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Species Composition of Saproxylic Fungal Communities on Decaying Logs in the Boreal Forest

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Abstract Coarse woody debris supports large numbers of saproxylic fungal species. However, most of the current knowledge comes from Scandinavia and studies relating the effect of stand or log characteristics on the diversity and composition of decomposer fungi have not been conducted in Northeastern Canada. Logs from five tree species were sampled along a decomposition gradient in nine stands representing three successional stages of the boreal mixed forest of Northwestern Quebec, Canada. Using a molecular fingerprinting technique, we assessed fungal community Shannon–Weaver diversity index, richness, and composition. We used linear mixed models and multivariate analyses to link changes in fungal communities to log and stand characteristics. We found a total of 33 operational taxonomic units (OTUs) including an indicator species for balsam fir (similar to *Athelia* sp.) and one found only in aspen stands (similar to *Calocera cornea*). Spruce logs supported the highest fungal Shannon–Weaver diversity index and OTU number. Our results support the hypothesis that log species influences fungal richness and diversity. However, log decay class does not. Stand composition, volume of coarse woody debris, and log chemical composition were all involved in structuring fungal communities. Maintaining the diversity of wood-decomposing communities therefore requires the presence of dead wood from diverse log species.

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Introduction

Saproxylic [61] fungi are the main agents of wood decomposition and an essential component of forest ecosystem food webs [45], influencing nutrient cycling and carbon sequestration [24]. They represent a highly diverse group, for example over 1,500 species were identified from Finnish forests alone [56]. Forest harvesting decreases the amount of deadwood and species diversity of saproxylic fungi in managed fennoscandian forests [50, 58] and wood-decaying fungi are considered good indicators of effective conservation [40], as they are sensitive to changes in forest structure.

Wood structure and transformations in chemical composition over the course of decomposition can induce changes in fungal species composition, as some species utilize specific substrates for a limited time according to their biochemical requirements [5]. Succession of saproxylic fungal communities during decomposition has been described in spruce logs in natural boreal old-growth forests of Sweden [4] and in spruce and trembling aspen logs in the boreal mixedwood forest of Alberta (Canada) [41]. Log species [41] and stand characteristics, such as age [48] and abundance of deadwood [33], can also influence fungal composition and diversity.

Factors influencing fungal diversity in decomposing logs vary according to fungal species [28], fungal successional status (early vs. secondary) [31], and the scale of the study [4]. Also, some species, especially heart rot agents, prefer larger logs [49] and species diversity has been shown to increase with log size [14]. On the other hand, logs at intermediate decay stages have also been found to harbor

more species [26] due to the availability of multiple niches caused by heterogeneous decomposition [52].

However, much of the information available on boreal saproxilic fungal communities comes from Fennoscandian forests with a long history of forest management and poor tree diversity. To our knowledge, few studies on saproxilic fungal diversity in relation to deadwood characteristics have been conducted in the boreal forests of North America [41]. Moreover, fungal diversity and community structure of coarse woody debris (CWD) have, so far, mainly been assessed from observations of sporocarps and based on single-log species. For example, investigations of fungal communities in relation to habitat type [60], stand age [48], successional status and total volume of dead wood [33], and host tree species [67] were all based on fungal fructifications. Community fingerprinting techniques have recently been applied to studies of soil fungi and a few studies have used molecular techniques to study saproxylic fungal diversity on dead wood [35, 36]. Denaturing/temperature gradient gel electrophoresis (DGGE/TGGE) has been used successfully for evaluating changes in community composition in soils [2] and in wood [38]. Moreover, this technique allows DNA to be recovered and sequenced for fungal identification.

We used DGGE to separate fungal internal transcribed spacer (ITS) regions amplified from total CWD DNA to evaluate saproxylic fungal diversity of five species of decomposing logs from natural boreal stands of different successional status in relation to chemical and physical characteristics of CWD. Our objectives were (1) to characterize how diversity and species composition vary along a decomposition gradient for different wood species in natural stands, (2) to link fungal communities to morphological and chemical features of CWD and other stand features, and (3) to identify indicator species of log species, stand features, or environmental conditions. We hypothesized that the saproxylic fungal diversity of individual logs would be maximal at intermediate decay stages, and that higher diversity would occur in larger logs. We also expected fungal composition to be influenced by log species and stand conditions such as age and CWD volume.

Methods

Field Sites Description

The study area was located within the Lake Duparquet Research and Teaching Forest [25] in the Abitibi region of northwestern Québec, 45 km northwest of Rouyn-Noranda, Québec (48°86′N–48°32′N, 79°19′W–79°30′W). The climate is moist continental with a mean annual temperature of 0.8°C and annual precipitation is 890 mm (Environment Canada; Canadian climatic normals 1971–2000, www.climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html). The region was situated in the mixed-wood zone of the boreal shield. Forest succession on rich mesic sites generally begins with the establishment of pure or mixed stands of paper birch (*Betula papyrifera* Marsh.), trembling aspen (*Populus tremuloides* Michx.), and jack pine (*Pinus banksiana* Lamb.) that can maintain dominance for a period of over 100 years. In the absence of a major disturbance, these species are gradually replaced by a mixture of shade tolerant species such as white spruce (*Picea glauca* (Moench) Voss), black spruce (*Picea mariana* (Mill) B.S.P.), and balsam fir (*Abies balsamea* (L.) Mill.) [3].

Field Methods

Sampling took place in the control stands of the SAFE experiment in early summer [8]. The SAFE study is set in natural stands of fire origin representing a gradient of composition typical of the natural succession on rich mesic clayey sites. The oldest stand type (mixed post budworm outbreak stands) dates from a fire in 1760 and was later effected by the 1970–1987 spruce budworm outbreak (*Choristoneura fumiferana*) [47] resulting in a mixture of white birch, white spruce, and balsam fir (Table 1). The experimental design of the SAFE study includes three, 1–2 ha, unharvested plots in each stand type, for a total of nine experimental units. In all experimental units, five permanent circular sampling plots (radius=11.28 m) were established and all tree stems greater than 5.0 cm in diameter at breast height (dbh) were identified and measured (dbh) for basal area estimation.

The volume of downed wood was estimated using triangular transects. One triangle (30 m side, [64]) was sampled in each experimental plot. Along each transect line, the frequency of downed wood was recorded by species, diameter class (5 cm, 2.5–7.6 cm; 10 cm, 7.6–12.5 cm; 15 cm, 12.6–17.5 cm; and greater than 17.5 cm) and five decomposition classes [11].

Logs of spruce, paper birch, jack pine, balsam fir, and trembling aspen with diameter over 10 cm and from five decay classes were located and identified. Log length and diameter (both ends) were measured. Material for DNA extraction was collected by drilling one hole in each log in the selected decomposition class with a flat drill bit (12.7 mm). The bark and the uppermost layer of wood were first removed and precautions taken to prevent cross-contamination of samples; drill bits were cleaned, rinsed with sterile water, soaked in 95% ethanol and flame sterilized between samples. Additional samples from each log were taken for physical and chemical laboratory analyses. For well-decomposed logs, where visual species identification was impossible, an additional wood sample was

Table 1 Mean volume of CWD by decay class and tree species basal area in sampled stands of different successional status

	Post-spruce budworm outbreak stand (1760)	Mixed trembling aspen stand (1910)	Trembling aspen stand (1923)
	Stand CWD volume (m^3 ha^{-1})		
Decay class			
Fresh	8.8 (n=6)	10.3 (n=9)	7.3 (n=10)
Medium	55.4 (n=8)	15.4 (n=6)	48.9 (n=22)
Advanced	25.5 (n=20)	85.1 (n=10)	71.7 (n=11)
All categories	89.7	110.8	127.9
	Basal area (m^2 ha^{-1})		
Log species			
Trembling aspen	0.7	30.2	37.3
Paper birch	9.6	0.2	0.7
Spruce	6.1	4.0	1.4
Balsam fir	2.6	2.6	0.6

Number of logs sampled are given in parenthesis

Fresh wood: decomposition classes I and II

Medium decayed wood: decomposition class III

Advanced decay stage: decomposition classes IV and V

taken from a less decomposed part of the log for laboratory identification. All samples were kept frozen at −20°C until analyzed.

Of the 102 sampled logs, 43 were sampled in the youngest stands types (1923 fire), 34 in the oldest stands (1760 fire), and 25 in the second youngest stands (1910 fire). Not all combinations of species and decomposition classes could be found in all stand types. For example, no trembling aspen logs were found in the 1760 stand or jack pine in the 1910 stand.

Log Species Identification

Wood species were identified from structural and anatomical features from sub-samples cut using a microtome [27]. However, this technique did not allow us to distinguish between white and black spruce.

Wood Physical and Chemical Characteristics

Log samples were cut into 5×5 cm pieces. Wood density was estimated from volume, determined by water displacement after immersing samples in hot paraffin, and weight corrected for moisture content estimated from a second sample. The latter sample was then air dried and ground with a cutting mill (Retsch, SM2000) for chemical analyses.

Total N and C were measured by dry combustion using a LECO CNS 2000 analyzer (LECO Corporation, St. Joseph, MI, USA). Lignin and cellulose were determined by near infrared spectroscopy [17] using a FOSS NIRSystems (model 6500). A calibration equation was first developed following wet chemical measurements from 100 samples to predict the chemical composition of every sample (data not shown). The wet chemical analyses used to calibrate the multivariate spectroscopic method were based on the acid detergent fiber/neutral detergent fiber method [22]. Lignin determination was based on the method of Brinkmann [9].

DNA Extraction from Wood

Wood samples were lyophilized for 48 h before disruption in a Qiagen TissueLyser (QIAGEN, Mississauga, ON, Canada), then run for 2 min at 26 Hz, or until the wood was reduce to a fine powder. DNA was extracted with the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. DNA was eluted in 100 μL of elution buffer and stored at −20°C.

PCR Amplification of Fungal-Specific Genes

The ITS region of the fungal rDNA was polymerase chain reaction amplified using the fungal specific primers ITS1-F [20, 29] and ITS2 [66] to obtain a sequence of 280-bp length. A GC clamp (CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CC) was added to the 5′ end of the ITS1-F primer to avoid complete separation of DNA strands during the subsequent denaturing electrophoresis. Polymerase chain reactions (50 μL) contained 2 μL of template, 5 μL of PCR reaction buffer (ThermoPol, New England Biolabs), 1 μl (10 mM) of each dNTP, 1 μL of each primer (50 μM), 0.2 μL of Taq polymerase (5 U μL^{-1}, New England Biolabs). Cycling

parameters were an initial denaturation cycle of 3 min at 95°C followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min 15 s, ending by a final elongation at 72°C for 8 min [36]. Negative (no DNA) and positive controls (fungal DNA from pure culture) were included in each set of reactions. All amplification products were analyzed by electrophoresis with 1% (*w*/*v*) agarose gels in tris–acetate–EDTA buffer (TAE; 40 mM Tris-acetate, 1 mM ethylenediaminetetraacetic acid (EDTA)), stained with Gelgreen (Biotium) and visualized under UV light.

Separation of Fungal ITS Amplicons by DGGE

Electrophoresis was performed according to a slight modification from the protocol of Julien et al. [32]. We used a DCode universal mutation detection system (Bio-Rad Laboratories, Hercules, CA, USA) and an acrylamide gel (8% [wt/vol] acrylamide–bis-acrylamide, 37.5:1) with a linear vertical gradient of 20–55% denaturing agents (100% denaturant corresponding to 7 M urea and 40% [*v*/*v*] deionized formamide) and increasing in the direction of the electrophoretic run with a stacking gel (4% [*w*/*v*] acrylamide–bis-acrylamide, 37.5:1) on top. Approximately 400 ng of each PCR product was loaded and electrophoresis was performed in EDTA TAE buffer (40 mM Tris-acetate, 1 mM EDTA) at 75 V and 60°C for 16 h. Gels were stained for 15 min with SYBR gold (Invitrogen, Carlsbad, CA, USA), visualized under UV illumination, and digitized using a ChemiDoc XRS System molecular imager (Bio-Rad Laboratories, Hercules, CA, USA).

Gel Analysis

The software package GelCompar II (version 5.0, Applied Maths, Belgium) was used to analyze ITS DGGE banding patterns. In order to minimize migration differences and to normalize for distortions between gels, we aligned the gels using an external reference pattern comprised of mixed ITS amplicons from five different fungi. A band-matching process was used to obtain a presence–absence matrix for statistical analyses. A 5% band intensity threshold was set for the band selection process. Individual bands were grouped into classes based on melting behavior (positions in the gels). Each band class was then considered to be an operational taxonomic unit (OTU), allowing calculation of their frequency among log samples. We also calculated the relative intensity of each band, applying a value between 0 and 1 by dividing the intensity of the band by the sum of the intensity of all the bands within the lane, thus eliminating the variation in band intensity due to difference in amplification and amount of DNA loaded on the DGGE gel.

Cloning and Sequencing of Excised DGGE Bands

Amplicons which generated prominent DGGE bands were selected for cloning and sequencing. Bands were excised from DGGE gels and the DNA eluted in 20 μL of sterile deionized water and subsequently used as a template for PCR using the primers ITS1-F and ITS2 as described above. Amplicons (280 bp) obtained with the fungal specific primers were purified on agarose gels using the Geneclean Turbo kit as recommended by the manufacturer (Q-bio gene, USA). The purified DNA was cloned by ligation to the pGEM-T Easy vector system (Promega, Madison, WI, USA) and transformed into competent *Escherichia coli* cells (JM109) according to the manufacturer's instructions. Positive clones were selected on appropriate LB agar plates and their plasmids isolated with the Wizard Plus SVMinipreps system (Promega). Sequencing reactions were performed with a BigDye Terminator cycle sequencing kit 3.1 with a genetic analyzer (3130XL; Applied Biosystems, Foster City, CA, USA) by the Plate-forme d'Analyses Biomoléculaires (Université Laval, QC, Canada). All clones were sequenced in both directions using Sp6 and T7 universal primers. Sequences were aligned using Bioedit software [23] and vector plasmid-related sequence was removed. Sequence identity searches were performed using BLASTn against the GenBank database of the National Center for Biotechnology Information.

Statistical Analysis

A matrix of relative abundance (and presence/absence) was obtained for fungal species. From this, the number of OTUs (i.e., DGGE band classes per sample) and the Shannon–Weaver diversity index (H′) were calculated.

Shannon–Weaver indices were calculated according to Eichner et al. [15]. In order to link OTU number and H′ with explanatory variables, data were analyzed with a linear mixed-effects model using the "nlme" package (R package version 3.1-90; nlme: linear and nonlinear mixed effects models) from R software [53]. Five models were tested based upon our hypothesis (Table 2). The global statistical model (model 5) included all possible explanatory factors: wood density, log volume, species (paper birch, trembling aspen, balsam fir, spruce, jack pine), decay stage (fresh deadwood (class I and II); medium decay (class III) and well–decomposed wood (class IV and V)), stand deadwood volume, deciduous and coniferous basal area, lignocellulose index $(\mathrm{LCI} = \mathrm{lignin}/(\mathrm{lignin} + \mathrm{cellulose}))$ [51], and the carbon/nitrogen ratio. All other models are subsets of the global model each corresponding to different hypothesis. All explanatory variables were entered as fixed factors whereas stand, replicates, and experimental units were considered random factors—each one nested in the former.

Table 2 Linear mixed models relating species richness (OTU number) and Shannon–Weaver diversity index to stand and log characteristics

Model	Variables included in each model	Corresponding hypothesis
1	Log species	Effect of log species
2	Decomposition class + log volume + wood log density	Effect of logs physical variables
3	LCI + C/N ratio	Effect of log chemical variables
4	CWD volume + deciduous basal area + coniferous basal area	Effect of stand features
5	Model 1 + model 2 + model 3 + model 4	Global model (all variables)

Models were compared on the basis of Akaike's information criteria (AIC) [10]. The "best" model is the model with the lowest AIC and the highest Akaike weight. Akaike weights (w_i) indicate the level of support in favor of any given model being the most parsimonious and most probable among candidate models [42]. For model selection, we used "AICcmodavg" R package (R package version 1.01; AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c), http://CRAN.R-project.org/package=AICcmodavg).

Multivariate analyses of fungal community composition were computed using the "vegan" package (R package version 1.15-2; vegan: Community Ecology Package, http://CRAN.R-project.org/package=vegan). We used canonical correspondence analysis (CCA) [19, 54] because it positions DGGE profiles (OTU scores and logs/site scores) in relation to environmental factors and ordinates fungal communities in such a way that the relationship between samples, species, and environmental variables on the ordination diagram can be assessed from angles between vectors (quantitative environmental variables) or distances between points (samples and species) and vectors [55]. The analysis shows the way in which changes in fungal community composition are associated with changes in environmental variables. The method assumes a unimodal distribution of species. Quantitative environmental variables were standardized before performing the analysis by subtracting the mean and dividing by the standard deviation. CCA was done on the presence–absence matrix of fungal DGGE bands because it gave the best separation between different samples [13].

Finally, indicator species analysis was carried out with the duleg function of the "labdsv" package (R package version 1.3-1; labdsv: Ordination and Multivariate Analysis for Ecology, http://ecology.msu.montana.edu/labdsv/R). We also applied a Holm correction on these probabilities. All statistical analyses were called significant when $P<0.05$.

Nucleotide Sequence Accession Numbers

The sequences generated during this study have been deposited in GenBank under accession numbers HM195119 to HM195131.

Results

Stand Characteristics

Stand types were characterized by different amounts of deadwood (Table 1), as well as tree species composition. The 1923 fire trembling aspen stands had the largest amount of total deadwood while the largest volume of well decomposed wood was found in the mixed trembling aspen stands (1910 fire stands). The oldest stand types (mixed post budworm outbreak stands from the 1760 fire) had the lowest volume of CWD but the largest volume in the medium decay stage (Table 1).

Dead Wood Properties

Log species differed in their physical and chemical features (Table 3). Higher C/N ratios were found among spruce and jack pine logs, while the lignocellulose index (LCI) was higher in jack pine. The largest logs were spruce, jack pine, and trembling aspen in decreasing order. C/N ratio and wood density were higher in fresh wood and lower in well-decomposed wood. Inversely, LCI index and water content was higher for well-decomposed wood (data not shown).

Fungal Richness and Diversity

We founded a total of 33 different DGGE bands (OTUs) in the 102 logs sampled. The mean number of OTUs per log was 6.3, with a minimum of one and a maximum of 16 (Fig. 1). The maximum number of OTUs in the same log (16 OTUs), was from a non-decomposed (fresh wood) spruce log in the oldest stand (1760 fire) and the second highest (15 OTUs) was from a trembling aspen log of the medium decay class in the mixed aspen stands (1910 fire). Seven logs had only one OTU: two trembling aspen, one jack pine, and four balsam fir logs (Fig. 1). The mean number of OTUs per decomposition class was 6.6 for fresh wood, 7.0 for medium decayed logs, and 6.4 for advanced decay stage. With respect to log species, the mean OTU number was 9.0 for spruce, 6.7 for trembling aspen, 6.0 for paper birch, 5.8 for jack pine, and 5.5 for balsam fir (Fig. 2).

Table 3 Physical and chemical characteristics (mean and SD values) of decomposing logs by decay stage found in natural stands

Wood characteristics	Decay stage	Spruce	Balsam fir	Paper birch	Trembling aspen	Jack pine
Log volume (m^3)	Fresh	0.27±0.25	0.16±0.14	0.19±0.26	0.51±0.80	0.39±0.26
	Medium	0.36±0.19	0.25±0.19	0.11±0.22	0.16±0.14	0.14±0.09
	Advance	0.32±0.31	0.14±0.08	0.16±0.16	0.12±0.07	0.67±0.58
Density (g cm^{-3})	Fresh	0.32±0.10	0.31±0.05	0.31±0.15	0.37±0.05	0.32±0.06
	Medium	0.30±0.10	0.25±0.04	0.32±0.11	0.20±0.09	0.28±0.10
	Advance	0.20±0.09	0.20±0.06	0.19±0.10	0.19±0.07	0.29±0.09
LCI[a]	Fresh	0.39±0.03	0.39±0.08	0.23±0.07	0.18±0.03	0.44±0.03
	Medium	0.38±0.02	0.43±0.07	0.27±0.11	0.27±0.09	0.58±0.16
	Advance	0.53±0.11	0.46±0.10	0.44±0.15	0.34±0.10	0.54±0.07
C/N ratio	Fresh	1586±709	754±662	615±355	908±799	1805±862
	Medium	1812±787	660±260	570±238	597±376	945±230
	Advance	273±74	592±283	188±140	321±306	876±772

[a] Ligno-cellulose index: LCI = lignin/(lignin + cellulose)

Fresh correspond to the decay class 1 and 2, medium to the decay class 3, and advance to the decay class 4 and 5

We cloned and identified 13 of the 33 OTUs (Table 4), corresponding to the highest band intensity observed on the DGGE gels. Other bands were too weak for cloning and had less success at the sequencing steps. The distribution of OTUs was highly variable across log species on the left of Fig. 3, whereas OTUs on the right are more similar between log species. OTU «U» (98% similar to *Bjerkandera adusta*), was present in half of the sampled logs. Sequences similar to *Phlebia centrifuga* (OTU «T»), *Athelia* sp. (OTU «S»), *Ascocoryne* sp. (OTU «R»), and *Phellinus cinereus* (OTU «Q») were also common. OTUs Z and AB were related to ectomycorrhizal fungi, the latter being rare, with only 6.9% of all logs were colonized by these OTUs.

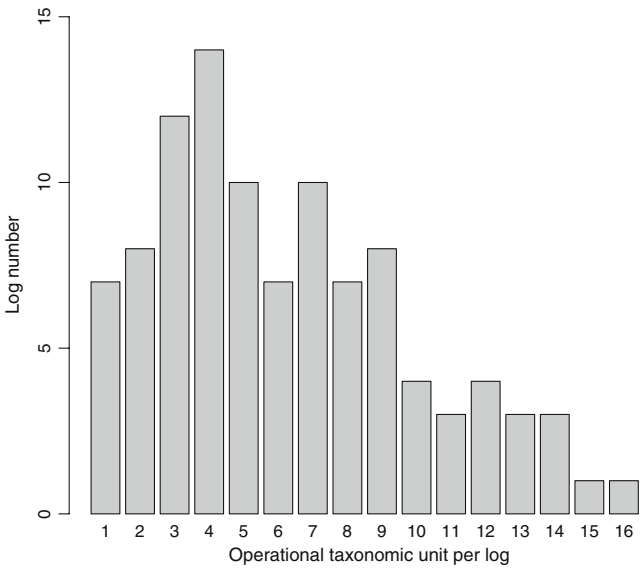

Figure 1 Histogram of the number of OTUs per log for the five log species obtained from PCR-DGGE profiles. A total of 102 logs were sampled in all natural stands (three successional stages) and decomposition classes

For fungal species richness, model 1 had the highest AICc weight (0.99; Table 5), indicating that the model based solely on log species was the most plausible model (i.e., model with lowest AIC) among the set of tested models (Table 2). The Akaike weight for model 1 was strong enough (i.e., w_i>0.9) to compute model estimates based solely on this model (Table 6). Spruce logs had the highest OTU number (species richness). We found no differences among balsam fir, jack pine, trembling aspen, and paper birch in terms of richness. Neither log volume, stand CWD volume, deciduous or coniferous basal area, decomposition class, or wood density were good predictors of the number of OTUs per log.

Concerning the Shannon–Weaver diversity index, model 1 (log species only) was also the most probable model with an Akaike weight higher than 0.9 (Table 5). Model estimates were computed based solely on this model (Table 6). The Shannon–Weaver diversity index varied strongly among log species, being lowest in balsam fir and highest in spruce. Jack pine, trembling aspen, and paper birch presented similar diversity indices. Hierarchical classification between log species based upon H′ is shown in (Fig. 2). Other measured characteristics (included in other models)—CWD volume, decomposition class, log volume, deciduous, or coniferous basal area and wood log density—were poor predictors of log Shannon–Weaver diversity index.

Saproxylic Fungal Community Structure

We assessed the relationship among the distributions of saproxylic fungi and selected environmental factors using CCA [63]. Variables included were deciduous and coniferous

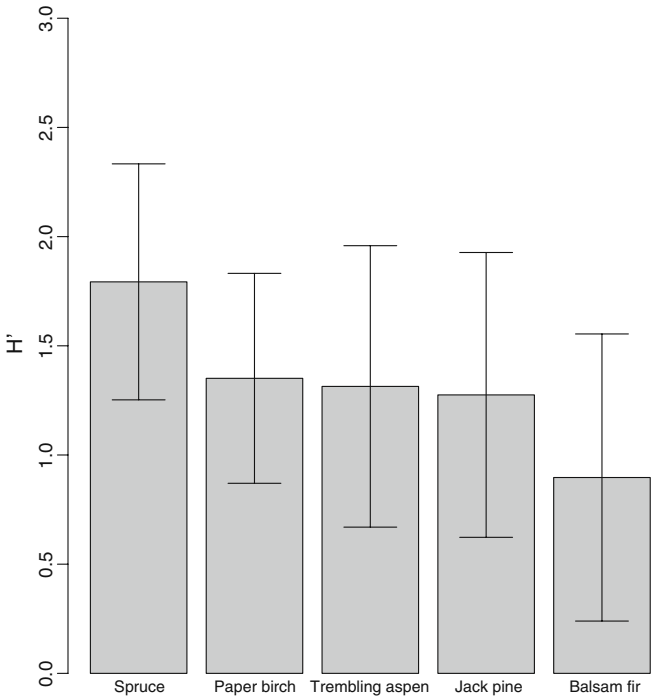

Figure 2 Shannon–Weaver diversity index (H′) in relation to log species

basal area, stand CWD volume, and percent lignin and hemicellulose in individual logs. Log species and decomposition category were also included as dummy variables (depicted as centroids on the CCA diagram). Collinearity between selected environmental variables was low (VIF < 10). The CCA produced an ordination in which the first axis was significant (p=0.046) and the test of significance for all canonical axes was also significant (p=0.039; assessed by

Table 4 Sequence analysis of bands excised from DGGE gels

OTU	Most closely related fungal sequence	Similarity (%)	Accession no. of related sequence	Environment from which related sequence was isolated	Occurrence (%)[a]
E	*Leptodontidium elatius* isolate A39WD232	97	FJ903294.1	Stump of *Picea abies* (Latvia)	7.8
F	Uncultured *Mortierella*	99	FJ553782.1	Forest soil (Canada)	11.8
G	*Calocera cornea*	99	AY789083	Culture collection	7.8
K	*Resinicium bicolor* strain JLL13731[b]	99	DQ826535	*Populus tremuloides* (Ontario, Canada)	17.7
O	*Ascocoryne cylichnium* isolate N31	99	FJ903373	Stump of *Picea abies* (Latvia)	25.5
Q	*Phellinus cinereus*[c]	99	AY340049	Strain isolated from fruiting body	36.3
R	*Ascocoryne* sp. isolate E2	97	FJ903331	Decayed wood of *Picea abies* (Latvia)	40.2
S	*Athelia neuhoffii*	95	U85798.1	Culture collection	39.2
T	*Phlebia centrifuga*	99	L43380.1	*Pinus ponderosa* (Arizona)	41.2
U	*Bjerkandera adusta*	98	FJ903353	Decayed wood of *Picea abies* (Latvia)	51.0
X	Uncultured fungus clone Singleton_ 24-2804_2353	86	FJ758813	Phyllosphere of *Quercus macrocarpa*	26.5
Z	Uncultured fungus	99	FM999613	Isolated from ectomycorrhizae in a mature beech maple forest	18.6
AB	Uncultured fungus clone Singleton_ (159-1104_0519)	83	FJ778188	Ectomycorrhizosphere of *Quercus* spp.	6.9

Most similar Genbank accession number and percent sequence similarities for the OTUs used in the multivariate analyses

[a] Proportion of logs on which this OTU was found

[b] Same similarity to FJ554463 (uncultured Agaricomycetes clone LTSP_EUKA_P6P23)

[c] Same similarity to *Phellinus nigricans* (AF200239)

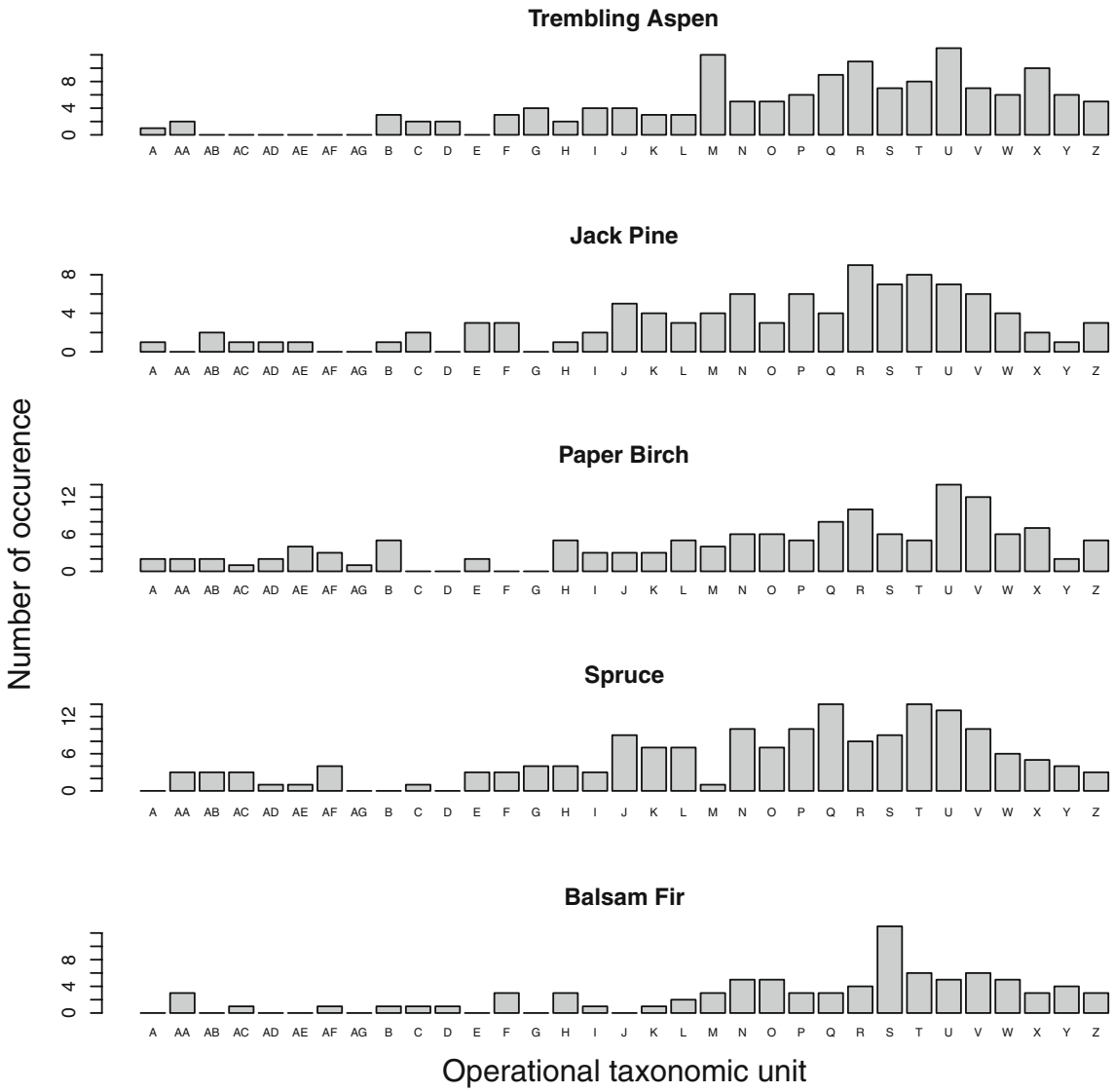

Figure 3 Histograms showing the number of occurrences of the 33 OTUs distinguished in DGGE profiles in relation to log species

permutation testing). The eigenvalues for the two first axes were 0.11 and 0.08 and the species–environment correlations for the two first axes were 0.66 and 0.63, respectively. Overall variance explained by environmental variables was 12.44%. The first three axes of the ordination explain 52.1% of this: 23.0% by the first axis and another 15.8% by the second axis.

The first axis was positively correlated with the deciduous basal area and CWD volume and negatively correlated with coniferous basal area (Figs. 4 and 5; intraset correlation=0.14, 0.25, and −0.24, respectively).

The second axis was positively correlated with percent lignin and negatively correlated with percent hemicellulose in individual logs and deciduous basal area (Figs. 4 and 5; intraset correlations of 0.33, −0.39, and −0.38). Hence, axis 2, which accounts for an important part of the variation, appears to represent the influence of both wood chemical composition and stand composition.

Indicator Species

After the indicator species analysis, we found three species characteristic of particular habitats: OTU «M» on trembling aspen logs (indicator value: 0.32, $P<0.05$), OTU «S» (sequence 95% similar to the Homobasidiomycete *Athelia* sp.) on balsam fir logs (indicator value: 0.40, $P<0.05$) and OTU «G» (sequence 99% similar to the Heterobasidiomycete *Calocera cornea*), indicative of the stand originating from the 1923 fire (indicator value: 0.19, $P<0.10$; Table 4 and Fig. 3). OTU «M» could not be re-amplified or cloned and therefore was not identified by sequencing.

Table 5 Akaike's Information Criterion (AICc) rank and weights (AICcWt) of models relating species richness (OTU number) and Shannon–Weaver diversity index to stand and log characteristics (see Table 1 for model description)

Models	K[a]	AICc	ΔAICc	w_i[b]	Cum.Wt[c]
OTU number					
Model 1: log species	9	557.56	0.00	0.99	0.99
Model 3: log chemical features	7	568.31	10.75	0.00	1.00
Model 5: global model	18	570.40	12.85	0.00	1.00
Model 4: stand features	8	570.43	12.87	0.00	1.00
Model 2: log physical features	9	572.31	14.75	0.00	1.00
Shannon–Weaver diversity index (H′)					
Model 1: log species	9	197.30	0.00	0.99	0.99
Model 5: global model	18	206.87	9.57	0.01	1.00
Model 4: stand features	8	212.47	15.17	0.00	1.00
Model 3: log chemical features	7	212.62	15.32	0.00	1.00
Model 2: log physical features	9	215.63	18.32	0.00	1.00

Models are listed from best to worst based on AICcWt

[a] K estimable number of parameters in the model

[b] Akaike weights, also known as model probabilities. These measures indicate the level of support in favor of any given model being the most parsimonious (i.e., the best explanatory model) among the candidate model set [43]

[c] Cumulative Akaike weights

Discussion

Although most dominant species (or OTUs) found in this study appeared to be generalists—being found on a large proportion of the sampled logs—our results indicate that log species was the main factor affecting the diversity, richness, and community composition of dominant fungal species at the log level. The influence of host tree species on saproxilic fungal communities is in agreement with other studies based on observations of fructifications [37, 67] and on culture-dependant methods [41]. Several authors have also identified decomposition stage as an important factor influencing saproxylic fungal community composition or species richness [26, 41]. Although we did not find direct evidence for this relationship, we did find that the composition of the saproxilic fungal community was related to log chemical composition, which, in turn is related to both log species and decay stage (Table 3, Strukelj Humphery, personal communication, [46]). Wood chemical composition was assessed from wood samples taken as close as possible to the material extracted for DNA analyses, while our assessment of decomposition stage was based on the entire log. The lignin and hemicellulose concentrations may be better indicators of decomposition than decay class estimated at the scale sampled because conditions within a single log are likely to be quite heterogeneous [52]. It is possible that differences detected between log species may have been influenced to some degree by variations in colonization patterns within individual logs (intra-log variability), which may have resulted

Table 6 Model estimates and standard error obtained from linear mixed model 1 relating species richness (OTUs number) and Shannon–Weaver diversity index to log species (n=102 logs)

	Estimated value	Standard error	DF[a]	P
OTU number				
Balsam fir	−4.421	1.131	57	< 0.001
Paper birch	−2.904	1.081	57	0.009
Trembling aspen	−2.730	1.081	57	0.014
Jack pine	−3.392	1.147	57	0.005
Shannon–Weaver diversity index (H′)				
Balsam fir	−0.896	0.194	57	< 0.001
Paper birch	−0.442	0.185	57	0.020
Trembling aspen	−0.479	0.185	57	0.012
Jack pine	−0.518	0.196	57	0.011

Significance values for each linear mixed effect are indicated considering spruce as a reference

[a] Degrees of freedom

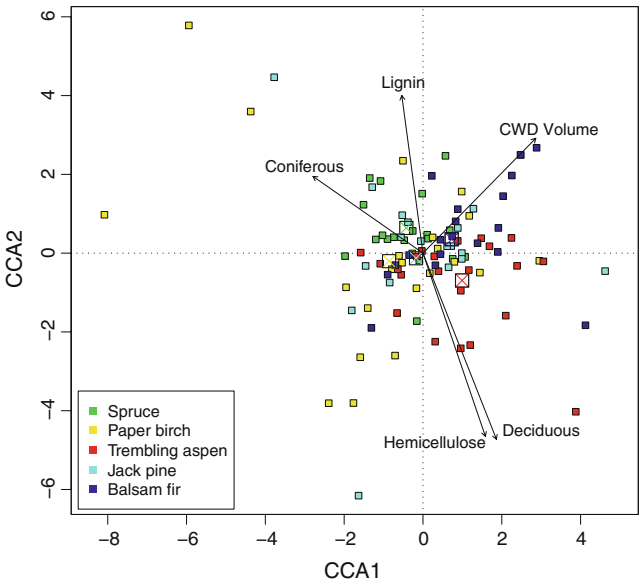

Figure 4 Canonical correspondence analysis of the saproxylic fungal communities (based on the DGGE profiling) of 102 logs of different wood species. *Multiplication symbol inside the box* centroids of each log species. Only sites scores are plotted. Axis 1 and 2 are displayed

in underestimates of fungal diversity for highly variable logs.

We also hypothesized that saproxilic fungal diversity would be highest in large logs. However, we found no influence of individual log volume on the fungal community or diversity. Our final hypothesis was that fungal composition would be influenced by stand conditions such as age and CWD volume. The influence of deadwood volume on saproxylic communities has been shown previously in Scandinavian boreal forests [50, 59] where variation in fungal species richness was best explained by the number and volume of dead trees [50]. In our study, the amount of CWD differed between the three successional stages (1760, 1910, and 1923 fires; Table 1). Therefore, stands may support somewhat different fungal communities due to the amount and tree species composition of CWD. This trend was also reported by Kubartová et al. [36], who noted that the dominant tree species had no significant influence on the richness of decomposer fungi but instead influences community composition. In our study, the proportion of coniferous trees (basal area) was correlated to the first axis of the CCA (Figs. 4 and 5) and was found to be higher in the 1760 stands than for the other successional stages (Table 1). From these results, we can infer that the oldest stand (1760) supports a specific saproxylic fungal composition due to differences in CWD amount and the proportion of coniferous trees. However, results may change with the fungal species involved. Jönsson et al. [31] found that different communities were affected differently by environmental variables. Early colonizers were influenced by the stage of decomposition, whereas secondary colonizers were influenced by other variables such as log diameter or connectivity. By analyzing the whole community without this distinction, we may have missed some differences among functional groups. Moreover, saproxylic fungal community patterns may be influenced by variables acting at a smaller scale. For example, De Bellis et al. [12] found that most of the variation in soil saprophytic microfungal composition was not correlated with overstory tree species, but with the composition of the understory herb layer. In the same way, microclimatic regime could have also been measured [26].

OTU «T» was 99% similar to *P. centrifuga* and found on 41.2% of all logs (Table 4). *P. centrifuga* prefers unmanaged habitats and is characteristic of old-growth spruce forests [18]. All our sampling sites were within natural stands and a third of our sites were old growth stand originated from a 1760 fire. The closest sequence match to OTU «Z» was that of an ectomycorrhizal fungus. The presence of mycorrhizal roots is not uncommon in decomposing logs [62, 65] and highlights the importance of deadwood for forest nutrition. The closest match for OTU «Q» was *P. cinereus*. This white rot fungus plays a role in CWD cycling and forest turnover, as it is saprophytic to hemiparasitic, causing trunk rot [7]. *P. cinereus* is nearly exclusively found on *Betula* in the northern hemisphere [16]. However, in our study, this species was found relatively frequently (Table 4) and seems to be a generalist without specific preference for log species. OTU «U» had the broadest distribution in our study, with the closest match being *B. adusta*—a white rot fungus with high

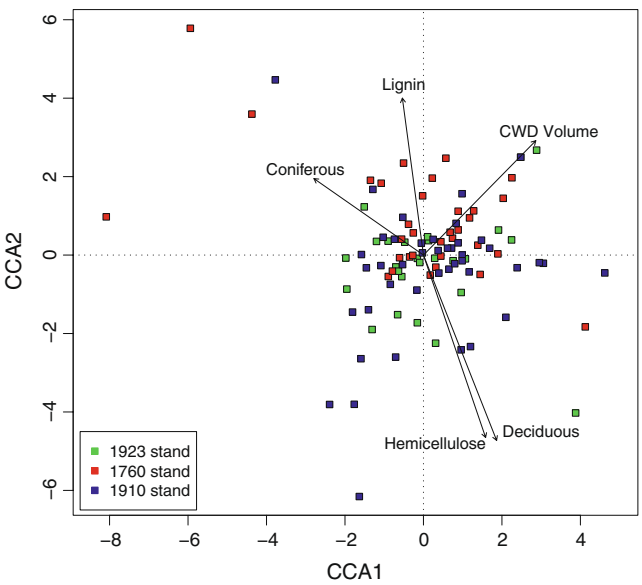

Figure 5 Canonical correspondence analysis of the saproxylic fungal communities (based on the DGGE profiling) of 102 logs in sites of similar stand type. Sites are displayed according to stand successional stage. Axis 1 and 2 are displayed

ligninolytic enzymatic activity [44] and a preference for aspen logs in northeastern America [7]. However, we found it to be ubiquitous on our sites (Fig. 3). OTU «G» (closest species match *C. cornea*)—an indicator for the youngest stands (1923 fire origin)—was found preferentially on highly exposed logs [39], a condition typical of our youngest and open stands (1923). The white rot fungus *Resinicum bicolor* (closest match for OTU «K ») is a heart wood hemiparasite. Although *R. bicolor* has preference for *Abies* and *Pinus* in northeastern North America [7], it was isolated on trembling aspen in Ontario, Canada (Table 4) and we found OTU «K» on all log species. The fact that molecular techniques allow us to detect fungal hyphae rather than only fructifications may explain differences between our observations on fungal host preferences and those of based on fruiting bodies. These studies may underestimate species distribution as some fungi may be non-host specific, but grow preferentially on particular log species. Similar asymmetries between direct molecular studies and fruiting occurrence have been widely reported for ectomycorrhizal fungi [21].

Sample size may be an issue when using molecular tools [1]. A large log can be up 21 m long and have a volume of up to 2.13 m^3, while our samples for DNA analysis consist of only 100 mg (wet weight) of wood. Many more samples per log would have been needed to obtain a more complete picture of the fungal communities. However, that would have been at the cost of a reduction in the total number of logs sampled. The fungal diversity reported here is consistent with previous studies using PCR-TGGE method on wood [38] or PCR-restriction fragment length polymorphism [30]. With respect to other identification methods, species richness found in our study is higher than some boreal studies based on fungal fructification [4] but lower than others [34]. However, comparison is difficult due to differences in the number of logs sampled and the sampling period. For example, more fungal species (i.e., 238) were found in a temperate Swiss forest [37] but 3,339 pieces of dead woody debris were sampled for fruit bodies of wood-inhabiting basidiomycetes, whereas we sampled 102 logs in our study. However, the focus of our study was on the variation in diversity between logs and not on total fungal diversity and any sampling biases remains constant between all of our samples.

The ecological significance of our results is valuable in the context of forest management. The rate and pattern of decomposition depends on fungal community composition and functioning of specific organisms under particular environmental conditions [6]. Because log species harbor different fungal communities, a change in tree diversity would lead to a change in the diversity of log species, thereby influencing important aspects of the decomposition process essential to forest productivity. In the same way, silvicultural practices decrease the quantity of coarse woody debris [8, 57]. Hence, saproxylic fungal community composition will be modified. To better understand the consequences of this change, studies are needed which link fungal community composition and ecological function (e.g. decomposition).

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