Norwegian University of Life Sciences
Faculty of Environmental Science
and Natural Resource Management

Philosophiae Doctor (PhD)
Thesis 2022:43

The importance of invertebrates on fungi in dead wood

Hvordan invertebrater påvirker sopp i død ved

Lisa Fagerli Lunde

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Det mugne må i muldet ned;
det rådne er det færskes næring; –
her hules slægtens bryst af tæring,
og kan ej ondet hostes op, –
så ned i kisten med dets krop.

What moulders, in the mould's its doom,
What rots must nourish what is fresh;
Their vitals canker and consume,
Let them cough up the imposthume,
Or to the grave with their dead flesh!

Brand (Ibsen 1866) line no 5,61; English translation by Herford (1894) 222-223

Fomitopsis pinicola fruit body with a thick layer of spores and insect traces. Photo by Tone Birkemoe.

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Lunde LF, Jacobsen RM, Kauserud H, Boddy L, Nybakken L, Sverdrup-Thygeson A and Birkemoe T (2022). "Legacies of invertebrate exclusion and tree secondary metabolites control fungal communities in dead wood". *Molecular Ecology*. DOI: 10.1111/mec.16448

Paper II

Lunde LF, Boddy L, Sverdrup-Thygeson A, Jacobsen RM, Kauserud H and Birkemoe T. "Beetles provide directed dispersal of viable spores of a keystone wood decay fungus". Submitted to *Fungal Ecology*.

Paper III

Lunde LF, Høye TT, Ferkingstad B, Wegger HH and Birkemoe T. “Quantification of invertebrates on fungal fruit bodies by the use of time-lapse cameras”. Manuscript.

Paper IV

Lunde LF, Birkemoe T, Kauserud H, Boddy L, Jacobsen RM, Morgado L, Sverdrup-Thygeson A and Maurice S (2022). "DNA metabarcoding reveals host-specific communities of arthropods residing in fungal fruit bodies". *Proceedings of the Royal Society B*, 289, 202112622. DOI: 10.1098/rspb.2021.2622

2 Summary

Dead wood is an important energy source in boreal forests and it hosts a high biodiversity dominated by fungi and invertebrates. Wood decay fungi are the initial decomposers of dead wood and invertebrates interact with them by, for instance, feeding on sporocarps (fungal fruit bodies) or dispersal of fungal spores. In this thesis, we investigate the interactions between fungi and invertebrates in dead wood and, specifically, look at how invertebrates affect wood decay fungi. In **paper I**, we consider the effects invertebrates have on fungal communities in aspen wood using DNA metabarcoding combined with an in-field invertebrate exclusion experiment. Exclusion affected fungal communities after 4.5 years of decomposition, even though the exclusion had ceased 3 years prior. We identified fungal OTUs that contributed to this compositional difference: some seemingly benefitted from invertebrate exclusion, while other were hampered by it. In **paper II** and **III**, we look at invertebrates on sporocarps of the keystone fungi *Fomitopsis pinicola*. Beetles were collected on sporocarps and newly felled spruce logs and put on unmated fungal cultures. We then showed that the beetles may provide directed dispersal of viable *F. pinicola* spores, on their exoskeletons and through their digestive tracts (**paper II**). Invertebrate activity on *F. pinicola* sporocarps was investigated and quantified by the use of time-lapse cameras (**paper III**). Beetles were the most common visitors, followed by true flies, spiders, slugs and centipedes. We combined the data from **paper II** and **III** to present a first estimate of three beetles species' spore dispersal effectiveness. Different invertebrate taxa varied in their visitation patterns and this could affect their potential as spore dispersers. In **paper IV**, we use DNA metabarcoding to identify arthropod communities inside living sporocarps of eleven species of wood decay fungi. We identified a diverse and host-specific community of arthropod OTUs which was structured by sporocarp traits (**paper IV**). We speculate that this specificity is because the fungi invest in physical and chemical defence to deter harmful fungivores. Fungivory is common among invertebrates in dead wood, but we know little of how it affects fungal fitness. Furthermore, as we have shown that fungivores could also disperse fungal spores, both mutualistic and antagonistic interactions must be considered if we are to fully understand how invertebrates affect wood decay fungi.

3 Sammendrag

Død ved er en viktig energikilde i boreale skoger og danner grunnlag for et stort biologisk mangfold dominert av sopp og invertebrater. Vednedbrytende sopp sørger for den initielle nedbrytninga av død ved og invertebrater kan interagere med soppen ved, for eksempel, å spre soppsporer eller spise sporokarper (soppens fruktlegemer). I denne oppgava har vi sett på interaksjonene mellom sopp og invertebrater i død ved, spesifikt hvordan invertebrater påvirker vednedbrytende sopp. I **artikkel I** brukte vi DNA-strekkoding og et ekslusjonseksperiment for å undersøke hvordan invertebrater påvirker soppsamfunnet i ospeved. Ekslusjon av invertebrater påvirket soppsamfunnet etter 4 ½ år med nedbrytning, selv om eksperimentet endte 3 år tidligere (**artikkel I**). I tillegg identifiserte vi sopp-OTUer som ble positivt eller negativt påvirket av eksklusjonen. I **artikkel II** samlet vi biller på rødrandkjuke og nylig hogde granstubber og kultiverte dem på uparret mycel. Vi fant ut at biller kan bidra til direkte spredning av vednedbrytende sopp. I **artikkel III** brukte vi kamerateknologi for å se på aktiviteten til invertebrater på sporokarper av rødrandkjuke. De vanligste invertebratene var biller, fulgt av tovinger, edderkopper, snegler og skolopendere. Vi kombinerte data fra **artikkel II** og **III** og regnet ut tre billers sporespredningseffektivitet. Ettersom ulike invertebrater varierte i deres besøksmønstre kan dette påvirke hvor effektive de eventuelt vil være som sporespredere. I **artikkel IV** brukte vi DNA-strekkoding for å identifisere artropodesamfunn i sporokarpene til elleve arter vednedbrytende sopp. Vi fant et diverst og vertsspesifikt samfunn. Vi diskuterer hvorvidt denne spesifisiteten kan være et resultat av soppens kjemiske og fysiske forsvar. Dette ville isåfall indikert at soppspisende invertebrater påvirker soppens fitness, men det må forskes mer på dette temaet for å trekke videre konklusjoner, ikke minst fordi resultatene våre viser at soppspisere også kan ha positive effekter på sopp i død ved.

4 Synopsis

4.1 Introduction

4.1.1 The dead wood ecosystem

Dead wood are quintessential parts of boreal forest ecosystems, and are pivotal for carbon sequestration and nutrient recycling (Goodale et al. 2002, Stokland et al. 2012). Highly diverse ecosystems exist in the dead wood, and around a fourth of species in boreal forests are *saproxylic*, i.e. dependent on dead wood (Siitonen 2001, Stokland et al. 2012). This high diversity can be explained, in part, by niche differentiation between species; as various components affect the physicochemical properties of the wood, e.g. tree species, mortality factor, decay stage and wood diameter, a large number of niches are available for different species (Dahlberg and Stokland 2004, Stokland et al. 2012). Furthermore, several saproxylic species are associated with different microhabitats, like tree hollows, roots or polypore sporocarps (fungal fruit bodies), further increasing the number of niches available in dead wood ecosystems.

The high diversity of saproxylic species can also be explained by the high amount of potential energy that can be rendered from dead wood. Wood makes up more than 90% of aboveground biomass in forests, thus representing an extremely important source of organic carbon in the forest (Rayner and Boddy 1988). However, wood is also built of a tough molecular complex of lignin, cellulose and hemicellulose, which is more or less unpalatable in its fresh state. Dead wood becomes accessible as a broad food source after the structural decomposition done by wood decay fungi, which colonise and degrade the wood as mycelium, and bacteria (Rayner and Boddy 1988, Johnston et al. 2016, Wilhelm et al. 2019, Tláskal et al. 2021). Wood decay fungi are therefore essential for dead wood food webs because: (1) several species (e.g. other fungi, bacteria, insects with gut symbionts) feed on the residual compounds that are created during fungal decomposition of wood; and (2) fungivores, parasites and scavenger feed on the fungi themselves. Even wood-eating insects are dependent on some fungal biomass to complete their growth (Filipiak and Weiner 2014).

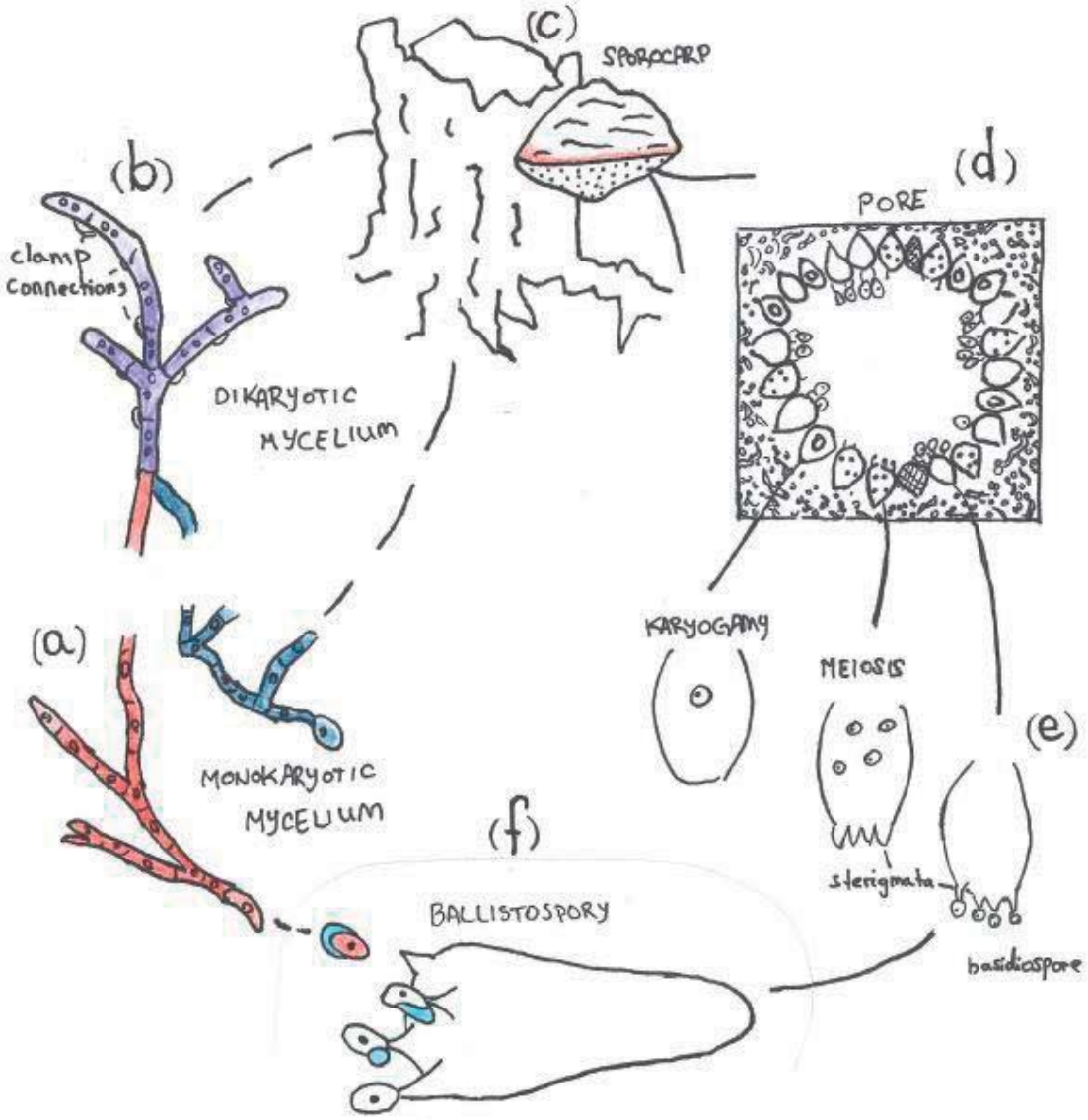
4.1.2 Wood decay fungi

The ability to decay wood has evolved several times within Basidiomycota (Fungi) (Floudas et al. 2012, Floudas et al. 2015) by species that inhabit and eat the wood as mycelium (Box 1). Polypores are important wood decayers and a polyphyletic group that is defined by producing sporocarps that protrude from the wood surface with a hymenium (i.e. spore-producing layer) that is developed within pores (Box 1). All polypores decompose cellulose and hemicellulose, but they differ in their strategies for decomposition of lignin – a recalcitrant aromatic-rich polymer that needs to be modified in order to access the wood carbon. White rot fungi degrade the lignin with specific enzymes while brown rot fungi only modify it by generating free reactive radicals in a Fenton reaction (Floudas 2021, Skrede 2021). The carbon that was bound inside the lignocellulose thus becomes available as cellulose or simple sugars that can be utilised by other agents of the ecosystem.

Antagonistic interactions occur frequently between fungi in dead wood and are among the main limitations to their growth (Boddy 2000). The fungi combat each other upon contact or at a distance by using chemical and physical warfare. The outcomes of such combats can be that one fungus partially of wholly replaces the other's territory (Woodward and Boddy 2008). However, as most of the fungal activity happen in the wood, it is not easily observed in the field. A hierarchical scheme can be drawn of fungal species' combative abilities by culturing and testing them pairwise on agar plates in the lab (Maynard et al. 2017). Yet, when three or more species are cultured together, the competitive hierarchy can be altered (Hiscox et al. 2017, Hiscox et al. 2018), illustrating a complexity of the system that it is not easily replicated in experiments. Species may behave differently *in vitro* than *in situ* (Crowther et al. 2018) whereas some species can not be cultured at all.

Field studies that utilise molecular techniques to identify fungi indicate that saproxylic beetles can act as vectors for wood decay fungi (Persson et al. 2011, Jacobsen et al. 2017, Seibold et al. 2019). Invertebrates can, nevertheless, also be antagonistic towards fungi, as shown in studies where invertebrate grazing reduced the combative abilities of wood decay fungi (Crowther et al. 2011, Rotheray et al. 2011). However, when these experiments were moved to the field, the effects of fungivory appeared to weaken (Crowther and A'Bear 2012, A'Bear et al. 2014). The insight gained from culturing studies are extremely valuable, but to get a full understanding of how invertebrates affect the fungal community in dead wood, these methods need to be complemented with field studies that apply molecular identification.

Box 1. The polypore life cycle.



Polypores can grow and decay wood as monokaryotic or dikaryotic mycelia, i.e. mycelium with one or two nuclei per hyphal cell, respectively. (a) Monokaryotic mycelium germinates from viable haploid spores. (b) Mating is initiated when two monokaryotic mycelia of compatible mating types meet and fuse to form a dikaryotic mycelium. These can be distinguished microscopically by the presence of clamp connections that form between hyphal cells during the migration of the second nucleus to adjacent cells. (c) Under the right environmental conditions, dikaryotic mycelia can initiate sporocarp formation. When the sporocarp matures, the hymenium develops with numerous pores, hollow cylinders where spore production occurs on *basidia*. (d) Every pore on a sporocarp is lined with basidia that can be at different stages; (e) inside the basidium, dikaryotic nuclei fuse (karyogamy) and divide (meiosis), and then basidiospores are formed on the tip of sterigmata. (f) Spores are liberated from the sterigma by the formation of Buller's drop, which culminates in a surface tension catapult (ballistospory). The spores are then transported by wind, animals or water to a deposition site that, hopefully, is suitable for germination.

Drawing by Lisa F. Lunde [d modified from Ingold (1971), e modified from Buller (1933)].

4.1.3 Effects invertebratese have on wood decay fungi

Fungi and invertebrates are the most diverse organismal groups that inhabit dead wood and they interact in many different ways, often involving feeding (fungivory) or dispersal (Birkemoe et al. 2018). The effects that invertebrates have on fungi can be positive, negative or negligible. The effects can also be indirect, for instance if beetle tunnelling or gnawing in dead wood facilitates colonisation for the fungus. In some cases, interactions with invertebrates can cause altered order and timing of fungal species' arrival (assembly history) into the dead wood, potentially causing long-term effects on the future fungal community, known as priority effects (Chase 2003, Fukami 2015). Invertebrates can cause lasting effects on fungal communities (Weslien et al. 2011, Jacobsen et al. 2015, Leopold et al. 2017, Jacobsen et al. 2018b), although the mechanisms behind it are not well known.

Invertebrate fungivores can be obligate or facultative, feeding on fungal mycelium, spores, or the sporocarp itself. Sporocarps form ephemeral microhabitats that persist between a few weeks to several years depending on the species (Elton 1966, Matthewman and Pielou 1971, O'Connell and Bolger 1997). In ephemeral habitats like carrion and dung, most inhabitants are generalists. Here, specialisation may be hampered due to unpredictable occurrences and small differences in the physical and chemical profiles of the host species (Hanski 1989). In comparison, sporocarps of wood decay fungi are persistent habitats. Also, they consist of living tissue, which means that the fungus might invest in physical or chemical defence against fungivores if these interactions are indeed antagonistic.

The evidence for overall negative effects of fungivory, or of fungi investing in defence against fungivores, is inconclusive. Those that eat mushrooms seem to be generalised in their host selection (Lacy 1984, Hanski 1989), perhaps because the sporocarps live for short time periods. In the polypore *Trametes versicolor*, however, Guevara et al. (2000) found that fungivory reduced the species' reproductive output. Potential defences that wood decay fungi invest in are sporocarp toughness – determined by hyphal system complexity and other factors (Corner 1932, Schigel et al. 2004) – which may control host selection in ciid beetle species (Paviour-Smith 1960). Jonsell and Nordlander (2004) reported higher specialisation among insects utilising living, rather than dead, sporocarps. They suggested this was because wood decay fungi invest in chemical defence that wanes off as the sporocarp ages and decays. Yet, the effects of fungivory on wood decay fungi may also be positive, as animals that feed on fungi could act as dispersers, like is common for mammals that eat ectomycorrhizal fungi (reviewed in Vašutová et al. 2019).

Dispersal plays a crucial role in maintaining geographic distributions and gene flow of populations (Wright 1969, Vellend 2010). Fungi depend on external vectors, e.g. wind or animals, to transport their spores to new habitats. Recent research has suggested that the dispersal strategies of fungi can be understood by looking at the spore morphology; typically, the spores of wind-dispersed species are small, thin-walled and produced in large amounts, while animal-dispersed spores are more enduring and ornamented (Calhim et al. 2018). However, the view that a species is «*either* wind- *or* animal-dispersed» might be obstructing our understanding of the true adaptations and plasticity that make up dispersal patterns. For instance, seeds of the invasive thistle *Carduus nutans* is primarily dispersed by wind, but incorporating animal vectors into models of dispersal might be essential to understand their spread (Jongejans et al. 2008, Rogers et al. 2019).

The spore morphology of wood decay fungi suggests that their primary dispersal is by wind, and this has also been the traditional view among mycologists (Ingold 1971). Exceptions are found in well-known mutualisms, like wood wasps and *Amylostereum areolatum* (Slippers et al. 2011). Bark beetles also disperse their fungal symbionts effectively, but at the same time they carry wood decay fungi (Castello et al. 1976, Lim et al. 2006) and even facilitate their establishment in dead wood (Strid et al. 2014). It is becoming increasingly clear that animals might provide important contributions to the overall dispersal of fungi, even though the fungus is primarily adapted to spread by wind (Talbot 1952, Jusino et al. 2016).

The main advantage with animal-vectored dispersal is that it can be directed because animals can navigate straight to favourable habitat for establishment (Wenny 2001). Dead wood, the preferred habitat of wood decay fungi, is patchily distributed in boreal forests and the probability of a spore landing on it is low when carried by wind currents (Galante et al. 2011, Norros 2013). Indeed, spore deposition is the limiting phase of wind-dispersed fungi (Calhim et al. 2018), which underlines the importance of mapping all possible dispersal vectors to understand their demographic patterns. In plant studies, *seed dispersal effectiveness* is used to quantify and compare the number of seeds that different animals can successfully disperse (Schupp 1993, Schupp et al. 2010). Effectiveness is based on a quantitative component (the number of seeds dispersed) and a qualitative component (the probability that the seed successfully establishes and survives) (Schupp 1993). The framework can be used for other mutualisms, as well, for example between plants and pollinators (Schupp et al. 2017, Valverde et al. 2019, Fuster et al. 2020). Birkemoe et al. (2018) modified it to fit insect-fungus interactions (spore dispersal effectiveness), although this has so far not been tested.

4.2 Objectives

In this thesis, we explore the interactions between wood decay fungi and invertebrates in dead wood, with a special emphasis on how the fungi are affected. Specifically, we look at the effects invertebrates have on fungal communities over 4.5 years of wood decomposition; on sporocarps of one polypore, the common brown rot species *Fomitopsis pinicola*; and lastly, we look at arthropod communities inside fungal sporocarps. we discuss whether the effects invertebrates have on fungi are positive or negative and we suggest future research prospects to gain a better understanding of the mechanisms underlying these effects. In four studies, we combine extensive data from the field with laboratory work that includes both traditional methods of fungal culturing and state-of-the-art molecular methods. My main objectives are to find out whether:

a) Invertebrate exclusion during the early phases of succession has contingent effects on fungal communities in aspen dead wood (paper I)

If these effects are detected, we will aim to identify the fungi that contribute to the difference in community composition and test whether they are negatively or positively affected by the exclusion.

b) Beetles disperse viable spores of *F. pinicola* (paper II)

We will test whether beetles that are collected from sporulating sporocarps or newly felled spruce logs carry viable spores of *F. pinicola*, on their exoskeletons or through their digestive tracts.

c) Invertebrates have different visitation patterns on sporocarps of *F. pinicola* (paper III)

We aim to identify the invertebrate community that visits sporocarps of *F. pinicola* and compare their visitation frequency, length of different invertebrates and how they respond to temperature, temporal variation and the presence of competitors/prey.

d) Arthropods in sporocarps of wood decay fungi are host specific (paper IV)

We will identify the arthropod community inside living sporocarps of 11 species of wood decay fungi and identify co-occurrences. We will look for patterns in arthropod community and diversity against sporocarp traits, such as toughness.

4.3 Study design and sampling

Detailed descriptions of materials and methods can be found within each respective manuscript.

Paper I

In April 2014, four aspen logs (originating from 17 felled trees) were placed at each of 30 sites in the boreal forests of Østmarka and Nordmarka (Southeastern Norway). An in-field experiment was conducted to exclude invertebrates from colonising aspen logs for 1.5 years (i.e. the first two summers). At each site, the four logs were placed on plastic sheets and subjected to one of four experimental treatments: (1) covered with a mesh cage (1 x 1 mm) to exclude invertebrates (*cage*), (2) covered with a mesh cage with large holes to control for microclimatic effects inside the cage (*cage control*), (3) baited with ethanol (1L, 96%) to attract wood-inhabiting insects (*positive control*), and (4) no treatment (*control*). In November 2015, the experimental treatments and plastic sheets were removed from all logs.

Measurements were made at three points in time: at tree felling (year 0), after 1.5 years (year 2), and after 4.5 years (year 5). DNA metabarcoding was applied to identify the fungal community at all three time points by targeting the ITS2 region. Two samples were taken per experimental log in year 2 and 5, while samples from year 0 were taken from separate, but adjacent, wood discs (Figure 1). Samples for wood density analysis were taken from the experimental logs in year 2 and 5 (pooled two and four samples per log, respectively). In year 0, chemical analyses were done on bark (3 months after felling) and the wood discs to quantify resource quality (carbon, nitrogen, phenolic acids, flavonoids, salicylates, methanol-soluble and -insoluble condensed tannins) (Figure 1).

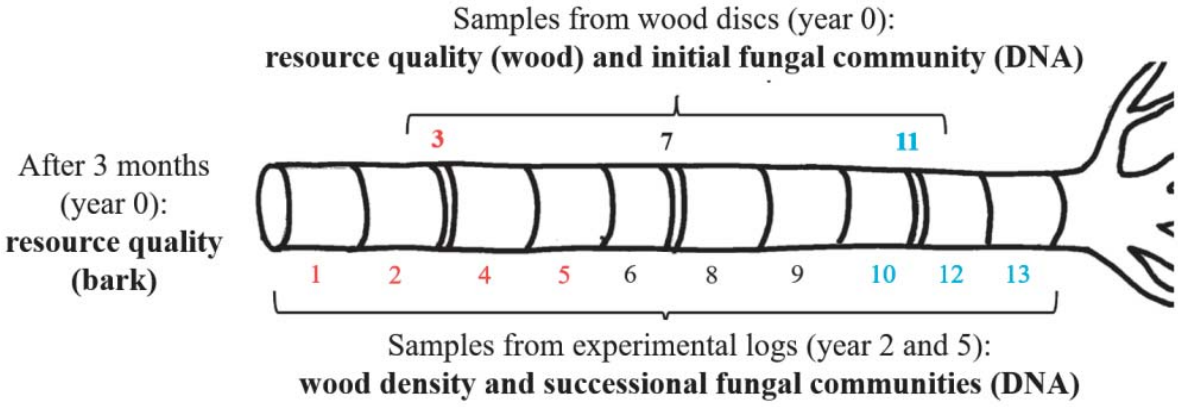


Figure 1. Explaining sampling scheme of fungal DNA (ITS metabarcoding), wood density and resource quality (C, N, phenolic compounds) in aspen dead wood. Samples were taken from different logs/discs derived from 17 felled aspen trees. Logs were sampled for wood density and fungal DNA (year 2 and 5). Wood discs were sampled right after felling for resource

quality and fungal DNA (year 0), and the values were linked with the nearest log in later analyses (same-coloured logs/discs in illustration). Bark was sampled after 3 months for resource quality (year 0) and the values were averaged per tree.

DNA analysis was done from sawdust samples using a modified CTAB protocol, pre-PCR cleaning (E.Z.N.A.® Soil DNA kit), PCR runs of year 0+2 (gITS7/ITS4, 2 libraries) and year 5 (fITS7/ITS4, 3 libraries), post-PCR cleaning (Wizard® Clean-Up System), magnetic beads purification (Agencourt AMPure XP), and Illumina Miseq sequencing (30% PhiX, 2 x 300 bp paired-ends, StarSEQ).

Paper II

Beetles were collected in the boreal forest of Nordre Pollen (Southeastern Norway) to investigate whether they could disperse viable spores of *F. pinicola*. The beetles were collected on the hymenium of *F. pinicola* sporocarps between May and June 2020, and on newly felled spruce logs (1 m long logs originating from one tree) in the same time period in 2021. Collected beetles were placed in sterile Petri dishes for 36 hours before sampling the head, elytra and faeces separately. Each sample was placed on a monokaryotic mycelium (i.e. unmated; Box 1) of *F. pinicola* that had been isolated from a different sporocarp in the same area. The fungal culture with a beetle sample on was inoculated in the dark at 25°C for 20 days and then checked for dikaryotisation by microscopic examination of clamp connections (Box 1), i.e. evidence that a viable spore of *F. pinicola* had been present on the beetle sample.

Paper III

Time-lapse cameras were mounted on the underside of *F. pinicola* sporocarps, facing upwards, to observe invertebrates on the hymenium (Figure 2). The study was situated in the boreal forests of Nordre Pollen and Østmarka (Southeastern Norway) between April and September 2019 (10 cameras) and 2020 (9 cameras). The cameras took pictures every ten minutes and had an in-built flash function. They were mounted ~20 cm from the hymenium, thus monitoring roughly the same hymenial area for all cameras (*ca*. 110 cm^2). A rubber ring and plexiglass was put on top of the lens to reduce dew, and frosted glass around it to reduce light reflection.

Figure 2. Time-lapse camera monitoring diurnal invertebrate activity on the hymenium of *Fomitopsis pinicola* sporocarps.

Paper IV

Living sporocarps of eleven species of wood decay fungi – *Amylocystis lapponica* (amylap), *Antrodia serialis* (antser), *F. pinicola* (fompin), *F. rosea* (fomros), *Gloeophyllum sepiarium* (glosep), *Phlebia centrifuga* (phecen), *Phellinus ferrugineofuscus* (phefer), *P. viticola* (phevit), *Phellopilus nigrolimitatus* (phenig), *Postia cyanescens* (poscae) and *Trichaptum abietinum* (triabi) – were sampled in the boreal forest of Issakka (Southeastern Finland) in October 2016 to identify the arthropod communities inside. The sporocarps were sampled aseptically (19-26 sporocarps per species), and by cutting out the outer layers (hymenium, etc.). Information on sporocarp traits, i.e. thickness, size, hymenial surface area, persistence, toughness and morphology, was compiled from the literature.

DNA analysis was done from sporocarp samples using a modified CTAB protocol, pre-PCR cleaning (E.Z.N.A.® Soil DNA kit), PCR (BF3/BR2, 3 libraries), normalisation (SequalPrep Normalization Plate kit), magnetic beads purification (Agencourt AMPure XP), and Illumina Miseq sequencing (20% PhiX, 2 x 300 bp paired-ends, StarSEQ).

4.4 Analyses

All statistical analyses were done in R (Team 2021), and figures were generated with base R, the ggplot2 (Wickham et al. 2016) and sjPlot (Lüdecke 2021) packages. Multivariate analyses were done with the vegan package (Oksanen et al. 2013). Overview of analyses for all papers are found in Table 1.

Paper I

In brief, amplicons were processed by demultiplexing (CUTADAPT); denoising, dereplication, chimera removal and merging (DADA2); removal of conserved regions (ITSx); clustering ASVs to OTUs (VSEARCH); post-curation (LULU) generating 1287 OTUs; taxonomic assignment against the UNITE+INSD database (88.13%, BLAST+); and guild assignment with FUNGUILD (58.81%).

Patterns of fungal OTU community composition was investigated with (1) hypothesis testing with constrained ordination (CCA, selection criterion: *p*), and (2) global non-metric multidimensional scaling (gNMDS), and subsequent interpretation of the axes by linear mixed models (LMM, site as random effect) and 'envfit'. Fungal OTU richness and wood density were investigated with LMMs and site as random effect. Categorical explanatory variables were invertebrate exclusion, landscape and log section. Continuous explanatory variables, transformed to zero skewness (Økland et al. 2001), were initial bark and wood resource quality, and initial fungal community (described by the first two axes of a DCA ordination). Bark variables were average values per tree, while wood variable values were linked to the closest wood disc that had been sampled between logs at each tree (Figure 2). OTUs contributing to fungal community dissimilarities between treatment levels were identified with a similarity percentage procedure (SIMPER; perm. = 999; Clarke 1993). The rarefied sequence abundance of identified OTUs were then fitted to generalised LMMs (GLMMs; Poisson distribution), to test whether they were more or less abundant in caged logs in year 2 and 5. Random effects were «site» and log «distance from tree base» nested in «tree identity». Although this was a case of multiple testing, Bonferroni correction of *p* values is often too conservative for genetic data (Lydersen 2021). Therefore, and because *p* values were not the main interest in these tests, a conservative significance value was set ($\alpha = 0.01$).

Paper III

Images from 19 cameras were filtered with an edge detection algorithm (variance of Laplacian to estimate image blur; Pech-Pacheco et al. 2000). Then, images were

manually looked through, and all visitors were annotated to the lowest possible taxon (Dutta and Zisserman 2019). GLMMs (Poisson/Binomial distribution) and model averaging were applied to test whether invertebrate observations were affected by temperature, *Gyrophaena boleti* clusters, time of day or season. Responses were observations of *Thymalus limbatus*, *Peltis ferruginea*, *Ipidia binotata*, *Lordithon lunulatus*, Diptera, Gastropoda, Araneae and Chilopoda. Random effects were camera ID (where possible) and an observation-level factor variable. Visitation was quantified by a count vector that added 1 when a new individual entered a frame (e.g. 0→1) in successive images, but not when they left (e.g. 2→1).

Paper IV

In brief, amplicons were processed by demultiplexing (CUTADAPT); denoising, dereplication, chimera removal and merging (DADA2); clustering ASVs to OTUs (VSEARCH); post-curation (LULU); and taxonomic assignment against the BOLD and NCBI databases (46%, BLAST+), resulting in 1664 arthropod OTUs.

Arthropod community composition was investigated with gNMDS, subsequent interpretation of axes with 'envift', hypothesis testing with constrained ordination (CCA, selection criterion: *p*) and PERMANOVA. Arthropod Shannon diversity was tested with LMMs and fungal host as random effect. Significant co-occurrences were identified with the indicspecies package (De Caceres et al. 2016) and visualised as a tripartite network with the igraph package (Csardi and Nepusz 2006).

Table 1. Overview of statistical analyses performed in all four papers in the thesis.

Paper	Response variables	Measurement	Statistical methods	Variables of interest	Random effects
I	β- and α-diversity (fungal OTUs), wood density	DNA metabarcoding (ITS2)	LMM, NMDS, CCA	Exclusion treatment. Wood (and bark) phenolic acids, flavonoids, salicylates, tannins, carbon, nitrogen and initial fungal community (DCA1, 2)	Site
II	Detection of viable *F. pinicola* spores	Beetle collection and culturing of monokaryotic mycelia	χ^2 contingency and Fisher's exact test	Beetle sample type (head, elytra, faeces)	-
III	Invertebrate observations on *F. pinicola* sporocarps (8 taxa)	Time-lapse cameras	GLMM	Temperature, time of day (sin + cos), day of year, presence of *G. boleti* cluster	Camera ID, observation-level
IV	β- and α-diversity (arthropod OTUs)	DNA metabarcoding (COI)	LMM, NMDS, CCA, PERMANOVA	Sporocarp size, thickness, hymenophore area, persistence, toughness, morphology	Fungal species

4.5 Main Results

a) Invertebrate exclusion during the early phases of succession has contingent effects on fungal communities in aspen dead wood (paper I)

Invertebrate exclusion affected fungal community composition in aspen dead wood in year 2 and 5 (Figure 3). Twenty-six fungal OTUs significantly contributed to differences in experimental treatments (Table S1), but only ten of these had a significant higher, or lower, sequence abundance in caged logs in year 2 or 5 (Table 2). Five fungal OTUs had more sequences in caged logs, while five OTUs had less sequences in caged logs, i.e. they were positively and negatively affected by invertebrate exclusion, respectively (Table 2). Three OTUs appeared to be indirectly affected by exclusion (i.e. affected in year 5, but not in year 2).

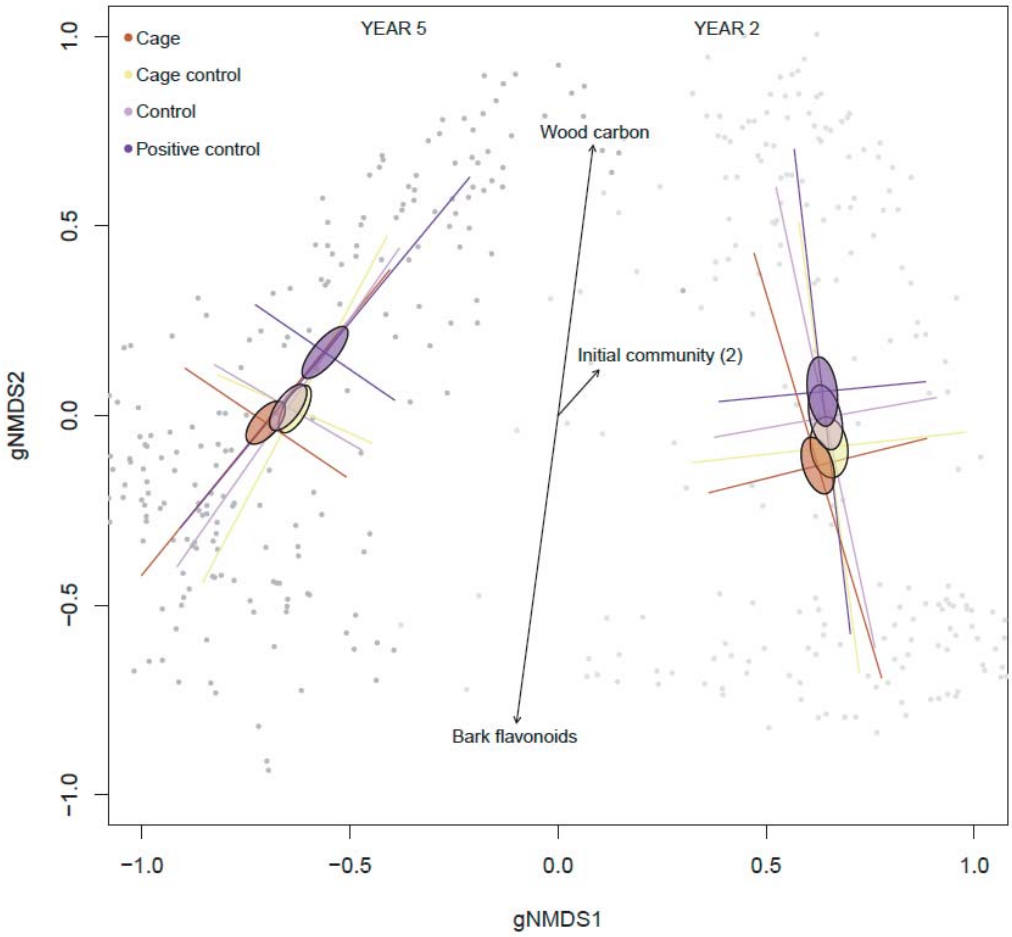


Figure 3. Global non-metric multidimensional scaling (gNMDS; Bray-Curtis, stress = 0.14) of fungal OTUs from dead aspen logs (n = 424) in year 2 (light grey) and 5 (dark grey) after tree felling. Logs were subjected to one of four invertebrate exclusion treatments until year 2. Coloured ellipses display the standard error of treatment levels, while error bars display the standard deviation. The initial fungal community (DCA2), concentration of wood carbon and bark flavonoids are fitted as vectors with 'envfit'.

Table 2. Fungal OTUs in aspen dead wood that were positively or negatively affected by invertebrate exclusion (caged logs) 2 and 5 years after tree felling. Sequence abundance (based on a rarefied dataset) of individual OTUs were fitted in GLMMs with $\alpha = 0.01$ (see Materials and Methods for details). Tested for all fungal OTUs that contributed to differential abundance in community composition between treatment groups identified with SIMPER. n.s. = not significant. Absent = not in dataset, filtered out or less than 1000 sequences.

		Abundance higher/lower in caged logs	
OTU ID	**Species**	**Year 2**	**Year 5**
OTU 9	*Annulohypoxylon multiforme*	n.s.	Lower
OTU 23	*Armillaria mellea*	- absent -	Higher
OTU 13	*Ascocoryne cylichnium*	Higher	Higher
OTU 10	*Cadophora* sp.	n.s.	Higher
OTU 15	*Coniochaeta* sp.	Higher	n.s.
OTU 29	*Coniochaeta* sp.	Lower	Lower
OTU 51	*Nakazawaea wyomingensis*	Higher	- absent -
OTU 18	*Peniophora incarnata*	Lower	- absent -
OTU 2	*Trametes ochracea*	Lower	Lower
OTU 47	*Trichoderma atroviride*	n.s.	Lower

Results discussed in **paper I**: Invertebrate exclusion did not affect decomposition rates or OTU richness. Initial wood and bark flavonoids and phenolic acids explained fungal community composition in year 5, while the initial fungal community affected communities in year 2, but not in year 5. Initial wood phenolic acid concentration had a positive effect on OTU richness in year 5. The initial concentration of bark methanol-insoluble condensed tannins impaired wood decomposition rates in year 2 and 5.

b) Beetles disperse viable spores of *F. pinicola* (paper II)

All eight beetle species that were collected from sporulating sporocarps of *F. pinicola* carried viable spores of the species – i.e. spores that germinated and dikaryotised with a monokaryotic mycelium of *F. pinicola* at least 36 hours after collection – through their digestive tracts and on their exoskeletons (Table 3). From newly felled spruce logs, 22 beetle species were collected, upon which eight carried viable spores on their exoskeletons and one in the faeces (Table 3).

Table 3. Beetles (Coleoptera) and whether they carry viable spores (in bold) of *Fomitopsis pinicola*. Collected on newly felled spruce logs or *F. pinicola* sporocarps. ^ Bark beetles.

Species	Family	Collected on	Individuals
Anthribus nebulosus	Antribidae	Spruce log	1
Platynus assimilis	Carabidae	Spruce log	1
Pterostichus melanarius	Carabidae	Spruce log	3
P. oblongopunctatus	**Carabidae**	**Spruce log**	**5**
Tetropium castaneum	**Cerambycidae**	**Spruce log**	**4**
Thanasimus formicarius	**Cleridae**	**Spruce log**	**8**
Atomaria vespertina	Cryptophagidae	Spruce log	2
Dryocoetes autographus^	**Curculionidae**	**Spruce log**	**2**
Hylobius excavatus	**Curculionidae**	**Spruce log**	**5**
Hylurgops palliatus^	**Curculionidae**	**Spruce log**	**4**
Polydrusus mollis	**Curculionidae**	**Spruce log**	**1**
Trypodendron lineatum^	**Curculionidae**	**Spruce log**	**5**
Ampedus nigrinus	Elateridae	Spruce log	1
Corticaria longicollis	Lathridiidae	Spruce log	1
Anisotoma humeralis	**Leiodidae**	**Both**	**21**
Hylecoetus dermestoides	**Lymexylidae**	**Spruce log**	**2**
***Epuraea* sp.**	**Nitidulidae**	**Both**	**26**
Ipidia binotata	**Nitidulidae**	**Sporocarp**	**44**
Ptinus subpilosus	Ptinidae	Spruce log	1
Rhizophagus dispar	**Rhizophagidae**	**Both**	**5**
Lordithon lunulatus	**Staphylinidae**	**Sporocarp**	**8**
Placusa incompleta	Staphylinidae	Spruce log	1
Sepedophilus littoreus	**Staphylinidae**	**Sporocarp**	**2**
Sepedophilus testaceus	Staphylinidae	Spruce log	2
Stenichnus collaris	Staphylinidae	Spruce log	1
Peltis ferruginea	**Trogossitidae**	**Sporocarp**	**34**
Thymalus limbatus	**Trogossitidae**	**Sporocarp**	**35**

c) Invertebrates have different visitation patterns on sporocarps of *F. pinicola* (paper III)

We recorded 6 248 invertebrate observations from 87 747 images (Figure 4) and the most common taxa were beetles (56.3%), spiders (11.3%), true flies (11.3%), slugs (6.4%) and centipedes (4.8%). The most frequent visitor was *T. limbatus* followed by true flies, and visitation length varied a lot between invertebrates (Table 5).

Results discussed in **paper III**: The invertebrate activity was structured by temperature, time of day (all showing a nocturnal pattern), time of season and the presence of *Gyrophaena boleti* clusters. The latter had a positive impact on the activity of predatory beetles *I. binotata* and *L. lunulatus*, as well as a negative effect on fungivorous *T. limbatus* and *P. ferruginea*.

Figure 4. Examples of images from time-lapse cameras where invertebrates were observed on the hymenium of *Fomitopsis pinicola* sporocarps (left: *Ipidia binotata* and a *Gyrophaena boleti* cluster; right: *Thymalus limbatus*, a slug and a *G. boleti* cluster).

Table 5. Number and length of visits of eight invertebrate taxa on the sporocarps of *Fomitopsis pinicola*, based on time-lapse camera images taking pictures every ten minutes. 1-2-3 are the first to third quartiles, i.e. Q2 = the median.

Taxon	Vis. frequency	Length, mean	Quartiles: 1-2-3
Thymalus limbatus	466	42.4 min	10-20-50 min
Peltis ferruginea	54	52.1 min	20-30-70 min
Lordithon lunulatus	122	21.2 min	10-10-30 min
Ipidia binotata	58	31.4 min	10-20-40 min
Diptera	222	30.2 min	10-10-30 min
Centipede	194	15.5 min	10-10-20 min
Gastropoda	127	30.3 min	10-10-40 min
Spiders	175	40.4 min	10-10-30 min

d) Arthropods in sporocarps of wood decay fungi are host specific (paper IV)

A diverse and host-specific community of arthropods was identified from inside living sporocarps of eleven species of wood-decay fungi. Nearly 75% of arthropod OTUs were specific to one or two fungal hosts (Figure 5a) and some had a preference for softer sporocarps (Figure 5b).

Results discussed in **paper IV**: The arthropod community was structured by species-specific sporocarp traits, namely size, thickness, hymenophore area, hyphal system complexity (i.e. toughness), persistence and morphology. Arthropod Shannon diversity was affected by sporocarp morphology, and diversity was expected to be highest in resupinate sporocarps, i.e. flat with large hymenial areas, rather than pileate sporocarps, i.e. thick and voluminous.

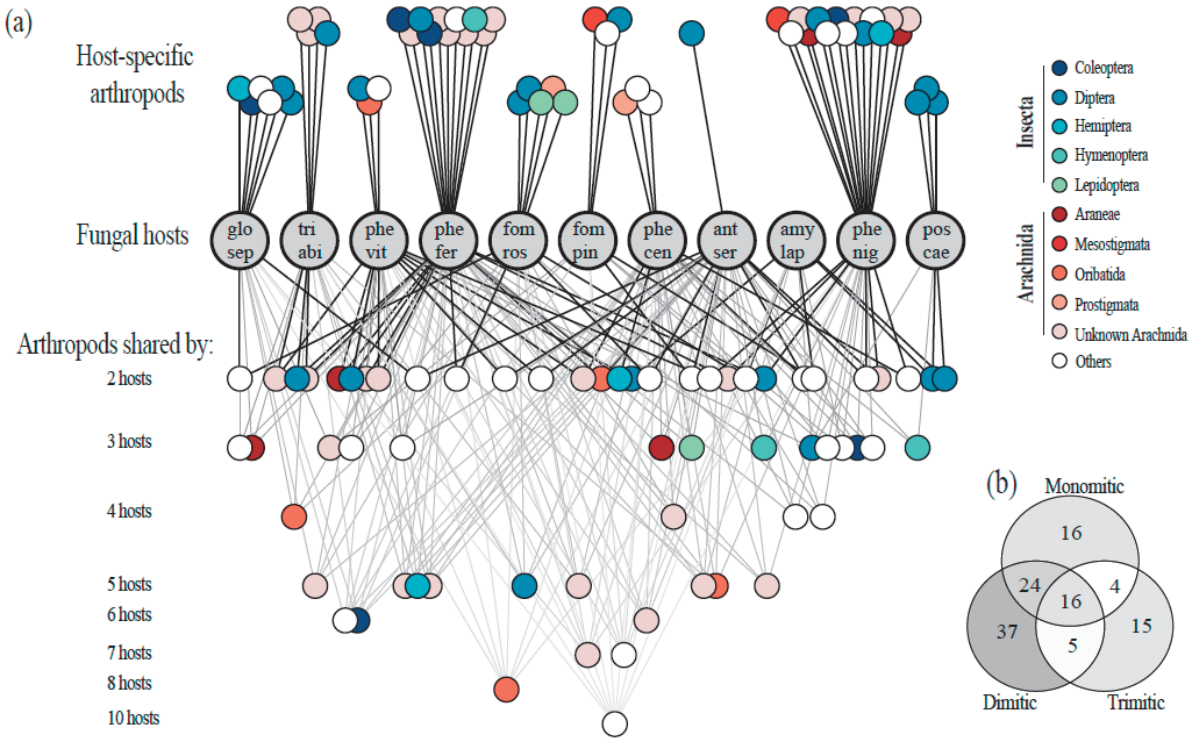


Figure 5. (a) Network displaying co-occurrence relationships between 117 arthropod OTUs and 11 fungal hosts (grey circles) identified with an indicator species analysis. Circles at the top are arthropods co-occurring with one fungal host, while circles underneath co-occur with 2-10 fungal hosts. Arthropods are coloured by orders (Arachnida in red, Insecta in blue/green). Network layout is manually adjusted from a tripartite Sugiyama layout algorithm. (b) Venn diagram showing the number of arthropod OTUs co-occurring with fungal hosts of differing hyphal systems (mono-, di- and trimitic).

4.6 Discussion

4.6.1 Invertebrates control fungal communities in dead wood through positive, negative and indirect effects

Invertebrate exclusion affected fungal community composition in aspen dead wood after 4.5 years of succession, even though invertebrates were only excluded for 1.5 years (**paper I**). Possibly, invertebrates can change the assembly history of fungi in wood by e.g. feeding on a dominant species (Fukami et al. 2016), facilitating establishment (Persson et al. 2011), or altering the competitive hierarchy between fungi (Chase et al. 2009, Crowther et al. 2011). Such priority effects caused by invertebrates have been shown in earlier studies (Weslien et al. 2011, Jacobsen et al. 2015, Leopold et al. 2017), and although our data are inadequate to determine whether these effect underlie our results, we can still discuss the nature of these underlying effects.

We identified 10 fungi contributing to the diverging effect in fungal community that were either negatively or positively affected by invertebrate exclusion (**paper I**). The fungi whose abundance was lower in caged treatments might benefit from invertebrates through dispersal. Indeed, DNA from all of these fungi have been found on wood-living beetles before (Jacobsen et al. 2017, Seibold et al. 2019). Fungi that benefitted from exclusion might get reduced survival through fungivory, for instance isopod grazing was reported to reduce the competitive abilities of two dominant fungal species in soil and wood (Crowther et al. 2011)

In addition, some of these fungi were absent during the first 1.5 years when invertebrates were excluded, and then more abundant in the formerly caged, or non-caged, logs after 4.5 years (**paper I**). One explanation for this indirect effect of the exclusion treatment is that some late colonising fungi are heavily affected by the presence of predecessors or antagonistic species. Furthermore, cord-forming fungi arriving via soil did not have access to the logs during the exclusion period, but are often strong competitors (Chapela and Boddy 1988, Hiscox et al. 2018). *Armillaria mellea* is a cord-forming species that got an indirect positive effect of the exclusion, arriving after 1.5 years possibly because the different community identity in formerly excluded logs might have given the species a competitive advantage.

Although these results are indications that invertebrates might directly or indirectly alter fungal community composition through priority effects, more

detailed studies of predecessor-successor relationships are needed to understand this fully. Furthermore, we need to identify the potentially positive or negative interactions that underlie these effects, e.g. whether invertebrates can disperse wood decay fungi and the extent to which fungivory affects fungal adaptations and fitness.

4.6.2 Beetles, and potentially other invertebrates, provide directed dispersal of wood decay fungi

We have demonstrated that beetles visiting sporocarps of *Fomitopsis pinicola* can disperse viable spores through their digestive tracts and on their exoskeletons (**paper II**). Using a subset of images from the time period when spore viability was demonstrated, we estimated the visitation frequency and length of three of these species (**paper III**). We assume that the product of these parameters and mean body size roughly represents the number of spores dispersed per species. We combine these data and present a tentative comparison of these beetles' *spore dispersal effectiveness* (Schupp 1993, Birkemoe et al. 2018), based on the expected number of spores dispersed (quantitative component) and the proportion of individuals carrying viable spores (qualitative component) (Table 6). The most effective disperser was *Thymalus limbatus* followed by *Peltis ferruginea*, both species being fungivores of the family Trogossitidae. As the larvae of these species live in rotten wood while adults eat sporocarp hymenium of different fungal species, *P. ferruginea* has even been suggested to disperse red-listed polypores that colonise *F. pinicola*-rotten wood (Schigel et al. 2004).

Table 6. Estimated spore dispersal effectiveness (SpDE) of three beetle species visiting *Fomitopsis pinicola* sporocarps between 4 May and 9 June 2020 in Nordre Pollen nature reserve. Beetle visitation frequency (number of visits per hour) and length (mean, minutes) are estimated from 5 sporocarps and 13 552 images (paper III). Mean body size is compiled from Hagge et al. (2021). Percentage viable spores is the proportion of elytra or faecal samples where viable *F. pinicola* spores were detected (paper II).

Species	**Visitation frequency**	**Visitation length**	**Mean body size**	**% viable spores (elytra, faeces)**	**SpDE (elytra, faeces)**
Ipidia binotata	0.0115	24 min	4.75 mm	(0.98, 0.64)	(0.1298, 0.0848)
Peltis ferruginea	0.0044	36 min	8.5 mm	(0.96, 1.00)	(0.1301, 0.1355)
Thymalus limbatus	0.0111	28 min	6 mm	(1.00, 0.94)	(0.1886, 0.1773)

The effectiveness of animal dispersers is a framework that was developed for plant-frugivore interactions to identify components that make up a good disperser. In the present study, the estimate of three beetles' spore dispersal effectiveness that we present must be interpreted with caution, most notably because we do not have a measure of the *proportion* of spores surviving on beetles during dispersal. However, this first attempt can hopefully act as a springboard to future research using the effectiveness framework to study spore dispersal, as suggested by Schupp et al. (2017) and Birkemoe et al. (2018). Further, it offers some solutions to known methodological challenges; although we cannot see that time-lapse cameras are better at detecting beetle diversity than manual observations are (Table S2), they certainly are less time-consuming if you wish to obtain quantitative data on visitation length and frequency. Moreover, although the fate of a dispersal propagule after it has been deposited, i.e. its subsequent survival and growth, is not estimated by effectiveness, we need to link the contributions of invertebrate dispersers to fungal fitness. This can prove a major empirical challenge (Rogers et al. 2019), and is also complicated as it depends on biotic interactions at the deposition site, for example density-dependent effects that can occur if clumps of spores are deposited at the same microsite (Spiegel and Nathan 2010).

Furthermore, we identified an invertebrate community, ranging from slugs to spiders, that visits the hymenium of *F. pinicola* sporocarps (**paper III**). Although only the beetles were identified to species and tested for spore-vectoring capacity, it is likely that other invertebrates also carry viable spores. Slugs have been shown to disperse fungal spores in earlier studies (Turchetti and Chelazzi 1984, Türke et al. 2010).

Beetles transported viable *F. pinicola* spores to newly felled spruce logs, which is a favourable habitat for spore deposition, and may as such provide directed dispersal of the fungus (**paper II**). Based on the spore morphology of most wood decay fungi, i.e. a large production of small and thin-walled spores, wind dispersal seems like the most likely adaptation (Calhim et al. 2018). However, given that dead wood is patchily distributed in the landscape, additional dispersal by animals could be advantageous by contributing to a more directed distribution of spores in the landscape. The relative importance of either dispersal strategies could depend on their potential for long-distance dispersal, which can be high with birds and mammals (Nathan et al. 2008, Golan and Pringle 2017), although whether this extends to invertebrates is less clear. However, studies on long-distance dispersal

are challenging and would require a huge effort in tracking and/or estimating movement patterns of spores and of invertebrate vectors (Rogers et al. 2019).

Combined efforts in the field and of camera monitoring revealed 21 beetle species visiting *F. pinicola* sporocarps, including 9 beetle species which have not been recorded on this fungus before (Table S2). Intriguingly, none of the beetles that we know visit *F. pinicola* sporocarps (Hågvar and Økland 1997, Hågvar 1999, Nikitsky and Schigel 2004, Schigel 2011) were also found carrying viable spores to spruce logs. The beetles on the logs may therefore have acquired the spores from the air whilst being transported by wind, or picked them up at another deposition site, e.g. close to the parent sporocarp. Indeed, all beetle species carrying viable spores to spruce logs in this study dwell in dead wood habitats.

Dispersal of plants often happens in sequence, for example by ants or scatter-hoarding rodents that pick up seeds that have been deposited relatively close to the parent (Vander Wall and Longland 2004, Jansen et al. 2012), or through predation on frugivores (Hämäläinen et al. 2017). Our findings suggest that dispersal by wood decay fungi could also be sequential, for example: primary dispersal by wind and then, secondary dispersal by invertebrates that passively pick up wind-dispersed spores. Or, primary dispersal by invertebrates visiting sporulating sporocarps, and then by predators that eat the invertebrates, which could have been the case for one predatory beetle we found on spruce logs that had viable spores in its faeces. In both cases, the sequences might continue, for instance by higher-order dispersal by other predators. Although sequential dispersal makes the tracking of propagules much more challenging, they can be extremely important contributions to the overall dispersal of a species (Vander Wall and Longland 2004, Rogers et al. 2019).

4.6.3 Wood decay fungi are important food and habitat for invertebrates, and may be negatively affected by fungivory

Dead wood is, in its fresh form, recalcitrant and its nutrient levels are too low to support growth and development of saproxylic invertebrates (Filipiak and Weiner 2014, Filipiak 2018). Wood decay fungi control nutrient dynamics in dead wood because they decompose the wood lignocellulose and translocate nutrients from outside the system (Rayner and Boddy 1988, Boddy et al. 2007). Saproxylic invertebrates thus benefit from fungal activities in the wood by eating the rotten wood – often containing some fungal bio- or necromass – or by eating the fungal mycelium, spore or sporocarps.

Sporocarps produce an enormous amount of nutritious spores, which are important additions to the diets of many invertebrates. Indeed, a large part of the invertebrate fauna we identified on *F. pinicola* sporocarps feed on spores or graze on the sporocarp itself; *Gyrophaena boleti*, which was common on *F. pinicola* sporocarps (**paper III**), is a specialist fungivore that spends all life stages on or in the hymenial pores (Staniec et al. 2016, Hågvar 2018); all beetles we collected from sporocarps had eaten spores (**paper II**), and the sporocarp-visiting slugs even left visible grazing marks that could be tracked in image sequences (**paper III**).

Sporocarps also form persistent microhabitats for invertebrates. We found a high degree of specificity between arthropods and sporocarps from 11 wood decay fungi (**paper IV**), a specificity that is supported by others (Jonsell and Nordlander 2004, Yamashita et al. 2015, Jacobsen et al. 2018a). Earlier studies have suggested that beetles have a preference for sporocarp toughness (Paviour-Smith 1960, Lawrence 1973, Lawrence 1989), which our results also indicate. However, a large number number of arthropods were specific to only one fungal species, which means that the arthropod network is also structured by other sporocarp traits that we did not measure, such as the chemical profile of the species. Others have reported that insects are more specialised on living, rather than dead, sporocarps (Jonsell and Nordlander 2004) and that grazing increases secondary metabolite production in the mould *Aspergillus nidulans* (Rohlfs 2015). These results suggest that sporocarp grazing by invertebrates can harm wood decay fungi, even so much that they invest in chemical and physical defence to wane them off. Indeed, Guevara et al. (2000) found that the reproductive output of *Trametes versicolor* was reduced due to sporocarp grazing. Also in this thesis, some fungal species were more common in aspen logs where invertebrates were excluded, which could mean that they are negatively affected by fungivory (**paper I**).

4.7 Concluding Remarks

From our results, it appears that invertebrates can have positive effects on fungi in dead wood. First, exclusion of invertebrates structured fungal communities over 4.5 years in aspen wood, and negatively affected the abundance of some fungal species (**paper I**). Further, we showed that beetles could disperse spores of wood decay fungi (**paper II**), but likely also other invertebrate fungivores (**paper III**). It seems like animal dispersal of wood decay fungi can happen in two ways: (1) from invertebrates that visit sporocarps, or (2) by dispersal sequences where an

invertebrate obtains spores that have already been transported by wind or a spore-feeding animal.

However, our results also suggest that invertebrates can have negative effects on wood decay fungi. Indeed, some fungi from aspen wood had higher abundances in aspen logs where invertebrates had been excluded (**paper I**). Although fungivores can contribute to dispersal of fungi, the many invertebrates we identified feeding on spores and sporocarps (**paper II, III**) could also potentially harm the fungus. Lastly, we identified a host-specific community of arthropods inside living sporocarps of wood decay fungi that was also structured by sporocarp traits, such as toughness (**paper IV**). These results indicate that wood decay fungi invest in defence against arthropods, something which would be expected if interactions were antagonistic.

If invertebrates can have both positive and negative effects on wood decay fungi, what are the overall effects they induce on fungi? This paradox is also debated in studies on seed-caching animals, as cachers act both as seed dispersers and predators. Insight from research on this topic show that these interactions are not exclusively positive or negative, but located along a mutualism-antagonism continuum that needs to be evaluated individually for each interaction (Gómez et al. 2019, Bogdziewicz et al. 2020). Furthermore, because of these divergent effects on plant fitness, adaptation to dispersal by seed-caching animals is often constrained (Gómez et al. 2019, 2022). Comparing to fungi and invertebrates, this could explain why wood decay fungi are (seemingly) adapted to wind dispersal, even though our results show that animal dispersal might be common too.

A demand going further will be to estimate the effects that different invertebrate species have on fungal fitness. This would require not only quantifying the effects of antagonistic and mutualistic interactions with invertebrates, but also to compare the relative importance of different dispersal modes. Even in plant ecology research, linking seed dispersal to plant fitness is a big empirical challenge, especially if the species rely on different modes of dispersal (Rogers et al. 2019).

It is essential to know the actual effects that invertebrates have on the fitness of wood decay fungi if we are to understand their evolutionary adaptations, but so far this question remains to be solved. In this thesis, we show that invertebrates can alter fungal community compositions (**paper I**), but we still do not understand how this happens on a population or organismal level. We suggest that directed dispersal by invertebrates can be an important contribution, but it also needs to be interpreted in light of any negative effects that invertebrates may have on fungi.

5 References

A'Bear, A. D., L. Boddy, E. Kandeler, L. Ruess, and T. H. Jones. 2014. Effects of isopod population density on woodland decomposer microbial community function. Soil Biology and Biochemistry **77**:112-120.

Birkemoe, T., R. M. Jacobsen, A. Sverdrup-Thygeson, and P. H. Biedermann. 2018. Insect-fungus interactions in dead wood systems. Pages 377-427 Saproxylic insects. Springer.

Boddy, L. 2000. Interspecific combative interactions between wood-decaying basidiomycetes. FEMS microbiology ecology **31**:185-194.

Boddy, L., J. Frankland, and P. Van West. 2007. Ecology of saprotrophic basidiomycetes. Elsevier.

Bogdziewicz, M., E. E. Crone, and R. Zwolak. 2020. Do benefits of seed dispersal and caching by scatterhoarders outweigh the costs of predation? An example with oaks and yellow-necked mice. Journal of Ecology **108**:1009-1018.

Buller, A. 1933. Researches on Fungi. Vol. V. . Longmans, Green and Co, London.

Calhim, S., P. Halme, J. H. Petersen, T. Læssøe, C. Bässler, and J. Heilmann-Clausen. 2018. Fungal spore diversity reflects substrate-specific deposition challenges. Scientific Reports **8**:1-9.

Castello, J. D., C. G. Shaw, and M. Furniss. 1976. Isolation of Cryptoporus volvatus and Fomes pinicola from Dendroctonus pseudotsugae. Phytopathology **66**:1431-1434.

Chapela, I., and L. Boddy. 1988. The fate of early fungal colonizers in beech branches decomposing on the forest floor. FEMS microbiology ecology **4**:273-283.

Chase, J. M. 2003. Community assembly: when should history matter? Oecologia **136**:489-498.

Chase, J. M., E. G. Biro, W. A. Ryberg, and K. G. Smith. 2009. Predators temper the relative importance of stochastic processes in the assembly of prey metacommunities. Ecology letters **12**:1210-1218.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian journal of ecology **18**:117-143.

Corner, E. 1932. A Fomes with two systems of hyphae. Transactions of the British Mycological Society **17**:51-81.

Crowther, T. W., and A. D. A'Bear. 2012. Impacts of grazing soil fauna on decomposer fungi are species-specific and density-dependent. Fungal ecology **5**:277-281.

Crowther, T. W., L. Boddy, and T. H. Jones. 2011. Outcomes of fungal interactions are determined by soil invertebrate grazers. Ecology letters **14**:1134-1142.

Crowther, T. W., L. Boddy, and D. S. Maynard. 2018. The use of artificial media in fungal ecology. Fungal ecology **32**:87-91.

Csardi, G., and T. Nepusz. 2006. The igraph software package for complex network research. InterJournal, complex systems **1695**:1-9.

Dahlberg, A., and J. N. Stokland. 2004. Vedlevande arters krav på substrat. Skogsstyrelsen, rapport **7**:1-74.

De Caceres, M., F. Jansen, and M. M. De Caceres. 2016. Package 'indicspecies'. indicators **8**:1.

Dutta, A., and A. Zisserman. 2019. The VIA annotation software for images, audio and video. Pages 2276-2279 *in* Proceedings of the 27th ACM international conference on multimedia.

Elton, C. S. 1966. Bracket Fungi and Toadstools. Pages 306-318 The Pattern of Animal Communities. Springer.

Filipiak, M. 2018. Nutrient dynamics in decomposing dead wood in the context of wood eater requirements: The ecological stoichiometry of saproxylophagous insects. Pages 429-469 Saproxylic Insects. Springer.

Filipiak, M., and J. Weiner. 2014. How to make a beetle out of wood: multi-elemental stoichiometry of wood decay, xylophagy and fungivory. PLoS One **9**:e115104.

Floudas, D. 2021. Evolution of lignin decomposition systems in fungi. Pages 37-76 Advances in Botanical Research. Elsevier.

Floudas, D., M. Binder, R. Riley, K. Barry, R. A. Blanchette, B. Henrissat, A. T. Martínez, R. Otillar, J. W. Spatafora, and J. S. Yadav. 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science **336**:1715-1719.

Floudas, D., B. W. Held, R. Riley, L. G. Nagy, G. Koehler, A. S. Ransdell, H. Younus, J. Chow, J. Chiniquy, and A. Lipzen. 2015. Evolution of novel wood decay mechanisms in Agaricales revealed by the genome sequences of Fistulina hepatica and Cylindrobasidium torrendii. Fungal Genetics and Biology **76**:78-92.

Fukami, T. 2015. Historical contingency in community assembly: integrating niches, species pools, and priority effects. Annual Review of Ecology, Evolution, and Systematics **46**:1-23.

Fukami, T., E. A. Mordecai, and A. Ostling. 2016. A framework for priority effects. Journal of Vegetation Science **27**:655-657.

Fuster, F., C. N. Kaiser-Bunbury, and A. Traveset. 2020. Pollination effectiveness of specialist and opportunistic nectar feeders influenced by invasive alien ants in the Seychelles. American Journal of Botany **107**:957-969.

Galante, T. E., T. R. Horton, and D. P. Swaney. 2011. 95% of basidiospores fall within 1 m of the cap: a field-and modeling-based study. Mycologia **103**:1175-1183.

Golan, J. J., and A. Pringle. 2017. Long-distance dispersal of fungi. Microbiology spectrum **5**:5.4. 03.

Gómez, J. M., E. W. Schupp, and P. Jordano. 2019. Synzoochory: the ecological and evolutionary relevance of a dual interaction. Biological reviews **94**:874-902.

Gómez, J. M., E. W. Schupp, and P. Jordano. 2022. The ecological and evolutionary significance of effectiveness landscapes in mutualistic interactions. Ecology letters **25**:264-277.

Goodale, C. L., M. J. Apps, R. A. Birdsey, C. B. Field, L. S. Heath, R. A. Houghton, J. C. Jenkins, G. H. Kohlmaier, W. Kurz, and S. Liu. 2002. Forest carbon sinks in the Northern Hemisphere. Ecological Applications **12**:891-899.

Guevara, R., A. D. Rayner, and S. E. Reynolds. 2000. Effects of fungivory by two specialist ciid beetles (Octotemnus glabriculus and Cis boleti) on the reproductive fitness of their host fungus, Coriolus versicolor. New Phytologist **145**:137-144.

Hagge, J., J. Müller, T. Birkemoe, J. Buse, R. H. B. Christensen, M. M. Gossner, A. Gruppe, C. Heibl, A. Jarzabek-Müller, and S. Seibold. 2021. What does a threatened saproxylic beetle look like? Modelling extinction risk using a new morphological trait database. Journal of Animal Ecology **90**:1934-1947.

Hanski, I. 1989. Fungivory: fungi, insects and ecology. Pages 25-68 *in* N. M. C. N. Wilding, P. M. Hammong, J. F. Webber, editor. Insect–fungus interactions. Academic Press.

Hiscox, J., J. O'leary, and L. Boddy. 2018. Fungus wars: basidiomycete battles in wood decay. Studies in mycology **89**:117-124.

Hiscox, J., M. Savoury, S. Toledo, J. Kingscott-Edmunds, A. Bettridge, N. A. Waili, and L. Boddy. 2017. Threesomes destabilise certain relationships: multispecies interactions between wood decay fungi in natural resources. FEMS microbiology ecology **93**.

Hämäläinen, A., K. Broadley, A. Droghini, J. A. Haines, C. T. Lamb, S. Boutin, and S. Gilbert. 2017. The ecological significance of secondary seed dispersal by carnivores. Ecosphere **8**:e01685.

Hågvar, S. 1999. Saproxylic beetles visiting living sporocarps of Fomitopsis pinicola and Fomes fomentarius. Norwegian Journal of Entomology **46**:25-32.

Hågvar, S. 2018. Contribution to the ecology of Gyrophaena boleti (Linnaeus, 1758)(Coleoptera, Staphylinidae) breeding in the pore layer of the fungus Fomitopsis pinicola (Fr.) Karst. Journal of Entomology **65**:108-114.

Hågvar, S., and B. Økland. 1997. Saproxylic beetle fauna associated with living sporocarps of *Fomitopsis pinicola* (Fr.) Karst. in four spruce forests with different management histories. Fauna Norvegica. Serie B, Norwegian journal of entomology **44**:95-105.

Ingold, C. T. 1971. Fungal spores. Their liberation and dispersal. Oxford University Press, London.

Jacobsen, R. M., T. Birkemoe, and A. Sverdrup-Thygeson. 2015. Priority effects of early successional insects influence late successional fungi in dead wood. Ecology and Evolution **5**:4896-4905.

Jacobsen, R. M., H. Kauserud, A. Sverdrup-Thygeson, M. M. Bjorbækmo, and T. Birkemoe. 2017. Wood-inhabiting insects can function as targeted vectors for decomposer fungi. Fungal ecology **29**:76-84.

Jacobsen, R. M., A. Sverdrup-Thygeson, H. Kauserud, and T. Birkemoe. 2018a. Revealing hidden insect–fungus interactions; moderately specialized, modular and anti-nested detritivore networks. Proc. R. Soc. B **285**:20172833.

Jacobsen, R. M., A. Sverdrup-Thygeson, H. Kauserud, S. Mundra, and T. Birkemoe. 2018b. Exclusion of invertebrates influences saprotrophic fungal community and wood decay rate in an experimental field study. Functional ecology **32**:2571-2582.

Jansen, P. A., B. T. Hirsch, W.-J. Emsens, V. Zamora-Gutierrez, M. Wikelski, and R. Kays. 2012. Thieving rodents as substitute dispersers of megafaunal seeds. Proceedings of the National Academy of Sciences **109**:12610-12615.

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

Jongejans, E., K. Shea, O. Skarpaas, D. Kelly, A. W. Sheppard, and T. L. Woodburn. 2008. Dispersal and demography contributions to population spread of Carduus nutans in its native and invaded ranges. Journal of Ecology **96**:687-697.

Jonsell, M., and G. Nordlander. 2004. Host selection patterns in insects breeding in bracket fungi. Ecological Entomology **29**:697-705.

Jusino, M. A., D. L. Lindner, M. T. Banik, K. R. Rose, and J. R. Walters. 2016. Experimental evidence of a symbiosis between red-cockaded woodpeckers and fungi. Proceedings of the Royal Society B: Biological Sciences **283**:20160106.

Lacy, R. C. 1984. Predictability, toxicity, and trophic niche breadth in fungus-feeding Drosophilidae (Diptera). Ecological Entomology **9**:43-54.

Lawrence, J. 1989. Mycophagy in the Coleoptera: Feeding strategies and morphological adaptations. Pages 1-23 Insect-fungus Interactions. Academic Press.

Lawrence, J. F. 1973. Host preference in ciid beetles (Coleoptera: Ciidae) inhabiting the fruiting bodies of Basidiomycetes in North America.

Leopold, D. R., J. P. Wilkie, I. A. Dickie, R. B. Allen, P. K. Buchanan, and T. Fukami. 2017. Priority effects are interactively regulated by top-down and bottom-up forces: evidence from wood decomposer communities. Ecology letters **20**:1054-1063.

Lim, Y. W., J.-J. Kim, M. Lu, and C. Breuil. 2006. Determining fungal diversity on Dendroctonus ponderosae and Ips pini affecting lodgepole pine using cultural and molecular methods.

Lüdecke, M. D. 2021. Package 'sjPlot'.

Lydersen, S. 2021. Adjustment of p-values for multiple hypotheses. Tidsskrift for Den norske legeforening.

Matthewman, W., and D. Pielou. 1971. Arthropods inhabiting the sporophores of Fomes fomentarius (Polyporaceae) in Gatineau Park, Quebec. The Canadian Entomologist **103**:775-847.

Maynard, D. S., M. A. Bradford, D. L. Lindner, L. T. van Diepen, S. D. Frey, J. A. Glaeser, and T. W. Crowther. 2017. Diversity begets diversity in competition for space. Nature ecology & evolution **1**:1-8.

Nathan, R., F. M. Schurr, O. Spiegel, O. Steinitz, A. Trakhtenbrot, and A. Tsoar. 2008. Mechanisms of long-distance seed dispersal. Trends in ecology & evolution **23**:638-647.

Nikitsky, N., and D. Schigel. 2004. Beetles in polypores of the Moscow region: checklist and ecological notes. Entomologica Fennica **15**:6–22-26–22.

Norros, V. 2013. Measuring and modelling airborne dispersal in wood decay fungi.

O'Connell, T., and T. Bolger. 1997. Fungal fruiting bodies and the structure of fungus-micro-arthropod assemblages. Pages 249-262 *in* Biology and Environment: Proceedings of the Royal Irish Academy. JSTOR.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. O'hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2013. Package 'vegan'. Community ecology package, version **2**:1-295.

Paviour-Smith, K. 1960. The fruiting-bodies of macrofungi as habitats for beetles of the family Ciidae (Coleoptera). Oikos **11**:43-71.

Pech-Pacheco, J. L., G. Cristóbal, J. Chamorro-Martinez, and J. Fernández-Valdivia. 2000. Diatom autofocusing in brightfield microscopy: a comparative study. Pages 314-317 *in* Proceedings 15th International Conference on Pattern Recognition. ICPR-2000. IEEE.

Persson, Y., K. Ihrmark, and J. Stenlid. 2011. Do bark beetles facilitate the establishment of rot fungi in Norway spruce? Fungal ecology **4**:262-269.

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

Rogers, H. S., N. G. Beckman, F. Hartig, J. S. Johnson, G. Pufal, K. Shea, D. Zurell, J. M. Bullock, R. S. Cantrell, and B. Loiselle. 2019. The total dispersal kernel: a review and future directions. AoB Plants **11**:plz042.

Rohlfs, M. 2015. Fungal secondary metabolite dynamics in fungus–grazer interactions: novel insights and unanswered questions. Frontiers in Microbiology **5**:788.

Rotheray, T. D., M. Chancellor, T. H. Jones, and L. Boddy. 2011. Grazing by collembola affects the outcome of interspecific mycelial interactions of cord-forming basidiomycetes. Fungal ecology **4**:42-55.

Schigel, D. S. 2011. Polypore—beetle associations in Finland. Pages 319-348 *in* Annales Zoologici Fennici. BioOne.

Schigel, D. S., T. Niemelä, M. Similä, J. Kinnunen, and O. Manninen. 2004. Polypores and associated beetles of the North Karelian Biosphere Reserve, eastern Finland. Karstenia **44**:35-56.

Schupp, E. W. 1993. Quantity, quality and the effectiveness of seed dispersal by animals. Vegetatio **107**:15-29.

Schupp, E. W., P. Jordano, and J. M. Gómez. 2010. Seed dispersal effectiveness revisited: a conceptual review. New Phytologist **188**:333-353.

Schupp, E. W., P. Jordano, and J. M. Gómez. 2017. A general framework for effectiveness concepts in mutualisms. Ecology letters **20**:577-590.

Seibold, S., J. Müller, P. Baldrian, M. W. Cadotte, M. Šťursová, P. H. Biedermann, F.-S. Krah, and C. Bässler. 2019. Fungi associated with beetles dispersing from dead wood–Let's take the beetle bus! Fungal ecology **39**:100-108.

Siitonen, J. 2001. Forest management, coarse woody debris and saproxylic organisms: Fennoscandian boreal forests as an example. Ecological bulletins:11-41.

Skrede, I. 2021. Diversity and distribution of ligninolytic fungi. Pages 1-36 Advances in Botanical Research. Elsevier.

Slippers, B., P. De Groot, and M. J. Wingfield. 2011. The Sirex Woodwasp and its Fungal Symbiont:: Research and Management of a Worldwide Invasive Pest. Springer Science & Business Media.

Spiegel, O., and R. Nathan. 2010. Incorporating density dependence into the directed-dispersal hypothesis. Ecology **91**:1538-1548.

Staniec, B., E. Pietrykowska-Tudruj, and K. Czepiel-Mil. 2016. Larva of Gyrophaena boleti (Linnaeus, 1758)(Coleoptera: Staphylinidae)—an obligatory Saproxylic and Mycophagous species associated with fomitopsis pinicola: notes on tergal gland system and behaviour. Pages 83-100 *in* Annales Zoologici. BioOne.

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

Strid, Y., M. Schroeder, B. Lindahl, K. Ihrmark, and J. Stenlid. 2014. Bark beetles have a decisive impact on fungal communities in Norway spruce stem sections. Fungal ecology **7**:47-58.

Talbot, P. 1952. Dispersal of fungus spores by small animals inhabiting wood and bark. Transactions of the British Mycological Society **35**:123-128.

Team, R. C. 2021. R: A language and environment for statistical computing.

Tláskal, V., V. Brabcová, T. Větrovský, M. Jomura, R. López-Mondéjar, L. M. Oliveira Monteiro, J. P. Saraiva, Z. R. Human, T. Cajthaml, and U. Nunes da Rocha. 2021. Complementary roles of wood-inhabiting fungi and bacteria facilitate deadwood decomposition. Msystems **6**:e01078-01020.

Turchetti, T., and G. Chelazzi. 1984. Possible role of slugs as vectors of the chestnut blight fungus. European journal of forest pathology **14**:125-127.

Türke, M., E. Heinze, K. Andreas, S. M. Svendsen, M. M. Gossner, and W. W. Weisser. 2010. Seed consumption and dispersal of ant-dispersed plants by slugs. Oecologia **163**:681-693.

Valverde, J., F. Perfectti, and J. M. Gómez. 2019. Pollination effectiveness in a generalist plant: adding the genetic component. New Phytologist **223**:354-365.

Vander Wall, S. B., and W. S. Longland. 2004. Diplochory: are two seed dispersers better than one? Trends in ecology & evolution **19**:155-161.

Vašutová, M., P. Mleczko, A. López-García, I. Maček, G. Boros, J. Ševčík, S. Fujii, D. Hackenberger, I. H. Tuf, and E. Hornung. 2019. Taxi drivers: the role of animals in transporting mycorrhizal fungi. Mycorrhiza **29**:413-434.

Vellend, M. 2010. Conceptual synthesis in community ecology. The Quarterly review of biology **85**:183-206.

Wenny, D. G. 2001. Advantages of seed dispersal: a re-evaluation of directed dispersal. Evolutionary Ecology Research **3**:37-50.

Weslien, J., L. B. Djupström, M. Schroeder, and O. Widenfalk. 2011. Long-term priority effects among insects and fungi colonizing decaying wood. Journal of Animal Ecology **80**:1155-1162.

Wickham, H., W. Chang, and M. H. Wickham. 2016. Package 'ggplot2'. Create Elegant Data Visualisations Using the Grammar of Graphics. Version **2**:1-189.

Wilhelm, R. C., R. Singh, L. D. Eltis, and W. W. Mohn. 2019. Bacterial contributions to delignification and lignocellulose degradation in forest soils with metagenomic and quantitative stable isotope probing. The ISME Journal **13**:413-429.

Woodward, S., and L. Boddy. 2008. Interactions between saprotrophic fungi. Pages 125-141 *in* British mycological society Symposia series. Elsevier.

Wright, S. 1969. Evolution and the genetics of populations: Vol. 2. The theory of gene frequencies.

Yamashita, S., K. Ando, H. Hoshina, N. Ito, Y. Katayama, M. Kawanabe, M. Maruyama, and T. Itioka. 2015. Food web structure of the fungivorous insect community on bracket fungi in a Bornean tropical rain forest. Ecological Entomology **40**:390-400.

Økland, R. H., T. Økland, and K. Rydgren. 2001. Vegetation-environment relationships of boreal spruce swamp forests in Østmarka Nature Reserve, SE Norway. Sommerfeltia **29**:1-1.

6 Supplementary Material

Table S1. Twenty-six fungal OTUs from aspen wood that contributed to differences in community composition between invertebrate exclusion treatment logs (from SIMPER). Each OTU was fitted separately in a GLMM to test whether their sequence abundance was different in caged and non-caged logs in year 2 and year 5 after tree felling.

otuid	species	2_intercept (cage)	2_Cage_control	2_Control	2_OH_Control
OTU_10	Cadophora_sp	5.40176	-0.069	-0.34652	-0.3865
OTU_11	Fungi_sp	absent	absent	absent	absent
OTU_13	Ascocoryne_cylichnium	2.8802	**-1.3148**	-0.7171	-0.2675
OTU_14	Resinicium_bicolor	absent	absent	absent	absent
OTU_15	Coniochaeta_sp	3.756	**-1.0621**	0.7554	-0.1471
OTU_18	Peniohphora_incarnata	3.1227	0.5998	0.1933	**0.9599**
OTU_19	Nakazawaea_anatomiae	4.7735	-0.7002	-0.7326	*-0.6327*
OTU_2	Trametes_ochracea	5.97075	**1.26668**	-0.02452	**1.07456**
OTU_23	Armillaria_mellea	absent	absent	absent	absent
OTU_26	Coniochaeta_sp	3.1155	**-0.6725**	-0.2675	-0.4549
OTU_27	Rhizoscyphus_sp	2.3441	**-1.5995**	**2.193**	0.1405
OTU_29	Coniochaeta_sp	2.68559	-0.17014	**0.857**	0.08295
OTU_30	Kuehneromyces_mutabilis	absent	absent	absent	absent
OTU_32	Coniochaeta_sp	2.5727	-0.12253	0.09459	-0.35302
OTU_34	Pleurotus_pulmonarius	1.64091	0.09003	0.10141	0.3958
OTU_35	Xylodon_radula	absent	absent	absent	absent
OTU_36	Ceriporiopsis_resinascens	absent	absent	absent	absent
OTU_47	Trichoderma_atroviride	1.3855	0.3962	0.6567	1.089
OTU_51	Nakazawaea_wyomingensis	2.7258	0.2933	**-1.6752**	*-0.8369*
OTU_59	Peniophorella_praetermissa	absent	absent	absent	absent
OTU_6	Brunnipila_palearum	5.0658	**-1.5746**	**2.227**	0.1327
OTU_62	Nakazawaea_populi	2.230417	0.05148	-1.11137	-0.87437
OTU_68	Peterozyma_toletana	1.15564	-0.183	-0.4476	-0.8607
OTU_69	Galzinia_sp	absent	absent	absent	absent
OTU_79	Bjerkandera_adusta	-0.49	0.6761	0.5943	0.8524
OTU_9	Annulohypoxylon_multiforme	4.818	-0.1709	0.5827	0.6555

5_intercept (cage	5_Cage_control	5_Control	5_OH_Control	SIMPER
1.0261	-0.3907	**-1.7567**	-0.901	cage_cagecontrol
2.2837	**-1.3816**	**3.9616**	0.5954	control_cagecontrol
6.0082	**-1.8644**	-0.2422	-0.5873	oh_cage
2.25058	0.08501	-0.61246	-0.31068	cage_control
5.7657	-0.2456	-0.4188	-0.2778	oh_cage
absent	absent	absent	absent	oh_control
absent	absent	absent	absent	cage_cagecontrol
4.23629	**0.0841**	**0.25265**	0.54583	oh_cagecontrol
3.9046	**-1.6106**	**-1.8425**	*-1.3753*	cage_cagecontrol
3.844557	**1.12064**	-0.1877	0.00648	cage_control
1.31	1.2271	**-2.5308**	-0.5925	oh_control
3.4232	0.7351	**0.9971**	0.6424	oh_cagecontrol
1.188	-0.7053	-0.6196	-0.6825	oh_cage
3.713	0.3932	-0.1298	-0.6547	cage_cagecontrol
-0.165	-0.288	-0.1021	0.5873	cage_control
0.4348	-1.1418	-0.2983	-1.5274	cage_control
-0.1223	0.06411	0.4083	1.2978	control_cagecontrol
-4.6859	**2.9409**	**3.4165**	**4.0822**	oh_control
absent	absent	absent	absent	cage_cagecontrol
-2.0711	-0.6917	1.8956	-0.8791	cage_cagecontrol
4.4557	0.2353	0.4514	*0.723*	oh_cage
absent	absent	absent	absent	cage_cagecontrol
-6.652	-1.3807	-0.9613	-2.4155	cage_cagecontrol
-5.57239	0.01651	-0.1821	-0.94424	cage_control
absent	absent	absent	absent	oh_control
3.0856	-0.4478	**2.0923**	-0.1768	oh_control

Table S2. List of beetle species found on living sporocarps of *Fomitopsis pinicola* by manual observation, by time-lapse cameras in Nordre Pollen nature reserve, May – June (September) 2020 and 2021 and in the literature (Hågvar and Økland 1997, Hågvar 1999, Nikitsky and Schigel 2004, Schigel 2011). Species in bold are new recordings on *F. pinicola* in this study.

Species	**Family**	**Manual**	**Camera**	**In the literature?**
***Pterostichus niger*[1]**	**Carabidae**	**Yes**	**No**	**No**
***Melanotus castanipes*[1]**	**Elateridae**	**No**	**Yes[2]**	**No**
Triplax russica	**Erotylidae**	**Yes**	**Yes[2, 3]**	**No**
***Mycetina cruciata*[1]**	**Endomychidae**	**Yes**	**No**	**No**
Anisotoma humeralis	Leiodidae	Yes	No	Yes
Rhizophagus dispar	Monotomidae	Yes	Yes	Yes
Epuraea variegata	Nitidulidae	Yes	No	Yes
Ipidia binotata	**Nitidulidae**	**Yes**	**Yes**	**No**
Pocadius ferrugineus[1]	Nitidulidae	Yes	No	Yes
Dendrophagus crenatus	Silvanidae	No	Yes[2]	Yes
Gyrophaena boleti	Staphylinidae	Yes	Yes	Yes
Lordithon lunulatus	Staphylinidae	Yes	Yes[2]	Yes
L. thoracicus	**Staphylinidae**	**Yes**	**No**	**No**
***Quedius mesomelinus*[1]**	**Staphylinidae**	**Yes**	**No**	**No**
Q. xanthopus[1]	Staphylinidae	Yes	No	Yes
Sepedophilus littoreus	Staphylinidae	Yes	?[4]	Yes
S. testaceus[1]	Staphylinidae	Yes	?[4]	Yes
S. thoracicus	**Staphylinidae**	**Yes**	**?[4]**	**No**
***Diaperis boleti*[1]**	**Tenebrionidae**	**Yes**	**No**	**No**
Peltis ferruginea	Trogossitidae	Yes	Yes	Yes
Thymalus limbatus	Trogossitidae	Yes	Yes	Yes

[1] collected manually, but not tested for spore viability. [2] Only in Østmarka forest holdings. [3] In 2019. [4] One unidentified *Sepedophilus* sp. in Østmarka.

Paper I
Lunde, L.F., Jacobsen, R.M., Kauserud, H., Boddy, L., Nybakken, L., Sverdrup-Thygeson, A. & Birkemoe, T. 2022. Legacies of invertebrate exclusion and tree secondary metabolites control fungal communities in dead wood. – Molecular Ecology. 13 pp.
DOI: 10.1111/mec.16448

Paper II
Lunde, L.F., Boddy, L., Sverdrup-Thygeson, A., Jacobsen, R.M., Kauserud, H. & Birkemoe, T. Beetles provide directed dispersal of viable spores of a keystone wood decay fungus. – Fungal Ecology.
(Submitted)

Paper III
Lunde, L.F., Høye, T.T., Ferkingstad, B., Wegger, H.H. & Birkemoe, T. Quantification of invertebrates on fungal fruit bodies by the use of time-lapse cameras.
(Manuscript)

Paper IV
Lunde, L.F., Birkemoe, T., Kauserud, H., Boddy, L., Jacobsen, R.M., Morgado, L., Sverdrup-Thygeson, A. & Maurice, S. 2022. DNA metabarcoding reveals host-specific communities of arthropods residing in fungal fruit bodies. – Proceedings of the Royal Society B 289: 202112622.
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