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Initial fungal colonizer affects mass loss and fungal community development in *Picea abies* logs 6 yr after inoculation

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ABSTRACT

Picea abies logs were inoculated with *Resinicium bicolor*, *Fomitopsis pinicola* or left un-inoculated and placed in an old-growth boreal forest. Mass loss and fungal community data were collected after 6 yr to test whether simplification of the fungal community *via* inoculation affects mass loss and fungal community development. Three techniques were used to survey communities: (1) observation of fruiting structures; (2) culturing on media; and (3) cloning and sequencing of ITS rDNA. Fruit body surveys detected the smallest number of species (18, 3.8 per log), DNA-based methods detected the most species (72, 31.7 per log), and culturing detected an intermediate number (23, 7.2 per log). Initial colonizer affected community development and inoculation with *F. pinicola* led to significantly greater mass loss. Relationships among fungal community composition, community richness and mass loss are complex and further work is needed to determine whether simplification of fungal communities affects carbon sequestration in forests.

Introduction

Wood and bark account for over 90 % of the above-ground biomass in all forest types (Cooke & Rayner 1984) and fungi are the only organisms capable of efficiently releasing the energy stored in this vast supply of lignified cellulose and hemicellulose (Gilbertson 1980; Hawksworth *et al.* 1995). Wood-inhabiting fungi therefore play a pivotal role in nutrient cycling. However, little is currently known about how disturbance events, such as forest management, affect fungal communities and decay rates of wood. Numerous studies indicate that forest management techniques are often associated with changes in fungal community composition and/or richness of wood-inhabiting fungal species (Wästerlund & Ingelög 1981; Bader *et al.* 1995; Høiland & Bendiksen 1997; Ohlson *et al.* 1997; Lindblad 1998; Junninen *et al.* 2006; Lindner *et al.* 2006; Nordén *et al.* 2008), however, it is unknown whether changes in fungal richness and community composition will affect mass loss and carbon sequestration at the ecosystem level.

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Although it is generally accepted that humans are affecting species composition of ecosystems and reducing overall species richness (Chapin *et al.* 2000; Ehrlich & Pringle 2008), it is difficult to predict the effects of these changes, especially for decomposers such as fungi. The majority of work examining the richness of decomposer fungi in relation to decomposition rates has been done in laboratory settings (e.g. in microcosms) and this work suggests the relationship between fungal richness and decay rates is complex (Blanchette & Shaw 1978; Tiunov & Scheu 2005; Fukami *et al.* 2010; LeBauer 2010) and dependent on environmental conditions such as temperature (Toljander *et al.* 2006) and moisture (Progar *et al.* 2000). The majority of work examining wood-decomposing fungal richness at the landscape level has relied on fruit body surveys to determine species composition (e.g. Bader *et al.* 1995; Nordén *et al.* 2004; Lindner *et al.* 2006; Nordén *et al.* 2008). Obtaining fungal cultures or extracting DNA from woody substrata as a means of determining species composition has not been widely used, primarily due to the cost, availability and technical complexity associated with cultural or DNA-based surveys at the scale of forest stands (Stenlid *et al.* 2008; Rajala *et al.* 2010). To date, no work has addressed whether changes in fungal community composition or diversity at the landscape level affect decomposition rates and carbon sequestration in forest systems.

The goal of this study was to bring together a field-based experimental design with a range of fungal identification techniques to determine whether fungal community composition and diversity affect decay rates in boreal systems. One-meter *Picea abies* logs were inoculated with one of two fungal species (a white-rot, *Resinicium bicolor*, or a brown-rot, *Fomitopsis pinicola*) or left un-inoculated and placed in an old-growth forest in Sweden for 6 yr. Surveys of fungal community development were then conducted using three different techniques: surveys of fruit bodies, culturing of fungi on artificial media, and identification of cloned ITS rDNA sequences from wood samples. This offered a unique opportunity to compare the advantages and disadvantages of these different survey methods while experimentally studying the effects of the initial fungal colonizer on subsequent development of the fungal community and mass loss in a boreal system.

Materials and methods

Field design and inoculation of logs

Two healthy *P. abies* trees growing near Knutby, Sweden were felled, branches removed and the main stems cut into 1 m logs. Logs included bark and ranged between approximately 25 and 35 cm diam. Logs were randomized and subjected to one of the following treatments: inoculation with *R. bicolor* ("*Resinicium*-inoculated"), inoculation with *F. pinicola* ("*Fomitopsis*-inoculated") or left un-inoculated as controls ("non-inoculated"). Fungal isolates were obtained from the culture collection of the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences (Uppsala, Sweden). Inoculum was produced by placing small (<1 cm) sterile *P. abies* wood chips on fully colonized Hagem agar plates until wood chips were covered with mycelium. Three holes approximately 1 cm diam. and 15 cm deep were drilled per log and filled to capacity with colonized wood chips. Non-inoculated logs were also drilled, but holes were filled with sterile wood chips. Ends of the logs were painted with paraffin to minimize moisture loss.

Following inoculation, logs were placed in the Fiby old-growth *P. abies* forest reserve (N 59°52.864′, E 17°21.192′) located approximately 15 km west of Uppsala, Sweden, for 6 yr (from summer 1997 to summer 2003). Six logs, representing two logs of each treatment, were placed at four different locations in the forest separated by approximately 100 m. Half of these logs (four *Fomitopsis*-inoculated logs, four *Resinicium*-inoculated logs, and four non-inoculated logs) were sampled for this present study while the other 12 logs were left in the forest for future studies.

Collection and identification of fruiting bodies

During Jul. of 2003, the entire surface (including the bottom surface) of the 12 logs was examined for fruit bodies. All fruit bodies that could be identified in the field were recorded, while all unknown fruit bodies were collected, dried and examined microscopically.

Culturing fungi from wood samples

After being examined for fruit bodies, logs were stripped of all bark and 3 cm discs were cut from the ends of each log. These discs were discarded and the remaining 94 cm log was cut into three sections approximately 30 cm in length (section length is approximate because material was lost due to the thickness of the saw blade) (Fig 1). Sampling of the sections, isolation and

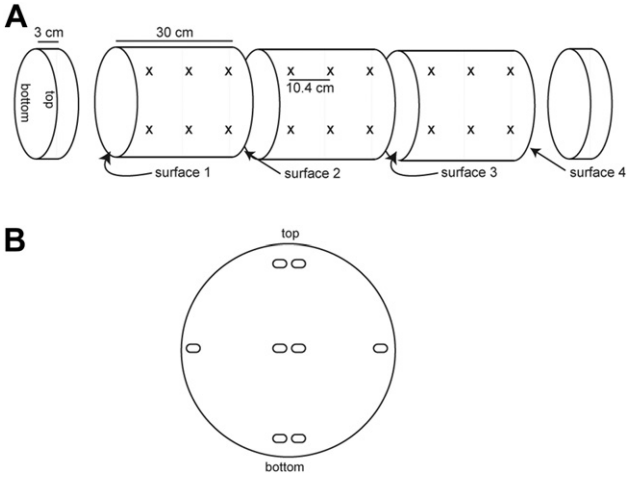

Fig 1 – Diagram of sampling within individual *Picea abies* logs for cultural and DNA analyses. (A) Diagram of entire 1-m log sections. Three-cm discs were removed from each end and discarded and the remaining portion was divided into three 30-cm sections. Cultures were taken from surfaces 1–4. The points at which drill samples were taken for DNA-analysis are marked with an "x". Eighteen drill samples were taken from the top of the log and 16 samples from the bottom. (B) Cross-section of logs with ovals representing the points at which cultural samples were taken. Eight samples were cultured from each surface with samples distributed across the top, bottom, side and central portions of the log.

identification of fungi was performed as in an earlier study (Vasiliauskas *et al.* 2005). Briefly, pieces of wood (about 5 × 5 × 10 mm in size) were cut off with a knife directly from the surface of respective sections (Fig 1). Wood samples were surface sterilized in a flame and placed onto Hagem agar medium in 9 cm Petri dishes and incubated at room temperature, and pure cultures of fungi were obtained by sub-culturing of distinct mycelia that grew out of wood (Vasiliauskas & Stenlid 1998a).

From each sample, one to five different isolates were obtained. They were either identified by observing morphological characteristics of the mycelium, or by comparing nuclear ribosomal ITS sequences with a library of sequences from identified fruit bodies and pure cultures. Morphological identification was partly done by the staff at the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands. All isolated strains (except for Zygomycota spp., *Penicillium* spp. and *Trichoderma* spp.) are deposited at the culture collection of the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences (Uppsala, Sweden). The molecular identification included DNA extraction, PCR amplifications and DNA sequencing and followed established protocols (Kåren *et al.* 1997). The ribosomal ITS region was sequenced using two primers (ITS1 and ITS4) for every culture (White *et al.* 1990). Sequences were checked against those available in a database at the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences (Uppsala, Sweden) and the GenBank database (Altschul *et al.* 1997). To check genetic identity and follow spread of individual mycelia inside the logs, pure cultures of the same species were subjected to vegetative compatibility tests (Vasiliauskas & Stenlid 1998b). First, isolates originating from the same log were tested and then different genotypes from all logs were confronted in all possible combinations.

Cloning and sequencing of fungal ITS sequences isolated directly from wood samples

DNA samples were collected by drilling 36 holes in each 1 m log (Fig 1) and collecting the resulting drill shavings for DNA extraction. For each of the three 30 cm sections from each log, six drill holes entered from the top of the log and six from the bottom, with holes separated by 10 cm (see Fig 1). Holes were drilled with 1 cm diam. lip and spur (brad point) bits to a depth of approximately 10 cm and bark was stripped before drilling. Bits were washed and DNA-sterilized between each use. DNA sterilization was accomplished by washing bits in water to remove all wood fragments and then exposing the entire surface of the bit to an open flame for at least 45 s, plunging in 70 % ethanol and then flaming again for 45 s. A test was run using a known DNA solution to confirm that this sterilization procedure removed DNA from drill bits. All drill shavings were collected within two weeks of logs being removed from the forest and logs were kept at 4 °C until sample collection. Drill shavings were collected in plastic bags and frozen at −20 °C until DNA was extracted. The samples used in this study were extracted within 10 months of collection. Drill shavings stored longer than 14 months at −20 °C did not consistently yield amplifiable fungal DNA despite repeated attempts.

DNA was extracted from drill shavings in 50 ml tubes by combining approximately 5 ml of drill shavings with enough filter-sterilized cell lysis solution (1.4 M NaCl, 0.1 M Tris—HCl, 20 mM EDTA, and 2 % hexadecyltrimethylammonium bromide) to bring the final volume to approximately 7.5 ml. Samples were then agitated and incubated at 65 °C for 2 hr. After incubation approximately 1.0 ml of supernatant was removed and transferred to a 1.5 ml microcentrifuge tube. Care was taken at this step to avoid pipetting larger wood fragments suspended in solution. Samples were then centrifuged at 16 000*g* for 6 min to remove all remaining wood fragments. Eight hundred microliters of the resulting supernatant was transferred to a clean 2.0 ml microcentrifuge tube and 1.0 ml of −20 °C 2-propanol (isopropanol) was added. The samples were then gently mixed by inversion and placed at −80 °C for 15 min followed by centrifuging for 20 min at 13 000*g* at 0 °C. Supernatants were discarded, 500 μl of 70 % ethanol (v/v) was added and tubes were centrifuged at 16 110*g* for 5 min at room temperature. Supernatants were removed, pellets air-dried at room temperature for 10 min and then resuspended in 50 μl water.

DNA in aqueous solution was then cleaned using GeneClean III kits (Qbiogene) following the manufacturer's protocol with the following modifications. Fifty microliters of aqueous DNA solution was combined with 150 μl of NaI solution and 3 μl of glassmilk provided with the kit. Samples were then agitated 3 times at 2-min intervals followed by centrifuging at 16 000*g* for 8 s. Supernatants were discarded and 500 μl of New Wash solution provided with the kit was added. Samples were washed by agitating in New Wash, centrifuging for 8 s at 16 000*g* and then discarding supernatants. This washing step was repeated two additional times. After final removal of New Wash, pellets were air-dried for 15 min and template DNA eluted in 50 μl of water in preparation for PCR.

All PCR, cloning and sequencing procedures followed Lindner & Banik (2009). In summary, the fungal-specific primer pair ITS1F and ITS4 (Gardes & Bruns 1993) was used for the initial PCR of DNA isolated from wood. PCR products were then cloned using pGEM-T Vector System II kits and JM109 competent cells from Promega (Madison, Wisconsin). For all drill samples for which PCR products were successfully obtained (108 total from 3 logs), 12 randomly selected clones were sequenced in one direction using ITS4. For 9 samples (3 from *Fomitopsis*-inoculated logs, 3 from *Resinicium*-inoculated logs and 3 from non-inoculated control logs), an additional 20 clones were sequenced.

Sequences were aligned using Sequencher 4.2 (GeneCodes Corporation) and chimeric sequences were detected by manually comparing ITS1 and ITS2 regions independently against GenBank data and noting conflicting phylogenetic placement within sequences. Sequences were assigned a putative identification based on BLAST comparisons to GenBank (NCBI) sequences. Sequences varying less than 2 % in base pair identity were considered the same and were designated as "taxa."

Fungal community and mass loss analyses

Completeness of sampling of fungal communities was investigated by calculating taxon accumulation curves for the culture- and DNA-based datasets. Taxon accumulation curves were not calculated for the fruit body dataset due to limited abundance data for each taxon per log. For the culture-based curves, taxon accumulation was plotted for each log.

Table 1 – Fruit bodies identified from four *Resinicium*-inoculated, four *Fomitopsis*-inoculated and four non-inoculated *Picea abies* logs with fungal species sorted by abundance

Species	Number of logs on which species was found (4 logs/treatment)			Total
	Non-inoculated control logs	*Fomitopsis*-inoculated	*Resinicium*-inoculated	
Basidiomycota				
Resinicium bicolor	2	0	4	6
Tubulicrinis subulatus	2	3	1	6
Athelia epiphylla	1	2	1	4
Fomitopsis pinicola	0	4	0	4
Trichaptum abietinum	2	0	2	4
Hyphoderma argillaceum	0	1	2	3
Hyphoderma praetermissum	1	1	1	3
Phlebiella pseudotsugae	0	2	1	3
Phlebiella vaga	1	2	0	3
Hyphodontia aspera	0	1	1	2
Antrodia serialis	1	0	0	1
Diplomitoporus lindbladii	1	0	0	1
Hyphodontia breviseta	0	1	0	1
Hyphodontia subalutacea	0	0	1	1
Sistotrema brinkmannii	1	0	0	1
Skeletocutis biguttulata	1	0	0	1
Skeletocutis carneogrisea	1	0	0	1
Tubulicrinis borealis	0	1	0	1

Abundance for each taxon was based on the number of wood samples from which each taxon was isolated, with a total of 32 samples per log. For the DNA-based data, two types of curves were calculated. The first type of curve was based on individual drill samples and plotted taxon accumulation *versus* the number of clones sequenced. These curves were only calculated for the 9 samples for which 32 clones were sequenced. The second type of curve was for individual logs and plotted taxon accumulation *versus* the number of drill holes. In these curves abundance of each taxon was based on the number of drill samples in which each taxon was found, with a total of 36 drill samples per log.

For culture-based taxon accumulation curves for individual logs, taxon accumulation values were averaged across the 4 logs of a given treatment to create an average curve. For the DNA-based accumulation curves for logs, data were available for only one log per treatment so it was not possible to calculate such curves. Taxon accumulation curves were calculated

Table 2 – Summary of wood density, fungal species richness and fungal Shannon diversity data for four *Resinicium*-inoculated, four *Fomitopsis*-inoculated and four non-inoculated *Picea abies* logs

Log number	Treatment	Average wood density after 6 yr (g/cm^{-3})	Species richness			Shannon diversity		
			Fruit bodies	Culturing	DNA	Fruit bodies	Culturing	DNA
1	Non-inoculated	0.265	5	7.0	–	1.61	0.88	–
2	control	0.245	2	8.0	51	0.69	1.61	3.44
3		0.246	3	5.0	–	1.04	1.08	–
4		0.224	4	11.0	–	1.33	1.71	–
Average controls		0.245 (A)[a]	3.5 (A)	7.75 (A)		1.17 (A)	1.32 (A)	
5	*Fomitopsis pinicola*	0.235	4	4.0	–	1.33	1.22	–
6	inoculated	0.235	7	5.0	19	1.83	1.22	1.84
7		0.228	4	4.0	–	1.39	1.05	–
8		0.204	3	4.0	–	1.10	1.13	–
Average *Fomitopsis*		0.225 (B)	4.5 (A)	4.25 (B)		1.41 (A)	1.16 (A)	
9	*Resinicium bicolor*	0.251	4	12.0	–	1.33	1.97	–
10	inoculated	0.248	3	9.0	25	1.05	1.34	2.34
11		0.273	4	9.0	–	1.24	1.65	–
12		0.252	3	8.0	–	1.10	1.21	–
Average *Resinicium*		0.256 (A)	3.5 (A)	9.5 (A)		1.18 (A)	1.54 (A)	
Average over all treatments		0.242	3.8	7.2	31.7	1.25	1.34	2.54
Treatment *p*-value		$p = 0.04$	$p = 0.48$	$p = 0.007$		$p = 0.45$	$p = 0.26$	

a Letters in parentheses following treatment means indicate which treatments were significantly different.

using Sanders' (1968) rarefaction equations as modified by Hurlbert (1971). These equations allow for the exact calculation of the mean taxon accumulation curve over all possible permutations of sampling order. Species richness and Shannon diversity index values (Magurran 1988) were calculated for each log and treatment and compared by one-way ANOVA (Minitab, Minitab®). Community composition was investigated relative to inoculation treatment by calculating CCA plots (Canoco, Microcomputer Power) using culture-based data.

Because initial dry-weight wood density measurements would be difficult to obtain on 1-m logs without destructive sampling, mass loss was investigated based on final dry-weight wood density. It was assumed that initial differences in log density were randomly distributed among treatments. Mass loss was investigated by taking three dry-weight wood density measurements for each 1 m log, with a single measurement taken for each of the three 30 cm wood segments. Density measurements were taken by splitting each 30 cm segment into 2–3 pieces and selecting the most uniformly shaped piece. Pieces for which density measurements were taken were wedge-shaped sections of a cylinder and were between 4 000 and 8 000 cm^3. Volumes were calculated using Archimedes' volume displacement method. Each section was placed in a thin plastic bag, submerged under water and the volume of the displaced water measured. A subset of sections was measured multiple times to ensure repeatability of measurements and blocks of known volumes were used to confirm accuracy of the method. Sections were then oven dried at approximately 45 °C until dry-weight measurements stabilized, which occurred after approximately 2 weeks. Dry weight was recorded for each segment and final dry-weight wood density was calculated as dry weight per unit volume (g/cm^{-3}). Relationships between final wood density and inoculation treatment were investigated by one-way ANOVA (Minitab, Minitab®).

Results

Collection and identification of fruiting bodies

A total of 18 species, all Basidiomycota, were observed (Table 1). The most abundant species were *R. bicolor* and *Tubulicrinis subulatus* (each found on six logs) and *Athelia epiphylla*, *F. pinicola* and *Trichaptum abietinum* (each found on four logs). *F. pinicola* fruit bodies occurred on all four *Fomitopsis*-inoculated logs and did not occur on any other logs. *R. bicolor* fruit bodies occurred on all four *Resinicium*-inoculated logs, as well as on two of the non-inoculated logs.

An average of 3.8 species of fruit bodies were observed per log (Table 2). Logs inoculated with *F. pinicola* displayed the highest

Table 3 – Fungal species isolated in culture from four *Resinicium*-inoculated, four *Fomitopsis*-inoculated and four non-inoculated *Picea abies* logs with fungal species sorted by abundance

Species	Number of samples from which species was isolated (32 samples/log, 4 logs/treatment)							Total
	By treatment			By location within log				
	Non-inoculated control logs	*Fomitopsis*-inoculated	*Resinicium*-inoculated	Central	Outer	Bottom	Top	
Basidiomycota								
Fomitopsis pinicola	0	120	3	32	31	30	30	123
Sistotrema brinkmanii	3	0	7	3	3	3	1	10
Resinicium bicolor	0	0	10	1	3	6	0	10
Antrodia serialis	0	0	5	1	0	0	4	5
Heterobasidion annosum	0	0	4	1	0	3	0	4
Trichaptum abietinum	1	0	1	0	0	0	2	2
Postia stiptica	1	0	0	1	0	0	0	1
Ascomycota								
Trichoderma viride	106	73	103	63	74	69	76	282
Penicillium sp.	16	46	10	19	15	18	20	72
Scytalidium lignicola	10	0	11	7	2	4	8	21
Ascocoryne sp.	3	0	0	3	0	0	0	3
Phialophora lagerbergii	1	0	1	2	0	0	0	2
Gyromitra infula	0	0	1	1	0	0	0	1
Phialocephala dimorphospora	0	0	1	0	1	0	0	1
Zygomycota								
Mucor sp.	39	11	27	12	20	26	19	77
Mortierella isabellina	13	1	2	4	5	4	3	16
Mortierella ramanniana	5	0	4	1	4	2	2	9
Unknown								
Dark unidentified	1	0	6	4	0	2	1	7
Light brown	1	0	0	1	0	0	0	1
Species 75	1	0	0	0	1	0	0	1
Species 338	1	0	0	1	0	0	0	1
Species 339	1	0	0	0	1	0	0	1
Species 621	0	1	0	0	0	1	0	1

species richness of fruit bodies (4.5 per log), while non-inoculated logs and *Resinicium*-inoculated logs displayed an average of 3.5 species per log; differences in richness were not statistically significant ($p = 0.48$). The logs inoculated with *F. pinicola* also displayed the highest Shannon diversity for fruit bodies (Table 2), but this result was not statistically significant ($p = 0.45$).

Culturing fungi from wood samples

A total of 384 isolation attempts were made across the 12 logs (32 isolation attempts per log, see Fig 1) and 651 fungal isolates were obtained in culture, averaging 1.7 species per isolation attempt. A total of 23 species were isolated into culture, including members of Basidiomycota (seven species), Ascomycota (seven species), Zygomycota (three species), and six unidentified species (Table 3). The most commonly isolated Basidiomycota species was *F. pinicola*, which was isolated 123 times out of a total of 384 isolation attempts (32 % of isolations). The next most commonly isolated Basidiomycota species were *Sistotrema brinkmanii* and *R. bicolor*, each isolated 10 times (3 % of samples). The most commonly isolated Ascomycota species were *Trichoderma viride* in 282 samples (73 %), *Penicillium* sp. in 72 samples (19 %), and *Scytalidium lignicola* in 21 samples (5 %). The most commonly isolated Zygomycota species were *Mucor* sp. in 77 samples (20 %) and *Mortierella isabellina* in 16 samples (4 %).

F. pinicola was most commonly isolated from *Fomitopsis*-inoculated logs, with 120 of the 123 *Fomitopsis* isolations (98 %) originating from *Fomitopsis*-inoculated logs (Table 3). All *R. bicolor* isolates (10 of 10) originated from *Resinicium*-inoculated logs. An analysis of species isolated more than 50 times (*F. pinicola*, *T. viride*, *Penicillium* sp., and *Mucor* sp.) indicated that these species were isolated equally from the central, outer, bottom and top portions of logs ($p > 0.05$ with chi-square for all species) (Table 3). *R. bicolor* was most commonly isolated from the bottom portion of the logs (6 of 10 isolates), but unfortunately this species was not isolated enough to determine statistical significance.

An average of 7.2 species per log were obtained with culturing (Table 2), with *Fomitopsis*-inoculated logs displaying significantly fewer ($p = 0.007$) species per log (4.3 species per log) than *Resinicium*-inoculated logs (9.5 species per log) or non-inoculated logs (7.8 species per log). Shannon diversity values were lowest in *Fomitopsis*-inoculated logs (1.16), although this was not statistically significant ($p = 0.26$) (Table 2). Culture-based taxon accumulation curves are presented in Fig 2.

Cloning and sequencing of fungal ITS sequences isolated directly from wood samples

The twelve random clones sequenced from 36 drill samples per log for one *Fomitopsis*-inoculated, one *Resinicium*-inoculated and one non-inoculated log, produced 1 296 sequences. Of these, 1 254 sequences (97 %) had interpretable chromatograms, while the remaining sequences were discarded due to low sequence quality. Using 98 % sequence similarity to define taxa, a total of 72 taxa were identified through DNA-analysis, 33 (46 %) of which had close matches (97 % sequence similarity over 90 % of length) in GenBank (taxa in bold, Table 4). Of the 33 taxa with close matches in GenBank, 29 are identified to the generic level; thus 40 % of the DNA-based taxa were identified to an approximate genus.

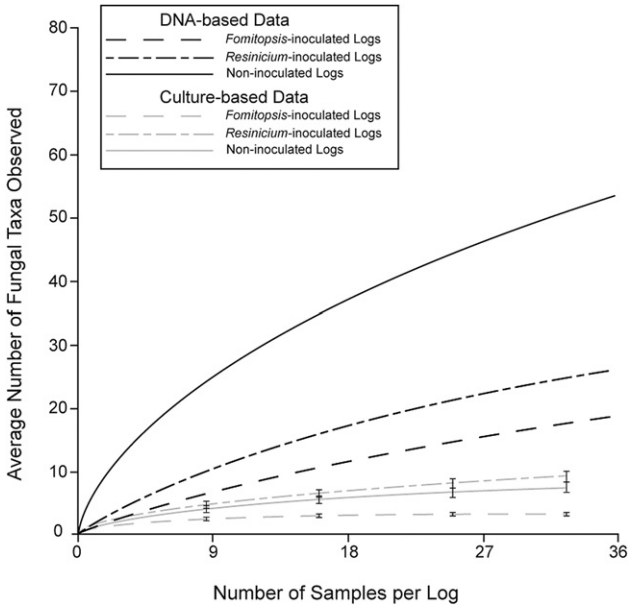

Fig 2 – DNA- and culture-based taxon accumulation curves for *Picea abies* logs. Each culture-based curve is the average of four curves generated from four separate logs, with error bars representing standard error. The DNA-based curves are based on single logs, with taxa defined based on 98 % sequence similarity.

An additional 20 clones were sequenced from three samples from each of the three logs to investigate the average number of taxa present in a single drill sample. Taxon accumulation curves from these samples (Fig 3) indicate that individual drill samples from inoculated logs contained on average four taxa, while non-inoculated samples contained on average 5.8 taxa. Fifty one taxa were found in the non-inoculated log, 25 taxa in the *Resinicium*-inoculated log, and 19 taxa in the *Fomitopsis*-inoculated log (Table 2; Fig 2). *R. bicolor* was identified in all 36 drill samples from the *Resinicium*-inoculated log and in 16 drill samples from the non-inoculated logs; *F. pinicola* was identified in 36 of the drill samples from the *Fomitopsis*-inoculated logs and was not identified in samples from any other logs (Table 4).

Fungal community analyses

The species used for inoculations appear to have successfully colonized logs and influenced fungal community development 6 yr after inoculation. This is evident from all three techniques used to survey the fungal community. Based on the DNA survey, *F. pinicola* was identified in all 36 samples taken from the *Fomitopsis*-inoculated log (Table 4), and *F. pinicola* fruit bodies were found on all four *Fomitopsis*-inoculated logs (Table 1). In addition, *F. pinicola* cultures dominated the cultures obtained from *Fomitopsis*-inoculated logs (Table 3) and an ordination of cultural data showed *F. pinicola* to be strongly associated with the *Fomitopsis*-inoculation treatment (Fig 4). Cultural data suggest that species richness was significantly

Table 4 – The 72 ITS sequence variants identified from one *Resinicium*-inoculated, one *Fomitopsis*-inoculated and one non-inoculated *Picea abies* log with fungal species sorted by abundance

GenBank accession number	Closest identified species based on BLAST of GenBank	% Base pair match	Number of drill samples in which ITS sequence was found (36 per log)			Total
			Non-inoculated control log	*Fomitopsis*-inoculated log	*Resinicium*-inoculated log	
Basidiomycota						
HQ611289	***Resinicium bicolor***[a] (FJ872065)	99.7 %	16	–	36	52
HQ611321	***Fomitopsis pinicola*** (AY854083)	99.0 %	–	36	–	36
HQ611281	***Calocera viscosa*** (DQ520102)	98.9 %	10	6	1	17
HQ611314	*Botryobasidium subcoronatum* (DQ200924)	90.3 %	9	–	2	11
HQ611280	***Sistotrema brinkmannii*** (FJ903297)	99.5 %	4	–	–	4
HQ611313	***Xeromphalina parvibulbosa*** (FJ596890)	100.0 %	2	1	1	4
HQ611292	*Hyphoderma setigerum* (AJ534259)	100.0 %	2	1	–	3
HQ611306	*Calocera cornea* (AY789083)	96.0 %	1	–	1	2
HQ611278	*Calocera* sp. (GQ411508)	88.5 %	2	–	–	2
HQ611343	*Calocera* sp. (FJ195751)	99.4 %	–	–	1	1
HQ611348	***Cortinarius casimiri*** (AJ889945)	99.6 %	–	1	–	1
HQ611325	***Guepinia spathularia*** (FJ478111)	93.4 %	–	–	1	1
HQ611346	***Irpex lacteus*** (EU273517)	98.6 %	–	1	–	1
HQ611345	*Limonomyces roseipellis* (EU622846)	89.4 %	–	1	–	1
HQ611312	*Pachnocybe* sp. (AY618669)	94.4 %	1	–	–	1
HQ611347	***Phanerochaete crassa*** (AY219341)	99.0 %	–	1	–	1
HQ611320	*Rhodotorula lignophila* (AF444513)	93.5 %	1	–	–	1
HQ611330	*Sporobolomyces singularis* (AF444600)	99.2 %	–	–	1	1
HQ611295	***Tremiscus* sp.** (DQ520100)	99.4 %	1	–	–	1
HQ611335	**Uncultured Agaricales clone** (FJ475676)	100.0 %	1	–	–	1
Ascomycota						
HQ611291	***Phialophora lagerbergii*** (AF083197)	99.1 %	17	–	6	23
HQ611284	*Ophiostoma grandicarpum* (AJ293884)	90.4 %	19	–	–	19
HQ611300	***Candida paludigena*** (DQ911451)	98.2 %	10	2	5	17
HQ611349	***Ascocoryne cylichnium*** (AY789395)	97.6 %	5	–	6	11
HQ611296	***Sporothrix* sp.** (AY618685)	97.9 %	11	–	–	11
HQ611305	*Lecythophora* sp. (FJ824625)	91.1 %	3	2	5	10
HQ611339	***Leptodontidium elatius*** (FJ903294)	99.8 %	9	–	–	9
HQ611325	*Orbilia delicatula* (U72593)	98.5 %	5	–	1	6
HQ611329	***Rhinocladiella atrovirens*** (AB091215)	98.6 %	–	2	3	5
HQ611285	**Uncultured Sordariomycetes** (FJ475666)	96.9 %	4	1	–	5
HQ611299	***Haplographium catenatum*** (FJ839621)	97.4 %	4	–	–	4
HQ611301	*Holwaya mucida* (DQ257357)	90.8 %	4	–	–	4
HQ611282	***Leptodontidium elatius*** (FJ903294)	97.7 %	4	–	–	4
HQ611309	***Phialophora* sp.** (FJ903315)	99.8 %	3	–	1	4
HQ611310	Xylariaceae sp. (EU009999)	97.0 %	4	–	–	4
HQ611308	Amphisphaeriaceae sp. (AY853246)	96.4 %	2	–	1	3
HQ611334	***Scytalidium lignicola*** (FJ903317)	92.7 %	1	–	2	3
HQ611302	***Ascocoryne sarcoides*** (GQ411510)	98.8 %	1	–	1	2
HQ611319	***Cenococcum geophilum*** (AY394919)	96.4 %	2	–	–	2
HQ611287	*Dactylella* sp. (DQ494371)	92.6 %	2	–	–	2
HQ611337	***Phialocephala dimorphospora*** (AY606303)	99.6 %	1	–	1	2
HQ611286	*Phialocephala dimorphospora* (AY606308)	98.8 %	1	–	1	2
HQ611322	*Phialocephala scopiformis* (AF486126)	99.6 %	–	2	–	2
HQ611303	**Uncultured Helotiales** (FJ475762)	97.7 %	2	–	–	2
HQ611297	Uncultured Pezizomycotina (FJ554391)	92.6 %	2	–	–	2
HQ611311	Xylariaceae sp. (EU009999)	95.8 %	2	–	–	2
HQ611336	*Candida* sp. (AB285026)	96.8 %	1	–	–	1
HQ611294	*Capronia pilosella* (DQ826737)	89.6 %	1	–	–	1
HQ611317	*Chalara* sp. (AY618226)	99.7 %	1	–	–	1
HQ611304	*Chloridium* sp. (GQ331985)	92.0 %	1	–	–	1
HQ611341	***Cladonia furcata*** (EU266080)	99.4 %	–	1	–	1
HQ611323	***Cladophialophora chaetospira*** (EU035405)	90.7 %	–	1	–	1
HQ611307	***Cladosporium* sp.** (AJ279487)	99.3 %	1	–	–	1
HQ611338	***Haplographium catenatum*** (FJ839620)	98.8 %	1	–	–	1
HQ611288	*Hyaloscypha aureliella* (EU940229)	99.6 %	1	–	–	1
HQ611324	*Hyaloscypha daedaleae* (AY789416)	96.6 %	–	1	–	1

(*continued on next page*)

Table 4 – (continued)

GenBank accession number	Closest identified species based on BLAST of GenBank	% Base pair match	Number of drill samples in which ITS sequence was found (36 per log)			Total
			Non-inoculated control log	*Fomitopsis*-inoculated log	*Resinicium*-inoculated log	
HQ611327	*Hyaloscypha daedaleae* (AY789416)	95.6 %	–	–	1	1
HQ611290	*Hymenoscyphus* sp. (AY354244)	98.7 %	1	–	–	1
HQ611326	*Isaria fumosorosea* (EU553306)	100.0 %	–	1	–	1
HQ611279	**Lecythophora sp.** (AY781228)	99.2 %	1	–	–	1
HQ611340	*Leptodontidium elatius* (FJ903294)	91.2 %	–	–	1	1
HQ611342	**Mollisia cinerea** (AJ430222)	99.1 %	–	1	–	1
HQ611293	*Nectria* sp. (AY805576)	97.9 %	1	–	–	1
HQ611331	*Phialocephala* sp. (FJ903314)	93.9 %	–	–	1	1
HQ611333	**Uncultured Sordariomycetidae** (FJ554106)	99.6 %	–	–	1	1
HQ611298	*Xenochalara* sp. (AY465471)	98.1 %	1	–	–	1
Glomeromycota						
HQ611283	Uncultured glomeromycete clone (EF619903)	90.0 %	3	–	–	3
HQ611316	Uncultured glomeromycete clone (EF619903)	90.3 %	1	–	–	1
HQ611318	Uncultured glomeromycete clone (EF619903)	90.3 %	1	–	–	1
Unknown						
HQ611315	Uncultured fungus clone (EF619885)	93.4 %	1	–	–	1
HQ611332	Uncultured fungus clone (FJ820507)	96.4 %	–	–	1	1
HQ611344	Uncultured fungus clone (EF619885)	97.0 %	–	1	–	1

a Taxa in bold had close matches in GenBank, defined as at least 97 % sequence similarity over 90 % of sequence length.

($p \leq 0.05$) depressed in *Fomitopsis*-inoculated logs (Table 2), and this is supported by DNA-based data, which indicate that samples from *Fomitopsis*-inoculated logs consistently displayed the fewest fungal taxa (Fig 3) and the *Fomitopsis*-inoculated log displayed the fewest taxa overall (Fig 2). Somatic compatibility tests among *F. pinicola* isolates indicate that all isolates were fully somatically compatible and thus derived from the same genet.

The picture for *Resinicium*-inoculated logs is not as clear as the *Fomitopsis*-logs, but *R. bicolor* was the dominant Basidiomycota species in these logs. The DNA-based data indicate the *Resinicium*-inoculated logs were strongly colonized by *R. bicolor* (Table 4), and *R. bicolor* fruit bodies were found on all four

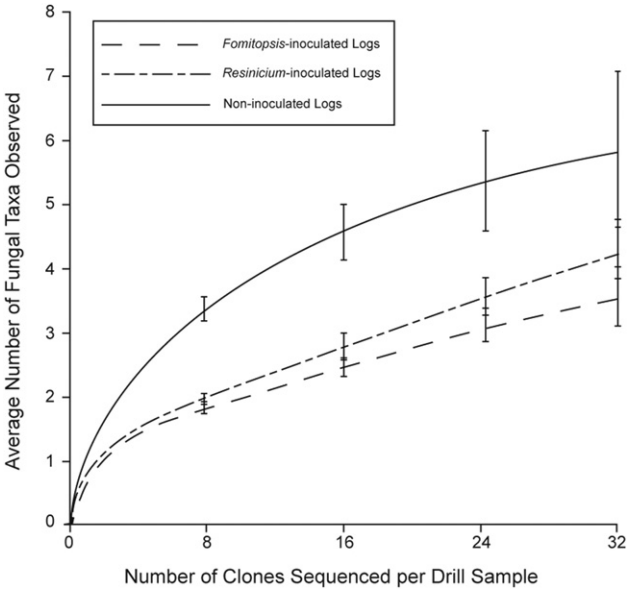

Fig 3 – DNA-based taxon accumulation curves from individual drill samples taken from *Picea abies* logs. Each curve is the average of three curves generated from three separate drill samples, with error bars representing standard error. DNA-based taxa were based on 98 % sequence similarity.

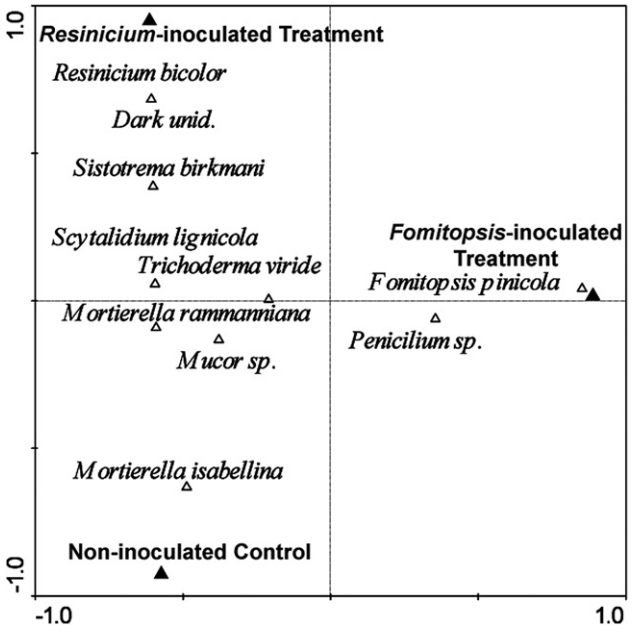

Fig 4 – CCA ordination plot of abundance of fungal species isolated in culture from four *Resinicium*-inoculated, four *Fomitopsis*-inoculated and four non-inoculated *Picea abies* logs.

Resinicium-inoculated logs. *R. bicolor* was only isolated ten times (3 % of isolations), but all ten of these isolates were from *Resinicium*-inoculated logs. Despite being colonized by *R. bicolor*, the *Resinicium*-inoculated logs displayed the highest culture-based species richness per log with an average of 9.5 taxa per log (Table 2). Somatic compatibility testing among *R. bicolor* isolates indicated that multiple genets were present in each log in which this species occurred.

The non-inoculated logs displayed intermediate levels of species richness based on fruit bodies and cultural data (Table 2), but were by far the most taxon rich based on the DNA-analysis. The non-inoculated logs displayed both the highest number of DNA-based taxa per drill sample (Fig 3) as well as the highest richness per log (Fig 2). The non-inoculated logs were not strongly colonized by any Basidiomycota species, although species of Zygomycota (*Mucor* sp. and *M. isabellina*) and Ascomycota (*T. viride* and *Penicillium* sp.) were commonly isolated from these logs.

Mass loss analyses

Wood density after 6 yr was significantly different ($p = 0.04$) between the *Fomitopsis*-inoculated logs and the *Resinicium*-inoculated and non-inoculated logs, with *Fomitopsis*-inoculated logs being significantly less dense on average than other logs (Table 2). An analysis of mass loss *versus* species richness of cultured fungi did not reveal any significant relationships, although a weak positive correlation was observed between species richness and increased mass loss in non-inoculated logs ($R = 0.415$, $p = 0.179$). An analysis of mass loss *versus* abundance of *F. pinicola* and *R. bicolor* isolates in culture did not reveal any significant relationships.

Discussion

This is the first experimental, field-based work to demonstrate that initial colonization events can significantly affect mass loss and fungal community development in large pieces of woody debris. This work complements a previous field study (Progar *et al.* 2000) suggesting that inoculation with decay fungi increases CO_2 respiration rates in *Pseudotsuga menziesii* logs in western Oregon. Although fungal communities were affected by inoculation and a significant difference was observed in mass loss in the present study, the mechanisms that resulted in differing levels of mass loss are still largely unknown. Likely potential mechanisms could be based on species composition of the fungal community (i.e., the presence or absence of strong decay species), species or genet richness of the fungal community, or some combination of these factors. All logs were in a similar physical environment, so it seems unlikely that environmental factors caused the differing levels of mass loss, but it is possible that particular fungal species affected the environmental conditions of the logs (e.g. moisture content) and thus the rate of decay. Each of these variables (species composition, species richness, etc.) would need to be individually manipulated to fully explain the greater mass loss in *Fomitopsis*-inoculated logs.

Regardless of the mechanism(s) involved, changes in mass loss over time may have significant implications for carbon cycling and nutrient turnover in forest systems. The *Fomitopsis*-inoculated logs, which had the most simplified fungal community in terms of both species and genets, decayed significantly faster, which may indicate that simplified fungal communities could lead to greater carbon respiration, at least during the initial stages of decay. This is important because intensive forest management procedures often reduce the species richness of fungi on large diameter debris at the site-level (Bader *et al.* 1995; Junninen *et al.* 2006; Lindner *et al.* 2006; Nordén *et al.* 2008). Unfortunately what is largely unknown is whether forest management affects the number of mycelial species and genets per log, which may directly affect the number of competitive interactions in decay columns and thus the rate of decay. Simplification of fungal communities, both at the species level as well as at the level of number of individuals in a population, may lead to an increase in mass loss in systems where competitive interactions are a dominating force (Fukami *et al.* 2010). Conversely, simplification of fungal communities could lead to decreased mass loss if synergistic interactions or species sampling effects contribute to faster rates of decay (Loreau & Hector 2001; LeBauer 2010). Such changes may also be dependent on environmental conditions (Progar *et al.* 2000; Toljander *et al.* 2006), thus complicating prediction of these variables given changes in mean annual temperatures or rates of precipitation at given latitudes.

Although richness of the fungal community may have affected decay rates, the composition of the fungal community probably also played a significant role. *Fomitopsis*-inoculated logs may have decayed faster because of the intrinsic decay abilities of *F. pinicola* relative to other decay species present in the logs. Previous work has demonstrated that brown-rot species such as *F. pinicola* can cause substantially more decay relative to white-rot species (Blanchette & Shaw 1978) in laboratory conditions, although this effect may be dependent on wood-quality (e.g. density, ring width, proportion of heartwood, etc.) (Edman *et al.* 2006). For accurate modelling of carbon turnover at ecosystem scales, more work is needed to determine whether brown- and white-rot fungi contribute differently to mass loss and carbon sequestration over longer periods of time (i.e., years or decades). In addition, more work is needed regarding the geographic distribution and prevalence of brown-rot *versus* white-rot species and whether these fungi react differently to changes in environmental conditions and wood-quality (Edman *et al.* 2006).

Although the *Fomitopsis*-inoculated logs decayed the fastest, there was a weak positive relationship between species richness and mass loss in non-inoculated logs. This suggests that in natural logs, increased species richness may lead to greater rates of decay, a relationship observed in some laboratory experiments (LeBauer 2010). The positive relationship between species richness and mass loss in the present study needs to be confirmed with additional work because this correlation was not statistically significant ($p = 0.179$) and this trend is dependent on a small number of data points in the analysis, rather than being a robust trend. Composition of the fungal community, including colonization rates of Basidiomycota species, probably also affected mass loss in non-inoculated logs. In the non-inoculated logs, Basidiomycota species appear to have colonized more slowly, which may have contributed to less mass loss. In addition, the presence of

a *Scytalidium* species in the non-inoculated and *Resinicium*-inoculated logs may have inhibited decay in these logs. *Scytalidium* species, which are known to inhibit a wide range of decay fungi (Cease *et al.* 1989), were isolated 10 or more times from non-inoculated and *Resinicium*-inoculated logs but were entirely absent from *Fomitopsis*-inoculated logs.

In the *Resinicium*-inoculated logs, a Basidiomycete decay species was presumably present for the entire 6 yr but mass loss was not significantly increased. This result may be based on the fact that *R. bicolor* has been characterized in laboratory studies as a strong competitor but a relatively slow decay species (Kirby *et al.* 1990; Holmer & Stenlid 1993, 1996). Alternatively, this result may have been due to the large number of *R. bicolor* genets in each log, thus producing a large number of intra-species competitive interactions and less resource allocation to wood decay. Since a single genet of *R. bicolor* was introduced into the logs at the beginning of the experiment, it seems likely that *R. bicolor* experienced di-mon mating (i.e., the Buller phenomenon) in the field, which led to a mosaic of genets in each log. Natural strains of *R. bicolor* colonized some of the non-inoculated logs, indicating that *R. bicolor* actively colonizes logs in these environments. The colonization by natural genets of *R. bicolor* along with the intrinsic differences in decay abilities of this species are likely factors influencing mass loss in *Resinicium*-inoculated logs.

Although mass loss was greatest in *Fomitopsis*-inoculated logs, all logs lost approximately half of their mass after 6 yr based on reported densities of undecayed *P. abies* wood (Mäkinen *et al.* 2002). This indicates that mass loss was only moderately affected by the inoculation treatments over the time period investigated. Tracking trends in mass loss over longer periods of time will help to answer whether these effects become more significant in later stages of decay. Although after 6 yr mass loss, and thus presumably carbon loss, was greatest in *Fomitopsis*-inoculated logs, it is possible that in the later stages of decay these logs will contribute the most to carbon sequestration due to the recalcitrant nature of brown-rot decay residues.

The three methods used to survey the fungal community each had advantages and disadvantages. Fruit body surveys were completed in the shortest period of time and were the least expensive to conduct, but required specialized literature and knowledge for accurate identifications. This method identified species that were actively reproducing on the logs, thus demonstrating the logs were important for completion of a species' lifecycle, but this method also identified the fewest species overall and the fewest species per log. Fruit body surveys were also significantly biased toward Basidiomycota species, which produce larger fruit bodies relative to most Ascomycota and Zygomycota species. Fruit body surveys may be the most cost effective for surveying a large number of logs over a large area and may identify the key Basidiomycota species important for decay, but this method is probably the least appropriate for identifying the fungal species present in individual logs, as there was not always a strong correlation between fruit bodies and presence of particular species in culture or with DNA. This may not be a significant drawback if the goal is to compile a list of species in an area (e.g. for conservation studies), but this could be problematic for studies connecting fungal communities to specific decay processes in individual logs.

Culturing produced the largest dataset covering all logs, but this method requires laboratory facilities for isolating and growing cultures. In addition, fungal cultures can be difficult or impossible to identify without DNA-based methods, making this method relatively resource and time intensive. Culturing was biased toward Ascomycota and Zygomycota species, which may influence the decay process in large woody debris, but which are generally of minimal importance as direct agents of wood decay (Stenlid *et al.* 2008). An advantage of culturing was that living cultures were available for determining somatic compatibility, which provided a level of genetic resolution difficult or impossible to obtain with other methods, and these isolates are available for further experimentation.

DNA-based methods produced an extensive and unparalleled view of the fungal community in individual logs. However, this method consumed the most resources and time of all methods, despite being applied to only three of the 12 logs. Molecular lab facilities are expensive and not available in many locations and molecular reagents and supplies are significantly more expensive than the supplies needed for culturing or identification of fruit bodies. Nonetheless, the costs of molecular techniques continue to decline and their availability and throughput are increasing, making them more and more attractive. Perhaps one of the largest drawbacks of this technique is that DNA-sequence identification is dependent on publically available databases, which often contain errors (Nilsson *et al.* 2006). While specific databases are available for the identification of ecto-mycorrhizal fungi (Kõljalg *et al.* 2005), comparable databases are not yet available for wood-inhabiting fungi.

It was observed during the course of this study that DNA became difficult to extract from drilled wood samples if they were stored for longer periods of time (more than 10 months). This was likely due to the fact that drill samples for this study were collected in plastic bags and frozen at −20 °C, thus fracturing fungal cells and exposing their cellular contents to air and products of wood decay for long periods of time. Preliminary testing (data not shown) indicates that freezing drill samples in a buffered 2 % CTAB solution significantly increases the length of time samples can be stored for molecular analysis.

Although this study presented a unique opportunity to compare different surveying methods for fungal communities, it is unfortunately impossible to directly compare these methods for a variety of reasons. One of the most fundamental problems for such comparisons is that each method characterizes the abundance of species in different ways and characterizes communities at different scales. In addition, it is difficult or impossible to apply all methods to exactly the same samples and each method is sensitive to different components of the fungal community. For example, culturing produced a large number of Ascomycota and Zygomycota species (e.g. *Trichoderma*, *Penicillium*, and *Mucor* spp.) that were rare or entirely absent in the DNA-based surveys. Culturing also may have missed some species that were difficult to isolate, such as *R. bicolor*, which was found in every sample of the *Resinicium*-inoculated log with DNA-based methods but only rarely observed with culturing. This may be partly due to the spatial distribution of *R. bicolor* mycelium, which is known to colonize wood superficially (Allmér *et al.* 2009). If *R. bicolor* was most common near the outer edge of the log, the DNA-based sampling method (i.e., drilling from the outer to

the central part of the log) may have more effectively sampled all spatial parts of the log relative to cultural methods. *F. pinicola*, in contrast, was observed uniformly with culturing, DNA-based methods and in the fruit body surveys. An earlier study using a combination of culturing and DNA-based identification of fungi colonizing root systems of *P. abies* growing in undisturbed parts of the same forest (Johannesson & Stenlid 1999) indicated that *F. pinicola* and *R. bicolor* occur in wood dead for approximately 5 yr, confirming the representativeness of the fungi observed in the present study.

More work is needed to fully characterize the biases of DNA-based methods relative to traditional methods such as culturing and fruit body identification (Stenlid *et al.* 2008) and additional manipulative studies are needed to probe the fundamental mechanisms that govern mass loss and fungal community development in woody debris. The logs used in the current study are part of a larger ongoing study and represent only half of the logs originally inoculated and placed in the forest in 1997. The other logs have been maintained for future sampling with next generation sequencing techniques to determine whether advances in molecular methods will provide a better understanding of the relationship between wood-inhabiting fungal communities and carbon turnover in forests. Understanding the diversity, distribution and function of wood-inhabiting fungi will be crucial to understanding carbon cycling and patterns of carbon sequestration in forests, especially in relation to issues such as forest management and climate change.

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REFERENCES

Allmér J, Stenlid J, Dahlberg A, 2009. Logging-residue extraction does not reduce the diversity of litter-layer saprotrophic fungi in three Swedish coniferous stands after 25 years. *Canadian Journal of Forest Research* **39**: 1737–1748.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ, 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389–3402.

Bader P, Jansson S, Jonsson BG, 1995. Wood-inhabiting fungi and substratum decline in selectively logged boreal spruce forests. *Biological Conservation* **72**: 355–362.

Blanchette RA, Shaw CG, 1978. Associations among bacteria, yeasts and basidiomycetes during wood decay. *Phytopathology* **68**: 631–637.

Cease KR, Blanchette RA, Highley TL, 1989. Interactions between *Scytalidium* species and brown- or white-rot basidiomycetes in birch wood. *Wood Science and Technology* **23**: 151–161.

Chapin III FS, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Díaz S, 2000. Consequences of changing biodiversity. *Nature* **405**: 234–242.

Cooke RC, Rayner A, 1984. *Ecology of Saprotrophic Fungi*. Longman Group Limited, New York.

Edman M, Möller R, Ericson L, 2006. Effects of enhanced tree growth on the decay capacities of three saprotrophic wood-fungi. *Forest Ecology and Management* **232**: 12–18.

Ehrlich PR, Pringle RM, 2008. Colloquium paper: where does biodiversity go from here? A grim business-as-usual forecast and a hopeful portfolio of partial solutions. *Proceedings of the National Academy of Science* **105**: 11579–11586.

Fukami T, Dickie IA, Wilkie JP, Paulus BC, Park D, Roberts A, Buchanan PK, Allen RB, 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. *Ecology Letters* **13**: 675–684.

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

Gilbertson RL, 1980. Wood-rotting fungi of North America. *Mycologia* **72**: 1–49.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN, 1995. *Ainsworth and Bisby's Dictionary of the Fungi*, 8th edn. CAB International, Cambridge, UK.

Høiland K, Bendiksen E, 1997. Biodiversity of wood-inhabiting fungi in a boreal coniferous forest in Sør-Trøndelag County, central Norway. *Nordic Journal of Botany* **16**: 643–659.

Holmer L, Stenlid J, 1993. The importance of inoculum size for the competitive ability of wood decomposing fungi. *FEMS Microbiology Ecology* **12**: 169–176.

Holmer L, Stenlid J, 1996. Diffuse competition for heterogeneous substrate in soil among six species of wood-decomposing basidiomycetes. *Oecologia* **106**: 531–538.

Hurlbert SH, 1971. The nonconcept of species diversity: a critique and alternative parameters. *Ecology* **52**: 577–586.

Johannesson H, Stenlid J, 1999. Molecular identification of wood-inhabiting fungi from a primeval forest in Sweden. *Forest Ecology and Management* **115**: 203–211.

Junninen K, Similä M, Kouki J, Kotiranta H, 2006. Assemblages of wood-inhabiting fungi along the gradients of succession and naturalness in boreal pine-dominated forests in Fennoscandia. *Ecography* **29**: 75–83.

Kåren O, Högberg N, Dahlberg A, Jonsson L, Nylund JE, 1997. Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. *New Phytologist* **136**: 313–325.

Kirby JJH, Stenlid J, Holdenrieder O, 1990. Population structure and responses to disturbance of the basidiomycete *Resinicium bicolor*. *Oecologia* **85**: 178–184.

Kõljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T, Ursing BM, 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytologist* **166**: 1063–1068.

LeBauer DS, 2010. Litter degradation rate and ß-glucosidase activity increase with fungal diversity. *Canadian Journal of Forest Research* **40**: 1076–1085.

Lindblad I, 1998. Wood-inhabiting fungi on fallen logs of Norway spruce: relations to forest management and substrate quality. *Nordic Journal of Botany* **18**: 243–255.

Lindner DL, Banik MT, 2009. Effects of cloning and root-tip size on observations of fungal ITS sequences from *Picea glauca* roots. *Mycologia* **101**: 157–165.

Lindner DL, Burdsall Jr HH, Stanosz G, 2006. Species diversity of polyporoid and corticioid fungi in northern hardwood forests with differing management histories. *Mycologia* **98**: 195–217.

Loreau M, Hector A, 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**: 72–76.

Magurran AE, 1988. *Ecological Diversity and Its Measurement*. Princeton University Press, Princeton, New Jersey.

Mäkinen H, Saranpää P, Linder S, 2002. Wood-density variation of Norway spruce in relation to nutrient optimization and fibre dimensions. *Canadian Journal of Forestry Research* **32**: 185–194.

Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson KH, Kõljalg U, 2006. Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. *PLoS One* **1**: e59.

Nordén B, Götmark F, Ryberg M, Paltto H, Allmér J, 2008. Partial cutting reduces species richness of fungi on woody debris in oak-rich forests. *Canadian Journal of Forestry Research* **38**: 1807–1816.

Nordén B, Ryberg M, Götmark F, Olausson B, 2004. Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests. *Biological Conservation* **117**: 1–10.

Ohlson M, Söderström L, Hörnberg G, Zackrisson O, Hermansson J, 1997. Habitat qualities versus long-term continuity as determinants of biodiversity in boreal old-growth swamp forests. *Biological Conservation* **81**: 221–231.

Progar RA, Schowalter TD, Freitag CM, Morrell JJ, 2000. Respiration from coarse woody debris as affected by moisture and saprotroph functional diversity in western Oregon. *Oecologia* **124**: 426–431.

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

Sanders HL, 1968. Marine benthic diversity: a comparative study. *American Naturalist* **102**: 243–282.

Stenlid J, Penttilä R, Dahlberg A, 2008. Wood-decay basidiomycetes in boreal forests: distribution and community development. In: Boddy L, Frankland JC, van West P (eds), *Ecology of Saprotrophic Basidiomycetes*. Academic Press, London and Oxford, UK, pp. 239–262.

Tiunov AV, Scheu S, 2005. Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities. *Ecology Letters* **8**: 618–625.

Toljander YK, Lindahl BD, Holmer L, Högberg NOS, 2006. Environmental fluctuations facilitate species co-existence and increase decomposition in communities of wood decay fungi. *Oecologia* **148**: 625–631.

Vasiliauskas R, Stenlid J, 1998a. Fungi inhabiting stems of *Picea abies* in a managed stand in Lithuania. *Forest Ecology and Management* **109**: 119–126.

Vasiliauskas R, Stenlid J, 1998b. Influence of spatial scale on population structure of *Stereum sanguinolentum* in northern Europe. *Mycological Research* **102**: 93–98.

Vasiliauskas R, Lygis V, Larsson K-H, Stenlid J, 2005. Airborne fungal colonisation of coarse woody debris in north-temperate *Picea abies* forest: impact of season and local spatial scale. *Mycological Research* **109**: 487–496.

Wästerlund I, Ingelög T, 1981. Fruit body production of larger fungi in some young Swedish forests with special reference to logging waste. *Forest Ecology and Management* **3**: 269–294.

White TJ, Bruns TD, Lee SB, Taylor JW, 1990. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. PCR Protocols – A Guide to Methods and Applications*. Academic Press, New York, pp. 315–322.