Running head: Invertebrate exclusion affects fungal communities

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

Rannveig M. Jacobsen* [a, b], Anne Sverdrup-Thygeson [a], Håvard Kauserud [c], Sunil Mundra [c], Tone Birkemoe [a]

[a] Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Høgskoleveien 12, 1433 Ås, Norway

[b] The Norwegian Institute for Nature Research, Gaustadalléen 21, 0349 Oslo, Norway

[c] Section for Genetics and Evolutionary Biology (EVOGENE), University of Oslo, Blindernveien 31, 0316 Oslo, Norway

* Corresponding author: rannveig.jacobsen@nina.no

Abstract

1. Decomposer communities perform an essential ecosystem function by recycling nutrients. However, the effect of higher trophic levels on microbial decomposer communities and rate of decomposition is poorly understood. We therefore 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.

2. 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.

3. 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. The strongest effect on fungal community composition, however, was attributed to differing baseline conditions in the individual trees. When accounting for these baseline differences, caged logs had significantly higher wood density than control logs after two years, indicating lower rates of wood decay in caged logs.

4. Further studies, spanning several years, are required to fully understand the influence of invertebrates on fungi and wood decay. However, our results indicate that invertebrates influence both the composition of saprotrophic communities in dead wood and their decomposition function, which is vital to forest ecosystems.

Key words

Top-down; saproxylic; insects; decomposition; dead wood; community assembly; 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 the composition of saprotrophic communities and how this influences ecosystem processes is an important task for ecologists. Decomposer community composition has been shown to influence the rate of decomposition and nutrient cycling,

resulting in indirect effects of decomposer organisms on plant diversity and primary production (Wardle *et al.* 2004; Wagg *et al.* 2014). Carbon cycling (Clemmensen *et al.* 2015; van der Wal, Ottosson & de Boer 2015) and denitrification (Cavigelli & Robertson 2000) can also be affected by the 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, Siitonen & Jonsson 2012), and are the key agents of wood decomposition (Cornwell *et al.* 2009; Bradford *et al.* 2014; 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, Wagner & Mulrooney 2014; van der Wal, Ottosson & de Boer 2015; Ulyshen 2016). As such, community composition of saprotrophic fungi in dead wood has been shown to significantly affect the rate of wood decay (Dickie *et al.* 2012; Kubartová, Ottosson & Stenlid 2015; van der Wal, Ottosson & de Boer 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 (Crowther, Boddy & Jones 2011; A'Bear *et al.* 2013). Such top-down effects on fungal community composition have also been found to affect the rate of decomposition (reviewed in A'Bear et al. 2014). However,

top-down effects on fungi have mainly been studied in soil microcosms, and the significance under realistic conditions in the field remains unclear (A'Bear, Jones & Boddy 2014). Field studies have indicated that invertebrates might also affect saprotrophic fungi by altering the substrate (Leach, Orr & Christensen 1937; Weslien *et al.* 2011; Jacobsen, Birkemoe & Sverdrup-Thygeson 2015) or dispersing fungal propagules (Lilleskov & Bruns 2005; Seres, Bakonyi & Posta 2007; Strid *et al.* 2014; Jacobsen *et al.* 2017), 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 the 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 the community composition of wood saprotrophic fungi and wood decay rate (Fukami *et al.* 2010; Dickie *et al.* 2012; 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 (Montgomery & Wargo 1983; Allison, Borden & Seybold 2004; Bouget *et al.* 2009). 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. As invertebrates seem to influence the fungal community in a species-specific manner (A'Bear, Jones & Boddy 2014; Strid *et al.* 2014; Jacobsen, Birkemoe & Sverdrup-Thygeson 2015), the paucity of studies on these interactions in relation to the overwhelming number of species makes it difficult to predict the compositional change in the fungal community. As for wood decay, previous studies have shown that even in areas without termites, exclusion of invertebrates generally decreases rate of wood decay (Ulyshen & Wagner 2013). Our main hypotheses were, therefore, as follows; the exclusion of invertebrates larger than 1 mm (1) alters the composition of fungal communities in dead wood and (2) reduces 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, Weslien & Ehnström 1998; Tikkanen *et al.* 2006) and its relatively fast decay rate (Angers, Drapeau & Bergeron 2012; 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 -80^{o}C.

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. Termites do not exist at these latitudes, so beetles (Coleoptera) are usually the functionally and numerically dominant invertebrates within dead wood in boreal forests (Stokland 2012).

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 (Montgomery & Wargo 1983; Allison, Borden & Seybold 2004; Bouget *et al.* 2009). 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 (Stokland 2012). 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 103^{o}C 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 (Supporting Information 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 98^{o}C for 30 seconds, then denaturation at 98^{o}C for 10 sec, annealing at 56^{o}C for 30 sec and elongation at 72^{o}C for 15 sec repeated 30 times, followed by a final elongation step at 72^{o}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) 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.

2.3 Bioinformatic analysis

We received 30 214 354 paired-end forward and reverse sequences from the two Miseq sequencing runs. The sequences were processed for quality filtering, assembling and demultiplexing, as described in detail in Supporting Information S2. Sequences were also checked for presence of both primers, ITS regions were extracted, singleton sequences were removed, and sequences were clustered and analysed for chimeras (Supporting Information S2). To minimize the impact of rare OTUs resulting from sequencing and PCR errors, we removed all OTUs with < 10 sequences (Nguyen *et al.* 2015) and 1878 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 1743 OTUs (21 196 269 sequences) were further classified into their ecological guild using *FUNGUILD* (Nguyen *et al.* 2016). After correction based on PCR negatives and technical replicates (see Supporting Information S2 for details), 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).

We used ordination to analyse composition of the fungal community in terms of abundance (number of sequences) or presence/absence of OTUs. We investigated the effect of experimental treatments and other explanatory variables on OTU composition with redundancy analysis (RDA) of Hellinger-transformed abundance data (Borcard, Gillet & Legendre 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. When fresh wood samples were included, the constraining variable was treatment (including fresh wood as a treatment), while tree identity and tree section 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 for differences between experimental treatments in 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 wood decay fungi found to be influential in the ordinations (Supporting Information S3: Table S1). Treatment, log section and diameter were included as fixed effects, while site and tree section nested under tree identity were included as random effects.

For analysis of number of OTUs, 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). We used the function "rrarefy.perm" from the package EcolUtils v 0.1 (based on function "rrarefy" from the vegan package) to randomly rarefy the number of sequences 100 times, using the mean community data for further analysis of OTU richness.

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 and tree section nested under tree identity as random effects. Multiple comparisons between modelled treatment means were conducted by general linear hypotheses using the "glht"-function in the multcomp-package v 1.4-8.

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 (Supporting Information S4: 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, estimate = 0.01, standard error = 0.005 (arcsine-transformed proportion as response), p-value = 0.07 in linear mixed models).

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. The fungal community composition of fresh wood samples, in terms of abundance

(number of sequences) of fungal OTUs, did differ significantly from the treatment samples (Supporting Information S3: Fig. 2). 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 Supporting Information S4: Table S2) was significantly lower in the fresh wood samples (Fig. 3B). There were no significant differences in OTU richness between the experimental treatments.

3.1 Effect of invertebrate exclusion on fungal community composition

The fungal community composition, in terms of abundance (Fig. 4, Table 1) or presence/absence (Supporting Information S3: Fig. 3) of fungal OTUs, was significantly affected by the experimental treatments. The ordination analysis showed that all experimental treatments differed from each other to some degree 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 (Fig. 4), explained significant gradients of variation (total variance = 0.52, RDA1; variance = 0.010, p-value = 0.001 and RDA2; variance = 0.004, 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). The invertebrate exclusion treatment, i.e. caged logs, had lower scores for RDA1 than the other treatments (Fig. 4), 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* (Supporting Information S3: Table S1). 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 (Supporting Information S3: Table S2 and S3). Abundance of *C. purpureum* was not found to differ significantly between treatments, but it was more abundant in the mid section of the logs (Supporting Information S3: Table S4).

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). 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 (Supporting Information S3: Table S1).

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).

3.2 Effect of invertebrate exclusion on wood decay

No invertebrate tunnels were visible in any of the wood cores, nor were any entrance holes visible on the bark. Nevertheless, 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). The higher wood density of caged logs was only significant in comparison with the control logs (Supporting Information S3: Table S5), although cage control and ethanol-baited logs also had slightly lower density on average (average wood density; caged logs = 0.389 g/cm^3, control logs = 0.387 g/cm^3,

cage control logs = 0.384 g/cm^3, ethanol-baited logs = 0.386 g/cm^3). Based on predicted values for otherwise identical logs, the wood density of control logs was approximately 2% lower than that of caged logs after less than two years of wood decay.

The variability in wood density attributed to tree identity (the individual tree each log stemmed from) or tree section (the part of the tree each log stemmed from) was relatively high, and these factors were therefore included as random effects in the model (Table 2).

4. Discussion

Our results, stemming from a field experiment repeated at thirty sites across two different landscapes, strongly suggest 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 the community composition of lower trophic levels such as primary producers (Schädler *et al.* 2004; Stein *et al.* 2010) and decomposers (A'Bear, Jones & Boddy 2014; Strid *et al.* 2014; Ulyshen, Diehl & Jeremic 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 mesocosms, 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 the 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 slightly 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 (Montgomery & Wargo 1983; Allison, Borden & Seybold 2004; Bouget *et al.* 2009).

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 (Montgomery & Wargo 1983; Bouget *et al.* 2009), while other species

might prefer (or avoid) the shaded microclimate of cage control logs (Jonsell, Weslien & Ehnström 1998; Sverdrup-Thygeson & Ims 2002; Seibold *et al.* 2016). 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, it is not uncommon for explanatory variables to account for a relatively low proportion of the compositional variation in fungal community data stemming from high-throughput sequencing (Dumbrell *et al.* 2010; Tedersoo *et al.* 2013; Mueller, Belnap & Kuske 2015; Varenius, Lindahl & Dahlberg 2017). High-throughput sequencing results in large and complex datasets, including a multitude of different taxa likely to exhibit contrasting responses. Although a single variable might not explain a large proportion of the total variation in community composition, the taxa influenced by this variable might nevertheless be functionally important and thus the effect of the variable can be ecologically significant. As is likely the case for the experimental treatments in the current study, which influenced functionally important taxa such as *T. versicolor* and other wood decay fungi.

Furthermore, the logs had only been subject to a little less than two years of wood decay following tree felling, which is a short time-frame for experimental treatments to influence fungal community composition. As such, we consider the significant differences between the treatments in the present study to be very interesting, especially since slight differences in the composition of fungi at the time of community assembly can result in increasing differences during succession due to priority effects favouring early arrivals (Fukami *et al.* 2010; Dickie *et al.* 2012; Ottosson *et al.* 2014; Hiscox *et al.* 2015). Early arrival can enable wood saprotrophic

fungi to colonize large wood volumes prior to the arrival of competitors, thus increasing their competitive ability (Holmer & Stenlid 1993).

Studies manipulating the 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 (Fukami *et al.* 2010; Dickie *et al.* 2012; 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.

Invertebrates can affect fungi through preferential grazing (A'Bear, Jones & Boddy 2014), substrate alterations (Jacobsen, Birkemoe & Sverdrup-Thygeson 2015) and propagule dispersal (Jacobsen *et al.* 2017). Excluding invertebrates thereby excludes all these mechanisms, and we cannot determine the exact invertebrate-fungus interaction responsible for the influence on the fungal communities. Preferential grazing has mainly been studied for soil invertebrates, which are incapable of grazing within wood and therefore have limited effects on community composition of wood saprotrophic fungi (Crowther, Boddy & Jones 2011). As for substrate alteration, experimentally drilling holes in logs to mimic insect tunnels has been shown to have little effect on the fungal community (Strid *et al.* 2014). Propagule dispersal resulting in priority effects (Jacobsen, Birkemoe & Sverdrup-Thygeson 2015) 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. Wood density of the caged logs was only two percent higher on average. However, decomposition of dead wood can take decades (Alban & Pastor 1993), and as such we were surprised to find a significant difference between the treatments after only two years of wood decay and two seasons of experimental treatment. We hope to resample the logs after additional years of wood decay to study the development of decay rate and the fungal communities.

Invertebrate exclusion might reduce decay rate by precluding direct effects of invertebrates on wood decomposition (Ulyshen, Wagner & Mulrooney 2014), but measuring wood density by water displacement does not register wood loss due to invertebrate excavations. That would have required additional measurements, but there were no visible invertebrate tunnels or entrance holes on the logs. We do recognize that small volumes of wood consumption by invertebrates might have been overlooked by our method for measuring wood decay, and so our estimate of the difference in decay rate between logs accessible and inaccessible to invertebrates might be conservative. However, mass loss due to wood consumption by invertebrates other than termites seems to be relatively low (Ulyshen & Wagner 2013; Ulyshen 2016), and termites do not exist in our study areas. Invertebrates have been found to significantly influence wood decay in areas where termites are absent (Müller *et al.* 2002; Kahl *et al.* 2017), but it is unclear whether this effect is due to direct or indirect effects. Our results strongly indicate that invertebrates can have a significant indirect effect on rate of wood decay, since we found that invertebrates seemed to

affect fungal community composition, and several previous studies have demonstrated that fungal community composition influences rate of wood decay (Kubartová, Ottosson & Stenlid 2015; van der Wal, Ottosson & de Boer 2015; Hoppe *et al.* 2016).

The influence of fungal community composition on wood decay is complex, as certain species combinations might result in facilitation and increased rates of wood decay, while competition between species might result in energy and resources being diverted to combative interactions, reducing rates of wood decay (van der Wal *et al.* 2013; Yang *et al.* 2016). Thus, the greater abundance of certain wood saprotrophs such as *T. versicolor* and *T. ochracea* in ethanol-baited logs might not result in higher rates of wood decay relative to the other treatments if competition is also more intense. Interestingly, the treatment with the least manipulation of natural conditions, i.e. the control treatment, seemed to result in the fungal community with greatest capacity for wood decay, at least at this point in the decomposition process.

While the effect on wood decay of caged logs 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 Fresh wood from different trees has different baseline conditions

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 (the individual tree each log stemmed from) 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 (Latta *et al.* 2000; Cornwell *et al.* 2009). Whatever the cause, we found that differences between individual trees clearly impacted the development of saprotrophic fungal communities after tree death. This was further underlined by the high variability in wood density after two years of decay between individual trees and between sections of their trunks, which would have masked treatment effects in our study if not accounted for in the models.

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, our results suggest 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

458 invertebrates, through their effect on the fungal community, can influence processes such as
459 nutrient cycling, carbon storage and productivity in forest ecosystems. This underlines the
460 importance of the dead wood community for the functioning of forest ecosystems. We therefore
461 call for long-term field studies of the interactions between invertebrates and fungi in the dead
462 wood community, and the influence of these interactions on ecosystem processes such as
463 decomposition and forest productivity.

464 5. Authors' Contributions

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

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7. Conflict of Interest

The authors declare no competing financial interests.

8. Data Accessibility

Sequence data, mapping files and associated metadata are available in Dryad public repository:

http//doi.org/10.5061/dryad.mb756c7, (Jacobsen *et al.* 2018).

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Tables

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		

Table 2) Linear mixed model fit by restricted maximum likelihood (REML) explaining density of wood core samples by experimental treatment (cage in the intercept, additional comparisons between treatments are available in Supporting Information S3: Table S5), 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				

Figure legends

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.

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.

Figure 3. Average number per sample ± standard error of the mean (SEM) of all OTUs (A) or wood decay OTUs (see Supporting Information S4: Table S1) (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.

Figure 4. Ordination plots for treatment samples showing centroids ± standard error of the mean (SEM) 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 Supporting Information S3: Table S1 for species scores of fungal OTUs.