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Life after tree death: Does restored dead wood host different fungal communities to natural woody substrates?

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ABSTRACT

In Europe, enhancing the amount of dead wood, and thereby promoting habitats for saproxylic species, is one of the most commonly applied restorative treatments in intensively managed forests. This study examines whether the different tree-level treatments utilized to increase the amount of dead wood (girdling, chainsaw felling, and uprooting) have an effect on the wood-inhabiting fungi in the logs of Scots pine (*Pinus sylvestris*). We also investigate whether the structure (diversity and composition) of these communities differ from those that occur in pine wood substrates that have been uprooted naturally. The study was conducted within currently protected forests that have been previously managed for timber production. Both fungal DNA (for sequence-based identification) and the presence of sporocarps of polypore fungi were surveyed from the logs. Based on the results, greater number of species were associated with the girdled logs compared to the other types of dead wood. The method of felling the trees (uprooting vs. chainsaw-felling) also resulted in differences in community composition, but this mainly concerned the sporocarp occurrence of polypore fungi. Fungal communities on naturally uprooted dead wood had more variation to the restored logs. Overall, our results suggest that restoration of dead wood can provide substrates for many fungi, including Red Listed polypores, and successfully contribute to achieving some of the restoration targets. However, to capture most of the variation in natural fungal communities, several methods should be used together when artificially increasing the amount of dead wood in forest ecosystems.

1. Introduction

Ecological restoration has become a globally applied approach to slow down the ongoing decline of biological diversity in human-modified and degraded forest ecosystems (Bullock et al., 2011; Halme et al., 2013; Stanturf et al., 2014). The main reasons for historical forest degradation (both spatial and temporal) vary globally, which calls for a wide array of strategies and methods for restoration of these ecosystems (Hobbs & Cramer 2008; Van Andel & Aronson 2012; Stanturf 2016). In northern Europe, the main cause of forest degradation is intensive management for industrial wood production (Kuuluvainen et al., 2012; Gauthier et al., 2015). Perhaps the most notable structural change has been the decline in the amount of dead wood (by 90–98%) in intensively managed forests (Siitonen 2001).

An estimated 20–25% of forest-associated species in the boreal forests of Europe depends on dead wood (Siitonen 2001; Stokland et al., 2012). Consequently, increasing the amount of dead wood is one of the key objectives in current restoration projects (Jonsson et al., 2005; Halme et al., 2013). For instance, approximately 16,000 ha of forests on mineral soils in Finland over the last two decades have been restored by the creation of dead wood. The most widely used methods for dead wood restoration (besides prescribed burning) have been to kill or damage living trees by chainsaw felling, girdling, or the uprooting of trees with an excavator (Similä & Junninen 2012; Halme et al., 2013; Komonen et al., 2014). Chainsaw felling and uprooting of trees can be regarded as analogs to wind-caused disturbances where the trunks are either snapped or uprooted, whereas girdling of trees creates dead wood that is characteristic of trees killed by insects or fungi while standing.

Wood-decaying fungi are one of the largest groups of species

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dependent on dead wood, and they play an important role in the carbon dynamics and nutrient cycling in forests (Harmon et al., 1986; Bradford et al., 2014). These fungi often have preferences for the substrate type that they utilize, and favor or require, for instance, a specific tree species, tree diameter or decay stage (Bader et al., 1995; Junninen & Komonen 2011; Rajala et al., 2012; Seibold et al., 2015). In addition, fungal community development in a particular type of substrate appears to be determined by several stochastic (and often disturbance-related) factors (Hiscox et al., 2015; Seibold et al., 2015). Based on earlier studies, there are strong indications that the mortality factor of a tree also plays a role in the development of fungal communities in decomposing logs, which probably has to do with the different life-history strategies of fungi (Stenlid et al., 2008; Komonen et al., 2014; Ottosson et al., 2015; Boddy & Hiscox 2016).

The presence of primary decay species (priority effect), the position of the trunk after death, and the vigor of the tree at the time of death are proposed as the most important tree mortality related factors that determine fungal community development in dead wood (Fukami et al., 2010; Stokland et al., 2012). The primary decayer species can affect fungal communities, for example, through substrate modification or through biotic interaction (Renvall 1995; Niemelä et al., 1995; Lindner et al., 2011; Ottosson et al., 2014). Position of the trunk (snag vs. log), in turn, affects the abiotic conditions in the trunk; e.g. fallen trunks are likely to retain moisture better than those that remain standing (Boddy & Heilmann-Clausen 2008). Moreover, logs in contact with the forest floor can more readily be colonized by soil-borne mycelia, in addition to windborne or insect-vectored spores (Stenlid et al., 2008; Boddy & Hiscox 2016). The vigor of a tree before its death is probably related to nutrient levels and/or chemical composition in the inner bark and the sapwood of the trunk due to weakened defense mechanisms, but the extent to which this affects fungal communities is, as yet, largely unknown (Stokland et al., 2012; Venugopal et al., 2015).

The patterns that determine the assembly of fungal communities on natural dead wood suggest that a safe strategy in forest management – including restoration – is to account also for the different mortality factors of trees. However, only little empirical data are available on the longer-term effects of tree mortality on fungal succession, particularly in cases when dead wood amounts are artificially increased as a conservation strategy (Komonen et al., 2014; Seibold et al., 2015).

In this study, we examined wood-inhabiting fungi in restored and natural dead wood. In the experiment, trees of Scots pine (*Pinus sylvestris*) were either: (1) girdled, (2) felled using a chainsaw, (3) or uprooted with machinery. We hypothesized that the way the tree has been killed would cause differences in the qualitative properties of the restored substrates, and result also in differences in which fungal species occupy the logs. We tested the hypothesis by examining fungi in three commonly used restoration treatments. In addition, we compared restored trees with naturally uprooted logs (at a similar decay stage and of a comparable size). Sampling was conducted ca. 10 years after the treatments using DNA sequence-based and sporocarp inventory methods. As a methodological question, we also examined if the two sampling methods produced similar results for an important group of wood-inhabiting species, the polypore fungi.

2. Material and methods

2.1. Study areas

The study was conducted in three previously managed but currently protected forest areas in eastern Finland (Fig. 1): Patvinsuo (63°12′ N, 30°71′ E, 160 m a.s.l.), Petkeljärvi (62°61′ N, 31°12′ E, 160 m a.s.l.), and Polvikoski (62°94′ N, 31°43′ E, 170 m a.s.l.). The studied stands were boreal Scots pine-dominated *Vaccinium*-type forests, according to the Finnish site type classification (Cajander, 1949) where the field layer of the forest was mainly dominated by *Vaccinium vitis-idaea* and the ground layer by *Pleurozium schreberi* and *Dicranum polysetum*.

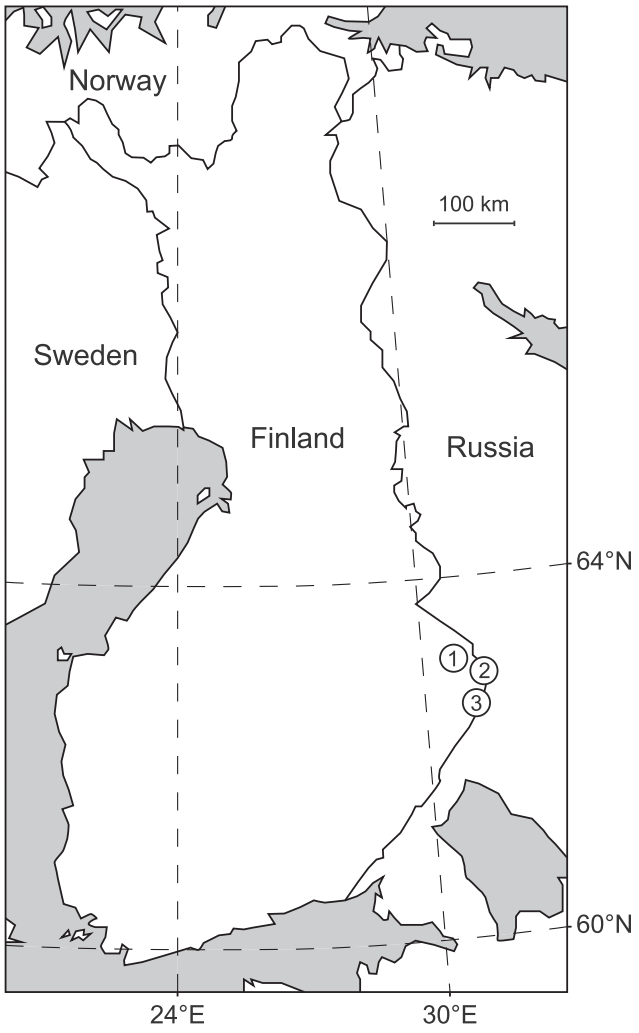

Fig. 1. Geographic locations of the study areas (numbered circles): Patvinsuo (1), Polvikoski (2), and Petkeljärvi (3).

Vaccinium-type forests cover approximately 25% of the forest area on mineral soils in Finland.

2.2. Study design

Restoration treatments of Scots pine; girdling, chainsaw felling, and uprooting trees with an excavator (henceforth referred to as artificially uprooted logs) were carried out approximately 10 years prior to the field study. In each study area, 10 logs within each treatment category (henceforth referred to as dead wood type) were randomly chosen for the study (Table 1). The diameter of sampled logs was at least 16 cm at the base and they represented decay stage 2 in a five-point scale (where 1 is *least decayed*, and 5 is *most decayed*, according to Renvall (1995)). A log within decay stage 2 is mostly decorticated and a hand-pushed knife is able to penetrate ca. 1–2 cm into the wood. The total number of studied logs was 120 and represented four different types of dead wood: (1) chainsaw-felled trees (n = 30), (2) girdled trees that had subsequently fallen (n = 30), (3) artificially uprooted logs (n = 30), and (4) naturally uprooted logs (n = 30). Decay stage of the naturally uprooted logs was assessed in the field and only those logs that belonged to decay stage 2 and were of comparable size to the restored logs were included in the sampling. Ideally, natural dead wood of different origin (i.e. snapped trees and trees that have died while standing), would have been useful as additional control types of dead wood. However, as the areas had been previously used for timber production, sufficient

Table 1
Mean values (± SD) for the measured log characteristics in the three study areas. Diameter (cm) of the uprooted logs was measured above the root collar and from the base of the log for the other dead wood types. Sample size is 40 for each location and 30 for each dead wood type.

Location	Dead wood type	Diameter (cm)	Length (m)	Bark cover (%)	Ground contact (%)	Max height from the ground (cm)
Polvikoski	Artificially uprooted	21.6 ± 2.1	16.7 ± 1.3	10.0 ± 9.4	52.5 ± 29.7	27.0 ± 16.6
	Chainsaw-felled	23.2 ± 3.2	16.3 ± 2.2	6.0 ± 9.1	51.0 ± 24.5	22.5 ± 11.6
	Girdled	19.2 ± 2.2	15.1 ± 1.7	17.0 ± 18.4	36.5 ± 29.3	23.4 ± 29.3
	Naturally uprooted	21.1 ± 3.8	14.4 ± 1.9	19.0 ± 20.0	59.5 ± 26.4	18.2 ± 13.6
Patvinsuo	Artificially uprooted	21.4 ± 2.6	18.9 ± 2.0	11.0 ± 9.1	21.5 ± 15.6	31.2 ± 15.8
	Chainsaw-felled	21.1 ± 1.7	16.8 ± 1.6	22.0 ± 12.1	15.5 ± 10.1	34.0 ± 17.3
	Girdled	20.3 ± 2.7	14.4 ± 2.6	10.5 ± 11.9	23.5 ± 32.1	30.6 ± 14.2
	Naturally uprooted	20.7 ± 2.6	17.5 ± 2.2	32.0 ± 17.7	54.0 ± 29.4	25.4 ± 22.9
Petkeljärvi	Artificially uprooted	21.1 ± 1.6	19.4 ± 1.1	9.0 ± 8.1	34.0 ± 31.5	30.1 ± 21.7
	Chainsaw-felled	23.5 ± 2.9	18.1 ± 2.7	13.5 ± 13.1	76.5 ± 25.5	12.1 ± 10.8
	Girdled	20.7 ± 3.1	16.4 ± 3.0	24.5 ± 22.3	77.5 ± 26.7	10.0 ± 13.0
	Naturally uprooted	22.4 ± 4.2	19.6 ± 2.3	30.0 ± 29.9	50.5 ± 31.2	20.0 ± 11.2

quantity of representative logs was not available.

2.3. Field sampling

Logs were sampled in late September and October 2015. Diameter (cm), length (m), bark cover (%), proportion of ground contact (%), and maximum height from the underside of the log to the ground (cm) were recorded for each log (Table 1).

Fungal communities in the trunks were sampled with two methods: (1) A sample of the whole fungal community, including Basidiomycota, Ascomycota and Zygomycota, was obtained from wood dust from which the fungi were identified using molecular high-throughput DNA sequencing, and (2) polypore fungi (a morphological group of Basidiomycetes) were sampled based on the visible sporocarps on the trunks.

DNA within the logs was sampled using a drill borer and 8 mm drill bit to obtain a wood dust sample. Wood dust was obtained from 10 positions (number of drilled holes was determined from Ovaskainen et al., 2010) along the log at equally spaced distances. Approximately 5 cm^3 of wood dust sample was obtained from each position by drilling until the drill bit reached the approximate center of the log. If insufficient material was collected from one drilling (in the upper part of the tree), an additional hole was drilled next to the first hole. Distance between the 10 drilling positions along the log was determined by first measuring the log from the base to the part of the tree where the diameter equals 7 cm and then dividing the length by 10. Drillings were made in a radial direction into the left, upper, and right side of the log. The first drilling position in the base of each log (left, up, or right) was randomly chosen and the order of the subsequent drillings followed in a clockwise direction. Prior to each drilling, 1–2 cm of the outer part of the log was removed with a small axe to avoid contamination of the wood dust sample with possible fungal tissue or spores on the surface of the log. The axe blade and the drill bit were sterilized between each drilling using 5% sodium hypochlorite (NaClO). The 10 wood dust samples from each log were pooled by packing them in the same resealable ziplock bag in the field. The samples were stored in a freezer (−20 °C) within the same day until DNA extraction.

Sporocarps of polypore fungi were recorded on the logs concurrently with the collection of the wood dust samples. Only the part of the trunk with a minimum diameter of 7 cm was sampled. All visually detectable live sporocarps were recorded, which included a careful inspection of the underside of the trunk. In the case of uncertainty in the identification of polypore species in the field, a sample was taken for further microscopic identification. Delineation and nomenclature for polypore species generally followed Niemelä (2016). The Red List categories are based on Kotiranta et al. (2010).

2.4. DNA extraction, PCR amplification, and sequencing

DNA was extracted one month after the field sampling. Approximately 0.5 mL of wood dust from each ziplock bag was subsampled in a separate 2 mL microcentrifuge tube. The sample tubes were freeze-dried overnight and pulverized using Precellys® 24 (Bertin Technologies) homogenizer. DNA was extracted using cetyltrimethylammonium bromide (CTAB) extraction buffer (3% CTAB, 150 mM Tris-HCl [pH 8 ± 0], 2.6 M NaCl, 2 mM EDTA), as described by Kubartová et al. (2012), and quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc.).

The internal transcribed spacer 2 (ITS2) was amplified using forward primer gITS7 (Ihrmark et al., 2012) and reverse primer ITS4 (White et al., 1990). Both primers included identification tags that were unique for each sample. Polymerase chain reaction (PCR) amplification was conducted in 50 μL reactions (by preparing three technical replicates) that consisted of 0.25 ng μL^{-1} template, 200 μM of each nucleotide, 0.5 μM of gITS7, 0.3 μM of ITS4, 2.75 mM $MgCl_2$, 0.025 U μL^{-1} polymerase (DreamTaq Green, Thermo Scientific, Waltham, MA) in a buffer solution.

The PCR was run on an Applied Biosystems® 2720 Thermal Cycler (Thermo Fisher Scientific Inc.). Initial denaturation at 95 °C for 5 min was followed by 31 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 57 °C and extension for 30 s at 72 °C, followed by 72 °C for 7 min. Negative controls were included during DNA extraction and PCR amplification. Amplification was confirmed by visualizing the DNA on a 1% agarose gel. Depending on the strength of the bands on the gel, the samples were re-amplified with 25 or 29 of cycles in the PCR. Final PCR products were purified with AMPure kit (Beckman Coulter) and quantified with Qubit® dsDNA HS Assay Kit and Qubit fluorometer (Thermo Fisher Scientific Inc.). The PCR products were pooled in equimolar proportions and sequenced using Pacific Biosciences RSII on 12 SMRT cells at SciLifeLab (NGI, Uppsala, Sweden).

2.5. Bioinformatics analysis

Obtained sequences were quality filtered and clustered into operational taxonomic units (OTUs) using bioinformatics pipeline SCATA (http://scata.mykopat.slu.se/). In quality filtering, short reads (< 200 bp), reads that were missing a primer or a tag, and reads that were of low quality (mean quality < 20 or containing bases with quality < 2) were removed. Of the total of 604,173 obtained sequences, 268,867 (45%) passed quality filtering and were clustered into OTUs using single-linkage clustering with a 98.8% similarity threshold using the USEARCH. If a cluster contained more than two sequences, the most common sequence was used to represent the OTU in the assignment of taxonomic affiliation. All OTUs with less than five reads were discarded.

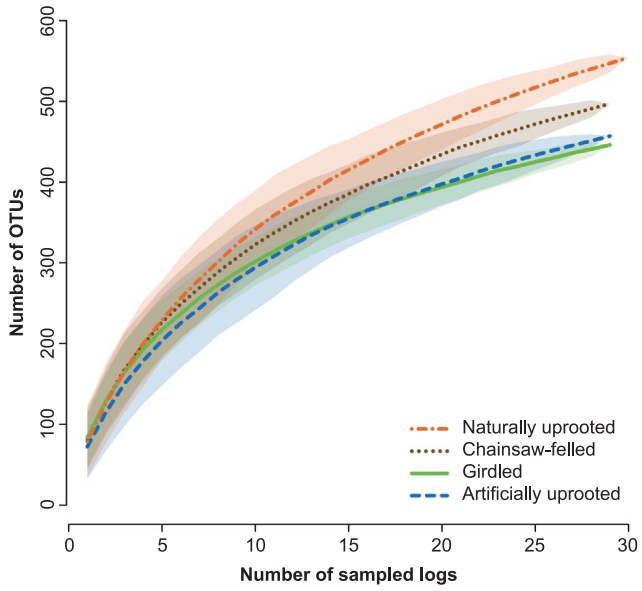

Fig. 2. Sample-based rarefaction curves showing the estimate for the cumulative number of fungal OTUs as a function of the number of the sampled logs for different dead wood types. Shaded areas around the curves illustrate 95% confidence intervals. The total number of OTUs was 557 in the naturally uprooted logs, 497 in the chainsaw-felled logs, 457 in the artificially uprooted logs, and 446 in the girdled logs.

The taxonomic affiliation of the OTUs (with a focus on the polypore fungi) was determined by including the entire UNITE database (Abarenkov et al., 2010; downloaded December 2016) in the clustering procedure. This also validated that species level was achieved in the clustering. The assignments of the polypore OTUs were double-checked against the UNITE database. In order to identify and remove non-fungal species, the OTUs were also subjected to a batch BLAST search against the NCBI GenBank (Altschul et al., 1997) and identification using the lowest common ancestor (LCA) algorithm in the software MEGAN version 6.4.10 (Huson et al. (2011)).

2.6. Statistical analyses

All statistical analyses were conducted using the software R version 3.2.3 (R Core Team, 2015). We used rarefaction curves to illustrate the sampling coverage and the accumulation of the number of OTUs in different dead wood types (function *specaccum* in the package *vegan* [Oksanen et al., 2016]). A linear mixed-effects model was used to examine the association between the fungal richness (OTU and sporocarp) and the measured log characteristics (function *lme* in package *nlme* [Pinheiro et al., 2015]). Dead wood type (naturally uprooted logs as the reference type), diameter (cm), length (m), bark cover (%), ground contact (%), and the max height from the ground (cm) were used as fixed effects and the study area as a random effect. Pairwise comparisons between the dead wood types in the models were done with function *glht* in package *multcomp* (Hothorn et al., 2008).

Community structure of fungi was examined using non-metric multidimensional scaling (NMDS) (package *vegan*; function *metaMDS*). To account for the unequal sampling depth, we used the relative abundance of the sequence reads in the sample-OTU matrices when all fungal species were included in the NMDS, and presence-absence data when assessing the polypore communities separately. For the NMDS on abundance data, we used the Bray-Curtis dissimilarity index. The data was standardized using the Wisconsin double standardization and square root transformation to improve the quality of the ordination. For the polypore data, the Sørensen dissimilarity index was used. Logs with less than two species, as well as species with only one observation were excluded from the polypore data to decrease the possible stochastic effects involved. To assess the statistical difference in the community structure (difference in group centroids in relation to dead wood type), we applied permutational multivariate analysis of variance (PERMANOVA; with study area as a strata term) using the function *adonis* (package *vegan*) (Anderson and Walsh, 2013).

As the interpretation of NMDS is largely based on visual appearance, we also compared community dissimilarity (beta diversity) between the dead wood types with the analysis of variance (followed by Tukey's HSD *post hoc* test for pairwise comparison). For an estimation of within-group beta diversity, standardized (0–1) dissimilarity indices were calculated for each dead wood type using the function *vegdist* (package *vegan*). The Sørensen dissimilarity index was used for the polypore data (presence-absence) and the Bray-Curtis dissimilarity index when all fungal species (abundance data) were analyzed.

To examine whether some of the species have association with the dead wood types, package *indicspecies* (De Caceres & Legendre 2009) was used for the indicator species analysis without including group combinations (function *multipatt*).

Three samples with low and deviant total number of reads (58, 67, and 75 reads; sample numbers A6, A12, and A21 in the Supplementary file S3) were removed from all the analyses and were considered as outliers. The low number of reads in the outlier samples was thought to originate from inefficient DNA extraction, as these samples showed only weak bands after the PCR (with 31 cycles) when visualized on agarose gel. The mean number of reads for the remaining samples was 2223 (range between 239 and 4952 reads).

In all boxplots, the bottom and top of the box represents the first and third quartiles, the vertical T-bars mark the range (if there are no outliers), the horizontal line inside the boxes is the second quartile (median), and the additional plus symbols mark the mean. Open circles are outliers. Horizonal brackets on top of the boxes denote statistical differences between the groups. Significance level of 0.05 was used in all statistical analyses.

3. Results

3.1. Number of fungal species in the logs

A total of 691 fungal OTUs (consisting of 257,440 sequences) were recorded in the studied logs. Taxonomic affiliation could be assigned to 124 (18%) of the OTUs (Supplementary file S2), which accounts for 50% of all the reads. Of the identified OTUs, 77 (62%) were Basidiomycota, 38 (31%) Ascomycota, and 4 (3%) Zygomycota. Based on the rarefaction curves, the total number of fungal OTUs in the naturally uprooted logs was higher than in logs with a restoration origin, and it could be expected that the difference would increase if more logs had been included in the sampling (Fig. 2).

We found no significant differences in the mean number of OTUs per log between the dead wood types (Table 2). Measured log characteristics did not have an effect on the mean number of OTUs per log.

Table 2
Results of the linear mixed model (study area as the random effect) on the effect of dead wood type (naturally uprooted logs as a reference group) and log characteristics on the mean number of fungal OTUs in the logs. Pairwise comparison between the dead wood types (factor) did not reveal any significant differences.

Fixed effects	Value	Std. Error	DF	t-value	p-value
(Intercept)	88.15	17.65	106	4.99	< 0.01
Treatment (artificially uprooted)	−7.1	5.72	106	−1.2	0.22
Treatment (chainsaw-felled)	4.09	5.51	106	0.74	0.46
Treatment (girdled)	3.68	5.54	106	0.66	0.51
Diameter	0.42	0.77	106	0.54	0.59
Length	−0.52	0.87	106	−0.59	0.55
Bark cover	−0.15	0.12	106	−1.31	0.19
Ground contact	−0.12	0.08	106	−1.45	0.15
Max height from the ground	−0.08	0.16	106	−0.50	0.62

Table 3
Polypore species and the number of records (observations) in different dead wood types. Values without parentheses indicate the number of records based on sporocarps and the values within parentheses are the number of records detected in wood dust samples.

Polypore species	Dead wood type			
	Chainsaw-felled	Artificially uprooted	Girdled	Naturally uprooted
Fomitopsis pinicola	21 (21)	7 (16)	11 (22)	13 (21)
Antrodia sinuosa	10 (15)	6 (13)	10 (12)	3 (6)
Butyrea luteoalba	6 (9)	1 (15)	(9)	2 (11)
Skeletocutis amorpha	4 (2)	7 (4)	3 (1)	6 (4)
Rhodonia placenta	(7)	(5)	(11)	(7)
Trichaptum fuscoviolaceum	(1)	1 (1)	5 (4)	6 (8)
Ischnoderma benzoinum	5 (14)	(1)		(5)
Antrodia xantha	5 (4)	1 (2)	4 (6)	
Skeletocutis kuehneri	(8)	(6)	(2)	(2)
Dichomitus squalens	(4)	2 (4)		1 (1)
Trichaptum abietinum	1	2	5	4
Postia leucomallella	(2)	(3)		1 (3)
Gloeophyllum sepiarium	2	1	2	2
Postia tephroleuca	3	1		2
Leptoporus erubescens		1	2	1
Oligoporus sericeomollis	(1)	(1)		(2)
Skeletocutis carneogrisea	1	2		1
Fibroporia norrlandica	(1)	(2)		
Heterobasidion annosum		1	1	1
Postia caesia	(1)		1 (1)	
Antrodia piceata	1		(2)	
Meruliopsis taxicola			(1)	1
Oligoporus rennyi			1	1
Postia fragilis			1	(1)
Skeletocutis biguttulata			1	
Total number of species	11 (14)	13 (13)	13 (11)	15 (12)
Total number of records	59 (90)	33 (73)	47 (71)	45 (71)

The mean OTU number (± SEM) per sampled log was 83.3 ± 3.7 in the chainsaw-felled logs, 72.3 ± 3.8 in the artificially uprooted logs, 82.4 ± 3.3 in the girdled logs, and 76.0 ± 4.0 in the naturally uprooted logs.

In total, 25 polypore species were recorded in the studied logs (Table 3). Of these, 21 species (184 records) were found based on sporocarp inventories and 17 species (305 records) based on the wood dust samples (22% of all the reads). Two of the recorded species are currently classified as threatened in Finland: *Antrodia piceata* (Endangered), and *Dichomitus squalens* (Vulnerable) (Kotiranta et al., 2010).

Based on sporocarp occurrence, the highest number of different polypore species was found on the naturally uprooted logs, although the polypore abundance was highest on the chainsaw-felled logs. Naturally uprooted logs shared 80% of the species with the artificially uprooted logs, 60% with the girdled, and 53% with the chainsaw-felled logs. Based on DNA occurrence, the highest number of species and occurrences were found in the chainsaw-felled logs. Naturally uprooted logs shared 92% of the species with both the artificially uprooted logs and the chainsaw-felled logs, and 58% with the girdled logs.

The mean number of polypore species based on sporocarps (Fig. 3A) was higher on the chainsaw-felled logs (2.0 ± 0.2 species per log) compared to the artificially uprooted logs (1.1 ± 0.1 species per log) ($p = .01$). Pairwise comparison did not reveal other significant differences between the dead wood types. The mean number of polypore OTUs per log based on wood dust samples (Fig. 3B) was not significantly affected by the dead wood type.

3.2. Community composition

NMDS ordination based on the OTUs illustrated that the fungal communities in logs with a different origin mostly overlapped (Fig. 4A–C). Naturally uprooted logs, in particular, had a higher within-treatment community dissimilarity (higher species turnover from log to log), compared to the other dead wood types (Fig. 4D). From the restored logs, the chainsaw-felled trees had a higher community dissimilarity compared to the artificially uprooted and the girdled logs. According to PERMANOVA, the dead wood type ($F_{3,113} = 3.34$, $p < .01$) was a significant predictor in explaining the community composition in the logs.

NMDS ordination based on the sporocarp observations indicated that the polypore communities that occupy the trees cut with a chainsaw have less variation in their composition compared to the communities that occupy the other dead wood types (Fig. 5A). This was also demonstrated by lower community dissimilarity in the chainsaw-felled trees compared to the other dead wood types (Fig. 5C). Statistically group centroids did not differ from each other.

NMDS ordination on the polypore species that were recorded based on the presence of DNA showed a slightly different pattern from the sporocarp observations and there was no indication that the chainsaw-felled logs differed from the other restored dead wood types in their polypore communities (Fig. 5B and 5D). There was no statistically significant difference in the group centroids. The only difference between the groups was related to higher beta diversity of the naturally uprooted logs compared to the girdled logs.

3.3. Indicator species

According to the indicator species analysis (full results in Supplementary file S1), 108 OTUs (16% out of all the OTUs) were associated with one of the dead wood types. Of the indicator species, more than half (61%) were associated with the girdled logs, 22% with the chainsaw-felled logs and 8% both with the naturally or the artificially uprooted logs.

Based on the DNA observations of polypores, the species associated with one of the dead wood types were *Ischnoderma benzoinum* (chainsaw-felled trees) and *Trichaptum fuscoviolaceum* (naturally uprooted logs). Of those polypores that were sampled based on sporocarps, three species were associated with the chainsaw-felled trees: *Fomitopsis pinicola*, *Ischnoderma benzoinum*, and *Butyrea luteoalba*, and none with the other dead wood types.

4. Discussion

In this study, fungi in Scots pine logs with a different restoration origin were examined and compared with naturally uprooted logs after a decade of tree death. Differences in the fungal communities were found between the restoration treatments, particularly depending on if the trees were initially left standing (girdled trees) or if they were felled. Compared to the restored logs, naturally uprooted logs hosted fungal communities that had a higher species turnover from log to log. Sporocarp occurrence and the presence of DNA of polypore fungi did not fully correspond with each other; sampling of sporocarps captured the total number of polypore species more efficiently compared to DNA-based sampling (21 vs. 17, respectively), although sporocarp observations for polypores covered only two-thirds of the observations recorded by DNA-based sampling.

4.1. Restoration treatment results in minor, but significant differences in fungal communities in the logs

Most of the sampled fungal OTUs were found in all of the

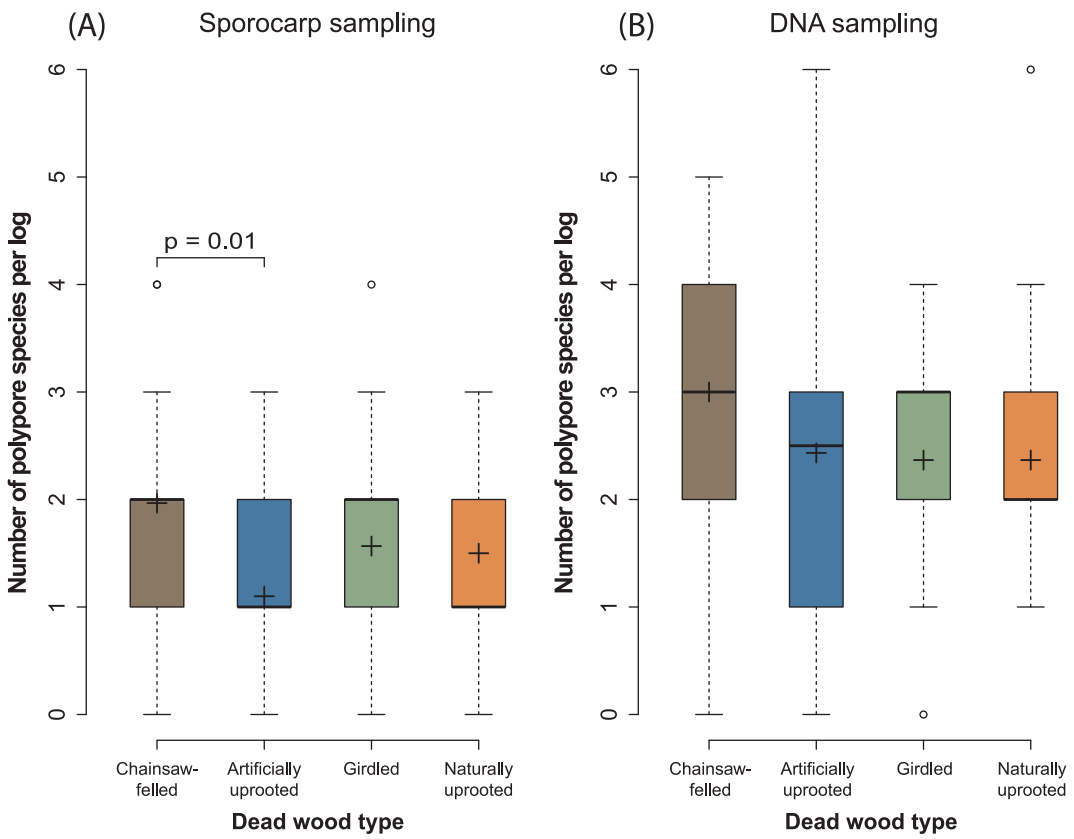

Fig. 3. Number of polypore species per log based on (A) the presence of sporocarps and (B) on DNA in different logs.

experimental groups. The way dead wood is restored, hence, does not appear to result in major differences in fungal communities on Scots pine logs in the first stages of their decomposition. When all fungal species are considered, community structure was mostly affected by tree position, particularly between the girdled trees and the artificially uprooted logs. In our data, this was seen as moderate horizontal separation in the ordination analysis, and by the greater number of species associated with the girdled logs (indicator species analysis) compared to the other types of restored dead wood. Deviation in fungal communities in the girdled logs compared with the felled logs, is likely caused by different moisture regimes in the snags immediately following the restoration treatments (Boddy & Heilmann-Clausen 2008). As the girdled trees die while standing, and often remain so for several years, the moisture content of the snags is most probably lower than the moisture content of the logs, which have had ground contact immediately after their mortality (Yatskov et al., 2003; Lindhe et al., 2004). Drier wood in snags can be expected to result in differences in how (and which) species are initially able to colonize the trunk, resulting in differences also after the tree has fallen down (Fukami et al., 2010; Schwarze et al., 2000; Zak & Wildman 2011). Moreover, it is possible that mechanical stress caused by the girdling treatment initiates induced defense mechanisms, such as resin, phenolic, and protein-based defenses before the tree dies, and results in differences in the chemical and physical properties of the logs (Eyles et al., 2010; Ebrahim et al., 2011).

Based on polypore sporocarp occurrence, the chainsaw-felled logs hosted, on average, a higher number of sporocarps per log but had less variation in community composition than the other dead wood types (e.g. chainsaw-felled logs shared ca. 50% of the species with the naturally uprooted logs). The higher number of species per chainsaw-felled log can be explained mostly by the frequent occurrence of *Fomitopsis pinicola*, *Ischnoderma benzoinum*, and *Butyrea luteoalba*, which all produced more sporocarps on this type of substrate. We speculate that the higher incidence of the mentioned species could be due to the cut surface that offers a favorable pathway in resource capture for certain ruderal species (Schwarze et al., 2000; Lindhe et al., 2004; Boddy & Hiscox 2016), in the same way as *Heterobasidion annosum s.l.* efficiently colonizes cut stumps in managed forests (Boddy & Heilmann-Clausen 2008). This could also provide an explanation for the lower variation in species turnover in the chainsaw-felled logs compared to the other dead wood types. Moreover, as the chainsaw-felled logs die instantly, they may have a richer supply of non-structural carbohydrates in their trunks that promotes sporocarp formation (Moore et al., 2008; Stokland et al., 2012).

4.2. Naturally uprooted logs host more diverse fungal communities compared to the restored ones

Naturally uprooted Scots pine logs had more variation in their fungal communities compared to the restored logs; demonstrated by the greater total number of OTUs and higher community dissimilarity. The result does not seem to originate from the fact that the logs with a natural origin were uprooted, as they also differed from the artificially uprooted logs. The finding may be partly explained by more complex mortality patterns in naturally uprooted logs, which in turn opens more variable decomposition pathways for fungi (Stokland et al., 2012). For example, there is a higher probability that the naturally uprooted logs have already been colonized by heart-rot fungi (Edman et al., 2007; Lännenpää et al., 2008), which results in differences in substrate quality and competition dynamics (Parfitt et al., 2010; Ottosson et al., 2014). Furthermore, the specific time of death of the naturally uprooted logs could not be confirmed in this study and death could have occurred over an extended period. Moreover, whether the trees had been alive or dead before uprooting could not be ascertained. Both these uncertainties may result in more variation in the colonization patterns of fungi.

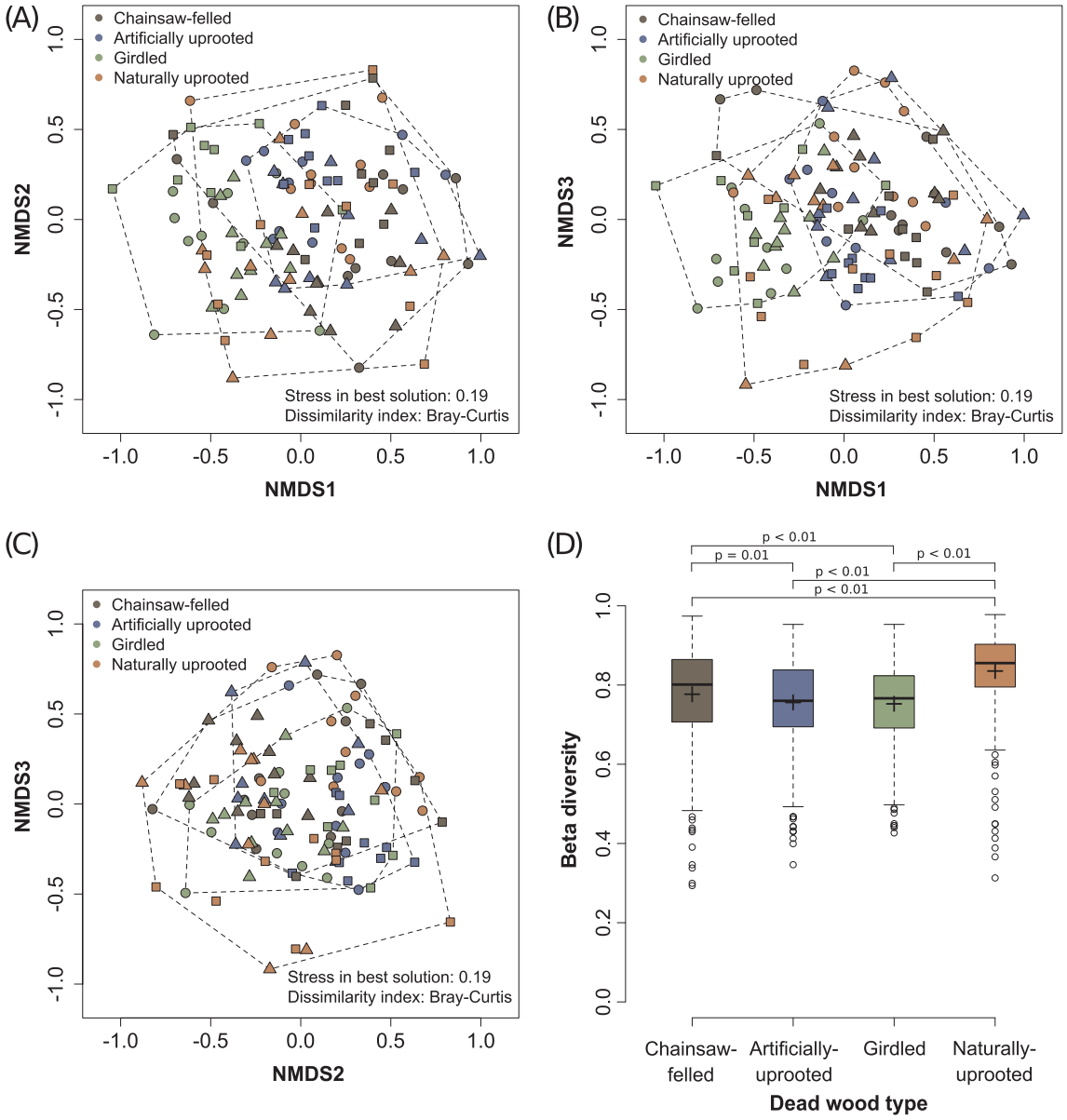

Fig. 4. Non-metric multidimensional scaling (NMDS; with 3-dimensional solution) ordination on the community composition (A–C) and boxplot (D) on the community dissimilarity (beta diversity) of fungal OTUs in the logs. Point shapes in the NMDS figure represent the study areas: Patvinsuo (filled square), Petkeljärvi (filled triangle), and Polvikoski (filled circle).

4.3. Sampling of sporocarps produces differences in results from sampling of DNA

Sporocarp occurrence and the presence of DNA of polypore fungi did not fully correspond with each other. Sampling of sporocarps captured ca. 60% of the DNA observations, although the total number of species was sampled more efficiently using sporocarp inventory (21 vs. 17 species). The latter result was somewhat unexpected, as one could assume to capture more species with DNA-based sampling (Ovaskainen et al., 2013). An explanation for the difference may be related to the fact that the outermost sapwood part of the trees was cut away prior to sampling the DNA. Certain species e.g. *Trichaptum abietinum* are known to have a relatively superficial distribution in wood and may have gone undetected. Also, as six species only had one sporocarp observation, it is possible that the mycelia of these species were not in the exact part of the log from where the drill-dust samples were taken (we used ca. 1.5 m distance between the drilling holes). Moreover, even though the drill-dust was carefully mixed before taking a smaller sample (0.5 mL) for DNA extraction, it is possible that records of some species were missed when the collected drill-dust samples were subsampled again in the laboratory.

Interestingly, based on the occurrence of sporocarps, polypore assemblages in the chainsaw-felled logs had less variation (and less species) compared to the other dead wood types. The same pattern could not be found from the DNA-based observations of polypores. As mentioned earlier, the difference could originate from the different aspects that the two sampling methods measure, as sporocarp occurrence may be more an indicator of the reproductive success of those fungi that strive well in a particular type of substrate (Ovaskainen et al., 2013; Runnel et al., 2015).

4.4. Conclusions and management implications

Clearly, restoration of dead wood can provide a habitat for many fungi, including red-listed polypores, and thus contribute in rehabilitating degraded forests closer to their natural state. However, the present study adds to evidence that several restoration treatments should be used together (e.g. creating both standing and downed dead

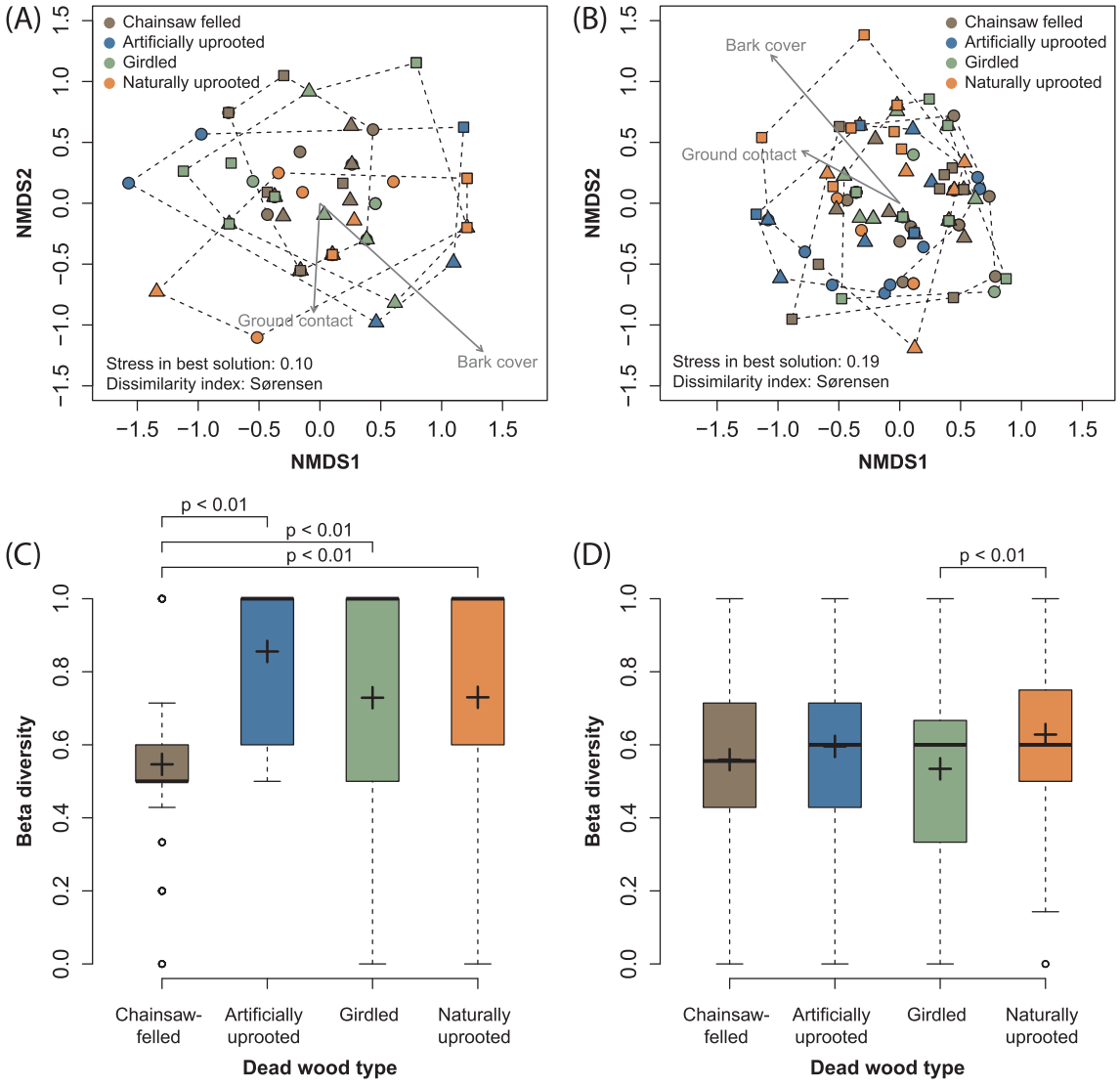

Fig. 5. Non-metric multidimensional scaling (NMDS; with 2-dimensional solution) ordinations of the polypore communities based on (A) the presence of sporocarps and (B) on the presence of DNA in the logs. Statistically significant log characteristics are plotted as arrows in the ordination plots. Box plots show the beta diversity based on (C) the occurrence of sporocarps and (D) on the occurrence of DNA in the logs. Point shapes in the NMDS figures represent the study areas: Patvinsuo (filled square), Petkeljärvi (filled triangle), and Polvikoski (filled circle).

wood) when enhancing the amount of dead wood in forests. The creation of fallen dead wood solely by using one method (e.g. chainsaw-felling) is not advisable as it may result in substrates that increase the competitive advantage of species that utilize a specific strategy in their resource capture. That is, different life-history strategies of fungi should also be considered in dead wood restoration. Our results suggest that all the variability in fungal communities that occurs in naturally uprooted substrates may be difficult to fully re-create with artificial treatments, and this may be due to more complex mortality patterns in the naturally uprooted logs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2017.12.021.

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