satisfactory.

When analysing presence-absence data, only beetle genera represented by at least nine individuals were included (N = 312). For analysis of the proportion data, which was restricted to individuals with fungal DNA, only insect genera represented by at least five individuals were included (N = 181). We used a slightly stricter limit for number of individuals in the presence-absence data, since each observation holds more information in continuous data than in binary data. When analysing wood-decay agaricomycetes, OTUs matching *C. purpureum* or its family Cyphellaceae were excluded in order to focus on agaricomycetes not already fruiting on the logs.

Variation in OTU composition of the beetle samples (all individuals with fungal DNA included, N = 187) was explored in ordinations using principal component analysis (PCA) on Hellinger-transformed abundance (number of sequences) data with vegan package v. 2.4–2 (Oksanen et al., 2017). We also performed PCA on Hellinger-transformed presence-absence data (Borcard et al., 2011) to corroborate the trends in the PCA of abundance data. The significance of explanatory variables for OTU composition (abundance data) was tested with permutation tests (999 permutations) of conditional redundancy analysis (RDA). Here, variation due to all

other variables than the one being tested was partialled out. Only insect genera represented by at least five individuals with fungal DNA were included in these tests (N = 181).

3. Results

3.1. Do insects bring fungi to dead wood?

Fungal DNA was amplified from 187 of the 343 beetle individuals that had been selected for DNA-analysis (Table S1). The beetle genera differed significantly in proportion of individuals with fungal DNA, and individuals of the genera *Glischrochilus, Rhizophagus, Xylita* and *Epuraea* frequently contained fungal DNA (Table 1). Additionally, beetles that had been rinsed or sampled from sticky traps were significantly less likely to contain fungal DNA (Table 1). For instance, the probability that an unrinsed, freely sampled *Glischrochilus* individual contained fungal DNA was 0.93, while the corresponding probability for a rinsed *Glischrochilus* individual sampled from a sticky trap was 0.66.

3.2. What kind of fungi do the insects carry?

After rarefying down to 10 000 ITS2 sequences per sample and removing low abundance OTUs comprising less than 20 sequences; 1069 fungal OTUs (1 714 063 sequences) remained for further analyses. Of these 51 OTUs were annotated to kingdom (fungi) level, 94 to phylum and 924 were annotated beyond phylum, including 468 to species or genus (Table S2). Ascomycetes constituted 77% of the sequences and 68% of the OTUs, whereas 20% of the sequences and 24% of the OTUs were basidiomycetes (Fig. 1). Forty-one percent of the OTUs (representing 65% of the sequences) were assigned to an ecological guild (Table S2). The majority of these were classified as decomposers (23% of the OTUs and 34% of the sequences; Fig. 1).

The proportional abundance of fungal guilds varied according to beetle genus (Fig. 1). There were significant differences between beetle genera in proportion of sequences from decomposer fungi, with individuals of genus *Endomychus* containing the largest proportions (Fig. 1, Table S4).

Among the decomposer fungi, there were 25 OTUs annotated as wood-decay agaricomycetes in addition to *C. purpureum* which was fruiting on the logs during insect sampling (Table S3). The proportion of individuals with DNA from these 25 wood-decay agaricomycetes differed significantly between beetle genera (Fig. 2, Table S5). Individuals of genus *Endomychus* contained DNA of wood-decay agaricomycetes most frequently (Fig. 2, Table S5).

Scanning electron microscopy showed that individuals of *E. coccineus* and *Rhizophagus* spp. carried fungal material attached to their exoskeletons (Fig. 3). This fungal material seemed to consist of yeast cells, spores and hyphae. All of the nine individuals that were investigated carried some fungal material, but it was most abundant on three of the five *E. coccineus* individuals.

Table 1
Generalized linear model (GLM, binomial distribution and logit link) with presence or absence of fungal DNA as response and insect genus (no. of individuals ≥9, genus *Agathidium* in the intercept), rinsing treatment and trap method as explanatory variables. Significant p-values marked in bold. N = 312.

Presence of fungal DNA	Estimate	SE	z-value	p-value
Intercept	0.23	0.42	0.55	0.584
Rinsed (Yes)	−1.21	0.36	−3.33	**0.001**
Trap (Sticky)	−0.70	0.40	−1.76	0.078
Insect genus:				
Endomychus	1.20	0.69	1.74	0.082
Epuraea	1.48	0.75	1.96	**0.050**
Glischrochilus	2.35	0.57	4.16	**<0.001**
Rhizophagus	1.36	0.60	2.26	**0.024**
Xylita	1.99	0.87	2.30	**0.021**
Fam. Staphylinidae;				
Acrulia	0.35	0.67	0.52	0.605
Anthophagus	0.34	0.59	0.58	0.562
Oxypoda	−0.91	0.54	−1.69	0.092
Quedius	0.42	0.65	0.65	0.518

Null deviance: 427.4 on 311 degrees of freedom.
Residual deviance: 301.1 on 300 degrees of freedom.

3.3. Does the composition of fungal OTUs depend on insect taxon?

Unconstrained ordination showed that composition of fungal OTUs was clearly influenced by beetle genus (Fig. 4). The first ordination axis (PC1) separated samples of *Glischrochilus* and partially *Rhizophagus* from most other beetle samples (Fig. 4A), while the third ordination axis (PC3) isolated samples of *Endomychus* in a cluster (Fig. 4B). PC1 represented 15.6% of the variation in fungal OTU composition, PC2 represented 7.6% and PC3 represented 6% of the variation. The *Glischrochilus-Rhizophagus* cluster was characterized by high abundance of sequences from the fungal OTUs annotated as *Phialophora bubakii, Candida mesenterica* and *Candida* sp. (Fig. 4C), whereas the *Endomychus* cluster had more sequences of *Cladosporium cladosporioides, Fusarium merismoides* and *C. purpureum* (Fig. 4D). Samples of the staphylinid genera and samples of the two *Glischrochilus* species did not show any substructuring.

Insect genus explained the largest proportion of the variation in fungal OTU composition in conditional constrained ordination (18.3%), whereas the factors related to sampling design explained much smaller proportions of the variance (0.9–5.7%, Table S6). To further assess the robustness of the patterns in Fig. 3, we repeated the unconstrained ordination without insect symbiont fungi, with only decomposer fungi (Fig. S1) and with presence-absence data for the fungal OTUs (Fig. S2). The same clusters of insect genera were apparent in these ordinations.

4. Discussion

This study demonstrates that several different species of wood-inhabiting insects bring fungi to recently dead wood, and that at least some of the fungal material is carried as propagules attached to the exoskeleton. While we do not know whether these propagules were viable, previous studies have shown that fungi may be vectored as spores or yeasts on insect exoskeletons or in insect guts without loss of viability (Lim, 1977; Tuno, 1998, 1999; Lilleskov and Bruns, 2005). Since we used fresh dead wood in our study, the beetles arrived at largely uncolonized habitat and the wood-inhabiting fungi (with the exception of *C. purpureum*) in the beetle samples were likely to have been brought to the logs by the beetles. Therefore, the beetle species that were found to frequently carry fungal material in the present study, i.e. *E. coccineus, Epuraea* spp., *Glischrochilus quadripunctatus, Glischrochilus hortensis, Rhizophagus* spp. and *Xylita laevigata*, are likely to disperse fungi. These beetle species are also known to be connected with fungi. The *Epuraea, Glischrochilus* and *Rhizophagus* species typically visit sporulating polypores (Kaila et al., 1994; Hågvar and Økland, 1997; Hågvar, 1999; Nikitsky and Schigel, 2004; Krasutskii, 2007a, 2010; Schigel, 2011), *E. coccineus* is a fungivore of *C. purpureum* (Schigel, 2012), and *X. laevigata* lives in fungus-colonised wood (Dahlberg and Stokland, 2004). This explains the high prevalence of fungal DNA in samples of these species. To our knowledge, this is the first study showing that several wood-inhabiting beetle species bring fungi to dead wood, not just the few well-studied species of bark

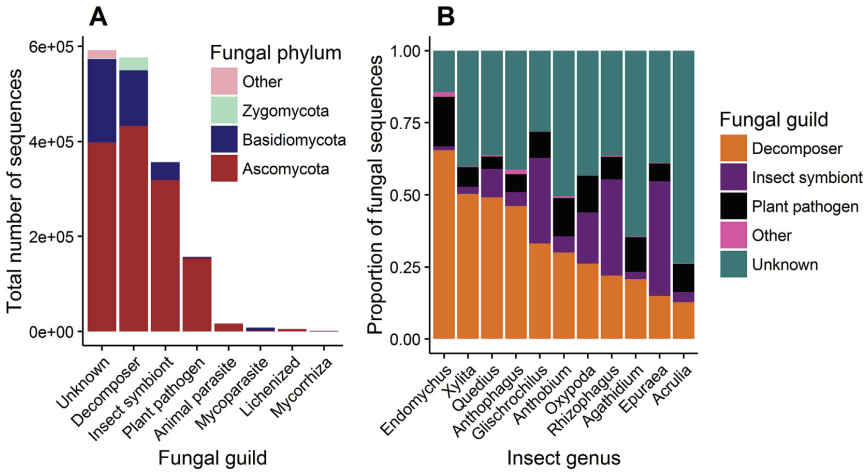

Fig. 1. (A) Abundance (no. of sequences) of fungal guilds and fungal phyla in the subsampled dataset used for analysis. (B) Proportion of the fungal guilds in the sequences extracted from insect samples of the different genera (no. of individuals with fungal DNA ≥5) in the subsampled dataset.

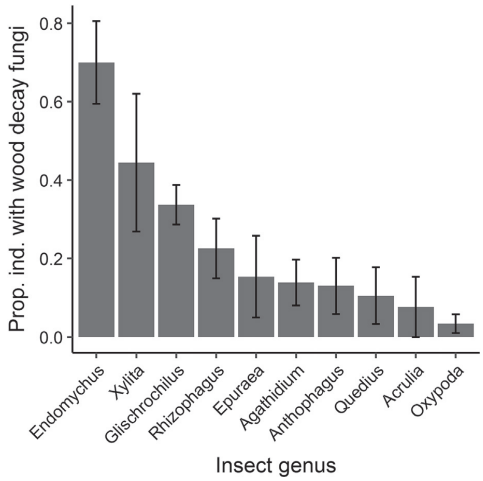

Fig. 2. Mean (±standard error) proportion of all analyzed beetle individuals (in genera represented by ≥ 9 individuals) containing DNA from wood-decay agaricomycete OTUs, *C. purpureum* excluded (Table S3).

and ambrosia beetles.

Although we do not know whether the fungi carried by these wood-inhabiting beetles actually colonized the dead wood, many of the fungal OTUs isolated from the beetles matched fungal genera previously isolated from wood samples, such as the widespread genera *Phialophora, Cladosporium* and *Oidiodendron* (Kubartová et al., 2012; Strid et al., 2014; Ottosson et al., 2015). Some OTUs also matched well-known wood-decay fungi such as *Fomes fomentarius, Fomitopsis pinicola* and *Trametes versicolor*. Basidiomycete wood-decay fungi seem to be responsible for the greatest mass loss during wood decay (Boddy, 2001; Kubartová et al., 2015), but other saprotrophs also contribute to decomposition of cellulose and carbohydrates (Rayner and Boddy, 1988; Rice and Currah, 2005; Rice et al., 2006; Ottosson et al., 2015). Furthermore, high-throughput sequencing of fungal DNA has shown that fungi with a variety of ecological roles are present in dead wood (Ottosson et al., 2015; van der Wal et al., 2015). It is, therefore, likely that several of the fungi vectored by the beetles in the present study could establish in the dead wood and contribute to wood decay.

The composition of fungal OTUs differed between beetle genera, meaning that arrival order of wood-inhabiting beetles might directly influence arrival order of wood-inhabiting fungi. Arrival order significantly affects the fungal community (Fukami et al., 2010; Dickie et al., 2012; Hiscox et al., 2016) since wood-decay fungi can be highly competitive (Holmer et al., 1997; Boddy, 2000). An early arrival might allow fungi to occupy larger wood volumes prior to the arrival of competitors, which has been shown to confer significant competitive advantages (Holmer and Stenlid, 1993). Early colonizing wood-inhabiting insects have been found to significantly affect the establishment and succession of the fungal community in dead wood (Müller et al., 2002; Weslien et al., 2011; Strid et al., 2014; Jacobsen et al., 2015) and our study indicates that these effects might stem from insect-vectored dispersal of fungal propagules. Beetle species like *E. coccineus* and *Glischrochilus* spp. might inadvertently inoculate fresh dead wood with their specific mix of decomposer fungi, potentially resulting in fungal communities that differ in terms of decay type and decomposition rate (Deacon et al., 2006; Hanson et al., 2008; Strickland et al., 2009; McGuire et al., 2010). If different species of wood-inhabiting beetles vector functionally different fungal communities, then diversity of wood-inhabiting beetles might promote functional diversity of wood-inhabiting fungi. Since functional diversity strongly affects ecosystem function (Cadotte et al., 2011; Mano and Tanaka, 2016; Pan et al., 2016), wood-inhabiting beetles might have a significant indirect effect on forest ecosystems through their effect on the fungal community in dead wood.

Several fungi have a very versatile ecology, where trophic mode and thus ecological guild varies depending on context (Nguyen et al., 2016). As such, our guild classification is highly simplified and uncertain for several taxa. For instance, fungi of the genus *Candida* were tentatively classified as insect symbionts in the present study since *Candida* species frequently have been isolated from insect guts (Suh et al., 2004; Suh and Blackwell, 2005; Suh et al., 2006). However, some of the *Candida* species seem to be more

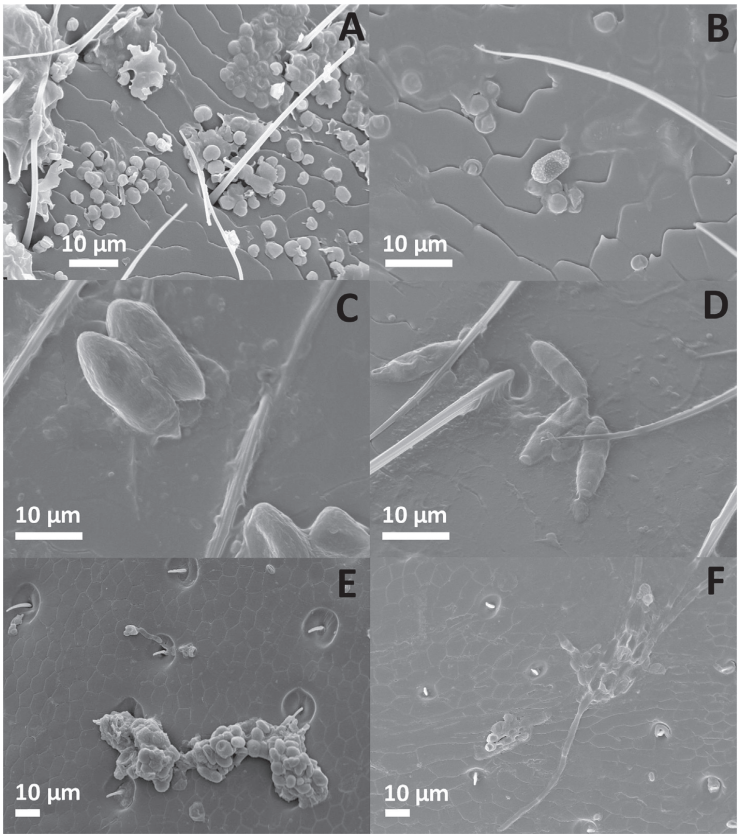

Fig. 3. Scanning electron microscope images of fungal structures on the exoskeletons of wood-inhabiting beetles. (A–D) Images from *E. coccineus*; (E–F) images from *Rhizophagus* sp. (A) Small, round structures were found in large numbers on two of five *E. coccineus* individuals and might be yeast cells. (B) The larger structure with ornamented exterior might be a spore from the polypore *Ganoderma applanatum* according to mycologist Leif Ryvarden (pers.com.). (C) The relatively large structures have a small stipe at one end, indicating that they might be basidiospores. (D) The fusiform shape and the horizontal cross-wall indicates that this might be spores of *Cladosporium* and *Cladophialophora* (Marie Davey, pers. com.). (E) The spore above to the left of the cluster seems to have sporulated on the exoskeleton. (F) The hyphae might form a conidiophore of a *Penicillium* species (Leif Ryvarden and Marie Davey, pers.com.).

closely associated with the insect habitat than the insects themselves (Suh and Blackwell, 2005), like *C. mesenterica* which was isolated in abundance from *Glischrochilus* beetles in the present study. *Candida* species have also been isolated from wood samples (Kubartová et al., 2012; Strid et al., 2014; Ottosson et al., 2015; van der Wal et al., 2015), so these species might be capable of colonizing dead wood. At any rate, guild classification can be used pragmatically as a tool to explore the composition of complex communities (Ottosson et al., 2015). We used the tentative insect symbiont classification to investigate whether fungi that might be insect symbionts were the main drivers of the difference in fungal OTU composition between insect genera, and found that this did not seem to be the case.

Most wood-inhabiting fungi are assumed to be dispersed by wind (Junninen and Komonen, 2011; Norros, 2013), but insect-vectored spore dispersal does not have to replace wind dispersal to be of importance for wood-inhabiting fungi. It is more likely to be complementary and especially beneficial under certain circumstances such as in weather conditions that are suboptimal for wind dispersal, or in fragmented habitats. Since volumes of dead wood have decreased drastically in managed forests in comparison with the few remnant old-growth forests (Siitonen, 2001), species living in dead wood frequently face a fragmented habitat. Still, some common wood-inhabiting fungi like *F. pinicola* and *Trichaptum abietinum* seem to have relatively high tolerance to fragmentation (Nordén et al., 2013) and high gene flow between populations (Nordén, 1997; Högberg et al., 1999; Kauserud and Schumacher, 2003). These species might be benefiting from efficient dispersal not only by wind, but also by wood-inhabiting insects. *F. pinicola* and *T. abietinum* were both isolated from wood-inhabiting insects in the present study and from bark beetles in a study by Persson et al. (2011). However, the benefit of insect-vectored dispersal in a fragmented landscape depends on the fragmentation tolerance of the insect vector. Consequently, if there are wood-inhabiting fungi that to a significant degree depend on dispersal by specific insect species, these species should be managed jointly in conservation efforts.

Understanding species interactions such as insect-vectored dispersal of fungi is becoming increasingly important due to the accelerating human-driven global changes (Cardinale et al., 2012;

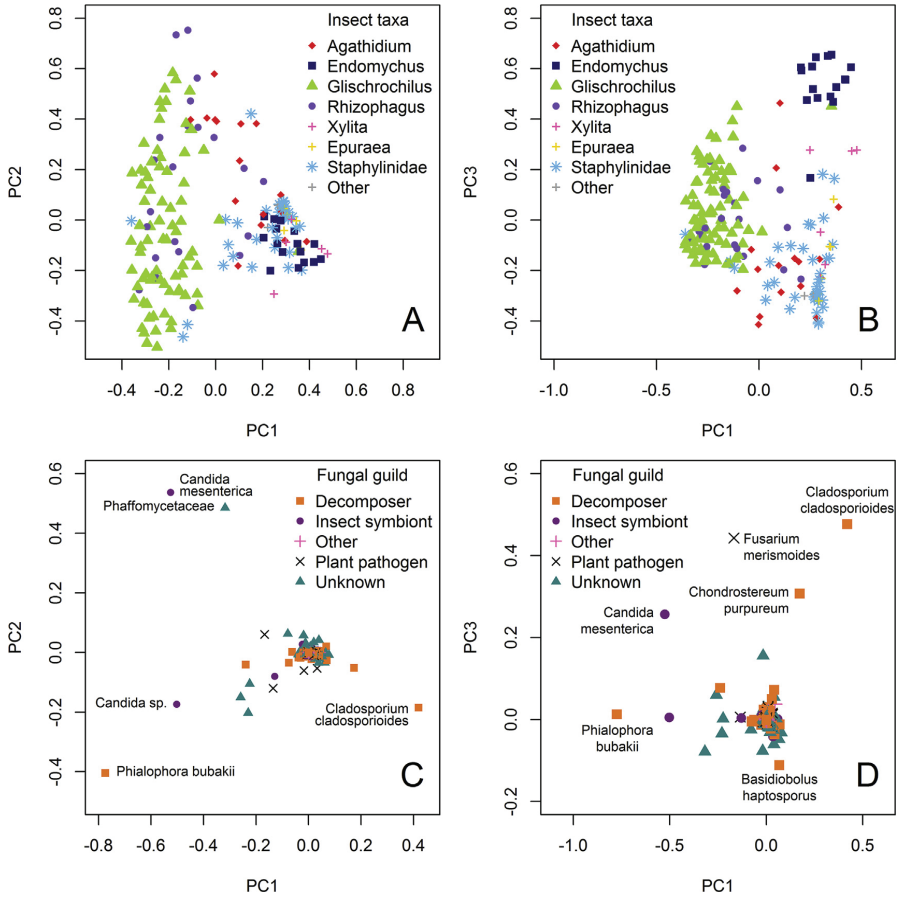

Fig. 4. Principal component analysis of the composition of fungal OTUs in the insect samples (all genera included, N = 187), based on Hellinger-transformed abundance data. (A–B) Insect scores, with symbols representing insect taxa (see Table S1 for full list of insect samples and Table S6 for PC scores summarized by insect taxa). (C–D) OTU scores, with symbols representing fungal guild of the OTUs and the most influential OTUs labelled with matching taxon identity. (A–C) Principal component axis 1 and 2; (B–D) principal component axis 1 and 3.

Haddad et al., 2015). Global change might disrupt existing interactions and facilitate novel interactions, through processes such as climate-driven range shifts, species extinctions and invasion by new species (Pimentel et al., 2000; Walther et al., 2002; Cardinale et al., 2012). For instance, the projected increase in precipitation in the northern hemisphere (IPCC, 2014) might benefit fungi through increased fruit body yields (Boddy et al., 2014), but the effect on insects will more likely be negative due to reduced dispersal (Sturtevant et al., 2013; Gough et al., 2015). Different responses of wood-inhabiting insects and fungi to climate change might de-couple interactions such as insect-vectored dispersal, potentially changing not only the dead wood community but also its ecosystem function. Without knowledge of the interactions currently shaping the dead wood community, our ability to understand and mitigate future changes will be severely impaired. We, therefore, recommend further research into insect-vectored dispersal of wood-inhabiting fungi, as our study suggests that this interaction might be of greater importance to forest ecosystems than previously assumed.

Authors' contributions

RMJ, TB, HK and AST conceived the ideas and designed the methodology; MMB did the bioinformatic analysis; RMJ did the field work, lab work, statistical analysis and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

Raw data (fastq-files), barcode and primer mapping file, OTU table and representative sequence files have been accessioned in Dryad with http://dx.doi.org/10.5061/dryad.3t2d4.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funeco.2017.06.006.

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PAPER III

Revealing hidden insect-fungus interactions in detritivore networks

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Abstract

Ecological communities consist of complex interaction networks that influence ecosystem structure and function. Fungi are the driving force for ecosystem processes such as decomposition and carbon sequestration in terrestrial habitats, and are strongly influenced by interactions with invertebrates. Yet, interactions in detritivore communities have rarely been considered from a network perspective. In the present study, we analyse the interaction networks between several functional guilds of fungi and insects sampled from dead wood.

We reveal a diversity of interactions differing in specificity in the detritivore networks. As predicted, plant pathogenic fungi were relatively unspecialized in their interactions with insects inhabiting dead wood, whereas the low degree of specialization for insect symbiont fungi was unexpected. Interactions between insects and wood-decay fungi exhibited the highest degree of specialization, which was similar to estimates for animal-mediated seed dispersal networks in previous studies. This supports the hypothesis that the interaction shaping this network could be spore feeding and subsequent spore dispersal by the insects. In general, the insect-fungus networks were significantly more specialized, more compartmentalized and less nested than randomized networks. Thus, our results indicate that detritivore networks share a general structure, but that degree of specialization depends on underlying interaction.

Introduction

Interactions between individuals and species shape ecological communities and drive evolution. Ecosystems therefore consist of complex networks that vary in structure depending on the specificity and frequency of the interactions. Highly specific interactions like pollination often result in very specialized networks with low robustness to species loss [1], where extinction of one species cascades to several connected species. As species are currently going extinct at an

alarmingly high rate [2], knowledge of ecological networks and interactions is becoming increasingly important in order to understand and hopefully prevent extinction cascades.

Several studies have underlined the importance of pollination and other well-known interactions such as predation, herbivory and animal-mediated seed dispersal for ecosystem structure and function (e.g. [3-5]). However, our knowledge of biotic interactions is highly skewed towards macroscopic organisms [6], and network studies have largely focused on well-known interactions such as pollination [7,8]. There are few studies of interactions between bacteria, fungi or invertebrates at the community level, despite their overwhelming abundance and species diversity [9-12]. Bacteria and fungi are integral to terrestrial and freshwater ecosystems through their roles as pathogens, symbionts and decomposers [13-17]. Up to 90% of terrestrial plant production enters the detrital food chain [18], where the microbiota of bacteria, fungi and invertebrates determine rate of decomposition and carbon sequestration [16,17].

Invertebrates can have a significant influence on ecosystem processes through interactions with bacteria or fungi, as demonstrated for rate of decomposition, nutrient cycling and mycorrhizal symbiosis in lab experiments [19-21]. However, the role of invertebrates in the detritivore community is rarely considered from a network perspective, in contrast with the intensively studied functional roles of invertebrates as pollinators or herbivores [7,8]. In the present study, we show that network analysis of understudied species groups such as insects and fungi can reveal hidden interactions and elucidate the structure of detritivore communities.

Ecological networks are shaped by the frequency of interactions between species, which in turn is determined by abundance of the species and their interaction specialization. Specialized species will interact with their preferred partner more frequently than what would be expected by chance encounters only governed by abundance. The tendency of species in a network to exhibit specialized interactions can be summed up at the network level as degree of specialization, a network metric that has been shown to differentiate between more or less

species-specific interactions [22,23]. For instance, pollinator networks in general have a higher degree of specialization than networks based on animal-mediated seed dispersal [22]. Degree of specialization can be compared between networks [24], which can help elucidate the form of interaction underlying networks where this is unknown.

Networks where species with few interactions mainly interact with species involved in many interactions have a so-called nested structure [25]. Nested networks are generally robust against random species loss [26], while networks with a high degree of specialization are more vulnerable [27]. Networks can also be organized into compartments called modules, in which species interact frequently within the modules and infrequently between modules. If within-module interactions are dominant, the network is said to have high modularity [28]. Modules might be the product of spatial or temporal variability in interactions, for instance if interaction frequency depends on overlap in phenology, or they might consist of closely related species or species with similar trait syndromes due to convergent evolution [29,30]. Thus, the structure of an interaction network can reveal selective pressures shaping the interactions and the degree of redundancy within the network.

In the present study, we analyse insect-fungus networks sampled from dead wood experimentally added to boreal forests. These networks are vital for the functioning of forest ecosystems, as they are the driving force for decomposition and nutrient cycling in these habitats [31-33]. Understanding how these networks are structured is therefore integral to understanding the basis for ecosystem processes in forests. We used DNA metabarcoding to identify fungi extracted from individual insects, which enabled us to include interactions involving microscopic fungal structures such as spores, hyphae or yeast. We compiled quantitative networks for interactions between insects inhabiting dead wood and three functional groups of fungi which we hypothesized would exhibit different network structures due to different underlying interactions. We hypothesized that the interaction networks with

wood-inhabiting insects would; 1) be highly specialized for insect symbiont fungi, 2) be intermediately specialized for wood-decay fungi, and 3) show little specialization for plant pathogenic fungi.

Results

Fungal DNA was obtained from 187 saproxylic beetle individuals of 17 species or genera (Supplementary Table S1). The DNA metabarcoding analyses resulted in 1069 fungal operational taxonomic units (OTUs) represented by more than 20 sequences and distributed on a total of 1 714 063 sequences. Low abundance OTUs with less than 20 sequences were not included, since we wanted to focus on widespread fungi more likely to be important in interactions. Of the included OTUs, 468 were annotated to species or genus level based on ITS homology of 100 - 98% for species and 97.9 – 95% for genus. Of these, 35 species or genera of fungi (356 279 sequences) were classified as insect symbionts, 22 (48 196 sequences) were classified as wood-decayers in the class Agaricomycetes and 60 (157 577 sequences) were classified as plant pathogens (Fig. 1). Excluding insect species represented by single individuals did not change the results and these species were therefore included in the network analysis.

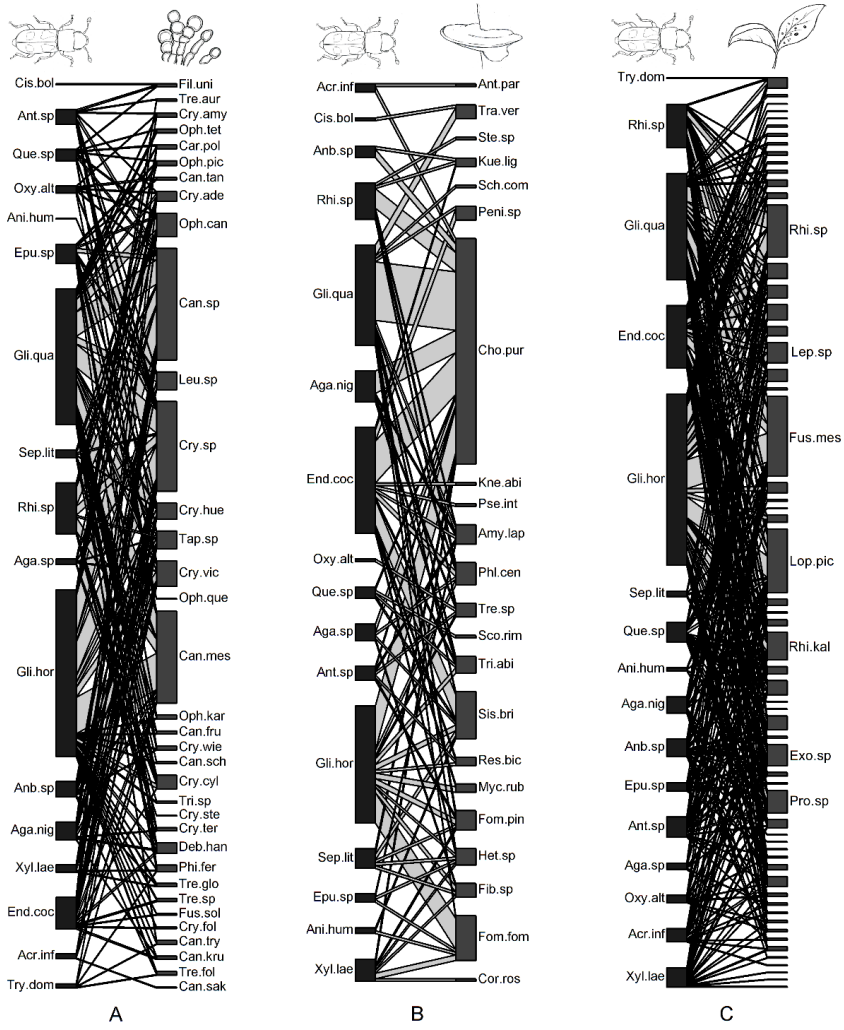

Figure 1) Insect-fungus quantitative bipartite networks. Networks of wood-inhabiting beetles and fungi classified as (A) insect symbionts, (B) wood-decayers or (C) plant pathogens. Sizes of boxes and interaction lines represent number of occurrences of the fungi in the insect samples. See Supplementary Tables S4, S5, S6, S7, S8 and S9 for full names of abbreviations.

All insect-fungus networks were significantly more specialized and less nested than the null model with randomized interactions, while the networks with insect symbiont fungi and wood-

decay fungi were also significantly more modular (Fig. 2). Surprisingly, the network with wood-decay fungi had the highest degree of specialization and modularity (H_2' = 0.21, Fig. 2). Correspondingly, it also had the lowest nestedness. We re-calculated the network metrics with OTUs annotated as *Chondrostereum purpureum* (Pers. : Fr.) Pouzar excluded from the network of wood-decayers, since this species was visibly fruiting on the logs during insect sampling and could have occurred in all samples indiscriminately. Indeed, DNA from *C. purpureum* was isolated from 43% of the insect samples, including 12 of 17 taxa. Excluding *C. purpureum* from the wood-decayer network resulted in even higher specialization (H_2' = 0.29, null model P 95% CI = 0.13 – 0.23), higher modularity (Q = 0.41, null model P 95% CI = 0.28 – 0.36) and lower nestedness (WNODF = 9.38, null model P 95% CI = 12.00 – 23.23). Without *C. purpureum*, the network between wood-inhabiting beetles and wood-decay fungi was organised in six modules (Fig. 3).

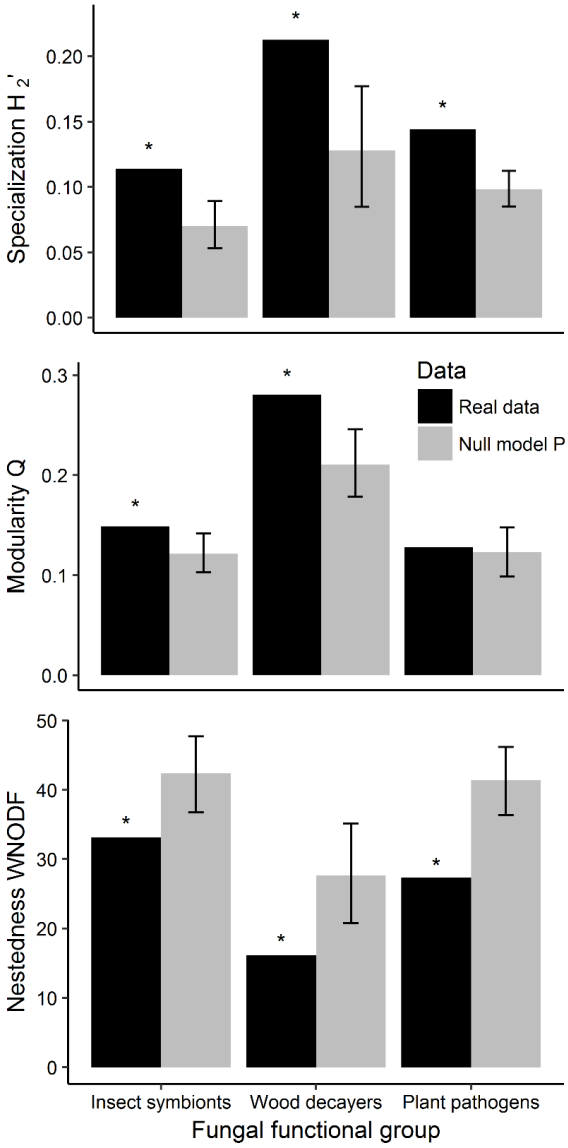

Figure 2) Estimated network metrics for real and randomized data. Network specialization (H_2' ranges from 0 for least specialized to 1 for most specialized, and reflects tendency for species to prefer certain interactions irrespective of partner abundance), modularity (Q ranges from 0 for least modular to 1 for most modular, and reflects tendency for interactions to be sorted into compartments) and weighted nestedness (WNODF ranges from 0 for least nested to 100 for

most nested, and reflects tendency for abundant species to be involved in most interactions) for networks between wood-inhabiting beetles and the fungal functional groups insect symbionts, wood-decayers and plant pathogens. Black bars represent the original networks, while grey bars represent networks randomized with constant marginal sums according to null model P [34] with 95% confidence intervals (CI). Asterisks (*) above the black bars signify significant differences between the original and the randomized networks.

We estimated specialization at the species level for the wood-decayer network. The insect species *Endomychus coccineus* (Linnaeus, 1758) was significantly (P-value = 0.005) more specialized and *Glischrochilus hortensis* (Geoffroy, 1785) was nearly significantly (P-value = 0.053) more specialized with regard to wood-decay fungi than expected from the null model (Supplementary Table S2), with index values (d') of 0.25 and 0.18, respectively (d' ranges from 0 for most generalized to 1 for most specialized). Among the wood-decay fungi, OTUs annotated as *Trametes versicolor* (L. : Fr.) Pilát., *Fomes fomentarius* (L. : Fr.) Fr. and *Sistotrema brinkmannii* (Bres.) J. Erikss. were significantly specialized with index values of 0.45, 0.38 and 0.24 (P-values < 0.05), respectively (Supplementary Table S3).

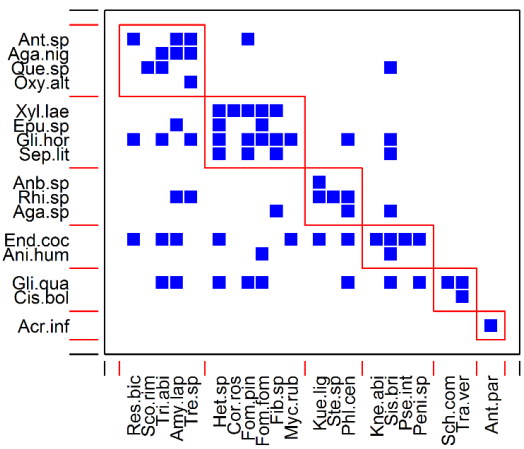

Figure 3) Module structure for the network between insects and wood-decay fungi. Modules in the network between wood-inhabiting beetles and wood-decay fungi with C. purpureum excluded, as organised by the QuanBiMo algorithm [28]. Lines demarcate modules, squares indicate interactions between insects and fungi. See Supplementary Tables S4, S5, S6, S7, S8 and S9 for full names of abbreviations.

Discussion

This study shows that species of the two dominant eukaryotic kingdoms in dead wood, insects and fungi, interact in structured networks. In general, the networks were not nested, they were specialized, though not to a high degree, and interacting species were compartmentalized in modules. Similar network structures have also been found for interactions between plants and mycorrhizal fungi [35-37], and further studies might corroborate whether this is a consistent pattern for quantitative networks (in contrast with qualitative networks [38]) involving diverse fungal communities. The lack of a nested network structure where abundant species are involved in most interactions indicates relatively low redundancy in the insect-fungus networks, although species within modules might fulfil similar interaction functions. Low redundancy could mean that the insect-fungus networks are vulnerable to species loss [7], although the relatively low degree of specialization (H_2'= 0.21 or less) might increase robustness [1]. However, to understand the degree of dependency between species, we need to know the underlying interactions of the networks.

We predicted that the fungal functional guilds would differ in the specificity of their interactions with the wood-inhabiting insects, and our predictions were based on the interaction type we assumed would be dominating for each fungal guild. We assumed that plant pathogenic fungi would mainly interact with the beetles through shared habitats and exhibit low specificity with regard to beetle species, since beetles inhabiting dead wood would be poor vectors for the plant

pathogens. This hypothesis was strengthened by the relatively low degree of specialization for the network with plant pathogenic fungi (H_2' = 0.14).

The network with fungi annotated as insect symbionts was predicted to be highly specialized, but had a very low degree of specialization (H_2' = 0.11). Most of these species were classified as insect symbionts based on previous isolation from beetle guts (references in Supplementary Table S4). In comparison, in a study by Shukla et al. [39] bacterial endosymbionts had a relatively high degree of specialization (H_2' = 0.35) even in an intraspecific network with males, females and larvae of one dung beetle species. Our results indicate that many of the fungal species found in insect guts might be unspecific symbionts, or simply contaminants from food or habitat that do not function as symbionts. Certainly, yeast fungi like *Candida* spp. and *Cryptococcus* spp. can occur in several different environments such as soil or dead wood [40-43], where insects are also abundant. Some of the fungi isolated from beetle guts do seem to be more closely associated with the habitat than with the beetle species [44]. However, endosymbionts can be relatively unspecific with regard to insect host species, especially if they are transmitted horizontally [15]. Further in-depth studies, including microscopy and experimentation, are required to clarify whether the fungal taxa annotated as insect symbionts in the present study can truly be classified as symbionts, despite their low specificity.

The network between wood-inhabiting beetles and wood-decay fungi was predicted to be intermediately specialized in comparison with the insect symbionts, but had the highest degree of specialization in this study (H_2' = 0.21). However, this is still much lower than the specialization of networks based on strong mutualisms such as pollination (H_2' = 0.60 [22]), ant-myrmecophyte networks (H_2' = 0.80 [22,45]) or legume-rhizobium bacteria networks (H_2' = 0.85 [46]). Instead, it was closer to that of networks based on more opportunistic interactions, such as ants harvesting honeydew from true bugs (H_2' = 0.43 [23]) or nectar from plants (H_2' = 0.25 [22]), or animal-mediated seed dispersal (H_2' = 0.18 – 0.47 [22,47,48]). This indicates that the network

between wood-inhabiting beetles and wood-decay fungi was based upon similarly opportunistic yet reciprocal interactions that would result in a moderate degree of specialization. Spore feeding and subsequent spore dispersal by the beetles could represent such an interaction. In line with this hypothesis, the nitidulid beetle *G. hortensis* has frequently been registered on sporulating fruit bodies of wood-decay fungi such as the polypore *F. fomentarius* [49,50], although its habitat is fresh dead wood [51]. In the present study, this beetle species was found to be significantly more specialized on wood-decay fungi than expected by chance, and *F. fomentarius* was isolated from eleven individuals of *G. hortensis*. This beetle species might therefore function as a moderately specific propagule vector for *F. fomentarius,* providing targeted dispersal to fresh dead wood [52].

Certain other wood-decay fungi also exhibited moderately species-specific interactions with the wood-inhabiting beetles. For instance, the polypore *T. versicolor* was isolated from just two beetle species; *Glischrochilus quadripunctatus* (Linnaeus, 1758) and *Cis boleti* (Scopoli, 1763). Although *C. boleti* was only represented by a single individual in our dataset, its connection with *T. versicolor* is well known as it is a fungivore with larval development in this polypore [53]. There was also a notable connection between the wood-decay fungus *S. brinkmannii* and the beetle *E. coccineus*, as we isolated OTUs annotated as *S. brinkmannii* from nine of sixteen *E. coccineus* individuals. This beetle is a fungivore with larval development on fruit bodies of *C. purpureum* [54], and fifteen of sixteen *E. coccineus* individuals carried DNA from *C. purpureum*. Even so, a variety of wood-decay fungi were isolated from the *E. coccineus* beetles, indicating a high degree of omnivory in the adult stage. This species might therefore be an efficient dispersal vector for several wood-decay fungi, since it will ultimately seek out fresh dead wood on which it can find its host fungus.

Although it can be argued that the network between wood-living beetles and wood-decay fungi could be a food web without dispersal benefits to the fungi, the beetles were sampled from dead

wood that had recently been cut and placed in these forests. The only wood-decay agaricomycete fruit bodies present on the logs during beetle sampling for the current study belonged to the species *C. purpureum*, which correspondingly had a high abundance of sequences in many beetle samples. Excluding *C. purpureum* from the network with wood-decay fungi increased the estimate of specialization (H_2' = 0.29). None of the other wood-decay fungi had a similar abundance or frequency in the beetle samples, indicating that there were no fruit bodies of these species sufficiently close to the sampling sites to exert a similar influence on the samples. This strongly suggests that these fungal species were brought to the logs by the beetles.

It should be noted that certain aspects of network structure can be subject to strong spatial and temporal variability [48,55,56]. Our networks were based on pooled datasets of beetles sampled over two seasons in two different landscapes, but the necessity of sampling beetles individually resulted in a sample size that was too low to explore spatial and temporal variability in network structure. However, the distribution of sampled individuals was relatively even between landscapes, and the majority of individuals were sampled during the second year. Module structure for the network with wood-decay fungi did not seem to reflect differences in sampling place or time, nor in species phylogeny, as closely related beetle species like *G. hortensis* and *G. quadripunctatus* were placed in different modules due to different affiliations with wood-decay fungi. Rather, the modularity of the wood-decayer network might reflect converging preferences of the beetles or similar olfactory cues of the fungi [57-59].

Specialization of the networks might reflect selective pressures acting on the insect-fungus interactions. If the network between wood-inhabiting beetles and wood-decay fungi was based on spore feeding and dispersal, its degree of specialization might be constrained by the same factors that limit specialization of animal-mediated seed dispersal networks [60]. Optimal dispersal of both spores and seeds requires the propagule vector to move away from the source

and deliver the propagule not to a conspecific, but to a suitable habitat. The propagule source has no means to direct the vector, its only chance is to attract vectors that share its habitat. Fungal odour has been shown to attract several different species of beetles inhabiting dead wood [59,61,62], and odour release increases during sporulation [57]. *F. fomentarius* and certain other polypore species also aggregate spores on top of their fruit bodies, which are visited by several wood-inhabiting insects [49]. Aggregation of spores and increased odour emission during sporulation thus seem to function as attractants to wood-inhabiting insects, in much the same way as brightly coloured fruits attract seed dispersing animals. As such, there is a basis for selection favouring a certain degree of reciprocity and specialization between wood-decay fungi and insects. However, spore dispersal effectiveness would be low if the insects were highly specialized spore-feeders that only moved between sporulating fruit bodies, without dispersing the spores to unoccupied substrates. For seed dispersal, it has been shown that generalist frugivores can be very effective seed dispersers [63,64] and that species in highly diverse frugivore assemblages fulfil complementary roles [65,66]. These mechanisms promote diversified interactions and generalized dispersal systems [67], restraining the degree of specialization in seed dispersal networks [22,47,48] and possibly in the potential spore dispersal network in the present study.

In conclusion, our results demonstrate that there is a diversity of interactions in detritivore networks. We show that wood-inhabiting beetles and wood-decay fungi engage in moderately specialized interactions that might be based on spore feeding and dispersal. These interactions could have significant influence on fungal communities in dead wood [43,68], and thereby affect important ecosystem functions such as carbon sequestration and decomposition [33]. We therefore underline the importance of revealing hidden interactions between functionally important species groups such as fungi and invertebrates, and encourage the use of molecular methods to include microscopic organisms in future network studies [6].

Methods

This study is based on data from Jacobsen et al. [52], where a more detailed description of insect sampling, DNA-analysis and bioinformatics can be found.

We sampled beetles from recently cut logs of aspen (*Populus tremula* L.) that had been placed at eight sites in two production forests in south-eastern Norway; Losby forest holdings (Lat. 55.98, Long.10.68, 150–300 m.a.s.l.) and Løvenskiold-Vækerø (LV) forest holdings (Lat. 54.49, Long. 21.24, 200–500 m.a.s.l.). Both forest landscapes lie within the southern boreal vegetation zone [69] and consist mainly of spruce (*Picea abies* (L.) H.Karst.), with pine (*Pinus sylvestris* L.), birch (*Betula pubescens* Ehrh.) and aspen as subdominants.

Beetles were sampled individually with tweezers directly from the logs or from sticky traps on the logs, on eleven occasions during May to August in 2014 and 2015. The sticky traps were exposed for one or two days prior to insect sampling. The tweezers were sterilized with ethanol and fire between handling of each insect. The insects were killed by freezing at – 80ºC and identified to species or genus in a sterile environment using sterilized equipment. Insects that could not be confidently identified at least to genus by the first author (RMJ) were not analysed further (< 20 individuals). We selected 343 wood-inhabiting beetle individuals, i.e. species or genera with larval development either in dead wood or in fungal fruit bodies on dead wood [51,70], for analysis of fungal DNA.

DNA was extracted from the beetles following a modified CTAB protocol [71] and amplified by polymerase chain reaction (PCR) on an Eppendorf Thermal Cycler (VWR, Radnor, USA) using primers ITS4 [72] and fITS7 [73]. The PCR products were cleaned using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, USA) and pooled according to strength of the bands in gel electrophoresis. Pooled samples were cleaned with the ChargeSwitch® kit (Invitrogen, California, USA), DNA-concentration was measured with the Qubit® BR DNA kit (Invitrogen,

California, USA), and the sample quality was confirmed by Nanodrop™ (Thermo Fisher Scientific, Madison, USA). The samples were submitted to GATC Biotech for adaptor-ligation and Illumina HiSeq Rapid Run 300bp paired-end sequencing. Quality control and clustering of the resulting sequences was conducted with the SCATA pipeline (https://scata.mykopat.slu.se/, accessed 5th of July 2016). The sequences were subsampled to 10 000 per beetle sample prior to clustering. Taxonomy was assigned to the representative sequences of each OTU taking the top hit of a Basic Local Alignment Search Tool (BLASTn [74]) search against the NCBI (National Centre for Biotechnology Information) and UNITE [75] databases. OTUs with e-values < e-10 and bit-scores > 100 were annotated to species level if ITS homology was 100 - 98%, genus for 97.9 - 95%, family or order for 94.9 – 80%, phylum for 79.9 – 70% and "Fungus" for lower homology or e-values > e-10 and bit-scores < 100. Taxonomy was updated according to the taxonomic database Dyntaxa (https://www.dyntaxa.se/, accessed 24th of February 2017).

Classification of fungal functional groups

Fungal OTUs annotated to species or genus level and represented by at least 20 sequences were classified into functional groups based on the FUNGuild database [76] and various literature (see Supplementary Table S5, S7 and S9). Groups were non-overlapping. We chose to focus on three functional groups hypothesized to interact with the wood-inhabiting beetles with differing specificity:

1. Insect symbionts (Supplementary Table S4); this group included known insect symbionts such as *Ophiostoma spp.* or *Phialophoropsis spp.*, and yeast species isolated from insect guts in previous studies such as *Candida spp.* and *Cryptococcus spp.,* that were assumed to be endosymbionts.

2. Wood-decayers (Supplementary Table S6); this group included fungi in the class Agaricomycetes known to inhabit dead wood, in which the majority of species produce large

fruit bodies and large quantities of spores that attract spore-feeding insects during sporulation (e.g. [49,50]).

3. Plant pathogens (Supplementary Table S8); this group included pathogens of living plants, except species that are known to be insect symbionts such as *Ophiostoma spp.*

Statistics

All analyses were conducted in R version 3.3.2 [77].

The number of beetle individuals in which each fungal OTU occurred was used as a basis for quantitative networks. Network specialization was estimated by the standardized two-dimensional Shannon entropy H_2' [78] using the package bipartite v. 2.07 [79]. This index defines the degree of specialization in a network as the deviation from the expected probability distribution of interactions, which assumes that a species interacts with another species in proportion to its total frequency of occurrence in the network (i.e. marginal sums). We estimated the species-level specialization by the standardized Kullback-Leibler distance d' [78]. The species-level specialization index is defined as the deviation of a species from the expected utilization of potential partners according to their availability in terms of marginal sums. Both H_2' and d' range from 0 for most generalized to 1 for most specialized.

Modularity of the networks was estimated with the QuanBiMo algorithm developed by Dormann and Strauss [28] and implemented as function "computeModules" in the bipartite package. Modularity Q ranges from 0, meaning that there are no more links between species in a module than expected by chance, to 1 which signifies maximum modularity for the network. To estimate nestedness of the network, we used the weighted version of the nestedness metric based on overlap and decreasing fill, abbreviated WNODF [80]. This metric ranges from 0 for networks without nested structure, to 100 for perfectly nested networks.

We tested the statistical significance of the metrics for each network by simulating null models (n=1000). Null model P followed Patefield's algorithm [34] as implemented in the function "r2dtable" in R, which randomises network interactions with the restriction of fixed marginal sums (i.e. the sum of interactions for each species was kept constant). We also tested null model V, which in addition to fixed marginal sums also keeps connectance (i.e. proportion of realised links between species) of the network constant as proposed by Vazquez et al. [81] and implemented in function "quasiswap_count" in the vegan package v. 2.4-2. However, as the results were relatively similar between the two null models, only those based on null model P are discussed (for results based on null model V, see Supplementary Fig. S1). We performed two-sided tests of the network metric value against the distribution of the null model metric values.

Data Availability

Raw data (fastq-files), barcode and primer mapping file, OTU table and representative sequence files have been accessioned in Dryad with http://dx.doi.org/10.5061/dryad.3t2d4.

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Author Contributions

RMJ, TB, HK and AST conceived the idea and designed the methodology. RMJ did the field work, lab work, analyses and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Additional Information

The authors declare no competing financial interests.

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Appendix

Supplementary information for "Revealing hidden insect-fungus interactions in detritivore networks" by Rannveig M. Jacobsen, Anne Sverdrup-Thygeson, Håvard Kauserud and Tone Birkemoe

Table S1) Number of individual wood-inhabiting beetles with fungal DNA sampled from Losby or Løvenskiold-Vækerø (LV) study landscape, and number sampled in the first (2014) and second (2015) year after the logs had been cut and placed in the study landscapes.

Insect species	**Losby**	**LV**	**2014**	**2015**	**Sum ind.**
Acrulia inflata	2	4	0	6	6
Agathidium nigripenne	6	5	0	11	11
Agathidium sp.	1	4	0	5	5
Anisotoma humeralis	1	0	0	1	1
Anthobium sp.	5	0	0	5	5
Anthophagus sp.	3	6	3	6	9
Cis boleti	0	1	1	0	1
Endomychus coccineus	4	12	0	16	16
Epuraea sp.	6	0	6	0	6
Glischrochilus hortensis	24	24	0	48	48
Glischrochilus quadripunctatus	22	9	0	31	31
Oxypoda alternans	7	1	1	7	8
Quedius sp.	3	4	4	3	7
Rhizophagus sp.	9	14	0	23	23
Sepedophilus littoreus	0	3	1	2	3

Trypodendron domesticum	1	0	0	1	1
Xylita laevigata	4	2	3	3	6
Total	98	89	19	168	187

Table S2) Species-level specialization indices (d') for the wood-inhabiting beetle species in the network with wood-decay agaricomycete fungi. Mean, lower and upper tails with p-values from two-sided tests are based on null model P with fixed marginal sums.

Insect species	No. of ind.	d'	Mean simulated d'	Lower tail (2.5%)	Upper tail (97.5%)	P-value
Acrulia inflata	6	0.28	0.19	0.00	0.61	0.552
Agathidium nigripenne	11	0.13	0.14	0.03	0.27	0.906
Agathidium sp.	5	0.07	0.17	0.03	0.42	0.353
Anisotoma humeralis	1	0.24	0.24	0.00	0.76	0.816
Anthobium sp.	5	0.16	0.19	0.02	0.51	1.000
Anthophagus sp.	9	0.29	0.19	0.04	0.46	0.331
Cis boleti	1	0.63	0.33	0.00	1.00	0.439
Endomychus coccineus	16	0.25	0.12	0.05	0.19	0.005
Epuraea sp.	6	0.36	0.20	0.00	0.62	0.353
Glischrochilus hortensis	48	0.18	0.11	0.06	0.18	0.053
Glischrochilus quadripunctatus	31	0.17	0.12	0.06	0.21	0.247
Oxypoda alternans	8	0.63	0.32	0.00	1.00	0.413
Quedius sp.	7	0.31	0.19	0.02	0.54	0.369
Rhizophagus sp.	23	0.20	0.13	0.04	0.25	0.225
Sepedophilus littoreus	3	0.14	0.15	0.02	0.37	0.986
Xylita laevigata	6	0.22	0.15	0.02	0.35	0.361

Table S3) Species-level specialization indices (d') for the wood-decay agaricomycete fungus species in the network with wood-inhabiting beetles. Mean, lower and upper tails with p-values from two-sided tests are based on null model P with fixed marginal sums.

Fungus species	No. of seq.	d'	Mean simulated d'	Lower tail (2.5%)	Upper tail (97.5%)	P-value
Amylocystis lapponica	61	0.17	0.14	0.02	0.34	0.714
Antrodiella parasitica	51	0.71	0.21	0.00	0.71	0.109
Chondrostereum purpureum	35 589	0.09	0.06	0.02	0.12	0.259
Corticium roseum	22	0.44	0.19	0.00	0.71	0.437
Fibulorhizoctonia sp.	753	0.25	0.17	0.01	0.40	0.433
Fomes fomentarius	1308	0.38	0.12	0.04	0.22	0.001
Fomitopsis pinicola	225	0.15	0.15	0.02	0.35	0.942
Heterobasidion sp.	687	0.12	0.16	0.01	0.39	0.744
Kneiffiella abieticola	43	0.03	0.20	0.00	0.81	0.900
Kuehneromyces lignicola	29	0.31	0.23	0.00	0.52	0.576
Mycena rubromarginata	42	0.11	0.23	0.00	0.51	0.347
Peniophora sp.	105	0.21	0.17	0.01	0.39	0.570
Phlebia centrifuga	213	0.07	0.14	0.03	0.31	0.409
Pseudochaete intricata	24	0.03	0.20	0.00	0.71	0.854
Resinicium bicolor	58	0.18	0.23	0.00	0.52	0.890
Schizophyllum commune	28	0.04	0.20	0.00	0.71	1.000
Scopuloides rimosa	29	0.63	0.19	0.00	0.81	0.203
Sistotrema brinkmannii	2347	0.24	0.12	0.04	0.22	0.033

Stereum sp.	1526	0.31	0.19	0.00	0.71	0.690
Trametes versicolor	4826	0.45	0.17	0.01	0.42	0.029
Trechispora sp.	171	0.35	0.17	0.01	0.42	0.115
Trichaptum abietinum	59	0.06	0.15	0.02	0.36	0.395

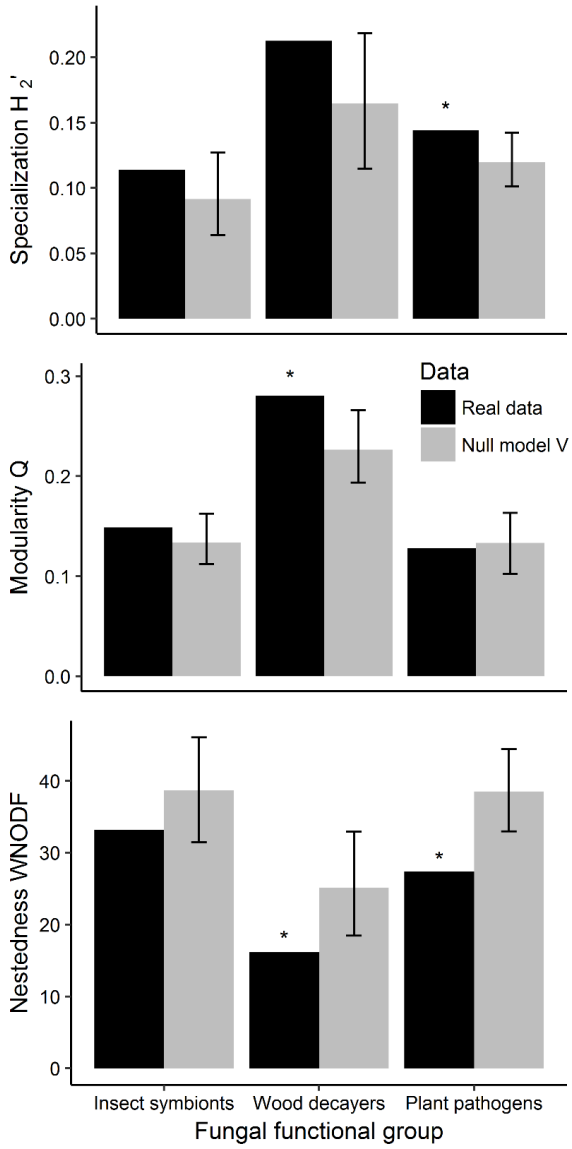

Fig. S1) Network specialization (H_2'), modularity (Q) and weighted nestedness (WNODF) for networks between wood-inhabiting beetles and the fungal functional groups insect symbionts, wood-decayers and plant pathogens. Black bars represent the original networks, while grey bars represent networks randomized with constant marginal sums and constant connectance according to null model V with 95% confidence intervals (CI). The weighted

connectance was 0.148 for the insect symbiont network, 0.14 for the wood-inhabiting agaricomycetes and 0.155 for the plant pathogen network. Asterisks (*) above the black bars signify significant (P-value < 0.05) differences between the original and the randomized networks.

Table S4) Network data for insect symbiont fungi isolated from wood-inhabiting beetles

Species/genus		Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi
Insects	Abbreviati	Can.sp	Can.fru	Can.kru	Can.mes	Can.sak	Can.sch	Can.tan	Can.try	Car.pol	Cry.sp
Acrulia inflata	Acr.inf	0	0	0	2	1	0	0	0	0	2
Agathidium nigripenne	Aga.nig	7	0	1	5	0	0	0	0	0	3
Agathidium sp	Aga.sp	1	0	0	0	0	0	0	0	0	1
Anisotoma humeralis	Ani.hum	0	0	0	0	0	0	0	0	0	1
Anthobium sp	Anb.sp	4	0	0	2	0	0	0	0	0	4
Anthophagus sp	Ant.sp	5	0	0	0	0	0	0	0	0	4
Cis boleti	Cis.bol	0	0	0	0	0	0	0	0	0	0
Endomychus coccineus	End.coc	8	0	2	7	0	0	0	0	1	5
Epuraea sp	Epu.sp	5	0	0	0	0	0	1	0	0	1
Glischrochilus hortensis	Gli.hor	48	1	1	45	0	1	0	1	1	42
Glischrochilus quadripunctatus	Gli.qua	30	3	0	30	0	1	0	3	2	29
Oxypoda alternans	Oxy.alt	1	0	0	1	0	0	2	0	0	2
Quedius sp	Que.sp	3	0	0	3	0	0	0	0	1	2
Rhizophagus sp	Rhi.sp	23	0	0	17	0	0	0	0	0	11
Sepedophilus littoreus	Sep.lit	2	0	0	1	0	0	0	0	0	2
Trypodendron domesticum	Try.dom	0	0	0	1	0	0	0	1	0	0
Xylita laevigata	Xyl.lae	1	0	0	0	0	0	0	0	0	2

Species/genus		Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi
	Abbreviati	Cry.ade	Cry.amy	Cry.cyl	Cry.fol	Cry.hue	Cry.ste	Cry.ter	Cry.vic	Cry.wie	Deb.han	Fil.uni	Fus.sol
Insects	Acr.inf	0	0	0	0	0	0	0	1	0	0	0	0
Insects	Aga.nig	0	0	0	0	0	0	1	0	0	2	0	0
Insects	Aga.sp	1	0	1	0	0	1	0	1	0	0	0	0
Insects	Ani.hum	0	0	0	0	0	0	0	0	0	0	0	0
Insects	Anb.sp	0	0	0	0	2	0	0	1	1	0	0	0
Insects	Ant.sp	1	1	0	0	1	0	0	1	0	0	2	0
Insects	Cis.bol	0	0	0	0	0	0	0	0	0	0	1	0
Insects	End.coc	1	0	1	1	2	0	0	3	0	3	0	1
Insects	Epu.sp	0	0	0	0	2	0	0	4	1	0	0	0
Insects	Gli.hor	1	1	5	1	8	0	1	12	1	4	0	1
Insects	Gli.qua	4	2	8	0	3	0	1	7	2	3	0	0
Insects	Oxy.alt	1	1	0	0	0	0	0	0	0	0	0	0
Insects	Que.sp	0	0	0	0	0	0	0	0	0	0	1	0
Insects	Rhi.sp	2	0	1	0	0	0	1	0	0	1	0	0
Insects	Sep.lit	1	0	1	0	1	0	0	0	0	0	0	0
Insects	Try.dom	0	0	0	0	0	0	0	0	0	0	0	0
Insects	Xyl.lae	0	0	0	0	1	0	0	2	0	0	0	0

Species/ge	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi
Abbreviati	Leu.sp	Oph.can	Oph.kar	Oph.pic	Oph.que	Oph.tet	Phi.fer	Tap.sp	Tre.glo	Tre.sp	Tre.aur	Tre.fol	Tri.sp
Acr.inf	0	0	0	0	0	0	0	0	0	0	0	0	0
Aga.nig	0	2	0	0	0	0	0	1	0	0	0	0	0
Aga.sp	0	0	0	0	0	0	0	1	0	0	0	0	0
Ani.hum	0	0	0	0	0	0	0	0	0	0	0	0	0
Anb.sp	1	0	0	0	0	0	0	2	1	0	0	1	0
Ant.sp	1	0	0	0	0	0	0	1	0	0	1	0	0
Cis.bol	0	0	0	0	0	0	0	0	0	0	0	0	0
End.coc	0	0	0	0	0	0	0	2	0	2	0	0	0
Epu.sp	2	2	2	2	1	0	1	0	0	0	0	0	0
Gli.hor	9	3	1	0	0	0	1	6	2	2	2	3	2
Gli.qua	9	15	2	2	0	4	3	5	0	0	0	0	0
Oxy.alt	0	1	0	0	0	0	0	0	0	0	0	0	0
Que.sp	0	2	0	1	0	1	0	0	0	0	0	0	0
Rhi.sp	0	3	0	1	0	0	0	2	0	0	0	0	1
Sep.lit	0	1	0	0	0	0	1	0	0	0	0	0	0
Try.dom	0	0	0	0	0	0	1	1	0	0	0	1	0
Xyl.lae	0	0	0	0	0	0	1	1	1	0	0	0	0

Table S5) Abbreviations and references for insect symbiont fungi

Insect symbiont fungi	Abbreviation
Candida	Can.sp
Candida fructus	Can.fru
Candida kruisii	Can.kru
Candida mesenterica	Can.mes
Candida sake	Can.sak
Candida schatavii	Can.sch
Candida tanzawaensis	Can.tan
Candida trypodendroni	Can.try
Carcinomyces polyporina	Car.pol
Cryptococcus	Cry.sp
Cryptococcus adeliensis	Cry.ade
Cryptococcus aff.amylolyticus	Cry.amy
Cryptococcus cylindricus	Cry.cyl
Cryptococcus foliicola	Cry.fol
Cryptococcus huempii	Cry.hue
Cryptococcus stepposus	Cry.ste
Cryptococcus terricola	Cry.ter
Cryptococcus victoriae	Cry.vic
Cryptococcus wieringae	Cry.wie
Debaryomyces hansenii	Deb.han
Filobasidium uniguttulatum	Fil.uni
Fusarium solani	Fus.sol
Leucosporidium	Leu.sp
Ophiostoma canum	Oph.can
Ophiostoma karelicum	Oph.kar
Ophiostoma piceae	Oph.pic
Ophiostoma quercus	Oph.que
Ophiostoma tetropii	Oph.tet
Phialophoropsis ferruginea	Phi.fer
Taphrina	Tap.sp
Tremella globospora	Tre.glo

Species	Reference
Candida	Gibson & Hunter 2010; Klimaszewski et al 2013; Grünwald et al 2010; Houseknecht et al 2011; Hu et al 2015; Zhang et al 2003; Nguyen et al 2006; Suh et al 2004; Suh et al 2005; Suh et al 2006; Suh et al 2013
Candida fructus	Gibson & Hunter 2010; Klimaszewski et al 2013; Grünwald et al 2010; Houseknecht et al 2011; Hu et al 2015; Zhang et al 2003; Nguyen et al 2006; Suh et al 2004; Suh et al 2005; Suh et al 2006; Suh et al 2013
Candida kruisii	Gibson & Hunter 2010; Klimaszewski et al 2013; Grünwald et al 2010; Houseknecht et al 2011; Hu et al 2015; Zhang et al 2003; Nguyen et al 2006; Suh et al 2004; Suh et al 2005; Suh et al 2006; Suh et al 2013
Candida mesenterica	Gibson & Hunter 2010; Klimaszewski et al 2013; Grünwald et al 2010; Houseknecht et al 2011; Hu et al 2015; Zhang et al 2003; Nguyen et al 2006; Suh et al 2004; Suh et al 2005; Suh et al 2006; Suh et al 2013
Candida sake	Gibson & Hunter 2010; Klimaszewski et al 2013; Grünwald et al 2010; Houseknecht et al 2011; Hu et al 2015; Zhang et al 2003; Nguyen et al 2006; Suh et al 2004; Suh et al 2005; Suh et al 2006; Suh et al 2013
Candida schatavii	Gibson & Hunter 2010; Klimaszewski et al 2013; Grünwald et al 2010; Houseknecht et al 2011; Hu et al 2015; Zhang et al 2003; Nguyen et al 2006; Suh et al 2004; Suh et al 2005; Suh et al 2006; Suh et al 2013
Candida tanzawaensis	Gibson & Hunter 2010; Klimaszewski et al 2013; Grünwald et al 2010; Houseknecht et al 2011; Hu et al 2015; Zhang et al 2003; Nguyen et al 2006; Suh et al 2004; Suh et al 2005; Suh et al 2006; Suh et al 2013
Candida trypodendroni	Gibson & Hunter 2010; Klimaszewski et al 2013; Grünwald et al 2010; Houseknecht et al 2011; Hu et al 2015; Zhang et al 2003; Nguyen et al 2006; Suh et al 2004; Suh et al 2005; Suh et al 2006; Suh et al 2013
Carcinomyces polyporina	Suh et al 2005
Cryptococcus	Suh et al 2005; Zhang et al 2003
Cryptococcus adeliensis	Suh et al 2005; Zhang et al 2003
Cryptococcus aff.amylolyticus	Suh et al 2005; Zhang et al 2003
Cryptococcus cylindricus	Suh et al 2005; Zhang et al 2003
Cryptococcus foliicola	Suh et al 2005; Zhang et al 2003
Cryptococcus huempii	Suh et al 2005; Zhang et al 2003
Cryptococcus stepposus	Suh et al 2005; Zhang et al 2003
Cryptococcus terricola	Suh et al 2005; Zhang et al 2003

Tremella	Tre.sp
Tremella aurantialba	Tre.aur
Tremella foliacea	Tre.fol
Trichosporon	Tri.sp

Cryptococcus victoriae	Suh et al 2005; Zhang et al 2003
Cryptococcus wieringae	Suh et al 2005; Zhang et al 2003
Debaryomyces hansenii	Suh et al 2005
Filobasidium uniguttulatum	Suh et al 2005; Zhang et al 2003
Fusarium solani	Six 2012
Leucosporidium	Suh et al 2005
Ophiostoma canum	Gibson & Hunter 2010; Kirisits 2007
Ophiostoma karelicum	Gibson & Hunter 2010; Kirisits 2007
Ophiostoma piceae	Six 2012; Kirisits 2007
Ophiostoma quercus	Gibson & Hunter 2010; Kirisits 2007
Ophiostoma tetropii	Gibson & Hunter 2010; Kirisits 2007
Phialophoropsis ferruginea	Vega & Blackwell 2005; Kirisits 2007
Taphrina	Suh et al 2005
Tremella	Suh et al 2005
Tremella aurantialba	Suh et al 2005
Tremella globospora	Suh et al 2005

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Table S6) Network data for wood-decay fungi in the class Agaricomycetes isolated from wood-inhabiting beetles

Species/genus		Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi
Insects	**Abbreviation**	Amy.lap	Ant.par	Cho.pur	Cor.ros	Fib.sp	Fom.fom	Fom.pin	Kne.abi	Kue.lig
Acrulia inflata	Acr.inf	0	1	2	0	0	0	0	0	0
Agathidium nigripenne	Aga.nig	1	0	8	0	0	0	0	0	0
Agathidium sp	Aga.sp	0	0	3	0	1	0	0	0	0
Anisotoma humeralis	Ani.hum	0	0	0	0	0	1	0	0	0
Anthobium sp	Anb.sp	0	0	3	0	0	0	0	0	1
Anthophagus sp	Ant.sp	1	0	1	0	0	0	1	0	0
Cis boleti	Cis.bol	0	0	0	0	0	0	0	0	0
Endomychus coccineus	End.coc	1	0	15	0	0	0	0	1	1
Epuraea sp	Epu.sp	1	0	0	0	0	1	0	0	0
Glischrochilus hortensis	Gli.hor	0	0	16	0	2	11	2	0	0
Glischrochilus quadripunctatus	Gli.qua	2	0	21	0	0	1	2	0	0
Oxypoda alternans	Oxy.alt	0	0	0	0	0	0	0	0	0
Quedius sp	Que.sp	0	0	1	0	0	0	0	0	0
Rhizophagus sp	Rhi.sp	1	0	7	0	0	0	0	0	1
Sepedophilus littoreus	Sep.lit	0	0	2	0	1	0	1	0	0
Trypodendron domesticum	Try.dom	0	0	0	0	0	0	0	0	0
Xylita laevigata	Xyl.lae	0	0	2	1	1	2	1	0	0

	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi
Insects	Myc.rub	Peni.sp	Phl.cen	Pse.int	Res.bic	Sch.com	Sco.rim	Sis.bri	Ste.sp	Tra.ver	Tre.sp	Tri.abi	Het.sp
Acr.inf	0	0	0	0	0	0	0	0	0	0	0	0	0
Aga.nig	0	0	0	0	0	0	0	0	0	0	1	1	0
Aga.sp	0	0	1	0	0	0	0	1	0	0	0	0	0
Ani.hum	0	0	0	0	0	0	0	1	0	0	0	0	0
Anb.sp	0	0	0	0	0	0	0	0	0	0	0	0	0
Ant.sp	0	0	0	0	1	0	0	0	0	0	1	0	0
Cis.bol	0	0	0	0	0	0	0	0	0	1	0	0	0
End.coc	1	4	2	1	1	0	0	9	0	0	0	1	1
Epu.sp	0	0	0	0	0	0	0	0	0	0	0	0	1
Gli.hor	2	0	2	0	1	0	0	2	0	0	1	2	1
Gli.qua	0	1	1	0	0	1	0	1	0	4	0	1	1
Oxy.alt	0	0	0	0	0	0	0	0	0	0	1	0	0
Que.sp	0	0	0	0	0	0	1	1	0	0	0	1	0
Rhi.sp	0	0	2	0	0	0	0	0	1	0	1	0	0
Sep.lit	0	0	0	0	0	0	0	2	0	0	0	0	1
Try.dom	0	0	0	0	0	0	0	0	0	0	0	0	0
Xyl.lae	0	0	0	0	0	0	0	0	0	0	0	0	1

Table S7) Abbreviations and references for wood-decay fungi

Wood-living fungi	Abbreviation	Guild reference
Amylocystis lapponica	Amy.lap	Ryvarden & Melo 2014
Antrodiella parasitica	Ant.par	Ryvarden & Melo 2014
Chondrostereum purpureum	Cho.pur	Bernicchia & Gorjón 2010
Corticium roseum	Cor.ros	Bernicchia & Gorjón 2010
Fibulorhizoctonia	Fib.sp	Nguyen et al 2016
Fomes fomentarius	Fom.fom	Ryvarden & Melo 2014
Fomitopsis pinicola	Fom.pin	Ryvarden & Melo 2014
Kneiffiella abieticola	Kne.abi	Ottosson et al 2015; Nguyen et al 2016
Kuehneromyces lignicola	Kue.lig	Ottosson et al 2015; Nguyen et al 2016
Mycena rubromarginata	Myc.rub	Knudsen & Vesterholt 2012
Peniophora	Peni.sp	Andreasen & Hallenberg 2009; Nguyen et al 2016
Phlebia centrifuga	Phl.cen	Ryvarden & Melo 2014
Pseudochaete intricata	Pse.int	Nguyen et al 2016
Resinicium bicolor	Res.bic	Ottosson et al 2015
Schizophyllum commune	Sch.com	Nguyen et al 2016
Scopuloides rimosa	Sco.rim	Nguyen et al 2016
Sistotrema brinkmannii	Sis.bri	Bernicchia & Gorjón 2010; Nguyen et al 2016
Stereum	Ste.sp	Nguyen et al 2016
Trametes versicolor	Tra.ver	Ryvarden & Melo 2014
Trechispora	Tre.sp	Nguyen et al 2016
Trichaptum abietinum	Tri.abi	Ryvarden & Melo 2014
Heterobasidion	Het.sp	Nguyen et al 2016

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Table S8) Network data for plant pathogenic fungi isolated from wood-inhabiting beetles

Species/genus		Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi
Insects	**Abbreviation**	Alt.alt	Alt.inf	Bot.cin	Cer.par	Cyt.chr	Dac.dim	Dev.sp	Exo.sp	Exo.are
Acrulia inflata	Acr.inf	1	0	0	0	0	0	0	1	4
Agathidium nigripenne	Aga.nig	0	0	0	0	0	3	0	0	1
Agathidium sp	Aga.sp	0	0	0	0	0	0	0	1	0
Anisotoma humeralis	Ani.hum	0	0	0	0	1	0	0	0	0
Anthobium sp	Anb.sp	0	0	1	0	0	1	0	2	0
Anthophagus sp	Ant.sp	1	0	1	0	0	0	0	3	1
Cis boleti	Cis.bol	0	0	0	0	0	0	0	0	0
Endomychus coccineus	End.coc	0	1	4	0	0	9	0	6	1
Epuraea sp	Epu.sp	0	0	1	1	0	0	0	2	0
Glischrochilus hortensis	Gli.hor	0	1	2	0	1	4	0	8	6
Glischrochilus quadripunctatus	Gli.qua	1	0	1	0	0	7	0	4	2
Oxypoda alternans	Oxy.alt	0	1	0	0	0	0	0	1	1
Quedius sp	Que.sp	0	0	1	1	0	0	0	1	0
Rhizophagus sp	Rhi.sp	0	0	0	0	0	1	0	1	0
Sepedophilus littoreus	Sep.lit	0	0	0	0	0	0	0	2	0
Trypodendron domesticum	Try.dom	0	0	0	0	0	0	0	0	0
Xylita laevigata	Xyl.lae	0	1	1	0	0	0	1	3	1

Species/genus		Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi
	Abbreviati	Exo.bis	Exo.mac	Fus.cil	Fus.mes	Fus.tri	Gro.cuc	Gro.fra	Hor.sp	Hya.sp	Ily.hub	Lepg.sp	Lepg.pir
Insects	Acr.inf	1	0	0	1	0	0	0	0	0	0	0	0
Insects	Aga.nig	0	0	0	7	0	0	0	0	0	0	0	0
Insects	Aga.sp	0	0	0	1	0	0	0	0	0	0	0	0
Insects	Ani.hum	1	0	0	1	0	0	0	0	0	0	0	0
Insects	Anb.sp	2	0	0	2	0	0	0	0	0	0	0	0
Insects	Ant.sp	1	0	0	5	0	0	0	2	0	0	0	0
Insects	Cis.bol	0	0	0	0	0	0	0	0	0	0	0	0
Insects	End.coc	0	0	0	16	2	0	0	0	2	1	0	3
Insects	Epu.sp	1	0	0	0	0	0	0	0	0	0	0	0
Insects	Gli.hor	7	0	3	48	2	1	0	3	2	6	0	2
Insects	Gli.qua	4	0	0	27	1	3	4	4	1	2	0	0
Insects	Oxy.alt	1	0	0	1	0	0	0	1	0	0	0	0
Insects	Que.sp	1	0	0	4	0	0	0	0	0	0	1	1
Insects	Rhi.sp	2	0	0	18	1	0	0	0	0	2	0	2
Insects	Sep.lit	1	0	0	0	0	0	0	0	0	0	0	1
Insects	Try.dom	0	0	0	0	0	0	0	0	0	0	0	0
Insects	Xyl.lae	1	1	0	1	0	0	0	0	0	0	0	0

Species/genus		Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi
	Abbreviati	Lep.sp	Lib.sp	Lir.yun	Lop.con	Lop.pic	Mel.sp	Mol.sp	Mon.sp	Mycc.ace	Neo.sp	Neo.fuc	Neo.obt
Insects	Acr.inf	0	0	0	1	4	0	0	0	0	0	0	0
Insects	Aga.nig	1	0	0	0	1	1	0	0	0	2	0	1
Insects	Aga.sp	0	0	0	0	1	0	0	0	0	1	0	1
Insects	Ani.hum	0	0	0	0	0	0	0	0	0	0	0	0
Insects	Anb.sp	2	1	0	0	5	0	1	0	0	0	1	0
Insects	Ant.sp	0	0	0	0	1	0	1	0	0	0	0	0
Insects	Cis.bol	0	0	0	0	0	0	0	0	0	0	0	0
Insects	End.coc	7	0	2	0	13	0	1	0	1	4	0	3
Insects	Epu.sp	0	0	1	0	3	0	0	0	0	0	0	0
Insects	Gli.hor	13	0	6	0	35	0	0	0	0	7	0	8
Insects	Gli.qua	7	0	0	0	22	0	0	0	0	4	2	7
Insects	Oxy.alt	1	0	0	0	1	0	0	0	0	0	0	0
Insects	Que.sp	0	0	3	0	5	0	0	1	0	0	0	0
Insects	Rhi.sp	3	0	0	0	10	0	0	0	0	2	0	1
Insects	Sep.lit	0	0	0	0	2	0	0	0	0	0	0	0
Insects	Try.dom	0	0	0	0	0	0	0	0	0	0	0	0
Insects	Xyl.lae	0	0	0	0	3	1	0	0	0	0	0	0

Species/genus		Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi
	Abbreviati	Neo.pun	Par.put	Pez.mel	Phac.lac	Phae.gae	Pod.sp	Pod.cla	Pol.sp	Pow.sp	Pro.sp	Pse.fra	Puc.are
Insects	Acr.inf	0	0	0	0	0	1	1	1	0	3	1	1
Insects	Aga.nig	0	1	0	0	0	0	0	1	0	2	0	1
Insects	Aga.sp	0	0	0	0	0	1	1	0	0	1	0	0
Insects	Ani.hum	0	0	0	0	0	0	0	0	0	1	0	0
Insects	Anb.sp	0	0	0	0	0	1	1	1	0	2	0	0
Insects	Ant.sp	0	0	0	0	1	0	0	1	1	3	2	0
Insects	Cis.bol	0	0	0	0	0	0	0	0	0	0	0	0
Insects	End.coc	1	0	1	2	0	0	0	0	2	1	4	0
Insects	Epu.sp	0	0	0	0	0	0	0	0	1	0	1	0
Insects	Gli.hor	1	0	0	5	0	0	0	2	6	15	7	1
Insects	Gli.qua	2	0	0	7	1	0	0	1	3	2	3	1
Insects	Oxy.alt	0	0	0	0	1	0	0	0	0	1	0	0
Insects	Que.sp	0	0	0	0	0	0	0	1	0	1	3	0
Insects	Rhi.sp	0	0	0	2	0	0	1	0	2	2	1	0
Insects	Sep.lit	0	0	0	0	0	0	0	0	0	0	0	0
Insects	Try.dom	0	0	0	1	0	0	0	0	0	0	0	0
Insects	Xyl.lae	0	0	0	0	0	0	0	1	0	3	2	2

Species/genus		Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi	Fungi
	Abbreviati	Rami.pin	Ramu.stel	Rhi.sp	Rhi.kal	Scl.sp	Sep.rib	Sep.sp	Sep.tan	Sir.sp	Spi.pse	Spo.bac	Sta.sp
Insects	Acr.inf	0	0	0	1	0	0	0	0	0	0	0	0
Insects	Aga.nig	0	0	1	1	1	0	0	0	1	0	0	0
Insects	Aga.sp	0	0	1	0	0	1	0	0	0	0	0	0
Insects	Ani.hum	0	0	0	0	0	0	0	0	0	1	0	0
Insects	Anb.sp	0	0	2	2	0	1	0	0	0	1	0	0
Insects	Ant.sp	0	1	1	4	1	2	0	0	0	1	0	0
Insects	Cis.bol	0	0	0	0	0	0	0	0	0	0	0	0
Insects	End.coc	0	1	3	5	1	1	0	1	0	0	0	2
Insects	Epu.sp	0	0	0	2	0	0	0	0	0	0	1	0
Insects	Gli.hor	0	4	35	15	13	0	0	0	1	7	0	1
Insects	Gli.qua	0	2	26	9	7	1	0	0	0	4	0	0
Insects	Oxy.alt	0	0	0	0	0	0	0	0	1	0	0	0
Insects	Que.sp	0	2	3	1	1	0	0	0	0	1	0	0
Insects	Rhi.sp	0	1	12	3	2	0	0	0	0	2	0	0
Insects	Sep.lit	0	0	2	0	0	0	1	0	0	0	0	0
Insects	Try.dom	0	0	0	0	0	0	0	0	0	0	0	0
Insects	Xyl.lae	1	3	0	3	0	0	0	0	1	0	0	0

Species/genus		Fungi	Fungi	Fungi
	Abbreviati	Try.pin	Ven.ina	Ver.sp
Insects	Acr.inf	0	0	0
Insects	Aga.nig	1	0	0
Insects	Aga.sp	0	0	0
Insects	Ani.hum	0	0	0
Insects	Anb.sp	0	0	0
Insects	Ant.sp	0	0	0
Insects	Cis.bol	0	0	0
Insects	End.coc	0	0	3
Insects	Epu.sp	0	0	0
Insects	Gli.hor	3	3	0
Insects	Gli.qua	4	0	0
Insects	Oxy.alt	0	1	0
Insects	Que.sp	0	0	0
Insects	Rhi.sp	1	0	0
Insects	Sep.lit	0	0	0
Insects	Try.dom	0	0	0
Insects	Xyl.lae	1	1	0

Table S9) Abbreviations and references for plant pathogenic fungi.

Plant pathogen fungi	Abbreviation	Guild reference
Alternaria alternata	Alt.alt	Tedersoo et al. 2014
Alternaria infectoria	Alt.inf	Tedersoo et al. 2014
Botrytis cinerea	Bot.cin	Tedersoo et al. 2014
Ceratocystis paradoxa	Cer.par	Tedersoo et al. 2014
Cytospora chrysosperma	Cyt.chr	Tedersoo et al. 2014
Dactylaria dimorphospora	Dac.dim	Nguyen et al. 2016
Devriesia	Dev.sp	Tedersoo et al. 2014
Exobasidium	Exo.sp	Tedersoo et al. 2014
Exobasidium arescens	Exo.are	Tedersoo et al. 2014
Exobasidium bisporum	Exo.bis	Tedersoo et al. 2014
Exobasidium maculosum	Exo.mac	Tedersoo et al. 2014
Fusarium ciliatum	Fus.cil	Tedersoo et al. 2014
Fusarium merismoides	Fus.mes	Tedersoo et al. 2014
Fusarium tricinctum	Fus.tri	Tedersoo et al. 2014
Grosmannia cucullata	Gro.cuc	Tedersoo et al. 2014
Grosmannia francke-grosmanniae	Gro.fra	Tedersoo et al. 2014
Hortaea	Hor.sp	Tedersoo et al. 2014
Hyalopeziza	Hya.sp	Nguyen et al. 2016
Ilyonectria hubeiensis	Ily.hub	Tedersoo et al. 2014
Leptographium	Lepg.sp	Nguyen et al. 2016
Leptographium piriforme	Lepg.pir	Nguyen et al. 2016
Leptosphaeria	Lep.sp	Tedersoo et al. 2014
Libertella	Lib.sp	Nguyen et al. 2016
Lirula yunnanensis	Lir.yun	Nguyen et al. 2016
Lophodermium conigenum	Lop.con	Tedersoo et al. 2014
Lophodermium piceae	Lop.pic	Tedersoo et al. 2014
Melampsora	Mel.sp	Tedersoo et al. 2014
Mollisia	Mol.sp	Tedersoo et al. 2014
Monilinia	Mon.sp	Tedersoo et al. 2014
Mycocentrospora acerina	Mycc.ace	Nguyen et al. 2016
Neonectria	Neo.sp	Tedersoo et al. 2014
Neonectria fuckeliana	Neo.fuc	Tedersoo et al. 2014
Neonectria obtusispora	Neo.obt	Tedersoo et al. 2014
Neonectria punicea	Neo.pun	Tedersoo et al. 2014
Parascedosporium putredinis	Par.put	Nguyen et al. 2016
Pezicula melanigena	Pez.mel	Tedersoo et al. 2014
Phacidium lacerum	Phac.lac	Nguyen et al. 2016
Phaeocryptopus gaeumannii	Phae.gae	Nguyen et al. 2016
Podosphaera	Pod.sp	Tedersoo et al. 2014
Podosphaera clandestina	Pod.cla	Tedersoo et al. 2014
Polyscytalum	Pol.sp	Tedersoo et al. 2014
Powellomyces	Pow.sp	Nguyen et al. 2016
Protomyces	Pro.sp	Tedersoo et al. 2014
Pseudocercosporella fraxini	Pse.fra	Tedersoo et al. 2014
Pucciniastrum areolatum	Puc.are	Tedersoo et al. 2014
Ramichloridium pini	Rami.pin	Tedersoo et al. 2014
Ramularia stellenboschensis	Ramu.stel	Tedersoo et al. 2014
Rhizosphaera	Rhi.sp	Nguyen et al. 2016

Rhizosphaera kalkhoffii	Rhi.kal	Nguyen et al. 2016
Scleroconidioma	Scl.sp	Tedersoo et al. 2014
Septoria ribis	Sep.rib	Tedersoo et al. 2014
Septoria	Sep.sp	Tedersoo et al. 2014
Septoria tanaceti	Sep.tan	Tedersoo et al. 2014
Sirococcus	Sir.sp	Tedersoo et al. 2014
Spizellomyces pseudodichotomus	Spi.pse	Tedersoo et al. 2014
Sporendocladia bactrospora	Spo.bac	Tedersoo et al. 2014
Stagonospora	Sta.sp	Tedersoo et al. 2014
Tryblidiopsis pinastri	Try.pin	Tedersoo et al. 2014
Venturia inaequalis	Ven.ina	Nguyen et al. 2016
Verticillium	Ver.sp	Tedersoo et al. 2014

Full reference

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q. & Suija, A. (2014) Global diversity and geography of soil fungi. Science, 346.

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PAPER IV

Running head: Invertebrate exclusion affects fungi

Exclusion of invertebrates influences saprotrophic fungal community and wood decay rate in an experimental field study

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Abstract

The effect of higher trophic levels on microbial decomposer communities and rate of decomposition is poorly understood. We conducted an exclusion experiment to test the effect of invertebrates on fungal decomposer communities in dead wood, repeated at 30 sites in two landscapes, and measured wood density to assess effect on decay rate. Invertebrates were excluded from recently cut logs by cages with a 1 mm mesh net, and fungal communities in caged logs were compared to logs accessible to invertebrates by DNA metabarcoding analyses. Accessible logs included control logs, cage control logs and positive control logs. We found that exclusion of invertebrates had a significant effect on fungal community composition. For example, the wood decay fungi *Trametes versicolor* and *T. ochracea* were significantly more abundant in accessible logs than in caged logs. Caged logs also had significantly higher wood density after two years, indicating lower rates of wood decay. Our results thereby indicate that

invertebrates influence not only the composition of saprotrophic fungi in dead wood, but also their function in the ecosystem.

Key words

exclusion experiment; invertebrates; insects; saproxylic; fungi; wood decay; decomposition; saprotrophs; dead wood; community composition; DNA; high-throughput sequencing

1. Introduction

The process of decomposition is integral to the functioning of all ecosystems. As such, understanding the factors that determine composition of saprotrophic communities and how this influences ecosystem processes is an important task for ecologists. Decomposer community composition has been shown to influence rate of decomposition and nutrient cycling, resulting in indirect effects of decomposer organisms on plant diversity and primary production (Wagg et al. 2014; Wardle et al. 2004). Carbon cycling (Clemmensen et al. 2015; van der Wal et al. 2015) and denitrification (Cavigelli & Robertson 2000) can also be affected by composition of decomposer communities, thereby influencing greenhouse gas emissions.

In terrestrial ecosystems, bacteria and fungi form the driving force of decomposition (Boer et al. 2005). Fungi are especially important for decomposition of plant material, due to their efficient enzymatic machinery for breakdown of recalcitrant components such as cellulose and lignin (Boer et al. 2005; Cornwell et al. 2009; Floudas et al. 2012). The ability to decompose lignin is restricted to certain Basidiomycetes and xylariaceous Ascomycetes, and these taxa are therefore integral to nutrient cycling and carbon dynamics in forest ecosystems (van der Wal et al. 2013). Fungi and invertebrates are the dominant eukaryote taxa colonizing dead wood in terms of both abundance and species richness (Stokland et al. 2012), and are the key agents of wood decomposition (Bradford et al. 2014; Cornwell et al. 2009; Kahl et al. 2017). However,

with the exception of termites, the direct effect of invertebrates on wood decay seems to be minor relative to that of fungi (Boddy 2001; Ulyshen et al. 2014; Ulyshen 2016; van der Wal et al. 2015). As such, community composition of saprotrophic fungi in dead wood has been shown to significantly affect rate of wood decay (Dickie et al. 2012; Kubartová et al. 2015; van der Wal et al. 2015).

Competitive interactions are important in shaping fungal communities (Boddy 2000; Fukami et al. 2010; Hiscox & Boddy 2017), but recent studies have shown that preferential grazing by macroinvertebrates can affect the competitive hierarchy of fungi in soil (A'Bear et al. 2013; Crowther et al. 2011). Such top-down effects on fungal community composition have also been found to affect rate of decomposition (reviewed in A'Bear et al. 2014). However, top-down effects on fungi have mainly been studied in soil microcosmoses, and the significance under realistic conditions in the field remains unclear (A'Bear et al. 2014). Field studies have indicated that invertebrates might also affect saprotrophic fungi by altering the substrate (Jacobsen et al. 2015; Leach et al. 1937; Weslien et al. 2011) or dispersing fungal propagules (Jacobsen et al. 2017; Lilleskov & Bruns 2005; Seres et al. 2007; Strid et al. 2014), but the effect on the fungal community as a whole is rarely explored (but see Ulyshen et al. 2016; Strid et al. 2014; Müller et al. 2002).

Our aim for this study was to experimentally test the influence of invertebrates on composition of fungal communities in dead wood and on wood decay rate, two years after tree death. Community assembly in the first years after tree death is especially interesting as arrival order has been shown to influence community composition of wood saprotrophic fungi and wood decay rate (Dickie et al. 2012; Fukami et al. 2010; Hiscox et al. 2015). The experimental treatments included; (i) exclusion of invertebrates larger than 1 mm from logs by fine mesh cages, (ii) control logs without cages, (iii) control logs with cages that did not exclude invertebrates (to control for microclimatic effects of the cage) and (iv) positive controls where

logs were baited with ethanol to attract wood-inhabiting invertebrates (Allison et al. 2004; Bouget et al. 2009; Montgomery & Wargo 1983). These treatments were hypothesized to form a gradient, where logs in cages would be colonized by very few invertebrates (i.e. only those smaller than 1 mm), control logs and cage control logs would be subject to natural invertebrate colonization, while ethanol-baited logs would be colonized by more invertebrates than the other logs. If the cage per se had a stronger effect on fungal community composition than exclusion of invertebrates, we expected that the fungal community of the cage control treatment would be similar to the cage treatment.

To our knowledge, this study is the first to experimentally test the effect of invertebrate exclusion on both wood decay and fungal community composition as described by DNA metabarcoding, thereby potentially linking these two responses. Our main hypotheses were as follows; exclusion of invertebrates larger than 1 mm alters (1) the composition of fungal communities in dead wood and (2) rate of wood decay, in comparison with dead wood that is accessible to invertebrates.

2. Methods

In March 2014, 17 aspen (*Populus tremula* L.) trees from the same stand in Ås municipality in Norway (Lat. 59.66, Long. 10.79, 92 m.a.s.l.) were felled and cut into 1 meter long logs, with diameters on average 27.6 cm (range 20.5 - 36.4 cm). Aspen was chosen due to its high diversity of wood-inhabiting species (Jonsell et al. 1998; Tikkanen et al. 2006) and its relatively fast decay rate (Angers et al. 2011; Kahl et al. 2017).

During felling, 53 fresh wood samples were taken from sections between every two or three logs (Fig. 1A). The wood samples were taken by drilling 10 cm into the wood after first removing the bark, at two different locations on the circumference of the section. Both the drill

bit (12 mm) and knife used for removing the bark were sterilized between samples using ethanol and a gas burner. Wood samples were stored at -80ºC.

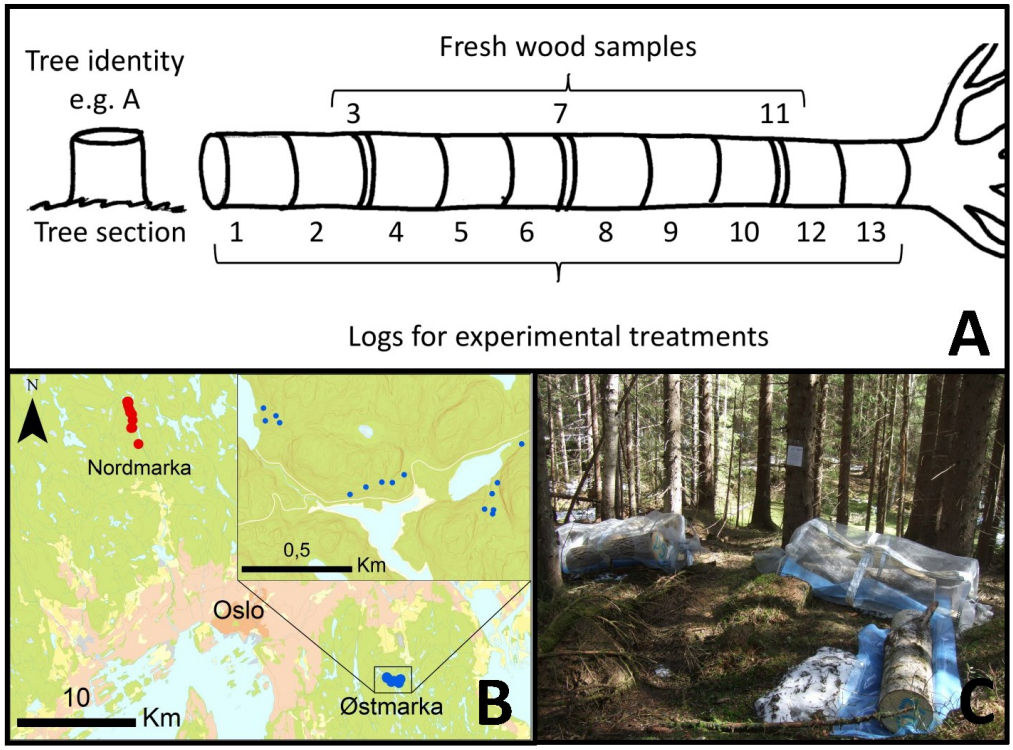

Figure 1. (A) Example of a felled tree divided into logs for experimental treatments with fresh wood samples collected between logs, and the classification of tree identity and tree section. (B) Study sites in the two landscapes in South-East Norway, Østmarka and Nordmarka, with a close-up of the sites in Østmarka. (C) Example of a study site with (from the left) cage control, cage and control treatments. The ethanol-baited log is not visible.

One hundred and twenty logs were distributed among two landscapes in South-East Norway (Fig. 1B); Losby forest holdings in Østmarka (Lat. 59.87, Long.10.97, 250–300 m.a.s.l.) and Løvenskiold-Vækerø (LV) forest holdings in Nordmarka (Lat. 60.08, Long. 10.58, 300–500 m.a.s.l.), both managed within the regulations of the PEFC (the Programme for the

Endorsement of Forest Certification schemes, Norway, pefcnorway.org). Both landscapes are within the south boreal vegetation zone (Moen 1998) and consisted of forest dominated by spruce (*Picea abies* (L.) H.Karst.), with pine (*Pinus sylvestris* L.), birch (*Betula pubescens* Ehrh.) and aspen as subdominants.

In each landscape, four logs were placed at each of 15 study sites in mature, semi-shaded forest (Fig. 1B). Distance between the sites varied due to transportation logistics, with a mean distance between sites of 120 meters in Østmarka and 276 meters in Nordmarka. At each site, the logs were assigned to one of four treatments; (i) cage, (ii) control, (iii) cage control and (iv) ethanol-baited positive control. The treatments were placed within a few meters or less of each other to ensure a similar microclimate, with the exception of the ethanol-baited logs which were placed approximately 10 meters from the other treatments.

2.1 Experimental treatments

(i) The cage treatment was designed to exclude invertebrates, and consisted of a fine polyester plastic mesh net (1x1 mm mesh size) suspended around the log by a scaffolding and a polyethylene plastic sheet beneath the log (Fig. 1C).

The plastic sheet was deemed necessary based on the experience of Müller and co-workers (2002), whose cages were penetrated by invertebrates in the soil. As the plastic sheet would also prevent colonization of fungi from the soil, it was included in all other treatments as well.

(ii) The control treatment therefore consisted of a log on a plastic sheet.

(iii) The cage control was designed to control for microclimatic effects of the cage and was identical to the cage treatment, with the exception of four large holes (20 cm diameter) cut in the mesh net to allow colonization by invertebrates.

(iv) The ethanol-baited treatment was designed to function as a positive control, as the evaporating ethanol would attract wood-inhabiting invertebrates (Allison et al. 2004; Bouget et al. 2009; Montgomery & Wargo 1983). The treatment consisted of a log on a plastic sheet, with a one liter bottle of 96% ethanol with small holes for evaporation attached to the log throughout the summer seasons.

While the cages for invertebrate exclusion would also exclude vertebrates, fresh aspen logs such as those used in this study do not function as habitat or resource for vertebrates, so their role in influencing the dead wood community would likely be minor. Furthermore, should the control logs mainly be influenced by vertebrates and not invertebrates, then the ethanol-baited logs should not differ from the control logs.

By the beginning of April 2014, all treatments had been installed in both study landscapes. Cages were removed in November 2014 to allow snow to fall naturally on all logs and set up again as soon as the snow had melted in 2015, i.e. by the end of March for logs in Østmarka and by the end of April for most sites in Nordmarka. Cages were removed and wood samples taken for analysis in November 2015.

Wood samples for DNA analysis were taken using the same method as described for fresh logs. For each log, wood samples were taken 25 cm (end sample) and 50 cm (mid sample) from the end of the log with least disturbance (i.e. least damage to the bark, cut branches etc.). Each end sample and mid sample consisted of wood chips from drilling into the log at three different locations on the circumference; the top and both sides. In total, there were 240 samples from the experimental treatments, stored at -80^{o}C.

Wood samples for density measurements were taken at the same positions as the DNA samples (25 cm and 50 cm from one end) with a core sample drill, in two replicates (top and side) pooled together for analysis. These samples were further sub-divided into the outer 5 cm

(without bark) and the inner 5 cm section of the sample. Green volume was measured by water displacement, followed by oven drying at 103ºC overnight and measurement of dry mass to calculate density (dry mass divided by green volume).

2.2 DNA analysis

DNA was extracted from the wood samples by following a CTAB protocol modified for large sample volumes (Appendix S1), as extraction was initiated with approximately 15 ml of wood chips from each sample.

After extraction, the DNA samples were cleaned using the E.Z.N.A. ® Soil DNA kit (Omega Bio-tek, Norcross, USA) as recommended by the manufacturers. DNA was eluted in two steps using 20 µl elution buffer in each step, resulting in approximately 40 µl suspended DNA. This was used in a 10x dilution for PCR.

PCR was run on an Eppendorf Mastercycler Nexus GSX1 (Eppendorf, Hamburg, Germany) in a total reaction volume of 20 µl consisting of 2 µl (5 mM) of primers ITS4 (White et al. 1990) and ITS7A (Ihrmark et al. 2012) each with an incorporated 12 bp molecular identifier, 2 µl (2 mM) dNTPs, 0.2 µl Phusion Hot Start II High-Fidelity DNA Polymerase and 4 µl 5X Phusion HF Buffer (Thermo Fisher Scientific, Waltham, USA), 1 µl bovine serum albumin (BSA), 0.6 µl dimethyl sulfoxide (DMSO), 6.2 µl milli-Q H_2O and 4 µl 10x-dilution of DNA template. PCR was run as follows; initial denaturation at 98ºC for 30 seconds, then denaturation at 98ºC for 10 sec, annealing at 56ºC for 30 sec and elongation at 72ºC for 15 sec repeated 30 times, followed by a final elongation step at 72ºC for 10 min. The PCR products were then frozen to deactivate the enzyme.

The PCR products were cleaned using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, USA) following a modified version of the manufacturer's protocol, with a longer centrifuge step after the final run-through of wash solution to avoid remnant ethanol. Samples

were combined in two pools with 162 and 158 samples, including 10 PCR negatives and 18 technical replicates, which were sequenced in two different paired-end (300 x 2) Illumina Miseq runs. Sequence data, mapping files and associated metadata are available in Dryad public repository (doi:XXXXXX).

2.3 Bioinformatic analysis

We received 30,214,354 paired-end forward and reverse sequences from the two Miseq sequencing runs. Sequences were passed for pre-joining quality filtering using the script provided by Bálint et al. (2014). Sequences with minimum average quality (Q) below 26 phred score were discarded, followed by truncation at 200 bp using *VSEARCH* v 2.0.3 (Rognes et al. 2016). A total of 27,273,503 quality filtered sequences were assembled using fastq-join method (Aronesty 2013), implemented in *QIIME* v 1.8.0 (Caporaso et al. 2010), with minimum overlap of 10 bp. Joined 26,204,367 sequences were passed for demultiplexing and quality control using *MOTHUR* v.1.31.2 (Schloss et al. 2009). Sequences with average quality score (Q) < 30, homopolymers > 8 bp, ambiguous base call > 0 and length < 100 bp were discarded. In addition, a 50 bp sliding window was used to identify regions of low sequence quality (average Q < 34) and truncate affected sequences at the beginning of the low-quality window. A total of 25,647,508 sequences were retained after this quality filtering. We checked presence of both forward and reverse primers using *FQGREP* v0.4.4 (https://github.com/indraniel/fqgrep/) with 1 and 0 mismatches allowed, respectively, and removed the sequences if the primer was absent. Both primers were truncated using FASTX-Toolkit (A. Gordon, http://hannonlab.cshl.edu/fastx_toolkit/) and remaining 25,047,388 sequences were reoriented in the same direction. ITS regions of the sequences were extracted using ITSx v1.0.11 (Nilsson et al. 2010) and sequences with >100 bp were removed. We used *VSEARCH* for dereplication of the ITS extracted dataset (24,609,443 sequences), and removed 933,142 global singletons. The same program was employed for clustering of the sequences at 97% similarity threshold

using --cluister_size function, which generated 10,541 clusters. The most abundant sequence of each cluster was designated as the representative sequence. Chimera analysis was performed on representative sequences using --uchime_denovo algorithm (Edgar et al. 2011), implemented in *VSEARCH*, with the minimum divergence parameter = 0.8, abundance skew = 2 and minimum difference in segment = 3. Of the 10,541 clusters, 6,650 (440,780 sequences; 1.9% of total sequences) were flagged as chimeric and removed from the dataset. To minimize the impact of rare OTUs resulting from sequencing and PCR errors, we removed all OTUs with < 10 sequences (Nguyen et al. 2014) and 1,878 OTUs (24,195,167 sequences) were retained. The representative sequence of each cluster was subjected to BLASTn search against the quality-checked UNITE+INSD fungal ITS sequence database (released 20 November 2016), containing both identified and unidentified sequences (Kõljalg et al. 2013). OTUs with no blast hit (101 OTUs; 88,753 sequences) or with similarity to plant sequences (34 OTUs; 2,910,145 sequences) were excluded from further analysis. Remaining 1,743 OTUs (21,196,269 sequences) were further classified into their ecological guild using *FUNGUILD* (Nguyen et al. 2016). For each OTU found in PCR negatives, the average number of sequences in the negatives was subtracted from each wood sample (in total, the ten negatives contained 73,120 sequences from 770 OTUs). Technical replicates were checked for consistency (Appendix S3: Fig. S1), and the one with lowest number of sequences was removed. Finally, 1737 OTUs (18,455,289 sequences) remained for analysis.

2.4 Statistical analysis

All statistical analysis was conducted in R version 3.3.2 (R Core Team 2016).

For analysis of OTU richness, number of sequences per sample was rarefied down to 18 000, which was the second lowest number of sequences isolated from a treatment wood sample (the treatment sample with lowest number of sequences was an outlier with only 2333 sequences).

Composition of the fungal community in terms of abundance (number of sequences) of OTUs was analysed using the full dataset, but we controlled that similar results were obtained in ordinations of the rarefied data as well as with presence-absence data. We investigated the effect of experimental treatments and other explanatory variables on OTU composition with redundancy analysis (RDA) of Hellinger-transformed abundance data (Borcard et al. 2011) using the vegan package v. 2.4-2 (Oksanen et al. 2017). When analysing the wood samples from the experimental treatments (n=239, one cage control wood sample was lost during processing), the constraining variables were treatment and log section (mid/end), while tree identity, tree section, site and log diameter were conditional variables.

To estimate the proportion of variance in fungal OTU composition explained by each of the variables, we used partial RDA with one constraining variable and all other variables included as conditional variables. Permutation (999 permutations) with the "anova.cca"- function from the vegan package was used to test the significance of RDA models and axes.

We used linear mixed models fit by restricted maximum likelihood (REML) to test whether number of OTUs, proportion of OTUs (arcsine-transformed as in Crawley (2012)) annotated as wood saprotrophs or abundance of OTUs (log-transformed number of sequences to meet the assumption of normal distribution) annotated as specific species of fungi differed between experimental treatments. Treatment, log section and diameter were included as fixed effects, while site, tree identity and tree section nested under tree identity were included as random effects.

Linear mixed models (fit by REML) were used to test whether density of wood core samples differed between experimental treatments (n=480), with treatment, section of the wood core sample (outer/inner), log section and log diameter as fixed effects and site, tree identity and tree section nested under tree identity as random effects.

3. Results

Of the 1737 fungal OTUs (18,455,289 sequences) obtained from the wood samples, 798 (14,920,438 sequences) were annotated to genus or species level (Appendix S2: Table S1). The majority of the OTUs were annotated to phylum Ascomycota (824 OTUs and 5,329,879 sequences), while the majority of the sequences belonged to phylum Basidiomycota (351 OTUs and 11,359,102 sequences). Fewer sequences of fungal DNA were obtained from the fresh wood samples collected directly after tree felling (mean 13 938 ± 3705 sequences), in comparison with wood samples from the experimental treatments collected after two years of wood decay (mean 73 819 ± 7735 sequences). The largest proportion of sequences in the treatment samples was classified as wood saprotrophs (Fig. 2A) and annotated as order Polyporales (Fig. 2B). The ethanol-baited treatment had a slightly larger proportion of wood saprotroph OTUs than the other experimental treatments (Fig. 2A, p-value = 0.07 in linear mixed models).

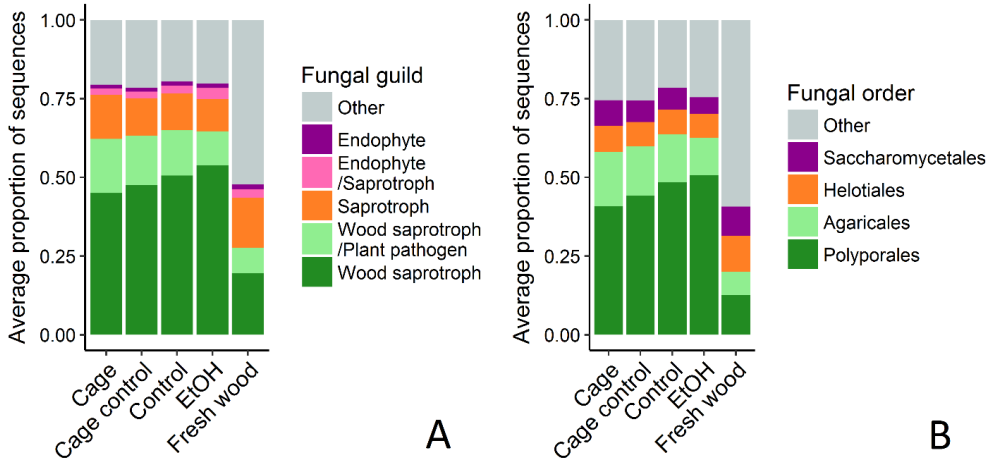

Figure 2. Average proportion of sequences annotated to different fungal guilds (A) or fungal orders (B) in samples from the experimental treatments (cage for invertebrate exclusion,

cage control, control and ethanol-baited (EtOH) positive control), and fresh wood samples collected directly after tree felling.

A total of 1735 OTUs were isolated from the experimental samples and 1586 OTUs were isolated from the fresh wood samples, of which two OTUs were only found in fresh wood samples. After rarefying down to 18 000 sequences per sample the average number of OTUs was significantly higher in samples from fresh wood (Fig. 3A). However, the average number of wood-decay fungal OTUs (including mixed guilds such as wood saprotroph/plant pathogen, see Appendix S2: Table S2) was significantly lower in the fresh wood samples (Fig. 3B). There were no significant differences in OTU richness between the experimental treatments.

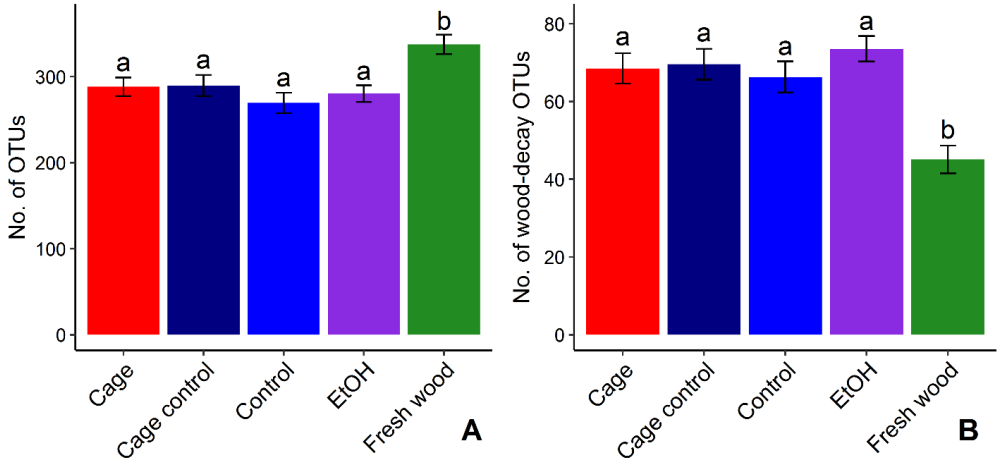

Figure 3. Average number per sample ± standard error of the mean (SEM) of all OTUs (A) or wood-decay OTUs (see Appendix S2: Table S2) (B) for the different experimental treatments (cage for invertebrate exclusion, cage control, control and ethanol-baited (EtOH) positive control), and fresh wood samples collected directly after tree felling. Different letters above columns denote significant differences (p-values <0.05 in linear mixed models). Number of sequences per sample rarefied to 18 000.

3.1 Effect of invertebrate exclusion on fungal community composition

The fungal community composition, in terms of abundance (number of sequences) of fungal OTUs, was significantly affected by the experimental treatments (Fig. 4, Table 1, Appendix S3: Table S1). The ordination analysis showed that all experimental treatments differed from each other and formed a gradient in community composition spanning from the invertebrate exclusion treatment (cage) to the ethanol-baited treatment (EtOH), with control and cage control treatments being intermediate (Fig. 4). The first two ordination axes, RDA1 and RDA2, explained significant gradients of variation (RDA1 p-value = 0.001 and RDA2 p-value = 0.010 based on 999 permutations).

The fungal communities in cage control and control logs were similar along the first gradient of variation (RDA1, Fig. 4, Appendix S3: Table S1). The invertebrate exclusion treatment, i.e. caged logs, had lower scores for RDA1 than the other treatments (Fig. 4, Appendix S3: Table S1), signifying a lower abundance of fungal OTUs annotated to species *Trametes ochracea* and *T. versicolor* and a higher abundance of e.g. fungal OTUs annotated to species *Chondrostereum purpureum* (Appendix S3: Table S2). This was confirmed by linear mixed models, showing that *T. ochracea* was significantly more abundant in wood samples from ethanol-baited logs relative to caged logs, and *T. versicolor* was significantly more abundant in both ethanol-baited and cage control logs (Appendix S3: Table S3 and S4). Abundance of *C. purpureum* was not found to differ significantly between treatments, but it was more abundant in the mid section of the logs (Appendix S3: Table S5).

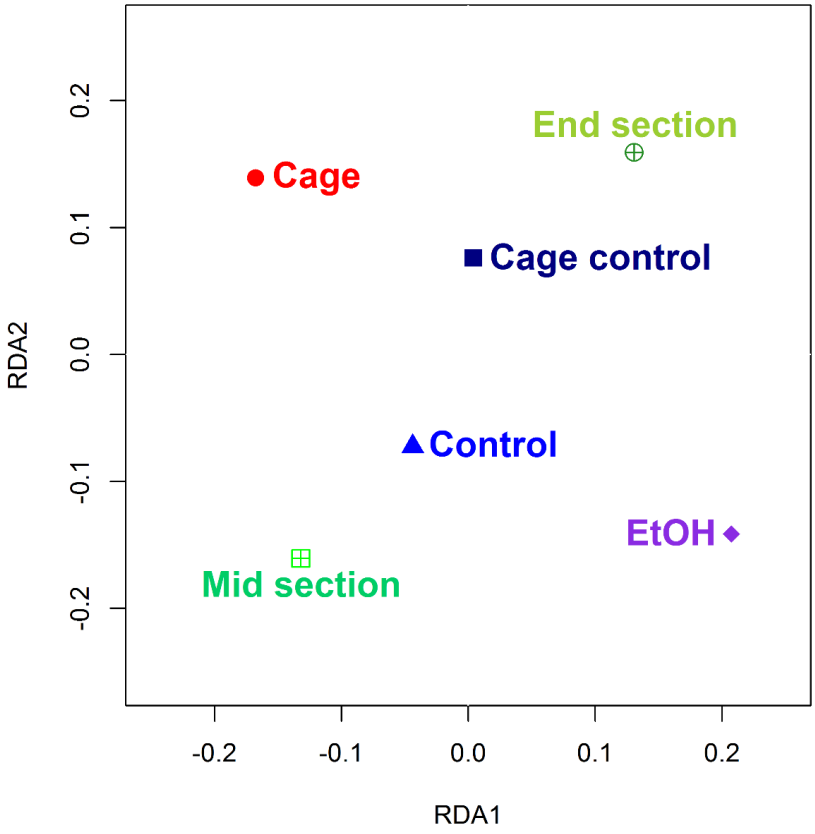

Figure 4. Ordination plots for treatment samples showing centroids (see also Appendix S3: Table S1) of constraining variables (log section (end or mid) and experimental treatments; cage (for invertebrate exclusion), cage control, control and ethanol-baited (EtOH) positive controls) in redundancy analysis of Hellinger-transformed abundance of fungal OTUs, with tree identity, tree section, log diameter, landscape and site as conditional variables. See Appendix S3: Table S2 for species scores of fungal OTUs.

Along the second gradient of variation (RDA2), caged logs were most similar to cage control logs, indicating an effect of the cage per se on the fungal community (Fig. 4, Appendix S3: Table S1). Several ascomycetes, e.g. *Penicillium* spp. and *Ascocoryne* sp., were among the

fungal OTUs with high scores for RDA2, while polypores such as *T. ochracea* had low scores (Appendix S3: Table S2).

In total, the experimental treatments explained a relatively small, but significant proportion of the variance in fungal community composition in the wood samples (Table 1). The identity of the tree from which the logs had been cut explained the largest proportion of the variance in fungal community composition (Table 1).

Table 1) Variance in OTU composition of the wood samples from experimental treatments partitioned between explanatory variables. Significance is tested by permutations (n=999) of redundancy analyses constrained by one explanatory variable while all other variables are conditional, thus partialling out variance explained by those variables including explained variance shared with the constraining variable. In the full model, all explanatory variables are included.

Variable	**Variance**	**Adjusted R^2**	**P-value**
Treatment	0.010	0.016	0.001
Log section	0.006	0.012	0.001
Tree identity	0.089	0.158	0.001
Tree section	0.031	0.034	0.001
Diameter	0.003	0.005	0.006
Site	0.065	0.057	0.001
Landscape	0.000	0.000	NA
Full model	0.271	0.352	0.001
Residual	0.249		

3.2 Effect of invertebrate exclusion on wood decay

The invertebrate exclusion treatment (cage) resulted in a significantly higher wood density of core samples in comparison with the control treatment, implying that the exclusion treatment reduced wood decay rate (Table 2).

Table 2) Linear mixed model fit by restricted maximum likelihood (REML) explaining density of wood core samples by experimental treatment (cage in the intercept), sample section (inner/outer), log section (mid/end) and log diameter as fixed effects and site, tree identity and tree section nested under tree identity as random effects.

Fixed effects	**Estimate**	**Std. error**	**t-value**	**p-value**
Intercept	*0.349*	*0.014*	*25.75*	*<0.001*
Cage control logs	-0.003	0.004	-0.81	0.418
Control logs	-0.008	0.004	-2.04	0.041
Ethanol-baited logs	-0.002	0.004	-0.60	0.546
Sample section (Outer)	0.015	0.002	8.63	<0.001
Log section (Mid)	0.002	0.002	0.98	0.328
Diameter	0.001	<0.001	2.62	0.009
Random effects	***Variance***	***Std. deviance***		
Site	*0*	*0*		
Tree identity (ID)	*0.001*	*0.024*		
Tree ID/Tree section	*<0.001*	*0.011*		
Residual	*<0.001*	*0.019*		
REML criterion at convergence: -2210.4				

4. Discussion

Our results, stemming from a field experiment repeated at thirty sites across two different landscapes, provide evidence that invertebrates have a significant effect on decomposer communities in dead wood and their function in the field. Exclusion of invertebrates larger than 1 mm from recently cut logs significantly affected fungal community composition, confirming our initial hypothesis. This corresponds with previous studies that demonstrate an effect of invertebrates on community composition of lower trophic levels such as primary producers (Schädler et al. 2004; Stein et al. 2010) and decomposers (A'Bear et al. 2014; Strid et al. 2014; Ulyshen et al. 2016). Our results also indicated that invertebrate exclusion decreased the rate of wood decay, since the wood density was significantly higher for caged logs relative to control logs. The effect of invertebrate exclusion on wood decay in the present study might have been mediated through the effect on the fungal community, which corresponds with previous studies of soil communities in laboratory micro- and mesocosmoses, where invertebrates have been found to indirectly affect wood decay through their effect on the fungal community (reviewed in A'Bear et al. 2014). The present study shows that invertebrate exclusion affects both wood decay rates and composition of complex and highly diverse fungal communities in the field.

4.1 Effect of the exclusion treatment

The fungal community of caged logs differed from that of cage control logs along the main gradient of compositional variation explained by the experimental treatments. Thus, although the similarity of cage and cage control treatments along the second gradient also indicated an effect of the cage per se, the absence or presence of invertebrates larger than 1 mm seemed to have a stronger effect on fungal community composition within logs. The ethanol-baited treatment seemed to increase this effect, indicating an important role of wood-inhabiting

invertebrates attracted to the ethanol-smell of decaying wood (Allison et al. 2004; Bouget et al. 2009; Montgomery & Wargo 1983).

We were not able to assess degree of invertebrate colonization of the different logs as there were no clear marks of insect activity that could be registered without destructive sampling, which would prevent future studies of the logs. However, in an experiment demonstrating that bark beetles influence the fungal communities in spruce logs, Strid et al. (2014) excluded invertebrates using cages similar to those in our study and found no signs of bark beetles or other wood-boring insects on logs within cages. Thus, it is highly likely that the cages used in our study at the very least significantly reduced invertebrate colonization of the logs.

In addition to the effect of experimental treatments on the abundance of invertebrates colonizing the logs, the species composition of invertebrates colonizing control, cage control and ethanol-baited logs might have differed. Some wood-inhabiting beetles seem to have an especially strong attraction to ethanol (Bouget et al. 2009; Montgomery & Wargo 1983), while other species might prefer (or avoid) the shaded microclimate of cage control logs (Jonsell et al. 1998; Seibold et al. 2016; Sverdrup-Thygeson & Ims 2002). Different invertebrate communities might in turn have resulted in different fungal communities, as we found in a previous study that insects carry a taxon-specific mix of fungi (Jacobsen et al. 2017).

4.2 Effect of invertebrate exclusion on fungal community composition

Experimental treatment explained a significant, but small proportion of the variation in fungal community composition between logs. However, the logs had only been subject to a little less than two years of wood decay, and slight differences in composition of fungi at the time of community assembly can result in increasing differences during succession due to priority effects favoring early arrivals (Dickie et al. 2012; Fukami et al. 2010; Hiscox et al. 2015; Ottosson et al. 2014). Early arrival can enable wood saprotrophic fungi to colonize large wood

volumes prior to arrival of competitors, thus increasing their competitive ability (Holmer & Stenlid 1993).

Studies manipulating arrival order of wood saprotrophic fungi have found that the polypore *T. versicolor* seems relatively dependent on early arrival to persist in dead wood, and that it affects the subsequent development of the fungal community (Dickie et al. 2012; Fukami et al. 2010; Leopold et al. 2017). Here we found that abundance of *T. versicolor* and the closely related *T. ochracea* was significantly reduced by the exclusion of invertebrates larger than 1 mm from dead wood. In a previous study we isolated DNA of *T. versicolor* from several beetles sampled from recently cut aspen logs (Jacobsen et al. 2017). That study was conducted in the same landscapes during the same years as the present study, so it is likely that the insects sampled by Jacobsen et al. (2017) are representative of those that colonized the logs in the present study. Thus, the reduced abundance of *T. versicolor* in caged logs in the present study could stem from lack of propagule dispersal by invertebrates.

Our experimental design does not enable us to determine whether invertebrates affected the fungal community through preferential grazing, substrate alterations or propagule dispersal, nor are these mechanisms mutually exclusive. However, preferential grazing by invertebrates has as of yet not been demonstrated to alter composition of fungi within dead wood (Crowther et al. 2011) and experimentally drilling holes in logs to mimic insect tunnels has been shown to have little effect on the fungal community (Strid et al. 2014). Thus, propagule dispersal might be a more likely mechanism to influence the fungal communities at this early stage of wood decay, though further studies are necessary to clarify the relative importance of different insect-fungus interactions in dead wood.

4.3 Effect of invertebrate exclusion on wood decay

Exclusion of invertebrates larger than 1 mm resulted in significantly higher wood density in caged logs than control logs, implying a lower rate of wood decay in caged logs. This could be due to a direct effect of invertebrates on wood decay, although mass loss due to wood-consumption by invertebrates other than termites seems to be relatively low (Ulyshen & Wagner 2013; Ulyshen 2016). Invertebrates have been found to significantly influence wood decay in areas where termites are absent (Kahl et al. 2017; Müller et al. 2002), but it is unclear whether this effect is due to direct or indirect effects. Our study shows that invertebrates might indirectly affect wood decay rates through their effect on fungal community composition (Hoppe et al. 2016; Kubartová et al. 2015; van der Wal et al. 2015). While the effect on wood decay in our study could also stem from the cage per se, Stoklosa et al. (2016) found that mesh bags increased decomposition of woody material. Thus, the decrease in decay rate of caged logs in the present study might be a conservative estimate of the effect of invertebrate exclusion on wood decay. This implies that species loss or reduced abundance of wood-inhabiting invertebrates might result in decreased rates of wood decay and nutrient cycling in forest ecosystems, although further long-term studies are required to test this hypothesis.

4.4 Fungal community in fresh wood

OTU richness was not significantly affected by experimental treatment, but it was surprisingly high in the fresh wood that was sampled directly after felling the trees, i.e. samples that essentially represented the fungal community in the living trees. These samples also contained, albeit in low abundance, several wood saprotrophic fungi. This corresponds with previous studies that found wood saprotrophic fungi in living trees (Parfitt et al. 2010; Song et al. 2017). Tree identity explained the largest proportion of variation in community composition in our study, which may reflect the influence of fungi already established in the living trees on the development of the fungal community. However, variation between individual trees in e.g.

nitrogen to carbon ratio or content of defensive compounds could also play a role (Cornwell et al. 2009; Latta et al. 2000). Whatever the cause, our results demonstrate the importance of accounting for differences between individual trees when studying fungal communities in wood.

4.5 Conclusion

We have shown that exclusion of invertebrates for two years in the field significantly influences both wood decay rates and the fungal community in dead wood. Two years is a short time frame for wood decay in boreal forests, which might account for the low effect size of the experimental treatments. Nevertheless, we show that variation in invertebrate colonization will lead to establishment of different fungal communities, which is likely to also influence subsequent succession of both invertebrates and fungi in dead wood. The interaction between wood-inhabiting invertebrates and fungi during community assembly might therefore contribute to the variability and diversity of dead wood communities. Furthermore, the effect of invertebrate exclusion on wood decay rates documented in our study indicates that wood-inhabiting invertebrates, either directly or indirectly through their effect on the fungal community, can influence processes such as nutrient cycling, carbon storage and productivity in forest ecosystems. This underlines the importance of the dead wood community for the functioning of forest ecosystems. We therefore call for long-term field studies of the interactions between invertebrates and fungi in the dead wood community, and the influence of these interactions on ecosystem processes such as decomposition and forest productivity.

5. Authors' Contributions

RMJ, TB, HK and AST conceived the ideas and designed the methodology; SM did the bioinformatic analysis; RMJ and TB did the field work, RMJ did the lab work, statistical

analysis and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Appendix

Appendix S1: CTAB protocol modified for large sample volumes

Extraction was initiated with approximately 15 ml of wood chips for each sample. Working in 50 ml Falcon tubes, 15 ml of 2% CTAB lysis buffer (100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2% CTAB) and 7 stainless steel beads (5 mm diameter) was added to the sample, which was homogenized by grinding on a FastPrep 24 at 4.5 Hz for 30 seconds. The tubes were then placed in a -80ºC freezer for 30 min before incubation over night at 54ºC. The following day the samples were inverted and allowed to cool before adding 15 ml of chloroform and vortexing. The tubes were centrifuged for 15 min at 14 000 rpm, before pipetting 5 ml of the upper layer into 15 ml Falcon tubes containing 5 ml cold isopropanol. The Falcon tubes were inverted and placed in a -20ºC freezer for 30 min to allow the DNA to precipitate. The tubes were then centrifuged for 10 min at 14 000 rpm before pouring off the isopropanol, adding 1000 µl cold 70% ethanol, vortexing, centrifuging at 14 000 rpm for 3 min and finally pouring off the ethanol. The tubes were incubated at 60ºC until the remaining ethanol had evaporated, then 60 µl of milli-Q H_2O was added to resuspend the DNA.

Appendix S2: Table S2

OTU ID	Taxon	Guild_in_paper	Wood_decay_OTU
OTU_469	Acremonium	Unknown	0
OTU_1196	Acremonium	Unknown	0
OTU_2438	Acremonium	Unknown	0
OTU_2922	Acremonium	Unknown	0
OTU_3497	Acremonium	Unknown	0
OTU_3771	Acremonium	Unknown	0
OTU_4057	Acremonium	Unknown	0
OTU_3601	Alatospora	Saprotroph	0
OTU_1888	Aleurodiscus	Saprotroph	0
OTU_879	Allantophomopsis	Plant Pathogen	0
OTU_1664	Alternaria	Unknown	0
OTU_9	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_425	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_517	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_573	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_583	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_823	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_1407	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_1910	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_2301	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_2383	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_2595	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_2865	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_3029	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_3067	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_3438	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_4016	Annulohypoxylon	Endophyte_Saprotroph	0
OTU_2288	Armillaria	Wood Saprotroph	1
OTU_6522	Armillaria	Wood Saprotroph	1
OTU_1480	Articulospora	Saprotroph	0
OTU_6	Ascocoryne	Wood Saprotroph	1
OTU_74	Ascocoryne	Wood Saprotroph	1
OTU_597	Ascocoryne	Wood Saprotroph	1
OTU_768	Ascocoryne	Wood Saprotroph	1
OTU_817	Ascocoryne	Wood Saprotroph	1
OTU_831	Ascocoryne	Wood Saprotroph	1
OTU_1300	Ascocoryne	Wood Saprotroph	1
OTU_1351	Ascocoryne	Wood Saprotroph	1
OTU_1701	Ascocoryne	Wood Saprotroph	1
OTU_2100	Ascocoryne	Wood Saprotroph	1
OTU_2177	Ascocoryne	Wood Saprotroph	1
OTU_2281	Ascocoryne	Wood Saprotroph	1
OTU_2549	Ascocoryne	Wood Saprotroph	1
OTU_2726	Ascocoryne	Wood Saprotroph	1
OTU_3236	Ascocoryne	Wood Saprotroph	1
OTU_3331	Ascocoryne	Wood Saprotroph	1
OTU_4133	Ascocoryne	Wood Saprotroph	1
OTU_4558	Ascocoryne	Wood Saprotroph	1

OTU_4592	Ascocoryne	Wood Saprotroph	1
OTU_4750	Ascocoryne	Wood Saprotroph	1
OTU_4775	Ascocoryne	Wood Saprotroph	1
OTU_5366	Ascocoryne	Wood Saprotroph	1
OTU_6427	Ascocoryne	Wood Saprotroph	1
OTU_7279	Ascocoryne	Wood Saprotroph	1
OTU_7449	Ascocoryne	Wood Saprotroph	1
OTU_9177	Ascocoryne	Wood Saprotroph	1
OTU_327	Aspergillus flavus	Unknown	0
OTU_520	Athallia	Lichenized	0
OTU_191	Atractium	Wood Saprotroph	1
OTU_2057	Atractium	Wood Saprotroph	1
OTU_812	Aureobasidium	Unknown	0
OTU_659	Aureobasidium pullulans	Unknown	0
OTU_157	Bacidia	Lichenized	0
OTU_239	Barbatosphaeria	Wood Saprotroph	1
OTU_2024	Bisporella	Wood Saprotroph	1
OTU_1	Bjerkandera	Wood Saprotroph	1
OTU_118	Bjerkandera	Wood Saprotroph	1
OTU_268	Bjerkandera	Wood Saprotroph	1
OTU_308	Bjerkandera	Wood Saprotroph	1
OTU_477	Bjerkandera	Wood Saprotroph	1
OTU_754	Bjerkandera	Wood Saprotroph	1
OTU_1101	Bjerkandera	Wood Saprotroph	1
OTU_1107	Bjerkandera	Wood Saprotroph	1
OTU_1630	Bjerkandera	Wood Saprotroph	1
OTU_1675	Bjerkandera	Wood Saprotroph	1
OTU_1866	Bjerkandera	Wood Saprotroph	1
OTU_1971	Bjerkandera	Wood Saprotroph	1
OTU_1996	Bjerkandera	Wood Saprotroph	1
OTU_2044	Bjerkandera	Wood Saprotroph	1
OTU_2094	Bjerkandera	Wood Saprotroph	1
OTU_2127	Bjerkandera	Wood Saprotroph	1
OTU_2645	Bjerkandera	Wood Saprotroph	1
OTU_2817	Bjerkandera	Wood Saprotroph	1
OTU_2957	Bjerkandera	Wood Saprotroph	1
OTU_3108	Bjerkandera	Wood Saprotroph	1
OTU_3215	Bjerkandera	Wood Saprotroph	1
OTU_3928	Bjerkandera	Wood Saprotroph	1
OTU_3956	Bjerkandera	Wood Saprotroph	1
OTU_3961	Bjerkandera	Wood Saprotroph	1
OTU_3982	Bjerkandera	Wood Saprotroph	1
OTU_4026	Bjerkandera	Wood Saprotroph	1
OTU_4386	Bjerkandera	Wood Saprotroph	1
OTU_5079	Bjerkandera	Wood Saprotroph	1
OTU_5375	Bjerkandera	Wood Saprotroph	1
OTU_5521	Bjerkandera	Wood Saprotroph	1
OTU_5555	Bjerkandera	Wood Saprotroph	1
OTU_5745	Bjerkandera	Wood Saprotroph	1
OTU_5893	Bjerkandera	Wood Saprotroph	1

OTU_6004	Bjerkandera	Wood Saprotroph	1
OTU_6284	Bjerkandera	Wood Saprotroph	1
OTU_6524	Bjerkandera	Wood Saprotroph	1
OTU_6630	Bjerkandera	Wood Saprotroph	1
OTU_6669	Bjerkandera	Wood Saprotroph	1
OTU_7569	Bjerkandera	Wood Saprotroph	1
OTU_7868	Bjerkandera	Wood Saprotroph	1
OTU_8589	Bjerkandera	Wood Saprotroph	1
OTU_8655	Bjerkandera	Wood Saprotroph	1
OTU_8927	Bjerkandera	Wood Saprotroph	1
OTU_9148	Bjerkandera	Wood Saprotroph	1
OTU_353	Botryosphaeria	Plant Pathogen	0
OTU_1089	Botryosphaeria	Plant Pathogen	0
OTU_13	Cadophora	Endophyte	0
OTU_34	Cadophora	Endophyte	0
OTU_90	Cadophora	Endophyte	0
OTU_153	Cadophora	Endophyte	0
OTU_189	Cadophora	Endophyte	0
OTU_240	Cadophora	Endophyte	0
OTU_246	Cadophora	Endophyte	0
OTU_275	Cadophora	Endophyte	0
OTU_480	Cadophora	Endophyte	0
OTU_600	Cadophora	Endophyte	0
OTU_631	Cadophora	Endophyte	0
OTU_675	Cadophora	Endophyte	0
OTU_779	Cadophora	Endophyte	0
OTU_789	Cadophora	Endophyte	0
OTU_1048	Cadophora	Endophyte	0
OTU_1092	Cadophora	Endophyte	0
OTU_1203	Cadophora	Endophyte	0
OTU_1328	Cadophora	Endophyte	0
OTU_1382	Cadophora	Endophyte	0
OTU_1462	Cadophora	Endophyte	0
OTU_1487	Cadophora	Endophyte	0
OTU_1552	Cadophora	Endophyte	0
OTU_1645	Cadophora	Endophyte	0
OTU_2133	Cadophora	Endophyte	0
OTU_2423	Cadophora	Endophyte	0
OTU_2797	Cadophora	Endophyte	0
OTU_2863	Cadophora	Endophyte	0
OTU_2918	Cadophora	Endophyte	0
OTU_2982	Cadophora	Endophyte	0
OTU_2995	Cadophora	Endophyte	0
OTU_3238	Cadophora	Endophyte	0
OTU_3352	Cadophora	Endophyte	0
OTU_3507	Cadophora	Endophyte	0
OTU_3573	Cadophora	Endophyte	0
OTU_3868	Cadophora	Endophyte	0
OTU_4091	Cadophora	Endophyte	0
OTU_5142	Cadophora	Endophyte	0

OTU_5380	Cadophora	Endophyte	0
OTU_5483	Cadophora	Endophyte	0
OTU_5943	Cadophora	Endophyte	0
OTU_6237	Cadophora	Endophyte	0
OTU_6722	Cadophora	Endophyte	0
OTU_6796	Cadophora	Endophyte	0
OTU_6822	Cadophora	Endophyte	0
OTU_7682	Cadophora	Endophyte	0
OTU_8575	Cadophora	Endophyte	0
OTU_1744	Candelaria	Lichenized	0
OTU_1846	Candelaria	Lichenized	0
OTU_66	Candelariella	Lichenized	0
OTU_111	Capronia	Endophyte	0
OTU_241	Capronia	Endophyte	0
OTU_516	Capronia	Endophyte	0
OTU_607	Capronia	Endophyte	0
OTU_1174	Capronia	Endophyte	0
OTU_1192	Capronia	Endophyte	0
OTU_1221	Capronia	Endophyte	0
OTU_1756	Capronia	Endophyte	0
OTU_2869	Catenulifera	Saprotroph	0
OTU_896	Catillaria	Lichenized	0
OTU_1940	Catillaria	Lichenized	0
OTU_542	Cenococcum	Mycorrhizal	0
OTU_847	Ceramothyrium	Saprotroph	0
OTU_866	Ceratobasidium	Mycorrhizal	0
OTU_98	Ceratocystiopsis	Saprotroph	0
OTU_2482	Ceratocystiopsis	Saprotroph	0
OTU_3527	Ceratocystiopsis	Saprotroph	0
OTU_8388	Ceratocystiopsis	Saprotroph	0
OTU_1610	Ceriporiopsis	Wood Saprotroph	1
OTU_26	Cerrena	Wood Saprotroph	1
OTU_3312	Cerrena	Wood Saprotroph	1
OTU_6762	Cerrena	Wood Saprotroph	1
OTU_1932	Chaetosphaeria	Saprotroph	0
OTU_443	Chalara	Endophyte_Wood Saprotroph	1
OTU_853	Chalara	Endophyte_Wood Saprotroph	1
OTU_995	Chalara	Endophyte_Wood Saprotroph	1
OTU_1178	Chalara	Endophyte_Wood Saprotroph	1
OTU_3	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_394	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_485	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_498	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_868	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_1142	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_1403	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_1874	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_1943	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_2222	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_2243	Chondrostereum	Plant Pathogen-Wood Saprotroph	1

OTU_2255	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_2524	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_2602	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_3163	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_3408	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_3430	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_3476	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_3609	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_3777	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_4164	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_4188	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_4236	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_4266	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_4332	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_4664	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_4895	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_4898	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_4967	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_4976	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_5154	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_5240	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_5282	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_5620	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_5805	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_5838	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_6099	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_6260	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_6336	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_6349	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_6501	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_7034	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_7136	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_9739	Chondrostereum	Plant Pathogen-Wood Saprotroph	1
OTU_141	Cistella	Saprotroph	0
OTU_1984	Cistella	Saprotroph	0
OTU_529	Cladonia	Lichenized	0
OTU_937	Cladonia	Lichenized	0
OTU_2493	Cladonia	Lichenized	0
OTU_862	Cladophialophora	Saprotroph	0
OTU_2565	Cladophialophora	Saprotroph	0
OTU_2967	Cladosporium	Endophyte_Plant pathogen	0
OTU_2417	Climacocystis	Wood Saprotroph	1
OTU_1505	Colletotrichum	Endophyte_Plant pathogen	0
OTU_746	Collophora	Plant Pathogen	0
OTU_18	Coniochaeta	Unknown	0
OTU_900	Coniochaeta	Unknown	0
OTU_1189	Coniochaeta	Unknown	0
OTU_2832	Coniochaeta	Unknown	0
OTU_5078	Coniochaeta	Unknown	0
OTU_7165	Coniochaeta	Unknown	0

OTU_2237	Coniosporium	Unknown	0
OTU_201	Coprinellus	Saprotroph	0
OTU_427	Coprinellus	Saprotroph	0
OTU_94	Corticium	Wood Saprotroph	1
OTU_4265	Corticium	Wood Saprotroph	1
OTU_589	Cortinarius	Mycorrhizal	0
OTU_1481	Corynespora	Saprotroph	0
OTU_832	Cosmospora	Fungal Parasite	0
OTU_3724	Cosmospora	Fungal Parasite	0
OTU_4821	Cosmospora	Fungal Parasite	0
OTU_7876	Cosmospora	Fungal Parasite	0
OTU_772	Crepidotus	Wood Saprotroph	1
OTU_2340	Crocicreas	Saprotroph	0
OTU_889	Cryptodiscus	Saprotroph	0
OTU_144	Cylindrocarpon	Plant Pathogen	0
OTU_187	Cylindrocarpon	Plant Pathogen	0
OTU_4514	Cyphellopsis	Saprotroph	0
OTU_5099	Cystobasidium	Fungal Parasite	0
OTU_229	Cystostereum	Saprotroph	0
OTU_1916	Cytospora	Endophyte_Wood Saprotroph	1
OTU_174	Dactylaria	Plant Pathogen	0
OTU_333	Dactylaria	Plant Pathogen	0
OTU_765	Dactylaria	Plant Pathogen	0
OTU_557	Dactylella	Saprotroph	0
OTU_730	Dactylellina	Saprotroph	0
OTU_946	Dactylellina	Saprotroph	0
OTU_2070	Dactylellina	Saprotroph	0
OTU_281	Dasyscyphus	Saprotroph	0
OTU_2297	Dermea	Plant Pathogen	0
OTU_674	Devriesia	Plant Pathogen	0
OTU_1045	Diaporthe	Endophyte_Plant pathogen	0
OTU_638	Dothiora	Saprotroph	0
OTU_1601	Dothiorella	Plant Pathogen	0
OTU_163	Efibulobasidium	Mycorrhizal	0
OTU_299	Endoconidioma	Saprotroph	0
OTU_1445	Endosporium	Saprotroph	0
OTU_1753	Endosporium	Saprotroph	0
OTU_1991	Eucasphaeria	Saprotroph	0
OTU_76	Eurotiales	Saprotroph	0
OTU_109	Eurotiales	Saprotroph	0
OTU_146	Eurotiales	Saprotroph	0
OTU_340	Eurotiales	Saprotroph	0
OTU_696	Eurotiales	Saprotroph	0
OTU_704	Eurotiales	Saprotroph	0
OTU_1043	Eurotiales	Saprotroph	0
OTU_1752	Eurotiales	Saprotroph	0
OTU_2700	Eurotiales	Saprotroph	0
OTU_2782	Eurotiales	Saprotroph	0
OTU_3150	Eurotiales	Saprotroph	0
OTU_4160	Eurotiales	Saprotroph	0

OTU_4199	Eurotiales	Saprotroph	0
OTU_6135	Eurotiales	Saprotroph	0
OTU_7076	Eurotiales	Saprotroph	0
OTU_7790	Eurotiales	Saprotroph	0
OTU_8145	Eurotiales	Saprotroph	0
OTU_571	Eutypa	Plant Pathogen	0
OTU_70	Exidia	Saprotroph	0
OTU_283	Exidia	Saprotroph	0
OTU_2291	Exidia	Saprotroph	0
OTU_3869	Exidia	Saprotroph	0
OTU_6990	Exidia	Saprotroph	0
OTU_9078	Exidia	Saprotroph	0
OTU_2459	Exobasidium	Plant Pathogen	0
OTU_2474	Exobasidium	Plant Pathogen	0
OTU_790	Exophiala	Saprotroph	0
OTU_1276	Exophiala	Saprotroph	0
OTU_2429	Exophiala	Saprotroph	0
OTU_850	Fellhaneropsis	Lichenized	0
OTU_2052	Fusarium	Plant Pathogen-Wood Saprotroph	1
OTU_2543	Fusarium	Plant Pathogen-Wood Saprotroph	1
OTU_1546	Fusicladium	Plant Pathogen	0
OTU_30	Graphium	Saprotroph	0
OTU_1628	Graphium	Saprotroph	0
OTU_4159	Graphium	Saprotroph	0
OTU_7028	Graphium	Saprotroph	0
OTU_54	Grosmannia	Plant Pathogen	0
OTU_6988	Grosmannia	Plant Pathogen	0
OTU_2589	Haptocillium	Animal Pathogen	0
OTU_923	Helicodendron	Saprotroph	0
OTU_59	Helicoma	Saprotroph	0
OTU_1458	Helicoma	Saprotroph	0
OTU_2271	Helicoma	Saprotroph	0
OTU_1121	Hemiphacidium	Plant Pathogen	0
OTU_234	Herpotrichia	Saprotroph	0
OTU_259	Herpotrichia	Saprotroph	0
OTU_1657	Heyderia	Saprotroph	0
OTU_130	Hyalopeziza	Saprotroph	0
OTU_1260	Hyphodiscus	Fungal Parasite	0
OTU_1301	Hyphodiscus	Fungal Parasite	0
OTU_1760	Hyphodiscus	Fungal Parasite	0
OTU_2211	Hypholoma	Saprotroph	0
OTU_1439	Hyphozyma variabilis	Endophyte	0
OTU_25	Hypocreales	Saprotroph	0
OTU_29	Hypocreales	Saprotroph	0
OTU_53	Hypocreales	Saprotroph	0
OTU_211	Hypocreales	Saprotroph	0
OTU_280	Hypocreales	Saprotroph	0
OTU_332	Hypocreales	Saprotroph	0
OTU_365	Hypocreales	Saprotroph	0
OTU_395	Hypocreales	Saprotroph	0

OTU_432	Hypocreales	Saprotroph	0
OTU_479	Hypocreales	Saprotroph	0
OTU_574	Hypocreales	Saprotroph	0
OTU_666	Hypocreales	Saprotroph	0
OTU_697	Hypocreales	Saprotroph	0
OTU_726	Hypocreales	Saprotroph	0
OTU_781	Hypocreales	Saprotroph	0
OTU_808	Hypocreales	Saprotroph	0
OTU_915	Hypocreales	Saprotroph	0
OTU_1084	Hypocreales	Saprotroph	0
OTU_1171	Hypocreales	Saprotroph	0
OTU_1229	Hypocreales	Saprotroph	0
OTU_1337	Hypocreales	Saprotroph	0
OTU_1556	Hypocreales	Saprotroph	0
OTU_1894	Hypocreales	Saprotroph	0
OTU_2029	Hypocreales	Saprotroph	0
OTU_2158	Hypocreales	Saprotroph	0
OTU_2171	Hypocreales	Saprotroph	0
OTU_2180	Hypocreales	Saprotroph	0
OTU_2448	Hypocreales	Saprotroph	0
OTU_2473	Hypocreales	Saprotroph	0
OTU_2821	Hypocreales	Saprotroph	0
OTU_3042	Hypocreales	Saprotroph	0
OTU_3078	Hypocreales	Saprotroph	0
OTU_3262	Hypocreales	Saprotroph	0
OTU_3266	Hypocreales	Saprotroph	0
OTU_3556	Hypocreales	Saprotroph	0
OTU_3870	Hypocreales	Saprotroph	0
OTU_4439	Hypocreales	Saprotroph	0
OTU_5502	Hypocreales	Saprotroph	0
OTU_7703	Hypocreales	Saprotroph	0
OTU_7865	Hypocreales	Saprotroph	0
OTU_1587	Hypohelion	Plant Pathogen	0
OTU_103	Jattaea	Saprotroph	0
OTU_2511	Jattaea	Saprotroph	0
OTU_436	Knufia	Unknown	0
OTU_252	Kuehneromyces	Saprotroph	0
OTU_457	Lecania	Lichenized	0
OTU_1622	Lecania	Lichenized	0
OTU_1682	Lecania	Lichenized	0
OTU_828	Lecanicillium	Animal Pathogen	0
OTU_972	Lecanicillium	Animal Pathogen	0
OTU_279	Lecanora	Lichenized	0
OTU_328	Lecanora	Lichenized	0
OTU_598	Lecanora	Lichenized	0
OTU_977	Lecanora	Lichenized	0
OTU_1067	Lecanora	Lichenized	0
OTU_2357	Lecanora	Lichenized	0
OTU_124	Lecidella	Lichenized	0
OTU_270	Lecidella	Lichenized	0

OTU_387	Lecidella	Lichenized	0
OTU_27	Lenzites	Wood Saprotroph	1
OTU_487	Lenzites	Wood Saprotroph	1
OTU_1498	Lenzites	Wood Saprotroph	1
OTU_1875	Lenzites	Wood Saprotroph	1
OTU_2505	Lenzites	Wood Saprotroph	1
OTU_2556	Lenzites	Wood Saprotroph	1
OTU_3140	Lenzites	Wood Saprotroph	1
OTU_3520	Lenzites	Wood Saprotroph	1
OTU_4041	Lenzites	Wood Saprotroph	1
OTU_5680	Lenzites	Wood Saprotroph	1
OTU_5864	Lenzites	Wood Saprotroph	1
OTU_5973	Lenzites	Wood Saprotroph	1
OTU_6305	Lenzites	Wood Saprotroph	1
OTU_6401	Lenzites	Wood Saprotroph	1
OTU_7313	Lenzites	Wood Saprotroph	1
OTU_8110	Lenzites	Wood Saprotroph	1
OTU_1687	Lepraria	Lichenized	0
OTU_5009	Leptodontidium	Endophyte	0
OTU_596	Leptographium	Plant Pathogen	0
OTU_1139	Leptosphaeria	Plant Pathogen	0
OTU_1289	Leptosphaeria	Plant Pathogen	0
OTU_149	Lophiostoma	Saprotroph	0
OTU_1444	Lophiostoma	Saprotroph	0
OTU_841	Lophodermium	Plant Pathogen	0
OTU_2418	Malassezia	Unknown	0
OTU_1891	Meliniomyces	Mycorrhizal	0
OTU_1840	Micarea	Lichenized	0
OTU_2377	Microdochium	Endophyte_Plant pathogen	0
OTU_167	Mollisia	Endophyte_Plant pathogen	0
OTU_690	Mycena	Saprotroph	0
OTU_743	Mycena	Saprotroph	0
OTU_3552	Mycoarthris	Saprotroph	0
OTU_2426	Myxotrichum cancellatum	Saprotroph	0
OTU_1643	Neobulgaria	Saprotroph	0
OTU_2773	Neofabraea	Plant Pathogen	0
OTU_37	Neonectria	Plant Pathogen	0
OTU_236	Neonectria	Plant Pathogen	0
OTU_1454	Neonectria	Plant Pathogen	0
OTU_1502	Neonectria	Plant Pathogen	0
OTU_1621	Neonectria	Plant Pathogen	0
OTU_2436	Neonectria	Plant Pathogen	0
OTU_6106	Neonectria	Plant Pathogen	0
OTU_113	Ochroconis	Saprotroph	0
OTU_131	Ochroconis	Saprotroph	0
OTU_2608	Ochroconis	Saprotroph	0
OTU_2900	Ochroconis	Saprotroph	0
OTU_220	Orbilia	Saprotroph	0
OTU_3707	Orbilia	Saprotroph	0
OTU_49	Peniophora	Wood Saprotroph	1

OTU_501	Pezicula	Endophyte_Plant pathogen	0
OTU_274	Phaeomoniella	Plant Pathogen	0
OTU_497	Phaeophyscia	Lichenized	0
OTU_134	Phellinus	Plant Pathogen-Wood Saprotroph	1
OTU_2983	Phellinus	Plant Pathogen-Wood Saprotroph	1
OTU_162	Phialocephala	Endophyte	0
OTU_320	Phialocephala	Endophyte	0
OTU_1490	Phialocephala	Endophyte	0
OTU_2134	Phialocephala	Endophyte	0
OTU_536	Phialophora	Endophyte	0
OTU_556	Phialophora	Endophyte	0
OTU_749	Phialophora	Endophyte	0
OTU_1247	Phialophora	Endophyte	0
OTU_1286	Phialophora	Endophyte	0
OTU_1600	Phialophora	Endophyte	0
OTU_1615	Phialophora	Endophyte	0
OTU_2086	Phialophora	Endophyte	0
OTU_2442	Phialophora	Endophyte	0
OTU_2567	Phialophora	Endophyte	0
OTU_2898	Phialophora	Endophyte	0
OTU_3006	Phialophora	Endophyte	0
OTU_4081	Phialophora	Endophyte	0
OTU_4189	Phialophora	Endophyte	0
OTU_4341	Phialophora	Endophyte	0
OTU_4809	Phialophora	Endophyte	0
OTU_4812	Phialophora	Endophyte	0
OTU_5082	Phialophora	Endophyte	0
OTU_5227	Phialophora	Endophyte	0
OTU_6782	Phialophora	Endophyte	0
OTU_6795	Phialophora	Endophyte	0
OTU_8076	Phialophora	Endophyte	0
OTU_804	Phlebia	Wood Saprotroph	1
OTU_161	Phoma	Plant Pathogen-Wood Saprotroph	1
OTU_5300	Phoma	Plant Pathogen-Wood Saprotroph	1
OTU_759	Phyllactinia	Plant Pathogen	0
OTU_780	Phylliscum	Lichenized	0
OTU_1470	Physcia	Lichenized	0
OTU_2715	Physcia	Lichenized	0
OTU_1105	Physconia	Lichenized	0
OTU_2592	Platismatia	Lichenized	0
OTU_33	Pleurotus	Wood Saprotroph	1
OTU_2007	Pleurotus	Wood Saprotroph	1
OTU_2056	Pleurotus	Wood Saprotroph	1
OTU_60	Polyporus	Wood Saprotroph	1
OTU_205	Prosthemium	Saprotroph	0
OTU_1703	Prosthemium	Saprotroph	0
OTU_699	Protoparmeliopsis	Lichenized	0
OTU_1252	Pseudeurotium	Saprotroph	0
OTU_963	Pseudocercosporella	Plant Pathogen	0
OTU_1686	Pseudocercosporella	Plant Pathogen	0

OTU_620	Pseudogymnoascus	Unknown	0
OTU_1757	Pseudogymnoascus	Unknown	0
OTU_2552	Pyrenochaeta	Wood Saprotroph	1
OTU_331	Rhinocladiella	Wood Saprotroph	1
OTU_390	Rhinocladiella	Wood Saprotroph	1
OTU_1613	Rhinocladiella	Wood Saprotroph	1
OTU_1746	Rhinocladiella	Wood Saprotroph	1
OTU_1732	Rhizoctonia	Plant Pathogen	0
OTU_17	Rhizoscyphus	Mycorrhizal	0
OTU_1222	Rhizoscyphus	Mycorrhizal	0
OTU_1913	Rhizoscyphus	Mycorrhizal	0
OTU_1941	Rhizoscyphus	Mycorrhizal	0
OTU_1980	Rhizoscyphus	Mycorrhizal	0
OTU_2069	Rhizoscyphus	Mycorrhizal	0
OTU_3178	Rhizoscyphus	Mycorrhizal	0
OTU_3551	Rhizoscyphus	Mycorrhizal	0
OTU_3619	Rhizoscyphus	Mycorrhizal	0
OTU_4889	Rhizoscyphus	Mycorrhizal	0
OTU_67	Rhizosphaera	Plant Pathogen	0
OTU_306	Rhizosphaera	Plant Pathogen	0
OTU_456	Rhizosphaera	Plant Pathogen	0
OTU_2026	Saccharicola	Saprotroph	0
OTU_2209	Saccharicola	Saprotroph	0
OTU_5	Saccharomycetales	Saprotroph	0
OTU_11	Saccharomycetales	Saprotroph	0
OTU_44	Saccharomycetales	Saprotroph	0
OTU_45	Saccharomycetales	Saprotroph	0
OTU_47	Saccharomycetales	Saprotroph	0
OTU_55	Saccharomycetales	Saprotroph	0
OTU_101	Saccharomycetales	Saprotroph	0
OTU_315	Saccharomycetales	Saprotroph	0
OTU_364	Saccharomycetales	Saprotroph	0
OTU_402	Saccharomycetales	Saprotroph	0
OTU_414	Saccharomycetales	Saprotroph	0
OTU_667	Saccharomycetales	Saprotroph	0
OTU_706	Saccharomycetales	Saprotroph	0
OTU_740	Saccharomycetales	Saprotroph	0
OTU_770	Saccharomycetales	Saprotroph	0
OTU_835	Saccharomycetales	Saprotroph	0
OTU_871	Saccharomycetales	Saprotroph	0
OTU_948	Saccharomycetales	Saprotroph	0
OTU_954	Saccharomycetales	Saprotroph	0
OTU_1495	Saccharomycetales	Saprotroph	0
OTU_1611	Saccharomycetales	Saprotroph	0
OTU_1864	Saccharomycetales	Saprotroph	0
OTU_1926	Saccharomycetales	Saprotroph	0
OTU_2120	Saccharomycetales	Saprotroph	0
OTU_2155	Saccharomycetales	Saprotroph	0
OTU_2172	Saccharomycetales	Saprotroph	0
OTU_2347	Saccharomycetales	Saprotroph	0

OTU_2381	Saccharomycetales	Saprotroph	0
OTU_2497	Saccharomycetales	Saprotroph	0
OTU_3126	Saccharomycetales	Saprotroph	0
OTU_3182	Saccharomycetales	Saprotroph	0
OTU_3321	Saccharomycetales	Saprotroph	0
OTU_3450	Saccharomycetales	Saprotroph	0
OTU_3505	Saccharomycetales	Saprotroph	0
OTU_3582	Saccharomycetales	Saprotroph	0
OTU_3624	Saccharomycetales	Saprotroph	0
OTU_3643	Saccharomycetales	Saprotroph	0
OTU_3786	Saccharomycetales	Saprotroph	0
OTU_3798	Saccharomycetales	Saprotroph	0
OTU_4097	Saccharomycetales	Saprotroph	0
OTU_4307	Saccharomycetales	Saprotroph	0
OTU_4435	Saccharomycetales	Saprotroph	0
OTU_4644	Saccharomycetales	Saprotroph	0
OTU_4943	Saccharomycetales	Saprotroph	0
OTU_5157	Saccharomycetales	Saprotroph	0
OTU_5232	Saccharomycetales	Saprotroph	0
OTU_5370	Saccharomycetales	Saprotroph	0
OTU_5421	Saccharomycetales	Saprotroph	0
OTU_5777	Saccharomycetales	Saprotroph	0
OTU_6013	Saccharomycetales	Saprotroph	0
OTU_6081	Saccharomycetales	Saprotroph	0
OTU_6584	Saccharomycetales	Saprotroph	0
OTU_6969	Saccharomycetales	Saprotroph	0
OTU_6970	Saccharomycetales	Saprotroph	0
OTU_7055	Saccharomycetales	Saprotroph	0
OTU_7140	Saccharomycetales	Saprotroph	0
OTU_7161	Saccharomycetales	Saprotroph	0
OTU_7223	Saccharomycetales	Saprotroph	0
OTU_7468	Saccharomycetales	Saprotroph	0
OTU_8329	Saccharomycetales	Saprotroph	0
OTU_8370	Saccharomycetales	Saprotroph	0
OTU_8647	Saccharomycetales	Saprotroph	0
OTU_8898	Saccharomycetales	Saprotroph	0
OTU_9073	Saccharomycetales	Saprotroph	0
OTU_664	Scoliciosporum	Lichenized	0
OTU_57	Scutellinia	Saprotroph	0
OTU_77	Scutellinia	Saprotroph	0
OTU_89	Scutellinia	Saprotroph	0
OTU_105	Scutellinia	Saprotroph	0
OTU_127	Scutellinia	Saprotroph	0
OTU_193	Scutellinia	Saprotroph	0
OTU_210	Scutellinia	Saprotroph	0
OTU_468	Scutellinia	Saprotroph	0
OTU_1150	Scutellinia	Saprotroph	0
OTU_1163	Scutellinia	Saprotroph	0
OTU_1443	Scutellinia	Saprotroph	0
OTU_1520	Scutellinia	Saprotroph	0

OTU_1536	Scutellinia	Saprotroph	0
OTU_1646	Scutellinia	Saprotroph	0
OTU_1750	Scutellinia	Saprotroph	0
OTU_1958	Scutellinia	Saprotroph	0
OTU_2054	Scutellinia	Saprotroph	0
OTU_2298	Scutellinia	Saprotroph	0
OTU_2400	Scutellinia	Saprotroph	0
OTU_2513	Scutellinia	Saprotroph	0
OTU_2593	Scutellinia	Saprotroph	0
OTU_2703	Scutellinia	Saprotroph	0
OTU_2830	Scutellinia	Saprotroph	0
OTU_3488	Scutellinia	Saprotroph	0
OTU_4046	Scutellinia	Saprotroph	0
OTU_4069	Scutellinia	Saprotroph	0
OTU_4079	Scutellinia	Saprotroph	0
OTU_4111	Scutellinia	Saprotroph	0
OTU_4364	Scutellinia	Saprotroph	0
OTU_4673	Scutellinia	Saprotroph	0
OTU_4736	Scutellinia	Saprotroph	0
OTU_4852	Scutellinia	Saprotroph	0
OTU_5335	Scutellinia	Saprotroph	0
OTU_5417	Scutellinia	Saprotroph	0
OTU_6322	Scutellinia	Saprotroph	0
OTU_6858	Scutellinia	Saprotroph	0
OTU_1155	Sistotrema	Mycorrhizal_Wood Saprotroph	1
OTU_2327	Sistotrema	Mycorrhizal_Wood Saprotroph	1
OTU_3216	Sistotrema	Mycorrhizal_Wood Saprotroph	1
OTU_5432	Sistotrema	Mycorrhizal_Wood Saprotroph	1
OTU_9155	Sistotrema	Mycorrhizal_Wood Saprotroph	1
OTU_9460	Sistotrema	Mycorrhizal_Wood Saprotroph	1
OTU_2294	Sistotremastrum	Saprotroph	0
OTU_2535	Sphaerulina	Lichenized	0
OTU_899	Sporobolomyces	Fungal Parasite	0
OTU_334	Stereum	Saprotroph	0
OTU_657	Sydowia	Saprotroph	0
OTU_48	Tetracladium	Saprotroph	0
OTU_763	Tetracladium	Saprotroph	0
OTU_1173	Tetracladium	Saprotroph	0
OTU_1441	Tetracladium	Saprotroph	0
OTU_1938	Tetracladium	Saprotroph	0
OTU_2254	Tetracladium	Saprotroph	0
OTU_3194	Tetracladium	Saprotroph	0
OTU_4404	Tetracladium	Saprotroph	0
OTU_4589	Tetracladium	Saprotroph	0
OTU_8312	Tetracladium	Saprotroph	0
OTU_2366	Thanatephorus	Plant Pathogen	0
OTU_7129	Thanatephorus	Plant Pathogen	0
OTU_8643	Thanatephorus	Plant Pathogen	0
OTU_693	Thaxteriella	Saprotroph	0
OTU_1797	Thelonectria	Saprotroph	0

OTU_2275	Tolypocladium	Unknown	0
OTU_2	Trametes	Wood Saprotroph	1
OTU_4	Trametes	Wood Saprotroph	1
OTU_830	Trametes	Wood Saprotroph	1
OTU_837	Trametes	Wood Saprotroph	1
OTU_838	Trametes	Wood Saprotroph	1
OTU_857	Trametes	Wood Saprotroph	1
OTU_874	Trametes	Wood Saprotroph	1
OTU_927	Trametes	Wood Saprotroph	1
OTU_1058	Trametes	Wood Saprotroph	1
OTU_1076	Trametes	Wood Saprotroph	1
OTU_1205	Trametes	Wood Saprotroph	1
OTU_1256	Trametes	Wood Saprotroph	1
OTU_1304	Trametes	Wood Saprotroph	1
OTU_1436	Trametes	Wood Saprotroph	1
OTU_1537	Trametes	Wood Saprotroph	1
OTU_1574	Trametes	Wood Saprotroph	1
OTU_1832	Trametes	Wood Saprotroph	1
OTU_1859	Trametes	Wood Saprotroph	1
OTU_2036	Trametes	Wood Saprotroph	1
OTU_2338	Trametes	Wood Saprotroph	1
OTU_2341	Trametes	Wood Saprotroph	1
OTU_2349	Trametes	Wood Saprotroph	1
OTU_2363	Trametes	Wood Saprotroph	1
OTU_2407	Trametes	Wood Saprotroph	1
OTU_2495	Trametes	Wood Saprotroph	1
OTU_2506	Trametes	Wood Saprotroph	1
OTU_2644	Trametes	Wood Saprotroph	1
OTU_2678	Trametes	Wood Saprotroph	1
OTU_2707	Trametes	Wood Saprotroph	1
OTU_2870	Trametes	Wood Saprotroph	1
OTU_2947	Trametes	Wood Saprotroph	1
OTU_3087	Trametes	Wood Saprotroph	1
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OTU_3271	Trametes	Wood Saprotroph	1
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OTU_3373	Trametes	Wood Saprotroph	1
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OTU_3824	Trametes	Wood Saprotroph	1
OTU_3825	Trametes	Wood Saprotroph	1
OTU_3921	Trametes	Wood Saprotroph	1
OTU_3950	Trametes	Wood Saprotroph	1
OTU_3974	Trametes	Wood Saprotroph	1
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OTU_4064	Trametes	Wood Saprotroph	1
OTU_4135	Trametes	Wood Saprotroph	1
OTU_4138	Trametes	Wood Saprotroph	1

OTU_4203	Trametes	Wood Saprotroph	1
OTU_4376	Trametes	Wood Saprotroph	1
OTU_4405	Trametes	Wood Saprotroph	1
OTU_4418	Trametes	Wood Saprotroph	1
OTU_4660	Trametes	Wood Saprotroph	1
OTU_4662	Trametes	Wood Saprotroph	1
OTU_4930	Trametes	Wood Saprotroph	1
OTU_4968	Trametes	Wood Saprotroph	1
OTU_5054	Trametes	Wood Saprotroph	1
OTU_5160	Trametes	Wood Saprotroph	1
OTU_5368	Trametes	Wood Saprotroph	1
OTU_5419	Trametes	Wood Saprotroph	1
OTU_5516	Trametes	Wood Saprotroph	1
OTU_5642	Trametes	Wood Saprotroph	1
OTU_5709	Trametes	Wood Saprotroph	1
OTU_5722	Trametes	Wood Saprotroph	1
OTU_5750	Trametes	Wood Saprotroph	1
OTU_5757	Trametes	Wood Saprotroph	1
OTU_5786	Trametes	Wood Saprotroph	1
OTU_5824	Trametes	Wood Saprotroph	1
OTU_5876	Trametes	Wood Saprotroph	1
OTU_6119	Trametes	Wood Saprotroph	1
OTU_6217	Trametes	Wood Saprotroph	1
OTU_6302	Trametes	Wood Saprotroph	1
OTU_7075	Trametes	Wood Saprotroph	1
OTU_7088	Trametes	Wood Saprotroph	1
OTU_7187	Trametes	Wood Saprotroph	1
OTU_7224	Trametes	Wood Saprotroph	1
OTU_7332	Trametes	Wood Saprotroph	1
OTU_7410	Trametes	Wood Saprotroph	1
OTU_7464	Trametes	Wood Saprotroph	1
OTU_7557	Trametes	Wood Saprotroph	1
OTU_7766	Trametes	Wood Saprotroph	1
OTU_7817	Trametes	Wood Saprotroph	1
OTU_7878	Trametes	Wood Saprotroph	1
OTU_7954	Trametes	Wood Saprotroph	1
OTU_8021	Trametes	Wood Saprotroph	1
OTU_8264	Trametes	Wood Saprotroph	1
OTU_8339	Trametes	Wood Saprotroph	1
OTU_8384	Trametes	Wood Saprotroph	1
OTU_8457	Trametes	Wood Saprotroph	1
OTU_8503	Trametes	Wood Saprotroph	1
OTU_9010	Trametes	Wood Saprotroph	1
OTU_9033	Trametes	Wood Saprotroph	1
OTU_9044	Trametes	Wood Saprotroph	1
OTU_9579	Trametes	Wood Saprotroph	1
OTU_9596	Trametes	Wood Saprotroph	1
OTU_10156	Trametes	Wood Saprotroph	1
OTU_737	Tremella	Fungal Parasite	0
OTU_1727	Tremella	Fungal Parasite	0

OTU_64	Tremellales	Fungal Parasite_Saprotroph	0
OTU_217	Tremellales	Fungal Parasite_Saprotroph	0
OTU_450	Tremellales	Fungal Parasite_Saprotroph	0
OTU_720	Tremellales	Fungal Parasite_Saprotroph	0
OTU_979	Tremellales	Fungal Parasite_Saprotroph	0
OTU_1036	Tremellales	Fungal Parasite_Saprotroph	0
OTU_1066	Tremellales	Fungal Parasite_Saprotroph	0
OTU_1081	Tremellales	Fungal Parasite_Saprotroph	0
OTU_1116	Tremellales	Fungal Parasite_Saprotroph	0
OTU_1262	Tremellales	Fungal Parasite_Saprotroph	0
OTU_1563	Tremellales	Fungal Parasite_Saprotroph	0
OTU_1599	Tremellales	Fungal Parasite_Saprotroph	0
OTU_1606	Tremellales	Fungal Parasite_Saprotroph	0
OTU_1634	Tremellales	Fungal Parasite_Saprotroph	0
OTU_1802	Tremellales	Fungal Parasite_Saprotroph	0
OTU_2140	Tremellales	Fungal Parasite_Saprotroph	0
OTU_2250	Tremellales	Fungal Parasite_Saprotroph	0
OTU_2951	Tremellales	Fungal Parasite_Saprotroph	0
OTU_305	Trichoderma atroviride	Endophyte	0
OTU_5234	Trichoderma koningii	Endophyte_Plant pathogen	0
OTU_40	Trichoderma polysporum	Fungal Parasite	0
OTU_286	Trichoderma polysporum	Fungal Parasite	0
OTU_891	Trichoderma polysporum	Fungal Parasite	0
OTU_1612	Trichoderma polysporum	Fungal Parasite	0
OTU_2778	Trichoderma polysporum	Fungal Parasite	0
OTU_3466	Trichoderma polysporum	Fungal Parasite	0
OTU_3477	Trichoderma polysporum	Fungal Parasite	0
OTU_3762	Trichoderma polysporum	Fungal Parasite	0
OTU_5742	Trichoderma polysporum	Fungal Parasite	0
OTU_2718	Trichomerium	Endophyte	0
OTU_1653	Trichosporon	Animal Pathogen	0
OTU_2744	Trichosporon	Animal Pathogen	0
OTU_28	Tricladium	Saprotroph	0
OTU_171	Tricladium	Saprotroph	0
OTU_1798	Tricladium	Saprotroph	0
OTU_2042	Tricladium	Saprotroph	0
OTU_5137	Tricladium	Saprotroph	0
OTU_6210	Tricladium	Saprotroph	0
OTU_1018	Unguiculariopsis	Fungal Parasite	0
OTU_180	Valsa	Plant Pathogen	0
OTU_190	Valsa	Plant Pathogen	0
OTU_63	Varicosporium	Saprotroph	0
OTU_1775	Varicosporium	Saprotroph	0
OTU_267	Venturia	Plant Pathogen	0
OTU_1415	Verrucaria	Lichenized	0
OTU_1637	Verticillium	Plant Pathogen	0

Appendix S3

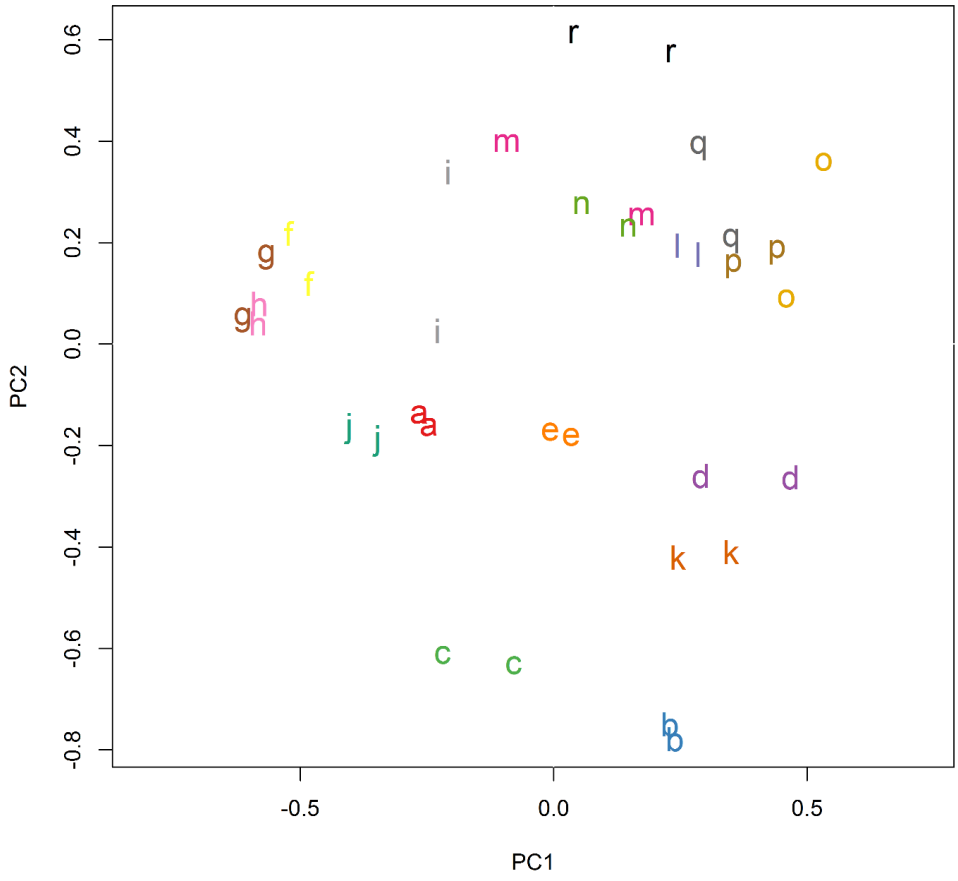

Figure S1) Composition of fungal OTUs in technical replicates in principal component analysis of Hellinger-tranformed abundance data. Identical letters and color signify replicates of the same sample.

Table S1) Centroids of constraining variables along the first (RDA1) and second (RDA2) axis of variation in redundancy analysis of Hellinger-transformed abundance of fungal OTUs in the treatment samples, with experimental treatment and log section as constraining variables, and tree identity, tree section, log diameter, landscape and site as conditional variables.

	RDA1	RDA2
Cage	-0.167	0.139
Cage control	0.004	0.076
Control	-0.044	-0.073
Ethanol-baited	0.207	-0.141
Log section (End)	0.131	0.159
Log section (Mid)	-0.132	-0.161

Table S2) OTU ID, fungal guild and species scores of the ten fungal OTUs with highest and lowest scores for the two first gradients of variation (RDA1 p-value = 0.001 and RDA2 p-value = 0.010) in redundancy analysis of Hellinger-transformed abundance of fungal OTUs in the treatment samples, with experimental treatment and log section as constraining variables, and tree identity, tree section, log diameter, landscape and site as conditional variables.

Fungal OTUs with highest scores		
OTU ID	**Fungal guild**	**RDA1**
Trametes_ochracea_2	Wood Saprotroph	0.232
Trametes_ochracea_4	Wood Saprotroph	0.186
Fungi_sp_8	Unknown	0.077
Cadophora_sp_13	Endophyte	0.075
Trametes_ochracea_9596	Wood Saprotroph	0.057
Fungi_sp_15	Unknown	0.056
Polyporales_sp_22	Unknown	0.048
Fungi_sp_14	Unknown	0.048
Trametes_versicolor_4930	Wood Saprotroph	0.035
Trametes_versicolor_2678	Wood Saprotroph	0.033
OTU ID	**Fungal guild**	**RDA2**
Ascocoryne_sp_6	Wood Saprotroph	0.109
Fungi_sp_8	Unknown	0.092
Ascomycota_sp_7	Unknown	0.079
Nakazawaea_anatomiae_11	Saprotroph	0.073
Penicillium_sp_146	Saprotroph	0.057
Coniochaeta_sp_18	Unknown	0.052
Penicillium_sp_109	Saprotroph	0.052
Fungi_sp_14	Unknown	0.051
Fungi_sp_16	Unknown	0.048
Cadophora_sp_13	Endophyte	0.043

Fungal OTUs with lowest scores		
OTU ID	**Fungal guild**	**RDA1**
Chondrostereum_purpureum_3	Wood Saprotroph/Plant Pathogen	-0.181
Candida_sp_5	Saprotroph	-0.127
Bjerkandera_adusta_1	Wood Saprotroph	-0.113
Penicillium_sp_146	Saprotroph	-0.060
Annulohypoxylon_multiforme_9	Endophyte/Saprotroph	-0.050
Nakazawaea_anatomiae_11	Saprotroph	-0.037
Nakazawaea_anatomiae_45	Saprotroph	-0.030
Fungi_sp_19	Unknown	-0.027
Venturiaceae_sp_21	Unknown	-0.025
Nakazawaea_populi_55	Saprotroph	-0.025
OTU ID	**Fungal guild**	**RDA2**
Trametes_ochracea_2	Wood Saprotroph	-0.099
Annulohypoxylon_multiforme_9	Endophyte/Saprotroph	-0.098
Chondrostereum_purpureum_3	Wood Saprotroph/Plant Pathogen	-0.089
Trametes_ochracea_4	Wood Saprotroph	-0.045
Candida_sp_5	Saprotroph	-0.028
Trametes_ochracea_9596	Wood Saprotroph	-0.023
Bjerkandera_adusta_6669	Wood Saprotroph	-0.020
Trichoderma_viride_25	Saprotroph	-0.019
Polyporus_varius_60	Wood Saprotroph	-0.016
Cerrena_unicolor_26	Wood Saprotroph	-0.014

Table S3) Linear mixed model fit by restricted maximum likelihood (REML) explaining number of sequences of OTUs annotated as *Trametes ochracea* (log+1) in treatment wood samples with experimental treatment (cage in the intercept), log section and log diameter as fixed effects and site, tree identity and tree section nested under tree identity as random effects.

Fixed effects	**Estimate**	**Std. error**	**t-value**	**p-value**
Intercept	9.64	2.15	4.48	<0.001
Cage control logs	1.05	0.65	1.62	0.106
Control logs	0.42	0.62	0.67	0.502
Ethanol-baited logs	1.73	0.62	2.77	**0.006**
Log section (Mid)	-0.83	0.38	-2.19	**0.028**
Diameter	-0.10	0.07	-1.30	0.194
Random effects	***Variance***	***Std. deviance***		
Site	*0.85*	*0.92*		
Tree identity (ID)	*5.21*	*2.28*		
Tree ID/Tree section	*1.00*	*1.00*		
Residual	*8.54*	*2.92*		
REML criterion at convergence: 1258.3				

Table S4) Linear mixed model fit by restricted maximum likelihood (REML) explaining number of sequences of OTUs annotated as *Trametes versicolor* (log+1) in treatment wood samples with experimental treatment (cage in the intercept), log section and log diameter as fixed effects and site, tree identity and tree section nested under tree identity as random effects.

Fixed effects	**Estimate**	**Std. error**	**t-value**	**p-value**
Intercept	5.49	1.57	3.50	<0.001
Cage control logs	1.02	0.46	2.20	**0.028**
Control logs	0.53	0.45	1.18	0.237
Ethanol-baited logs	1.43	0.45	3.21	**0.001**
Log section (Mid)	-0.52	0.24	-2.15	**0.031**
Diameter	-0.04	0.05	-0.81	0.420
Random effects	***Variance***	***Std. deviance***		
Site	*0.84*	*0.92*		
Tree identity (ID)	*2.80*	*1.67*		
Tree ID/Tree section	*0.94*	*0.97*		
Residual	*3.52*	*1.88*		
REML criterion at convergence: 1085.4				

Table S5) Linear mixed model fit by restricted maximum likelihood (REML) explaining number of sequences of OTUs annotated as *Chondrostereum pupureum* (log+1) in treatment wood samples with experimental treatment (cage in the intercept), log section and log diameter as fixed effects and site, tree identity and tree section nested under tree identity as random effects.

Fixed effects	**Estimate**	**Std. error**	**t-value**	**p-value**
Intercept	8.52	0.77	11.09	<0.001
Cage control logs	-0.13	0.22	-0.60	0.546
Control logs	-0.18	0.21	-0.83	0.404
Ethanol-baited logs	-0.29	0.21	-1.38	0.168
Log section (Mid)	0.36	0.14	2.65	**0.008**
Diameter	-0.01	0.03	-0.38	0.703
Random effects	***Variance***	***Std. deviance***		
Site	*0.72*	*0.85*		
Tree identity (ID)	*0.32*	*0.57*		
Tree ID/Tree section	*0.06*	*0.25*		
Residual	*1.11*	*1.05*		
REML criterion at convergence: 795.6				

PAPER V

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Priority effects of early successional insects influence late successional fungi in dead wood

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Abstract

Community assembly is an integral process in all ecosystems, producing patterns of species distributions, biodiversity, and ecosystem functioning. Environmental filters and colonization history govern the assembly process, but their relative importance varies depending on the study system. Dead wood decomposition is a slow process, allowing decomposer communities to develop within a slowly changing substrate for decades. Despite this, there are few long-term studies of priority effects from colonization history in this ecosystem. In this study, we investigate the importance of insects in early succession of dead wood on the fungal community present one decade later. Sixty aspen trees were killed in two study landscapes, each tree producing one aspen high stump and log. Insects were sampled with flight interception traps during the first 4 years after tree death, and fungal fruiting bodies were registered in year twelve. We found positive priority effects of two fungivorous beetles, the sap beetle *Glischrochilus quadripunctatus* and the round fungus beetle *Agathidium nigripenne*, on the Artist's bracket (*Ganoderma applanatum*) and a positive priority effect of wood-boring beetles on the ascomycete Yellow fairy cup (*Bisporella citrina*). The Aspen bracket (*Phellinus tremulae*) did not respond to insects in early succession of the dead wood. Our results suggest that early successional insects can have significant, long-lasting effects on the late successional fungal community in dead wood. Also, the effect can be specific, with one fungus species depending on one or a few fungivorous beetle species. This has implications for decomposition and biodiversity in dead wood, as loss of early colonizing beetles may also affect the successional pathways they seem to initiate.

Introduction

To understand patterns in species distributions, biodiversity, and ecosystem function, it is vital to understand the process of community assembly. Community assembly can be considered a purely deterministic process governed by abiotic factors such as nutrient availability and climate, or it can be influenced by colonization history (Drake 1991). Colonization history introduces a stochastic element which might lead to multiple stable states for similar habitats and thus increase biodiversity on a large scale (Chase 2010). There are several studies showing a marked effect of colonization history, often called a priority effect (Alford and Wilbur 1985; Shorrocks and Bingley 1994; Ejrnæs et al. 2006; Kennedy et al. 2009; Chase 2010; Dickie et al. 2012; Rasmussen et al. 2014). Short-term experimental studies have shown that manipulating arrival order of species can strongly affect not only species composition and richness, but also ecosystem function (Fukami et al. 2010; Dickie et al. 2012). In most ecosystems, community assembly is probably affected by both abiotic factors and priority effects, but the relative importance varies (Chase 2003, 2010).

Depending on the system, colonization history might only influence the community initially (Cifuentes et al. 2010), or it can have more long-lasting effects (Chase 2010; van de Voorde et al. 2011; Weslien et al. 2011). Priority effects of species arriving early can be positive or negative for the late successional species, leading to facilitative or inhibitory succession (Connell and Slatyer 1977). Connell and Slatyer (1977) suggested decomposer communities as a system where species assemblages could develop through facilitative succession. Their reasoning was that initial decomposition by early successional

species might make the substrate more accessible for species in late succession. Whether facilitative or inhibitory, priority effects are likely to be strong in decomposer communities due to the changeable nature of the habitat.

The decomposer community associated with dead wood constitutes a major component of the biodiversity in boreal forests (Stokland et al. 2012), including a large number of endangered species (Gärdenfors 2010; Kålås et al. 2010; Rassi et al. 2010). The dead wood community is mainly composed of insects and fungi. Wood-decay fungi have been shown to compete intensely for resources both in laboratory trials and in the field (Boddy 2000), and the competitive balance is influenced by the volume of wood each competitor controls (Holmer and Stenlid 1993). Furthermore, consistent patterns in fungal succession have been documented, with successor species following specific predecessor species (Niemelä et al. 1995; Ottosson et al. 2014). Thus, it is not surprising that strong priority effects have been found between wood-decay fungi, affecting species richness and wood-decay rate (Fukami et al. 2010; Dickie et al. 2012). This indirectly affects wood-living insects, as several studies have shown a structuring effect of the fungal community on the species assemblage of wood-living insects (Kaila et al. 1994; Jonsell et al. 2005; Abrahamsson et al. 2008; Leather et al. 2013). However, fungivorous insects can also affect fungal colonization history by acting as vectors for spores (Lim 1977; Tuno 1999; Persson et al. 2009; Strid et al. 2014) and may shift the competitive balance between fungi by preferential grazing (Crowther et al. 2011). Furthermore, wood-boring insects can function as ecological engineers that alter the habitat by tunneling under the bark and into the wood, potentially affecting both insects and fungi (Buse et al. 2008; Weslien et al. 2011; Strid et al. 2014; Ulyshen 2014).

Current studies on the effect of insects on species composition of fungi mainly span a few years or less (Müller et al. 2002; Strid et al. 2014), while the process of decomposition and succession in dead wood can span decades (Mäkinen et al. 2006). In this study, we use a dataset spanning more than 10 years to investigate long-term priority effects of beetles in early succession on wood-decay fungi in late succession of aspen (*Populus tremula* L.) dead wood. To our knowledge, there has only been one previous study of long-term priority effects in dead wood communities (Weslien et al. 2011). Weslien et al. (2011) showed that early colonizing wood-boring beetles (Coleoptera) affect subsequent establishment of the common wood-decay fungus the Red-belt conk (*Fomitopsis pinicola* (Sw.: Fr.) P. Karst.) in dead wood of spruce (*Picea abies* (L.) H. Karst.). We advance upon this knowledge by studying three species of fungi with contrasting life-history strategies and their response to not only wood-boring beetles, but also fungivorous beetles. Furthermore, we include two different forest environments in our study design, which allows us to assess whether the priority effects are conditional upon surrounding environment. Thus, our study tests the generality of the hypothesis that beetles in early succession of dead wood exert priority effects on fungi in late succession.

Materials and Methods

The field study was conducted in two landscapes in southern Norway, in the south boreal vegetation zone (Moen 1998), Losby forest holdings in Østmarka (Lat. 55.98, Long. 10.68, 150–300 masl) and Løvenskiold-Vækerø forest holdings in Nordmarka (Lat. 54.49, Long. 21.24, 200–500 masl). Both forest holdings were managed as sustainable production forests within the regulations of the PEFC (the Programme for the Endorsement of Forest Certification schemes, Norway, pefcnorway.org). Both landscapes consisted of forest dominated by spruce (*Picea abies*), with pine (*Pinus sylvestris* L.), birch (*Betula pubescens* Ehrh.), and aspen (*Populus tremulae*) as subdominants.

In 2001, 60 study sites were chosen with a minimum distance of 100 m between the sites, each containing a mature aspen tree with diameter ≥20 cm at breast height (1.3 m above ground) (Sverdrup-Thygeson and Birkemoe 2009). Within each study landscape, 15 study sites were established in closed canopy forest (aged 90–120 years) and 15 study sites in open, clear-cut forest areas (2–4 years since clear-cutting), each site being surrounded by a minimum of 10 m of the relevant habitat type. In the late fall of 2001, all 60 trees were cut at about 4 m above ground using detonating chord. Thus, after 2001, each site contained one aspen log and one aspen high stump.

In spring 2002, trunk window traps (40 cm × 60 cm) were mounted on the aspen high stumps, facing south and with the lower edge of the window pane 1 m above ground. The window traps collected insects by flight interception from medio May to medio August for 4 years following tree death, that is from 2002 to 2005 (Fig. 1). All beetle (Coleoptera) individuals were identified to species and categorized according to tree species preference and feeding guild according to the literature (Hansen et al. 1908–1965; Palm 1959; Hågvar 1999; Schigel 2011) and The Saproxylic Database compiled by Dahlberg and Stokland (2004) (accessible at http://radon.uio.no/WDD/Login.aspx?ReturnUrl=%2Fwdd%2FDefault.aspx). Vindstad and colleagues (unpublished data) are conducting a thorough analysis of the beetle communities for later publication. In the current paper, data from all 4 years of insect sampling were pooled in the statistical analysis.

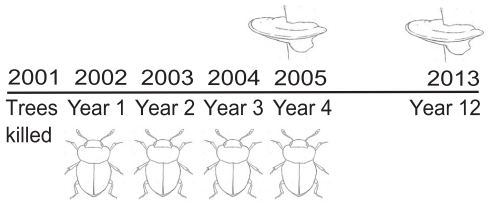

Figure 1. Time line showing time of tree death, followed by 4 years of insect sampling and registration of fungal fruiting bodies 4 years and 12 years after tree death.

In 2005, a precursory registration (presence/absence) of fungal fruiting bodies on high stumps and logs was conducted, identifying all polypores and a few other easily recognizable species. At the same time, proportion of bark left on the logs and high stumps was recorded.

In 2013, 12 years after tree death, fungal fruiting bodies of macrofungi on high stumps and logs, both Basidiomycetes and Ascomycetes, were registered (presence/absence) and identified to species. Only bark fungi that could be identified in the field were included. Fungi were categorized according to tree species preference recorded in the literature (Ryvarden and Melo 2014). High stumps and/or logs were missing at five sites in 2013, resulting in a total of 55 sites for analysis.

The fruiting body registration from 2013 was analyzed to explain distribution of certain fungus species, while the fruiting body registration from 2005 was only used to confirm whether these species had established at this point in succession and thus aid interpretation of the results.

Fruiting body surveys have certain methodological drawbacks, mainly the potential presence of a species as mycelium without fruiting body. However, high-throughput sequencing of mycelium in dead wood has shown that well-established species with high mycelial abundance tend to also have high fruiting rate (Ovaskainen et al. 2013). Thus, fruiting body surveys seem to be good indicators of dominating species.

Unless otherwise stated, all data were compiled to site level for analysis, combining fungal fruiting body registrations for high stumps and logs (presence at either high stump or log resulted in presence at site level).

Study species

Only five species of wood-decay fungi in late succession met the demands of occurrence at 10–45 sites (of 55) and preference for deciduous wood. Three of these species were chosen for their contrasting biology, in order to analyze for possible effect of early successional beetles; *Ganoderma applanatum* (Pers.) Pat., *Phellinus tremulae* (Bondartsev) Bondartsev & B.N. Borisov, and *Bisporella citrina* (Batsch: Fr.) Korf & S.E. Carp. *B. citrina* is an annual ascomycete, while *G. applanatum* and *P. tremulae* are both basidiomycetes and perennial polypores. Furthermore, *P. tremulae* often parasitically infects living aspen trees (Ryvarden and Melo 2014), while both *G. applanatum* and *B. citrina* usually only colonize the trees after death.

Of the beetles collected in the first 4 years after tree death, species of two feeding guilds were used to explain occurrence patterns for the three species of fungi in year 12; fungivorous and wood-boring beetles. Only species with a known affinity for dead wood of deciduous trees were included.

The fungivore guild included the fungivorous species in eight families: Ciidae, Endomychidae, Erotylidae, Latridiidae, Leiodidae, Ptinidae, Staphylinidae, and Nitidulidae. Interactions between fungivores and fungi depend on beetle feeding preferences, which can be species specific and are unknown for many species. Each species could not be tested separately, as that would lead to problems with multiple testing, so we initially tested for effect on the family level as a screening process for species-specific effects. If there was a near significant effect of family ($P < 0.1$), the most abundant species in the families were also tested for effect in separate analyses. For most of the families, a few species accounted for almost all of the sampled individuals.

The wood-borer guild included wood-feeding species in three families: Cerambycidae, Curculionidae, and Ptinidae, the latter including only *Ptilinus fuscus* (Geoffroy, 1785). The wood-borer guild was not partitioned further, as the hypothesized effect of wood borers as ecosystem engineers was expected to depend mostly on abundance of wood borers in general, and not on species-specific traits other than guild membership. For abundance of wood borers sampled in the window traps to affect the fungi in the aspen dead wood through habitat alteration, abundance had to reflect use of the substrate. This connection was confirmed for a subset of species, including the numerically dominant wood-borer *Rusticoclytus rusticus* (Linnaeus, 1785), in an earlier for study (Sverdrup-Thygeson and Birkemoe 2009).

Statistical methods

Generalized linear models (GLMs) with binomial distribution and logit link were used to test whether beetle abundance (fungivores or wood borers) affected the presence or absence of each of the three species of fungi. Wood-boring beetles and each family of fungivores were tested separately. Habitat type (open or closed forest), site

coordinates, and interaction between beetle abundance and habitat were included in all models, but the interaction was excluded if it was insignificant.

For fungivorous beetles significantly ($P < 0.05$) or near significantly ($P < 0.10$) associated with any of the three species of fungi, we also tested whether these fungivores were associated with the fungi registered in year 4 (with occurrences at 10–50 of 60 sites), to check whether the association with fungi in year 12 might be an indirect correlation due to attraction to fungi in year 4.

Effect of wood-borer abundance on bark loss from logs and high stumps in early stages of decay was tested by a GLM with mean bark cover of the aspen dead wood at each site as response variable. For fungi responding to wood-borer abundance, effect of bark cover in year 4 and dead wood object type (high stump or log) on occurrence of fruiting bodies in year 12 was also tested with GLMs. In these tests, the data for high stumps and logs were separated, resulting in two observations of all variables at most sites ($n = 106$).

All GLMs were evaluated with Pearson residual plots, Cooks distance, and the Hosmer–Lemeshow goodness of fit test (Hosmer and Lemeshow 2004). All analyses were conducted in R 3.1.1 (R Core Team 2014).

Results

In total, 552 beetle species (19 512 individuals) were sampled during the first 4 years after tree death, of which 277 species (13 476 individuals) were wood-living beetles associated with deciduous trees. The wood-borer guild consisted of 23 species (961 individuals) and the fungivore guild of 56 species (3456 individuals) (Table S1). Both beetle guilds were significantly more abundant in open, clear-cut forest than in the closed, mature forest (Fig. S1).

In the precursory registration of fungal fruiting bodies in year 4 after tree death, 14 species of fungi were registered. The most common species were *Trametes ochracea* (Pers.) Gilb. & Ryvarden (present at 50 of 60 sites) and *Chondrostereum purpureum* (Pers.: Fr.) Pouzar (present at 41 sites). Of the three fungus species from year 12 selected for analysis, only *P. tremulae* occurred already in year 4 (present at 24 of 60 sites).

In year 12 after tree death, 62 species of fungi were registered on the aspen high stumps and logs (including one species from the Norwegian Red List (Kålås et al. 2010), *Antrodia mellita* Niemelä & Penttilä). The most common species was *T. ochracea* which was present at 44 of 55 sites, followed by *B. citrina* at 41 sites (Table S2). *P. tremulae* was present at 19 sites and *G. applanatum* at 14 sites.

Effects of early fungivorous beetles on late successional fungi

Of the three species of fungi selected for analysis, only the saprotrophic polypore *G. applanatum* was affected by abundance of fungivores in the first 4 years after tree death. *G. applanatum* had a positive response to abundance of fungivorous sap beetles (Nitidulidae, $P = 0.06$) and round fungus beetles (Leiodidae, $P = 0.04$) (Table S3). The most abundant sap beetles were *Glischrochilus hortensis* (Geoffroy, 1785) (58% of the Nitidulidae individuals) and *G. quadripunctatus* (Linnaeus, 1758) (42%), and the most abundant round fungus beetles were *Agathidium nigripenne* (Fabricius, 1792) (51% of the Leiodidae individuals) and *Anisotoma humeralis* (Fabricius, 1792) (14%). Analyzing these four species separately showed that the polypore *G. applanatum* was more likely to be present 12 years after tree death at sites where the fungivorous beetles *G. quadripunctatus* ($P = 0.07$) and *A. nigripenne* ($P = 0.03$) had been abundant during the first 4 years after tree death, than on sites without this colonization history (Table 1, Figs 2 and 3).

Although not strictly significant, an increase in abundance of the fungivorous sap beetle *G. quadripunctatus* from 0 to 20 individuals in early succession increased probability of *G. applanatum* presence in late succession with as much as 43% in closed and 25% in open habitat (Fig. 2), as predicted from the model (Table 1). Increase in abundance of the round fungus beetle *A. nigripenne* from 0 to 20 individuals in early succession was predicted to increase probability of *G. applanatum* presence in late succession with about 35% in closed and 25% in open habitat (Fig. 3). Thus, although *P*-values were not very small, the effect sizes of the fungivores were noticeable.

The abundance of the fungivorous beetles *G. quadripunctatus* and *A. nigripenne* in year 1–4 was not correlated with any of the wood-decay fungi that were registered in year 4 (Table S4). Thus, these fungivores did not seem to be attracted to or hatching from any of the fungal fruiting bodies present in year 4 after tree death.

Effect of early wood-boring beetles on late successional fungi

Of the three species of fungi selected for analysis, only the ascomycete *B. citrina* was more likely to be present in year 12 after tree death at sites where wood-boring beetles had been abundant during the first 4 years after tree death (Table 2, Fig. 4).

An increase in abundance of wood-boring beetles from 0 to 20 individuals in early succession was predicted to increase the probability of *B. citrina* presence with about 13% in closed and 45% in open forest (Fig. 4).

Table 1. Presence of the basidiomycete *G. applanatum* in year 12 after tree death explained by abundance of the fungivores *G. quadripunctatus, G. hortensis, A. humeralis,* or *A. nigripenne* in year 1–4 after three death, habitat type (open/closed forest), and site coordinates in a generalized linear model (binomial distribution and logit link). $n = 55$.

	Estimate	Standard error	*z*-value	*P*-value
Intercept	−710.30	602.60	−1.18	0.239
Fungivorous beetle *G. quadripunctatus*	0.09	0.05	1.81	0.071
Habitat (Open forest)	−1.46	0.74	−1.97	0.048
x coordinate	1.1×10^{-4}	7.7×10^{-5}	1.36	0.173
y coordinate	9.7×10^{-5}	8.4×10^{-5}	1.16	0.248
Null deviance: 62.40 on 54 degrees of freedom				
Residual deviance: 55.44 on 50 degrees of freedom				
Intercept	−609.10	602.50	−1.01	0.312
Fungivorous beetle *G. hortensis*	0.04	0.03	1.31	0.191
Habitat (Open forest)	−1.34	0.73	−1.83	0.068
x coordinate	8.9×10^{-5}	7.6×10^{-5}	1.17	0.242
y coordinate	8.4×10^{-5}	8.4×10^{-5}	0.99	0.321
Null deviance: 62.40 on 54 degrees of freedom				
Residual deviance: 57.12 on 50 degrees of freedom				
Intercept	−792.40	622.50	−1.27	0.203
Fungivorous beetle *A. nigripenne*	0.08	0.04	2.13	0.034
Habitat (Open forest)	−0.83	0.70	−1.18	0.238
x coordinate	1.1×10^{-4}	7.9×10^{-5}	1.40	0.163
y coordinate	1.1×10^{-4}	8.7×10^{-5}	1.26	0.210
Null deviance: 62.40 on 54 degrees of freedom				
Residual deviance: 53.37 on 50 degrees of freedom				
Intercept	−570.90	588.90	−0.97	0.332
Fungivorous beetle *A. humeralis*	−0.01	0.14	−0.09	0.930
Habitat (Open forest)	−0.99	0.71	−1.40	0.160
x coordinate	8.2×10^{-5}	7.3×10^{-5}	1.12	0.264
y coordinate	7.8×10^{-5}	8.2×10^{-5}	0.95	0.341
Null deviance: 62.40 on 54 degrees of freedom				
Residual deviance: 58.79 on 50 degrees of freedom				

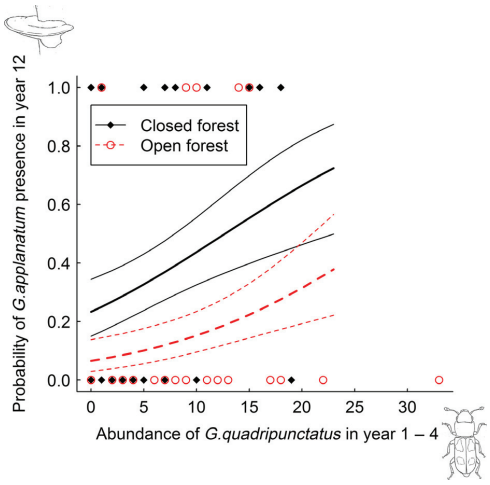

Figure 2. Observed presence of *G. applanatum* in year 12, with prediction lines and 95% confidence intervals based on the binomial GLM with abundance of *G. quadripunctatus* in the first 4 years after tree death as explanatory variable (Table 1). Prediction lines only extend to 23 individuals of *G. quadripunctatus*.

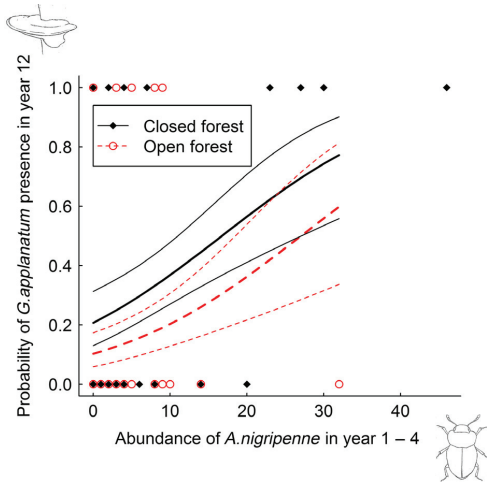

Figure 3. Observed presence of *G. applanatum* in year 12, with prediction lines and 95% confidence intervals based on the binomial GLM with abundance of *A. nigripenne* in year 1–4 after tree death as explanatory variable (Table 1). Prediction lines only extend to 32 individuals of *A. nigripenne*.

The aspen high stumps and logs had lost significantly more bark in year 4 at sites where wood-boring beetles had been abundant during the first 4 years after tree death (Fig. 5).

Although bark cover in year 4 did not affect *B. citrina* in year 12 if dead wood object (high stump/log) remained in the model, a negative interaction was found if dead wood object was excluded (Table 3). This was expected as the two variables were clearly correlated. Bark cover was significantly lower on logs than high stumps in early

Table 2. Presence of the ascomycete *B. citrina* in year 12 after tree death explained by abundance of wood-boring beetles in year 1–4, habitat type (open/closed forest) and site coordinates in a generalized linear model (binomial distribution and logit link). $n = 55$.

	Estimate	Standard error	*z*-value	*P*-value
Intercept	−2164.00	864.30	−2.50	0.012
Wood-boring beetles	0.10	0.05	2.04	0.042
Habitat (Open forest)	−2.32	1.10	−2.12	0.034
x coordinate	2.4×10^{-4}	9.5×10^{-5}	2.51	0.012
y coordinate	3.0×10^{-4}	1.2×10^{-4}	2.50	0.013
Null deviance: 62.40 on 54 degrees of freedom				
Residual deviance: 47.28 on 50 degrees of freedom				

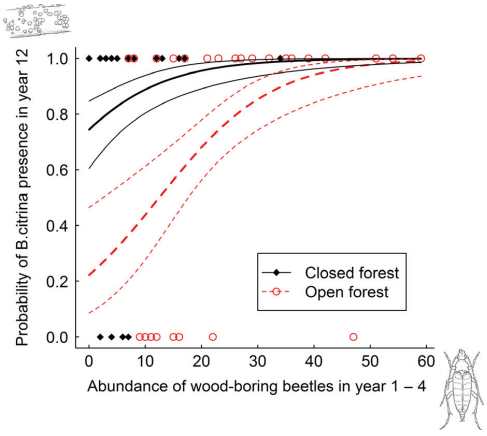

Figure 4. Observed presence of *B. citrina* in year 12, with prediction lines and 95% confidence intervals based the on binomial GLM (logit link) with abundance of wood-boring beetles in the first 4 years after tree death as explanatory variable (Table 2).

stages of decay (mean bark cover in year 4; logs = 45%, high stumps = 86%, Wilcoxon rank-sum test; $W = 423.50$, *P*-value <0.001), and *Bisporella citrina* occurred more often on logs than on high stumps in year 12 (Table 3).

Discussion

Our results strongly suggest that the establishment of fungi is affected by the colonization history of beetles in early succession of dead wood and that this priority effect is evident in two habitats with presumably quite different microclimates; closed and open forest. The predicted presence of the wood-decay fungi increased with as much

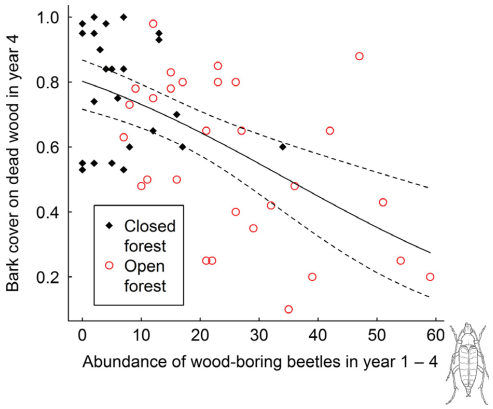

Figure 5. Bark cover (0–1, 1 = 100% cover) remaining in year 4 after tree death explained by abundance of wood-boring beetles during the first 4 years after tree death. Prediction line with 95% confidence intervals from binomial GLM (logit link) explaining bark cover by abundance of wood-boring beetles (estimate = −0.04 ± 0.02 standard error, *z*-value = −2.01, *P*-value = 0.045, $n = 55$).

Table 3. Presence of *B. citrina* in year 12 after tree death explained by bark cover (0–1, 1 = 100% cover) in year 4, dead wood object (high stump/log), and site coordinates in generalized linear models (binomial distribution and logit link). $n = 106$.

	Estimate	Standard error	*z*-value	*P*-value
Intercept	−1679.00	655.70	−2.56	0.010
Bark cover	0.98	1.15	0.86	0.392
Object (high stump)	−4.77	1.02	−4.66	<0.001
x coordinate	2.2×10^{-4}	8.5×10^{-5}	2.63	0.009
y coordinate	2.3×10^{-4}	9.1×10^{-5}	2.55	0.011
Null deviance: 139.46 on 105 degrees of freedom				
Residual deviance: 73.79 on 101 degrees of freedom				
Intercept	−416.40	428.00	−0.97	0.331
Bark cover	−0.03	0.07	−3.89	<0.001
x coordinate	3.8×10^{-5}	5.4×10^{-5}	0.70	0.482
y coordinate	5.9×10^{-5}	6.0×10^{-5}	0.99	0.321
Null deviance: 139.46 on 105 degrees of freedom				
Residual deviance: 117.23 on 102 degrees of freedom				

as 13–45% with increasing fungivore or wood-boring beetle abundance. The only fungus species with no response to insect colonization history was parasitic and could have infected the trees prior to tree death.

Our and previous research show that priority effects are important in heterotrophic communities (Shorrocks and Bingley 1994; Fukami et al. 2010; Weslien et al. 2011; Dickie et al. 2012; Ottosson et al. 2014). Comparisons of the strength of priority effects in different ecosystems are largely lacking but Chase (2010) showed that productivity

mediates the strength of priority effects in autotrophic ecosystems. While productivity might not be directly applicable to heterotrophic communities, patch size and patch continuity may have similar effects. Patch continuity is very long in the dead wood system and both our correlative field study and that of Weslien et al. (2011) indicate that priority effects from early colonizing wood-living beetles have a long-lasting and strong influence on the establishment of wood-decay fungi. Experimental or comparative studies including a wide range of short-lived and long-lived habitats are needed to establish whether priority effects in heterotrophic communities are modulated by patch continuity.

Strong priority effects can increase beta-diversity by leading to different species assemblages in similar habitats (Chase 2010). The dead wood community in boreal forests is remarkably species rich, with about 25% of all forest species associated with dead wood (Stokland et al. 2012). The priority effects in dead wood communities found in our and previous studies (Fukami et al. 2010; Weslien et al. 2011; Dickie et al. 2012; Ottosson et al. 2014) might contribute to this high biodiversity by increasing beta-diversity between habitat patches. If this is indeed the case, loss of early succession species would mean not only loss of the species themselves, but also of their priority effects and the subsequent successional pathways they might initiate. For instance, Weslien et al. (2011) found that colonization by the wood-boring beetle *Hylurgops palliatus* (Gyllenhal, 1813) in early succession had an indirect positive effect on the endangered beetle *Peltis grossa* (Linnaeus, 1758) in late succession of spruce dead wood. Thus, loss of *H. palliatus* from a region would presumably have a negative influence on *P. grossa*. Similarly, our study suggests that loss or reduced abundance of the round fungus beetle *A. nigripenne* in early succession might reduce the probability that the polypore *G. applanatum* will be present in late succession of aspen dead wood. This is important, as other studies indicate that the present forest management regime might lead to profound shifts in the abundance and composition of early succession beetle communities in a long time perspective (Kouki et al. 2012; Vindstad et al. unpublished data).

The fungivorous beetles *A. nigripenne* and *G. quadripunctatus* seemed to facilitate subsequent establishment of the wood-decay fungus *G. applanatum* and thereby follow the prediction of Connell and Slatyer (1977) that heterotrophic communities develop through facilitative succession. While Weslien et al. (2011) also found an example of inhibitory succession, all the priority effects in our study were positive. It seems that the nature of the priority effect depends on the biology of the study species. For instance, in both our study and the study of Weslien et al. (2011), the activity of wood-boring beetles seemed to facilitate bark loss, but whereas the polypore *F. pinicola* preferred higher bark cover and therefore responded negatively to wood-boring beetles (Weslien et al. 2011), *B. citrina* is known to prefer no bark cover (Hallingbäck and Aronsson 1998) and responded positively to wood-boring beetles in our study.

While wood-boring beetles can function as ecosystem engineers that alter the habitat and thereby affect species in late succession, fungivorous beetles do not impact the structure of the dead wood per se. Presumably, priority effects of fungivorous beetles in early succession on wood-decay fungi in late succession are mediated through spore dispersal or preferential grazing. While preferential grazing can have significant short-term effects on fungal communities (Crowther et al. 2011; A'Bear et al. 2014), this mechanism inherently facilitates one fungus species while inhibiting another. As we did not find any negative relationships between fungivores and fungi in year 4 or year 12, we consider preferential grazing to be a less likely explanation for the positive effect of fungivores. Spore dispersal seems to be the most likely mechanism in our study, and adults of both *A. nigripenne* and *G. quadripunctatus* are known to visit sporulating polypores (Hågvar and Økland 1997; Hågvar 1999; Økland 2002; Nikitsky and Schigel 2004; Schigel 2011), presumably to feed on spores.

There are certain well-known cases of spore dispersal by insects (Ingold 1953), such as the bark beetles that act as vectors for pathogenic fungi (Webber 2004). However, apart from these specialized relationships between specific species, the role of insects as spore dispersers is unclear. Several studies have shown that wood-living or fungivorous insects often carry large numbers of spores on their exoskeleton or in their gut (Lim 1977; Tuno 1999; Persson et al. 2009), but the effect of such incidental spore dispersal on distribution of fungi is difficult to assess. However, exclusion studies have shown that the fungal community that establishes in dead wood without insects is significantly different to the fungal community established when insects are present (Müller et al. 2002; Strid et al. 2014). In the study by Strid et al. (2014), they included a treatment with manufactured tunnels resembling those made by wood-boring beetles, and they found that these artificial tunnels only had a marginal effect. Thus, the effect of insects on the fungal community seemed to stem from something more than physical alteration of the substrate. Spore dispersal by fungivores in early succession should lead to strong positive priority effects such as those seen for *G. applanatum* in our study, as early arrival of fungal spores would enable the fungus to capture a large area of wood, increasing its competitive advantage against fungi arriving later (Holmer and Stenlid 1993).

Ganoderma applanatum was the only one of the three species of fungi tested that was positively associated with fungivores, and it is also the species most likely to be dispersed by fungivores. While the parasitic *P. tremulae* does not necessarily depend on dispersal after tree death and *B. citrina* produces small, annual fruiting bodies, *G. applanatum* is a saprotrophic polypore whose perennial fruiting bodies produce remarkable numbers of spores (Ingold 1953), and several insects have been recorded to visit its fruiting bodies (Kochetova et al. 2011; Schigel 2011; Ryvarden and Melo 2014). Tuno (1999) found that *Mycodrosophila* flies caught from fruiting bodies of *G. applanatum* both dropped and excreted large numbers of viable spores, and another *Ganoderma* species produces spores that only germinate after passage through insect intestines (Lim 1977), although reduced germination rate has also been found (Kadowaki, Leschen & Beggs 2011). For insects to function as vectors for spore dispersal, they must first contract the spores, presumably by visiting a fruiting body, and then deliver the spores in viable state to a suitable substrate. This mechanism is highly contingent upon species-specific traits, and it is therefore not surprising that the priority effects on *G. applanatum* were only found for fungivores in two of eight families tested.

It is possible that the fungivores in early succession and the fungi in late succession simply shared habitat preferences, resulting in a positive correlation. However, such indirect correlation through shared preferences offers no explanation for why *G. applanatum* was the only fungus species that responded to abundance of fungivores. Furthermore, at least with respect to the habitat types included in our design, fungi and insects exhibited opposite habitat preferences. The fungi tended to occur more often in closed forest, while both beetle guilds were more abundant in open habitats. Nevertheless, as this is an observational study, we can but suggest causal relationships. Future studies with greater control of environmental variables or of colonization history are necessary to verify the links underlying the priority effects observed in this study. Several studies of priority effects have experimentally manipulated the order of species arrival (Shorrocks and Bingley 1994; Ejrnæs et al. 2006; Kennedy et al. 2009; Chase 2010; Fukami et al. 2010; Dickie et al. 2012), which clarifies causality. On the other hand, effects that are discernible in field studies with natural colonization despite the increased variation in both colonization history and environment are more likely to be of significance for natural processes.

Conclusions

Our study strongly indicates that colonization history of insects in early succession has a significant, long-lasting influence on the fungal community in dead wood. Wood-boring beetles seemed to function as ecosystem engineers, as their activity increased bark loss from the dead wood in early decay, which facilitated the ascomycete *B. citrina* several years later. Furthermore, the positive priority effects of the fungivores *A. nigripenne* and *G. quadripunctatus* on the polypore *G. applanatum* suggest that there might be a mutual dependency between some species of fungivorous insects and fungi, possibly mediated by spore dispersal. This has important implications for conservation of wood-decay fungi, as some species might depend not only upon substrate availability, but also on facilitation by certain wood-living insects.

Data Accessibility

The data associated with this study have been submitted to Dryad digital repository (http://dx.doi.org/10.5061/dryad.jg2k4).

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Conflict of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Box plots showing the abundance of wood-boring and fungivorous beetles in closed and open forest habitat.

Table S1. Numbers of species and individuals of xylophages and fungivores sampled in 2002–2005 (year 1–4).

Table S2. Species inventory of all fungal fruiting bodies registered on aspen high stumps and logs in 2013 (year 12).

Table S3. GLM explaining presence of *G. applanatum* in year 12 by abundance of fungivores in family Nitidulidae or Leiodidae in year 1–4.

Table S4. Correlation between abundance of *G. quadripunctatus* or *A. nigripenne* in year 1–4 and presence of fungi in year 4 tested in GLMs.

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