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Litter heterogeneity modulates fungal activity, C mineralization and N retention in the boreal forest floor

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ABSTRACT

The implications of forest floor heterogeneity for fungal activity, nutrient retention and carbon sequestration within the forest floor remain poorly documented. This information would be particularly relevant to situations where large changes in fresh litter occur such as following stand replacing disturbances. Numerous laboratory studies have documented mycelial translocation of simple forms of C and nutrients between contrasting substrates, allowing fungi to overcome local deficiencies. In slightly more complex but less controlled conditions, we assessed how factors contributing to forest floor heterogeneity—decay state, litter origin and tree species—individually affect fungal activity during decomposition. We also assessed how the juxtaposition of litters of contrasting nutrient and C status (decay state) alter fungal activity within individual substrates. We expected fungal biomass to be reallocated to C-rich litters while lignocellulolytic activity would increase in all juxtaposed litters. A microcosm experiment was conducted in which wood and leaf litters of one softwood species (*Pinus banksiana*) and one hardwood species (*Populus tremuloïdes*) were incubated alone or in combination with litters of contrasting decay states. Litter mass loss, change in N content, C mineralization, fungal biomass, specific respiration rate and lignocellulolytic activity were measured after 15 and 30 weeks. The decay state of litter had the most pronounced and consistent effects on fungal activity, with higher fungal biomass and lignocellulolytic enzyme activity in well decomposed litters and higher mass loss, C mineralization and specific respiration rate in fresh litters. In juxtaposed litters, fungal biomass was initially reallocated to fresh litters when incubated with well decomposed litters. Cellulolytic activity also increased by 30% in juxtaposed fresh litters while Mn-peroxidase activity increased by 42% in both fresh and well decomposed litters. With the longer incubation period, C mineralization and specific respiration of fungal biomass increased in juxtaposed well decomposed wood, indicating an increase in overflow metabolism presumably in response to an increase in labile C. Fresh litters that were juxtaposed increased their N content while that of single litters decreased. A better understanding of fine scale mechanisms affecting litter decomposition could improve our ability to forecast ecosystem response to disturbance.

1. Introduction

Several processes supporting forest ecosystem functions and integrity are known to take place within the forest floor (Prescott et al., 2000; Rydgren et al., 2004; Boberg et al., 2014), including nutrient and carbon (C) retention and cycling (Prescott et al., 2000). The forest floor supports a large portion of forest biodiversity (Niemelä et al., 1996; Buée et al., 2009) and contributes to ecosystem stability (Ponge, 2003). Yet, whether changes in forest litter abundance and quality resulting from stand replacing disturbances (Strukelj et al., 2015) have any lasting effects on forest floor processes remains an open question.

The coexistence of tree species with contrasting litter quality in combination with variations in litter origin (leaves, wood, and roots) and decay state contribute to the chemical and structural heterogeneity of the forest floor. Moreover, within the forest floor of boreal forests, restrictive temperatures and acidity favor

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progressive organic matter (OM) accumulation (Deluca and Boisvenue, 2012). The resulting LFH horizon or MOR humus form (Prescott et al., 2000; Ponge, 2003) is characterized by a vertical gradient of decomposing material. Besides leaf and moss litter, more or less decayed logs and branches are found. With time, well decayed wood becomes buried within the LFH, adding to the heterogeneity of the forest floor (Kayahara et al., 1996; Brais and Drouin, 2012; Strukelj et al., 2013).

Because of their tolerance to high acidity (Rousk et al., 2010), their capacity to penetrate solid material (Money, 1995) and their ability to secrete extracellular enzymes (Baldrian, 2008), fungi are the main decomposers of boreal forest leaf and wood litters (Boer et al., 2005; Baldrian, 2008; Boddy and Heilmann-Clausen, 2008). Numerous studies have demonstrated the mycelial translocation of simple forms of C and nutrients between contrasting substrates (Frey et al., 2003; Boberg et al., 2010; Bonanomi et al., 2014; Philpott et al., 2014). Translocation allows filamentous fungi, mostly Basidiomycota, to overcome local C and/or nutrient depletion (Watkinson et al., 2006; Boberg et al., 2010; Philpott et al., 2014) and adjust to the complex geometry of nutrient and energy supply caused by litters of different quality and state of decay (Tedersoo et al., 2003; Lindahl and Olsson, 2004; Watkinson et al., 2006).

However, the bulk of these studies were laboratory experiments using single litter types (Boyle, 1998; Boberg et al., 2008; Philpott et al., 2014), single C and N sources (Allison et al., 2009; Boberg et al., 2011b) and/or single fungal species (Boberg et al., 2008). Working with a slightly more complex system, Boberg et al. (2014) reported that the juxtaposition of fresh and well-decomposed needles of *Pinus sylvestris* led to the reallocation of N and fungal biomass from well decomposed to fresh material whereas the decomposition of well decomposed needles decreased. Similarly, Bonanomi et al. (2014) observed N transfer from N rich to N poor paired substrates. Few studies have been conducted in the field (Frey et al., 2003) and the implications of litter heterogeneity for fungal activity, nutrient retention and carbon sequestration within the forest floor remain poorly documented.

The first objective of the study was to quantify how factors contributing to forest floor heterogeneity—state of decomposition (fresh vs. well decomposed), litter origin (wood vs. leaves) and tree species (jack pine (*Pinus banksiana* Lamb.) vs. trembling aspen (*Populous tremuloides* Michx.))—individually affect fungal activity during decomposition. The second objective was to assess whether the juxtaposition of fresh and well decomposed litters stimulates microbial activity and litter decomposition. Finally, given that mycelial translocation and lignin degradation are predominately associated with Basidiomycota (Baldrian, 2008; Boberg et al., 2014) and that little information is currently available regarding the saprophyte fungal communities of forest litters of the Eastern Canadian boreal forest (Kebli et al., 2014), we characterized the fungal community composition of sampled litters.

We conducted a laboratory microcosm experiment representing intermediary conditions between complex natural ecosystems and studies involving single litter or single fungal species. Fresh and well decomposed unsterilized litters with contrasting C/N ratios were incubated singly or juxtaposed for 15 and 30 weeks corresponding to 1 and 2 years of decomposition in the field. At the end of incubations, microbial activity and litter characteristics were measured.

We hypothesized that fresh litter (high C/N ratio) being rich in easily labile C would break down more quickly than well decomposed litter (low C/N). Under identical conditions, leaf litter is expected to initially decompose faster than wood litter (Strukelj et al., 2012). However, as decomposition proceeds, leaves and wood reach similar decomposition limits (Strukelj et al., 2012). Jack pine and trembling aspen are two boreal species which differ in their chemical properties and rate of decomposition (Brais et al., 2006; Strukelj et al., 2013). Because of differences between hardwood and softwood in chemical composition and fine scale cell anatomy (Cornwell et al., 2009), we expected fresh aspen litter to decompose faster than jack pine litter. However, the chemical composition of foliar litters of hardwoods and softwood are found to converge with decomposition (Strukelj et al., 2012) lessening initial differences in decomposition rate. Finally, we hypothesized that regardless of species or litter origin, the juxtaposition of fresh and well decomposed litter would result in a reallocation of fungal biomass and transfer of N from well decomposed litters to fresh ones (Boberg et al., 2014; Bonanomi et al., 2014). We also expected an increase in enzyme activity and decomposition rates of both fresh and well decomposed litter when juxtaposed.

2. Methods

2.1. Study area

Litter samples were collected in the Lake Duparquet Research and Teaching Forest (Harvey, 1999), 45 km northwest of Rouyn-Noranda in the Abitibi region of northwestern Quebec (48°30′N – 48° 32′N, 79°19′W – 79°30′W). Briefly, the region is located in the mixed boreal forest of the Canadian Shield area. The climate is continental with average annual temperatures of 1.0 °C and average total rainfall of 985 mm of which 663 mm fall as rain from April to November. Degree days >0 °C and degree days >5 °C average 2282 °C-day and 1891°C-day respectively (Mont Brun meteorological station (48°25′ N, 78°44′ W), http://climat.meteo.gc.ca/climate_normals). Litter samples were collected within a mixed trembling aspen-jack pine stand originating from a 1923 wildfire (Dansereau and Bergeron, 1993) where aspen patches are located within a matrix of jack pine (for a complete stand description see Brais and Drouin (2012)).

2.2. Litter sampling

Litter samples were collected in May 2013 just after snow melt under both trembling aspen and jack pine tree cover, at three different locations within the stand. Dead aspen leaves from the previous fall and fresh jack pine twigs were collected from the ground (L horizon). Fresh wood samples (2–5 cm disks) were collected from fresh snags that were brought down using a handsaw. Well decomposed wood samples were collected from well decomposed logs partly buried within the forest floor (buried depth > half the log diameter) after removing mosses at the surface of logs. The FH horizon originating from leaf material was sampled under each tree species using a trowel to a depth of 5–7 cm. The latter samples were free of any visible wood fibers. A total of 24 samples (3 locations × 2 tree species × 2 litter origins (wood vs. leaves) x 2 decomposition states) were collected.

2.3. Laboratory analyses

In the lab, leaves and needles were fragmented into 2–3 cm pieces. Fresh wood samples (10 cm × 2 cm × 2 mm) were cut from wood disks. Fresh sub-samples (1 g) from each of the 24 samples were set aside and stored at −20 °C until DNA extraction. The remaining material was placed in a refrigerator (4 °C, 3 weeks) pending microcosm construction.

2.4. Initial litter characterization

A sub-sample of each litter sample was air-dried and ground (250 μm). N-Kjeldahl was determined according to Bremner and

Mulvaney (1982) and organic C by wet oxidation (Yeomans and Bremner, 1988). The acid- and neutral-detergent fiber (ADF and NDF) concentration was determined according to Goering and Van Soest (1970) and the acid-unhydrolyzable residue (AUR lignin) concentration following Brinkmann et al. (2002). Cellulose concentration corresponds to ADF - AUR, that of hemicellulose to NDF - ADF and the non-structural fraction to 100 - NDF.

DNA extraction and amplicon sequencing was performed on 12 of the 24 original samples using the MoBio PowerSoil DNA isolation kit according to the manufacturer's protocol (http://www.mobio.com/). Amplifications were performed with primers ITS1 and ITS4 (Schoch et al., 2012). ITS4 is a reverse primer with high taxon coverage (Toju et al., 2012). A single-step, 30 cycle PCR was executed using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) under the following conditions: 94 °C (3 min) and 28 cycles of 94 °C (30 s); 53 °C (40 s) and 72 °C (1 min); with a final elongation step at 72 °C (5 min). Following PCR, all individually-tagged amplicon products from the 12 samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Samples were then sequenced with Roche 454 FLX titanium instruments following the manufacturer's guidelines.

The Q25 sequence data derived from the sequencing process was handled using the proprietary analysis pipeline of MR DNA, (www.mrdnalab.com; Shallowater, TX). Sequences were depleted of barcodes and primers. Sequences <200 bp, with ambiguous bases or with homopolymer runs exceeding 6bp were removed. Sequences were then denoised and chimeras were removed. Sequences with similarity ≥97% were grouped into operational taxonomic units (OTUs) following the removal of singleton sequences. OTUs were then taxonomically classified using BLASTn against a curated GreenGenes database (DeSantis et al., 2006) and compiled by taxonomic level into both "count" and "percentage" files.

2.5. Microcosm construction

Ninety-six microcosms (2 tree species (trembling aspen, jack pine) x 2 origins (leaves, wood) x 2 decay classes (fresh, well decomposed) x 3 repetitions (field/incubator location) x 2 incubation periods (15 and 30 weeks) x 2 treatments (single or juxtaposed) were prepared. Polyethylene containers (14 cm high × 9 cm diameter) were used. The bottom of each microcosm was covered with a 5 cm layer of sterile sand. Twenty grams of well-decomposed leaf litter, 30 g of well decomposed wood and 10 g of fresh wood and leaf litter were placed in 1 mm mesh pre-weighted fiber glass bags (10 × 8 cm). In each microcosm, one litter bag (single litter) or two litter bags (fresh and well decomposed juxtaposed litter) were placed on top of the sand layer for a total of 144 litter bags. Fresh litters were incubated with well decomposed litters of same tree species. Litter bags were placed vertically on the sand layer so that the bag walls touched while allowing for air circulation between bags. Finally, each microcosm was covered with a perforated lid to allow for gas exchange. Microcosms were grouped in an incubator (MA Electronics, Inc.) according to sampling location, but randomly distributed within location and were incubated in the dark at a constant temperature of 20 °C for 15 weeks (corresponding to a period of 2100° days > 0 °C or one year in the field) and 30 weeks. Because of inherent differences in water content between leaves and wood and between fresh and well decomposed material, litters were incubated at their initial field water content. The bottom sand layer of the microcosms was saturated with water to maintain a moist environment. To keep microcosm humidity constant during the experiment, microcosms were weighed at the start of incubation and on a regular basis. Any weight loss was corrected by adding water to the sand layer.

2.6. Measurements of fungal activity

At the end of both incubation periods, microbial respiration (mineralized C) was measured from each litter bag separately using the soda lime method (Keith and Wong, 2006). Litter bags were incubated for 24 h in sealed polyethylene jars with dried and weighed soda lime. The soda lime granules were then oven-dried at 105 °C for 14 h and weighed. To account for the CO_2 absorbed by the soda lime granules during the incubation, blank measurements were made in empty jars. Mineralized carbon (CO_2 (g)) over a 24 h period was based on the following equation:

CO_2 (g C g^{-1} litter day^{-1}) = {[(sample soda lime weight gain (g) − mean blank soda lime weight gain (g)) × 1.69]/litter bag dry weight (g)} × {24 (h)/time of exposure (h)} × {12/44}.

Following CO_2 measurements, the contents of each litter bag was coarsely ground with a small coffee grinder and divided into 5 sub-samples for subsequent analysis (moisture, fungal biomass, enzymatic activity (2 subsamples) and Kjeldahl N and organic C analyses). A first sub-sample was dried in the oven (65 °C, 48 h). The moisture content was used to convert the results of analyses conducted on fresh samples, including respiration.

We estimated fungal biomass by measuring ergosterol content according to Bååth (2001). Briefly, 0.25 g (fresh weight) of litter samples, 1 ml of cyclohexane and 4 ml KOH (10% in methanol) were placed in a test tube and treated for 15 min with ultrasound (47 kHz). The mixture was then placed in a heating block at 70 °C for 90 min. Subsequently, 1 ml of distilled water and 2 ml of cyclohexane were added and the mixture was vortexed for 30 s. Following centrifugation, the upper layer was recovered. This step was repeated three times. The three upper phases were combined, evaporated under N_2 at 40 °C and analyzed by high performance liquid chromatography (Galaxy Chromatography Data System) (UV detection at 282 nm) with methanol as the mobile phase. The system was calibrated using a pure commercial solution of ergosterol of known concentration (Sigma-Aldrich).

We measured the activity of two cellulolytic enzymes (1,4-β glucosidase (CE 3.2.1.21), cellobiohydrolase (EC 3.2.1.91)) and two ligninolytic enzymes, (laccase (EC 1.10.3.2) and Mn-peroxidase (CE 1.11.1.3)).

The activity of 1,4-β-glucosidase and cellobiohydrolase was measured using respectively ρ-nitrophenyl-β-D-glucopyranoside (25 mM PNG) and ρ-nitrophenyl-β-cellobioside (PNC 25 mM) as substrates (Tabatabai, 1994). The reaction mixture contained 1 g of fresh ground sample, 0.25 ml of toluene, 4 ml of modified universal buffer (pH 6) and 1 ml of the substrate solution (PNG or PNC) (Tabatabai, 1994). Test tubes were placed in an orbital shaker (100 rpm) for 1 h at 37 °C. After incubation, 1 ml $CaCl_2$ and 4 ml Tris buffer (0.1 M, pH 12) were added to the mixture to stop the reaction. The same procedure was followed for blank samples but the substrate was added after the addition of $CaCl_2$. The reaction mixture was filtered through a Whatman No. 5 filter paper and absorbance was read at 400 nm (Unico SQ-2800). One unit of enzyme activity was defined as the amount of enzyme releasing 1 μmol of substrate $hour^{-1}$. Values are expressed per gram dry mass of the litter samples.

For laccase and Mn-peroxidase extraction, 0.5 g fresh ground litter sub-samples were mixed with a 10 ml sodium acetate buffer (50 mM, pH 5) at 4 °C for 2 h on an orbital shaker (100 rpm). The reaction mixture was filtered through a Whatman No. 5 filter paper and the extract kept in an ice bath until enzyme activity analysis. Laccase activity was measured using 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) (Li et al., 2008). The reaction mixture contained 1.5 ml sodium acetate buffer (1 mM, pH

5), 1.5 ml ABTS (0.5 mM) and 1.5 ml enzyme extract. A control solution without ABTS was prepared. Absorbance was read at 420 mn. One unit of enzyme activity corresponds to the amount of enzyme releasing 1 μmol of oxidized substrate per minute.

Mn-peroxidase activity was assayed by shaking 0.3 ml enzyme extract, 0.3 ml sodium succinate buffer (0.5 M, pH 4.5), 0.3 ml guaiacol substrate solution, 0.6 ml magnesium sulfate and 1.2 ml distilled water. A guaiacol free control was prepared. Samples were incubated for 2 min at 30 °C. After this first incubation, 0.3 ml H_2O_2 (1%) was added and the absorbance was read at 465 nm (Mtui and Masalu, 2008) following a second incubation of 1 min. One unit of enzyme activity corresponds to the amount of enzyme releasing 1 μmol of oxidized substrate per minute.

2.7. Calculations and statistical analyses

All measurements are expressed per unit of dry weight except when specified otherwise. The lignocellulose index (LCI) is the ratio of lignin over the sum of lignin + cellulose + hemicellulose. Litter mass loss is the proportion of initial litter weight loss in the course of the incubation period. Absolute N content in each litter was calculated as N concentration multiplied by dry litter weight, and change in N content equals the difference between final and initial N content expressed as the proportion of initial content. Specific respiration rate (qCO_2) is the ratio of microbial respiration to fungal biomass.

The data were analyzed according to a complete random block design with 3 replications or blocks. The blocks corresponded to the locations where the material had been harvested and to microcosm locations within the incubator. Separate statistical analyses were performed for each incubation period. A mixed linear model was applied using SAS MIXED procedure (Littell et al., 2006). Blocks were treated as a random factor, and fixed factors were decomposition state (fresh vs. well decayed), origin (wood vs. leaves), species (jack pine vs. trembling aspen) and juxtaposition (single vs. juxtaposed). Two-way and three-way interactions between main factors were also included in the models. However, three-way interactions were removed from models when found to be not significant ($p > 0.100$). Variance estimates were based on the restricted maximum likelihood and significance of the fixed effects on the Type 1 test of hypothesis. Normality and homogeneity of residuals and their distribution in relation to predicted values were verified by visual assessment. When residuals were not normally distributed or presented a funnel-shaped pattern, logarithmic or square root transformations were applied. All variables were transformed with the exception of change in N content. To get a better view of how indicators of fungal activity were related to one another, simple correlations among measures of fungal activity were also estimated.

3. Results

3.1. Initial chemical characteristics of litter samples

Initial cellulose and hemicellulose concentrations were higher in fresh than in well decomposed litters, while lignin and non-structural element concentrations were lower (Table 1). The lignocellulosic index (LCI) increased and C/N decreased with decomposition. These trends were more pronounced in wood than in the leaf litters. Fresh wood had the highest, and well decomposed leaves from the FH horizon the lowest C/N ratio. On the other hand, the lowest LCI ratios were observed in fresh wood while comparably high LCI were observed in well decomposed litters regardless of tree species and litter origin.

3.2. Initial fungal community composition of litter samples

A total of 62,171 sequences were recovered with an average of 5181 per sample. Ascomycota represented the largest proportion of sequences in all but two samples (Table 2, for a complete taxonomic list see Table S1). Basidiomycota were present in all samples. Among these, the known ligninolytic fungi *Phellinus igniarius*, *Ganoderma applanatum*, *Lepista saeva*, *Mycena vitilis*, *Mycena silvaenigrae*, *Lactarius picinus* and *Lactarius tabidus* were found on at least one sample. Up to 24% of the sequences, depending on sample, could not be assigned to a phylum.

The two fresh leaf litter samples were characterized by a distinct mix of endophyte/epiphyte and early colonizer genera. Among these, *Dothideales* ssp., *Phoma* sp., *Sydowia polyspora*., *Lophodermium australe* and *Lophodermium conigenum* were identified on fresh leaf litter of both host species. *Phacidiopycnis washingtonensis* was found on jack pine litter only.

Ascomycota belonging to the Helotiales order, including members of the Dermateaceae family (42%), represented 85.3% of the sequences from the fresh aspen wood sample. Basidiomycota were represented by a single species (*Malassezia restricta*). Among the most frequent taxa extracted from the fresh jack pine wood sample were members of the orders Eurotiales (e.g. *Penicillium citreonigrum*, 21.8%), Chaetothyriales (e.g. *Capronia sp*. 8.0% and *Rhinocladiella* sp. 4.0%), Helotiales (e.g. *Pezicula eucrita*., 3.5%) and Leotiomycetes (3.8%).

Agaricales, Helotiales, Hypocreales, Leotiomycetes were found on all well decomposed litter samples. *Mortierellales* (Zygomicota) was also present in all well decomposed samples. Arbuscular mycorrhizal fungi (Glomeromycota) were identified in 6 of the 8 well decomposed litter samples, while Basidiomycota ectomycorrhizal fungi (e.g. *Tylospora fibrillosa*. and/or *Piloderma fallax*.) were found in 5 samples.

3.3. Fungal activity in decaying leaf and wood litters

Of the 48 litter samples incubated singly, all but one showed signs of Mn-P activity and all samples showed signs of BG, CBH and laccase activity. At the end of the 15-week incubation and regardless of incubation treatment, BG, CBH and Mn-P activity was positively correlated with fungal biomass ($r = 0.89$, 0.89 and 0.46 respectively, $p < 0.001$, $n = 72$) and negatively correlated with qCO_2 ($r = -0.43$, -0.43, -0.38, $p < 0.001$, $n = 72$ respectively) and decrease in N content ($r = -0.59$, -0.60, -0.27, $p < 0.020$, $n = 72$). No such correlation was observed for laccase activity, with the exception of a positive correlation with qCO_2 ($r = 0.26$, $p = 0.028$, $n = 72$). At the end of the 30-week incubation, few significant correlations between enzyme activity and other measurements of fungal activity were observed. CBH and Mn-P activity was correlated with the decrease in N content over the 30-week period ($r = -0.24$, -0.27, $p < 0.05$, $n = 72$).

The decomposition state of litter, regardless of litter origin or tree species, had the most pronounced and consistent effects on fungal activity. Hence, these effects are presented first. However, the effects of decomposition state varied with litter origin or host tree species as indicated by significant statistical interactions between main factors (Table 3). These interactions are described alongside the effects of litter origin or tree species when significant ($p < 0.100$). Effects of the juxtaposition of fresh and well decomposed litters on fungal activity are revealed by significant statistical interactions between juxtaposition and decomposition state or by three-way interactions between the latter and tree species or litter origin. They are presented last.

Table 1
Initial chemical properties of fresh and well decomposed trembling aspen and jack pine leaf and wood litters used in microcosm preparation (n = 3, ±standard error).

Tree species	Litter origin	Decomposition state	Lignin (mg g^{-1})	Cellulose (mg g^{-1})	Hemicellulose (mg g^{-1})	NSC (mg g^{-1})	LCI	C/N
Trembling aspen	Wood	Fresh	120 ± 20	580 ± 20	190 ± 20	110 ± 0	0.14 ± 0.03	246 ± 40
		Well decomp.	420 ± 90	160 ± 30	50 ± 30	370 ± 50	0.66 ± 0.10	57 ± 14
	Leaves	Fresh	320 ± 40	170 ± 20	50 ± 20	460 ± 10	0.59 ± 0.08	50 ± 6
		Well decomp.	380 ± 40	140 ± 30	30 ± 20	450 ± 10	0.69 ± 0.06	22 ± 3
Jack pine	Wood	Fresh	300 ± 60	460 ± 10	140 ± 50	110 ± 20	0.33 ± 0.07	303 ± 22
		Well decomp.	390 ± 60	200 ± 60	30 ± 10	380 ± 0	0.63 ± 0.09	80 ± 21
	Leaves	Fresh	250 ± 20	230 ± 20	40 ± 20	480 ± 20	0.48 ± 0.06	43 ± 3
		Well decomp.	400 ± 40	120 ± 80	50 ± 40	440 ± 10	0.71 ± 0.09	24 ± 2

NSC: Nonstructural compounds, LCI: Lignocellulose index = lignin/(lignin + cellulose + hemicellulose).

Table 2
Fungal community composition at various taxonomic levels (order or higher) of fresh and well decomposed trembling aspen and jack pine leaf and wood litter (N = 24) following 454 pyrosequencing of ITS1-ITS4 amplicons. Only groups representing >5% of sequences within at least one sample are presented. For a complete list see Table S1.

Taxa	Trembling aspen			Jack pine			Trembling aspen			Jack pine		
	Leaf litter						Wood					
	Fresh	Well decomposed		Fresh	Well decomposed		Fresh	Well decomposed		Fresh	Well decomposed	
						Ascomycota						
Chaetothyriales	0.0	4.4	2.1	0.0	0.4	0.0	0.0	1.5	15.7	16.4	31.7	5.9
Dothideales	31.6	0.0	0.0	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eurotiales	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.2	0.1	23.0	0.4	1.2
Helotiales	0.5	12.2	41.7	10.3	18.1	2.5	85.3	7.1	11.1	7.9	28.8	23.6
Hypocreales	0.1	2.1	3.9	0.0	4.8	5.3	0.0	1.1	0.5	0.4	0.6	2.0
Leotiomycetes	33.6	1.7	1.2	42.8	1.9	0.3	0.0	2.7	3.2	3.8	14.8	9.3
Pezizomycotina	8.7	1.7	1.6	2.2	1.0	0.0	0.0	2.5	3.4	0.4	0.5	0.2
Pleosporales	0.7	1.1	0.1	37.4	0.0	0.2	0.0	0.3	0.1	0.1	0.0	0.0
Unidentified Ascomycota	0.2	16.8	19.9	0.3	3.4	4.0	0.0	28.5	16.5	7.7	5.1	9.8
Total Ascomycota	**75.7**	**44.2**	**72.1**	**98.0**	**30.2**	**12.9**	**85.3**	**45.1**	**57.9**	**64.3**	**88.9**	**55.4**
						Basidiomycota						
Agaricales	0.0	6.1	10.4	0.0	0.4	0.5	0.0	2.2	1.6	0.0	2.2	3.0
Atheliales	0.0	1.4	0.3	0.0	1.6	6.3	0.0	6.0	0.0	0.0	0.0	0.0
Cystofilobasidiales	0.0	6.1	2.2	0.0	0.4	1.0	0.0	0.1	0.2	0.0	0.0	0.0
Hymenochaetales	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.9	0.0	0.0	0.0
Malasseziales	0.0	0.0	0.0	0.0	0.0	0.0	14.7	0.0	0.0	0.0	0.0	0.0
Basidiomycota Unidentified	4.1	22.6	8.1	0.9	10.4	19.8	0.0	2.8	1.1	10.0	1.5	3.8
Total Basidiomycota	4.1	**38.8**	**24.1**	**1.3**	**16.2**	**31.2**	**14.7**	**12.3**	**17.9**	**12.0**	**6.1**	**14.8**
						Chytridiomycota						
Total Chytridiomycota	0.0	**0.0**	**0.1**	**0.0**	**0.0**	**0.7**	**0.0**	**0.2**	**5.5**	**0.0**	**0.6**	**0.0**
						Glomeromycota						
Glomeromycota	0.0	0.2	1.4	0.0	0.0	7.6	0.0	1.1	0.0	0.0	0.0	0.0
Total Glomeromycota	0.0	**0.5**	**1.4**	**0.0**	**0.0**	**7.6**	**0.0**	**1.4**	**0.1**	**0.0**	**0.3**	**0.0**
						Zygomycota						
Mortierellales	0.0	4.8	1.9	0.0	51.4	45.9	0.0	34.9	0.8	0.0	3.4	17.4
Total Zygomycota	**0.0**	**4.9**	**2.0**	**0.0**	**51.4**	**46.1**	**0.0**	**35.0**	**0.8**	**0.0**	**3.4**	**19.2**
						Unidentified fungus						
	20.2	**11.6**	**0.2**	**0.7**	**2.2**	**1.4**	**0.0**	**5.9**	**17.7**	**23.7**	**0.7**	**10.5**

3.4. Fungal activity in decaying leaf and wood litters as a function of litter decomposition state

At the end of the 15-week incubation, decomposition state had significant effects on all response variables except mass loss (Table 3). Well decomposed litters were characterized by a higher fungal biomass (2.6 times), and greater 1,4 β-glucosidase (2.8 times), cellobiohydrolase (2.8 times), laccase (2,0 times), and Mn-peroxidase (2.6 times) activity than those of fresh litters (Table 4). On the other hand, C mineralization (CO_2 released over 24 h per litter unit) and the specific respiration rate of fungal biomass (qCO_2) were respectively 1.4 and 2.9 times higher for fresh than for well decomposed litters. After 15 weeks of incubation, well decomposed litters had lost 17% of their original N content while fresh ones had registered a gain of 21% (Table 4).

After 30 weeks of incubation, all response variables except laccase activity were significantly affected by litter decomposition state (Table 3). Fungal biomass and cellulolytic and Mn-P activity were still higher in well decomposed litters. Mn-P activity was fivefold higher in well decomposed than in fresh litters (Table 4). Carbon mineralization and qCO_2 were still higher in fresh litters by factors of 2.5 and 2.9 respectively. Fresh litters had lost twice as much of their dry mass as decomposed litters and, contrary to what was observed after 15 weeks, fresh litter N content had decreased.

3.5. Fungal activity in decaying litters as a function of litter origin (leaves vs wood)

At the end of the first 15 weeks of incubation, significant interactions were found between origin and decomposition state (D*O, Table 3) for fungal biomass and for BG, CBH and Mn-P activity. While fresh leaves and wood had similar values for these variables, biomass and enzyme activity were 1.2–1.6 times higher in well decomposed wood than in well decomposed leaf litter (Fig. 1). At the end of the period, C mineralization was similar between leaf and wood litters but leaf litter had a higher qCO2 and had lost a

Table 3
Effects of litter juxtaposition (J), decay state (D), origin (O) and tree host species (S) on fungal activity following microcosm incubation over 15 and 30 weeks. The significance of main factors and their interactions was tested using mixed linear models and is based on a Type 1 test of hypothesis.

	Fungal biomass	Enzyme activity				C and N mineralization				
		BG	CBH	Laccase	Mn-P	CO_2	qCO_2	Mass loss	ΔN	C/N
					15 week incubation period					
J					0.023					
D	<0.001	<0.001	<0.001	0.027	<0.001	0.009	<0.001		<0.001	<0.001
O							0.001	0.070	0.033	<0.001
S	0.002	0.006	0.006							0.023
D*O	0.076	0.013	0.012		0.017				0.005	<0.001
D*S	0.032	0.016	0.018		<0.001		0.075			0.073
O*S						0.023	0.006			<0.001
J*D	0.026	0.008	0.008	0.079						
J*O									0.067	
J*S										
J*D*O				0.035						
J*D*S								0.094		
J*O*S	0.072									
					30 week incubation period					
J									0.072	0.090
D	0.010	<0.001	<0.001		<0.0001	0.004	0.001	0.001	0.005	<0.001
O	<0.001			0.045		0.004	<0.001	0.004	0.090	<0.001
S	<0.001		0.068	<0.001	0.055		0.076			0.010
D*O				0.029	0.001			0.100		<0.001
D*S	0.039	0.002	0.002	<0.001						0.008
O*S				0.057	0.006					0.025
J*D						0.042	0.055		0.024	
J*O						0.098	0.069			
J*S										
J*D*O						0.099	0.095		0.090	
J*D*S										
J*O*S	0.098								0.085	

BG: 1,4 β Glucosidase; CBH: Cellobiohydrolase; Mn-P: Manganese peroxidase; CO_2: C mineralized over 24 h; qCO2: Biomass specific respiration rate; ΔN: Change in N content.

greater proportion of its dry mass than wood (Table 4). Fresh wood and fresh leaf litter N content had increased respectively by 33% and 9% (±6%) while well decomposed litters had lost similar amounts (14 and 20%, ±6%).

After 30 weeks of incubation, fungal biomass was significantly larger in wood than in leaf litter by a factor of 1.5 regardless of decomposition state (Tables 3 and 4). On the other hand, cellulolytic enzymes were not affected by litter origin. Laccase activity was greater in leaf litter than in wood but the difference was mostly caused by a higher activity (2.5 times) in fresh leaf than fresh wood litters (Fig. 1). In contrast, Mn-peroxidase activity was 3.2 times higher in well decomposed wood than in well decomposed leaf litter, but 1.8 higher in fresh leaves than in fresh wood. Regardless of decomposition state, C mineralization, qCO_2 and mass loss were significantly higher for leaf than for wood by factors of 2.4, 3.8 and 2.0, respectively (Table 4). Wood N content had decreased to a larger extent than that of leaves, but the difference was only marginally significant (Table 3).

3.6. Fungal activity in decaying litters as a function of tree host species

Following the first 15 weeks of incubation, higher values of fungal biomass and cellulolytic activity were observed for trembling aspen than for jack pine litters (Tables 3 and 4, Fig. 2) with larger differences observed for fresh than for well decomposed litters (Table 3, Fig. 2). Also higher values of Mn-P activity were observed in fresh litters of trembling aspen than in those of jack pine (Table 3, Fig. 2) while the reverse was true in well decomposed litters. No differences in mineralized C, mass loss or change in N content were observed among trembling aspen and jack pine litters. However, higher values of qCO_2 were observed in fresh jack pine litters (9.6 ± 1.6 mg C mg^{-1} ergosterol 24 h^{-1}) than in those of trembling aspen (5.7 ± 0.9 mg C mg^{-1} ergosterol 24 h^{-1}), while the difference was not significant for well decomposed litters (2.5 ± 0.4 mg C mg^{-1} ergosterol 24 h^{-1}).

At the end of the 30 week incubation, fungal biomass was 1.5 times higher in jack pine than in trembling aspen litters (Table 3 , Fig. 2). Higher cellulolytic enzyme and laccase activity was observed in trembling aspen than in jack pine fresh litters, while well decomposed litters of both species displayed comparable activity (Fig. 2). Otherwise, Mn-P activity and qCO_2 were respectively 2.2 and 1.8 times higher in trembling aspen than in jack pine litters, regardless of decomposition state (Tables 3 and 4). No significant differences in mineralized C, mass loss or change in N content were observed between tree host species.

3.7. Modification of microbial activity caused by the juxtaposition of fresh and well decomposed litters

The juxtaposition of fresh and well decomposed litters in itself had little effect on fungal activity, but significant juxtaposition effects were revealed through interactions between juxtaposition and litter decomposition state (Table 3). Nonetheless, at the end of the 15 week incubation, Mn-P activity was 40% higher in all juxtaposed litters (0.61 ± 0.05 U g^{-1}) than in litters incubated singly (0.43 ± 0.05 U g^{-1}), regardless of litter decomposition state, tree species or origin. At the end of the 30 week incubation, juxtaposed litter C/N was marginally less (53 ± 2) than that of litters incubated singly (60 ± 3).

At the end of the 15 week incubation, fungal biomass was 23% higher in fresh litters that were incubated with (juxtaposed to) well decomposed litters than in fresh litters that were incubated singly (Fig. 3). In contrast, fungal biomass was 16% lower in juxtaposed well decomposed litters than in single well decomposed litters. Similarly, cellulolytic enzyme activity was 30% higher in fresh

Table 4
Fungal activity in decaying forest litters as a function of litter decomposition state (fresh or well decomposed), origin (wood or leaves) and tree host species (trembling aspen or jack pine). Litters were incubated in microcosms over 15 and 30 weeks. Mean and standard error (lower boundary-upper boundary) estimates were obtained from the mixed linear models in Table 3.

	Fungal biomass	Enzyme activity				C and N mineralization				
		BG	CBH	Laccase	Mn-P	CO_2	qCO_2	Mass loss	Δ N	C/N
	mg ergo. g^{-1}	μmol PNP g^{-1} h^{-1}	μmol PNP g^{-1} h^{-1}	U g^{-1}	U g^{-1}	mg C-CO_2 g^{-1} 24 h^{-1}	mg C-CO_2 mg^{-1} ergo. 24 h^{-1}	%	%	
15-week incubation period										
Decomposition state										
Fresh	0.25	2.90	2.92	0.11	0.32	0.547	7.43	13	21	*75*
	0.02–0.01	0.13 0.13	0.13–0.13	0.02–0.02	0.03–0.03	0.062–0.056	0.94–0.83	2.8–2.5	4–4	3–3
Well decomp.	0.66	8.06	8.12	0.22	0.83	0.386	2.54	12	−17	38
	0.04–0.04	0.37–0.36	0.37–0.36	0.04–0.04	0.09–0.08	0.044–0.039	0.32–0.29	2.8–2.5	4–4	1–1
Litter origin										
Wood	0.43	5.05	5.06	0.13	0.53	0.441	3.29	10	7	110
	0.03–0.03	0.24–0.23	0.24–0.23	0.03–0.02	0.06–0.05	0.051–0.046	0.42–0.37	2.5–2.2	4–4	4–4
Leaves	0.39	4.63	4.68	0.18	0.49	0.479	5.75	16	−3	26
	0.02–0.02	0.22–0.21	0.22–0.21	0.04–0.03	0.05–0.05	0.055–0.050	0.74–0.66	3.1–2.8	4–4	1–1
Tree host species										
Trembling aspen	0.48	5.39	5.41	0.14	0.53	0.441	3.81	13	1	50
	0.03–0.03	0.26–0.25	0.26–0.25	0.03–0.03	0.06–0.05	0.052–0.047	0.51–0.45	2.9–2.6	4–4	2–2
Jack pine	0.34	4.34	4.38	0.18	0.49	0.479	4.96	13	3	57
	0.02–0.02	0.21–0.20	0.21–0.20	0.04–0.03	0.05–0.05	0.057–0.051	0.66–0.58	2.9–2.6	4–4	2–2
30-week incubation period										
Decomposition state										
Fresh	1.89	4.42	4.23	0.24	1.86	1.039	1.20	25	−8	81
	0.13–0.12	0.32–0.30	0.30–0.28	0.04–0.03	0.46–0.37	0.189–0.160	0.26–0.21	3.1–2.9	4–4	4–4
Well decomp.	2.32	7.25	7.06	0.23	9.73	0.421	0.41	12	−21	40
	0.16–0.15	0.53–0.50	0.50–0.47	0.04–0.03	2.40–1.92	0.077–0.065	0.09–0.07	2.2–2.0	4–4	2–2
Litter origin										
Wood	2.59	5.58	5.33	0.19	4.88	0.427	0.36	12.3	−20	137
	0.18–0.17	0.42–0.39	0.39–0.36	0.03–0.03	1.22–0.98	0.078–0.066	0.08–0.06	2.3–2.1	4–4	6–6
Leaves	1.70	5.74	5.60	0.29	3.70	1.025	1.37	24.7	−9	23
	0.12–0.11	0.43–0.40	0.40–0.38	0.04–0.04	0.93–0.74	0.187–0.158	0.30–0.25	3.0–2.8	4–4	1–1
Host species										
Trembling aspen	1.71	6.14	6.05	0.38	6.34	0.700	0.93	16.9	−9	50
	0.13–0.12	0.49–0.46	0.47–0.43	0.06–0.05	1.76–1.38	0.128–0.108	0.21–0.17	2.6–2.4	4–4	2–2
Jack pine	2.56	5.21	4.93	0.15	2.85	0.625	0.52	19.1	−20	63
	0.19–0.18	0.42–0.39	0.38–0.35	0.02–0.02	0.79–0.62	0.114–0.096	0.12–0.10	2.7–2.5	4–4	3–3

BG: 1,4 β Glucosidase; CBH: Cellobiohydrolase; Mn-P: Manganese peroxidase; CO_2: C mineralized over 24 h; qCO_2: Biomass specific respiration rate; ΔN: Change in N content; ergo: Ergostérol; PNP: p-nitrophénol.

juxtaposed litters than in fresh litters incubated alone (Table 3, Fig. 3), and cellulolytic activity had decreased by 9% in well decomposed juxtaposed litters when compared with well decomposed single litters. Well decomposed wood experienced higher laccase activity when incubated alone (($p = 0.035$, Table 3, result not shown) than when juxtaposed, but no such differences were observed for fresh wood or for fresh and well decomposed leaf litters. At the end of the 15-week incubation, no significant differences in mineralized C or qCO_2 were observed between juxtaposed and single litters. Mass loss was 10 ± 5% for single and 18 ± 5% for juxtaposed fresh jack pine litters while mass loss decreased from 18 ± 7% for single well decomposed jack pine litters to 7 ± 3% for juxtaposed well decomposed jack pine litters. No such trend was observed for trembling aspen litters ($p = 0.094$, Table 3).

At the end of the 30 week incubation, fungal biomass and enzyme activity were similar between single and juxtaposed litters (Table 3). However, significant differences were found between juxtaposed and single litters for mineralized C, qCO_2 and changes in N content (Table 3). These differences were somewhat modulated by litter origin as indicted by marginally significant three-way interactions between, juxtaposition, decomposition state and litter origin (Table 3).

Mineralized C was 24% lower in fresh juxtaposed litters when compared with fresh single litters while mineralized C was 113% higher in well decomposed litters when juxtaposed (Fig. 3). However, when factoring in the origin of litters, the increase in mineralization in well decomposed liters caused by juxtaposition was stronger for wood. A similar trend was observed for qCO_2 with reduction of 24% in fresh litters when juxtaposed and an increase of 134% in well decomposed juxtaposed litters (Fig. 3). When factoring in litter origin, the increase in qCO_2 observed in well decomposed juxtaposed litters was limited to wood and represented a 6.5 fold increase in qCO_2. At the end of the 30 week incubation, the largest reduction in N content was observed in fresh wood incubated singly (36 ± 10%). This contrasted with the unchanged N content of juxtaposed fresh wood. On the other hand, well decomposed litters lost between 15% and 30% of their initial N content whether they were juxtaposed or not (Fig. 3).

4. Discussion

Variations in substrate quality caused by different tree species, litter origin and state of decomposition create distinct patterns of fungal biomass, enzyme activity and C mineralization within the forest floor. Among these factors, state of decay had the largest impact. Moreover, juxtaposition of substrates with contrasting decomposition states alters microbial activity within these substrates. At the microcosm level, at least, litter heterogeneity promotes N retention.

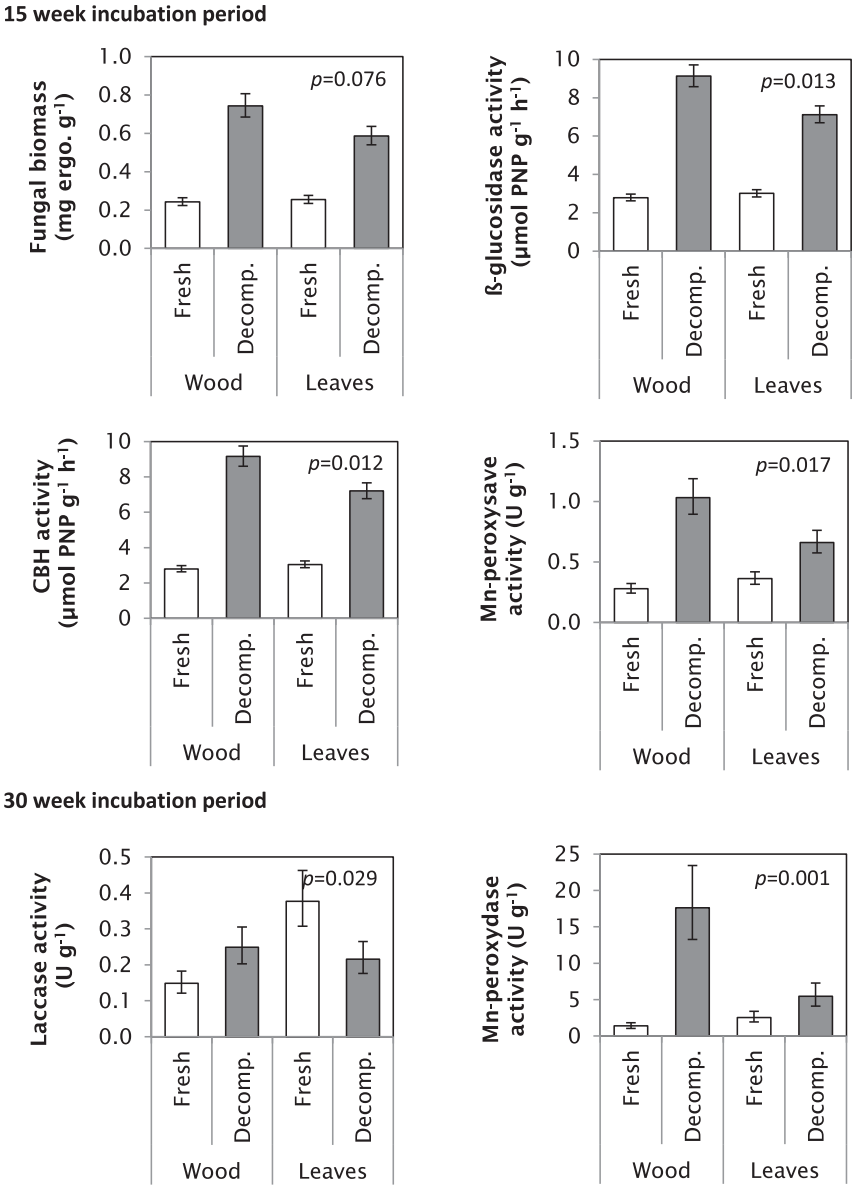

Fig. 1. Fungal biomass and lignocellulolytic enzyme activity in decaying leaf and wood litter as a function of litter decomposition state and origin after incubating in microcosms for 15 and 30 weeks. (CBH = cellobiohydrolase; *p* represents the significance of interactions between decomposition state and origin as in Table 3).

4.1. Fungal activity in relation to decay state

The sampled litters exhibited a large range of chemical characteristics, at least with regards to their C/N and LCI ratios, both indicators of decay state (Linkins et al., 1990; Moorhead et al., 2013). Moreover, well decomposed litters displayed LCI values at which decomposition is considered to be severely limited by energy requirements (Moorhead et al., 2013). Even after a 30 week incubation period, the equivalent to two years under field temperature conditions, large differences in fungal biomass and activity persisted between fresh and well decomposed litters.

Observed fungal biomass and enzyme activity were within the range of values reported by Allison and Vitousek (2004), Šnajdr et al., 2008, Allison et al. (2009) and, Větrovský et al. (2011). Lignocellulolytic enzymes are produced in response to energy requirements (Baldrian, 2008). Consistent with our findings, the activity of cellulolytic enzymes has been shown to increase during decomposition, reaching maximum values when mass loss reaches 20%–30% (Šnajdr et al., 2011) or at later stages of decay (Sinsabaugh et al., 2002b; Rinkes et al., 2013). Ligninolytic enzyme activity has also been found to increase at later stages of decomposition (Sinsabaugh et al., 2002a; Šnajdr et al., 2008; Šnajdr et al., 2011), while enzymatic activity is often correlated with fungal biomass (Fioretto et al., 2007; Papa et al., 2008).

However contrary to our own observations, Šnajdr et al., 2008 reported higher cellulolytic activity in the F than in the H horizon of a *Quercus petraea* forest soil and no differences in cellulase activity was observed among the L, F and H horizons of a *Fagus sylvatica* stand (Papa et al., 2014). Moreover, no relationships between cellulolytic activity and state of decomposition were observed for *Picea abies* needles (Žifčáková et al., 2011) or *Myrtus communis* and *Quercus ilex* leaves (Fioretto et al., 2007). Also, a number of studies have reported simultaneous degradation of holocelluloses and lignin in fresh snags and logs (Fioretto et al., 2005; Fukasawa et al., 2009; Strukelj et al., 2013) and fresh leaf litter (Fioretto et al., 2005; Osono et al., 2009), implying that both cellulolytic and ligninolytic

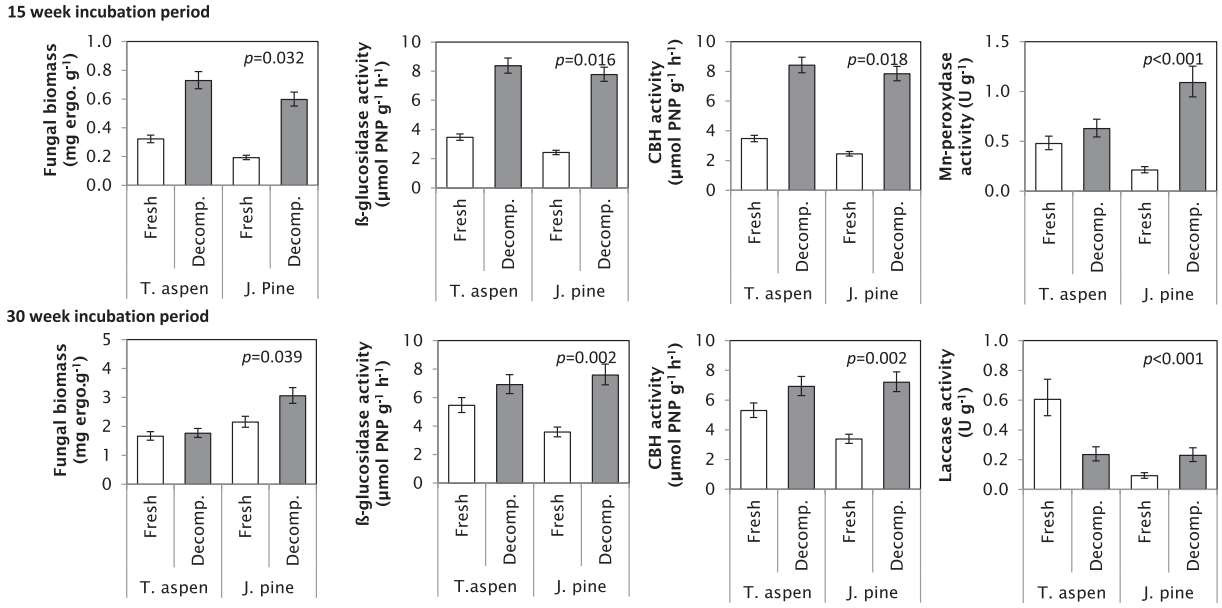

Fig. 2. Fungal biomass and lignocellulolytic enzyme activity in decaying litters as a function of litter decomposition state and host tree species after incubating in microcosms for 15 and 30 weeks (CBH = cellobiohydrolase; *p* represents the significance of interactions between decomposition state and tree species as in Table 3).

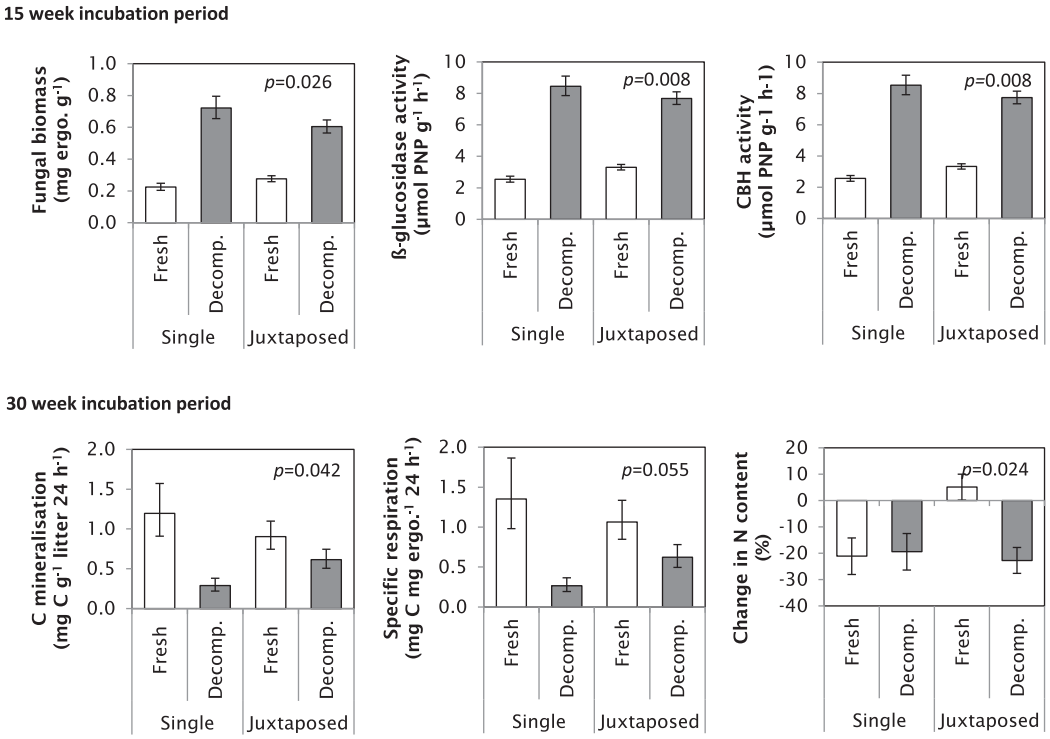

Fig. 3. Fungal biomass and activity in decaying litters as a function of litter juxtaposition (incubated singly or juxtaposed) and decomposition state after incubating in microcosms for 15 and 30 weeks. Fresh litters were incubated with well decomposed litters of same tree species (p represents the significance of two-way interactions as in Table 3.

enzymes can be active in fresh litters.

Fresh leaves or needles collected from the forest floor surface horizon (L) and fresh snags had undeniably been exposed to microbial activity before they were sampled (Strukelj et al., 2013; Rinkes et al., 2014). This initial decomposition phase mainly affects labile compounds, requires little enzymatic synthesis (Rinkes et al., 2014) and involves opportunistic fungi such as *Sydowia polyspora* (Boberg et al., 2011a) found in fresh leaf litter of both host species. Given the values for mass loss, C mineralization and enzyme activity observed during incubation, it is likely that the community of fungi active in fresh litters was metabolizing labile C compounds but also relying on extracellular enzymes for energy procurement. The diversity of taxa found on fresh litter samples would support different C acquisition strategies. Recent work has underlined the ability of endophytes such as *Lophodermium* sp (Yuan and Chen, 2014), found on fresh leaf samples, to synthetize

extracellular lignocellulolytic enzymes. Strains of the microfungi *Penicillium citreonigrum*, found on the fresh jack pine wood sample, can also synthesize cellulolytic enzymes (Baldrian et al., 2011). These fungi can cause rapid litter mass loss (Boberg et al., 2011a) or elevated C mineralization (Allison et al., 2009). Moreover, many cellulolytic strains of Ascomycetes can absorb simple forms of C (Baldrian et al., 2011) without exoenzyme production (Linkins et al., 1990).

The community of fungi active in fresh litter samples was characterized by a higher specific respiration rate or lower C-use efficiency (Hart, 1999; Spohn, 2015) than the community of well decomposed litters. The regulation of C-use efficiency via overflow metabolism (Schimel and Weintraub, 2003; Spohn, 2015) would allow decomposers on high C/N substrates to maintain their stoichiometric balance by excreting C in excess of their needs (Mooshammer et al., 2014). Alternatively, under these N limiting conditions, microorganisms could rapidly metabolize holocelluloses to access minerals, which would then cause them to quickly metabolize simple sugars in order to limit competition by non-cellulolytic organisms (Boyle, 1998).

In contrast to fresh litters, no mass loss was observed in the last 15 weeks of incubation for well decomposed litters, and their C mineralization rate remained lower than that of fresh litters. Also, a larger share of C metabolism was allocated to enzymatic synthesis and fungal biomass in well decomposed litters, thus reducing the community-specific respiration rate (Schimel and Weintraub, 2003). Similar differences in microbial C-use efficiency between fresh and well decomposed material have been reported by Dilly and Munch (1996) and Hart (1999). In a recent review of 14 studies, Spohn (2015) validated the correlation between qCO_2 and C/N, but could not conclude as to what mechanisms caused the relationship. Dilly et al. (2001) also suggested that high microbial C-use efficiency was associated with high ligninolytic activity.

Increases in litter N content in early stages of decomposition are generally followed by N mineralization at later stages (Laiho and Prescott, 2004; Fukasawa et al., 2009; Preston et al., 2009). The loss of nitrogen observed in both fresh and well decomposed litters at the end of the experiment coincides with visual observations of fungal senescence (yellowing mycelial cords). Also, mycelium foraging (Boddy, 1999) out of litter bags in response to depleted energy sources could explain N losses.

The high positive correlations between fungal biomass and lignocellulolytic activity observed at the end of the first 15 week incubation support our assumption that decomposition in these litters resulted mostly from fungal activity (Větrovský et al., 2011). While bacteria are also involved in the decomposition of forest litter (Dilly et al., 2001), it is generally recognized that filamentous fungi remain the primary decomposers in acidic organic-rich soil horizons (Frostegard and Baath, 1996; Blagodatskaya and Anderson, 1998) and in decaying wood (Boddy and Watkinson, 1995).

4.2. Differences in fungal activity between leaf and wood litters

Very few studies have compared the decomposition of leaf and wood litters of the same tree species. As predicted, higher mass losses and C mineralization rates were observed for leaf than for wood litters, while the fungal community of leaf litters was characterized by a lower C-use efficiency. The larger mass losses observed for leaf litters could have resulted from leaching or/and mineralization of non-structural compounds (McTiernan et al., 2003), labile C compounds or soluble tannins (Lorenz et al., 2000; Preston et al., 2009) as these compounds were more abundant in leaves than wood (Table 1 (Strukelj et al., 2012),). Also, the fragmentation of leaves during the preparation of the microcosms might have increased the leaves' exposure to fungal attack.

However, these mechanisms could hardly explain the higher weight losses in leaves than wood at more advanced states of decomposition. Decomposition enriches leaf litter in alkyl, phenolic and, carbonyl compounds, generating residual recalcitrant organic matter (Strukelj et al., 2012), and the well decomposed leaf material in our study had a higher LCI ratio than that of wood. Moreover, at the end of the 15 weeks of incubation, the well decomposed wood was characterized by higher fungal biomass and enzyme activity than the well decomposed leaves. It is generally recognized that saprophyte communities in leaf litter differ from those in wood (Liers et al., 2011), but very few studies have systematically compared the composition and activity of these communities. Some species have been shown to effectively degrade both substrates (Tanesaka et al., 1993; Osono and Takeda, 2002) and a large overlap in fungal composition was found here among well decomposed leaf and wood samples.

4.3. Differences in fungal activity between tree species

Jack pine and aspen litters differed primarily by the size of their fungal biomass and its specific respiration rate and enzyme activity. As anticipated, these differences were more pronounced in fresh than in well decomposed litters. Surprisingly, they had no implications for litter decomposition rate. Hardwood litter is richer in nutrients (Cornwell et al., 2009) which could initially favor enzymatic production, an expensive energy and N process (Schimel and Weintraub, 2003). However with decomposition, recalcitrant compounds increase in aspen leaves (Strukelj et al., 2012) and wood (Strukelj et al., 2013) faster than they do in conifer litters. This could explain the strong decline in fungal biomass and enzyme activity observed between fresh and well decomposed aspen litters.

4.4. Forest floor chemical heterogeneity and fungal activity

Carbon and N are both essential for fungal activity, especially the production of exoenzymes, which enable fungi to metabolize complex organic compounds (Allison and Vitousek, 2005). However, as highlighted by the wide range of C/N ratios found among the sampled litter types, C and N are unevenly distributed within the forest floor (Lindahl and Olsson, 2004). We anticipated that juxtaposing fresh and well decomposed litters would lead to the reallocation of fungal biomass and N from well decomposed litters with low C/N to fresh litters with high C/N. This hypothesis was supported by work conducted by Watkinson et al. (2006), Philpott et al. (2014) and Boberg et al. (2014), among others. While these studies involved cord-forming basidiomycetes, nutrient translocation by means of diffusion over short distances is performed by a wide range of filamentous fungi (Olsson and Jennings, 1991; Ritz, 1995). Given the predominance of Ascomycetes over Basidiomycetes initially found in the sampled litters, the proximity of litter bags in our microcosms and the relatively long incubation time, we assume translocation between litters was performed by both taxa.

During the first 15 weeks of incubation, juxtaposition induced a shift in resource allocation. Hence, fungal biomass growth was redirected from well decomposed litters to fresh ones. More resources were also dedicated to cellulolytic enzyme synthesis in fresh litters, presumably in response to mycelial translocation of N (Allison and Vitousek, 2005; Boberg et al., 2008; Allison et al., 2009) from well decomposed litters. Consistent with our hypothesis, juxtaposition also enhanced Mn-peroxidase activity in both litter types, likely increasing microbial access to energy-rich hollocelluloses (Šnajdr et al., 2010; Moorhead et al., 2013).

While fungal mycelium of juxtaposed litters initially responded by adjusting its lignocellulolytic activity, the longer term response

reflected changes in C dynamics or possibly fungal community. Hence, fresh litters juxtaposed with well decomposed liters for 30 weeks underwent a decrease in C mineralization and specific respiration rate. Without parallel changes in mass loss, fungal biomass and enzyme activity this could suggest increased translocation of C from fresh to well decomposed litters.

This presumed increase in available C in juxtaposed well decomposed litters was associated with an increase in CO_2 mineralization and specific respiration rates in decaying wood. This would suggest a lessening of C-use efficiency, which is surprising given the substrates were considered energy-limited as indicated by their elevated LCI and exoenzyme activity (notably that of Mg-peroxidase) (Burns et al., 2013). It is possible that the increased labile C availability in the well decomposed wood stimulated the growth of opportunistic fungi with low C-use efficiency (see discussion above). Exploring these concepts in the context of the "priming effect" (the increase of soil organic matter decomposition in response to fresh organic matter addition), Fontaine et al. (2003) suggested that K-strategists are poor competitors for labile C in the presence of faster growing r-strategists.

Over the long term, juxtaposition had little effect on litter mass loss. However, at the end of the 30 week incubation, juxtaposed litters, regardless of their decomposition state, were characterized by lower C/N ratios, indicating not increased decomposition but higher N retention (Boberg et al., 2010).

5. Conclusion

This study relied on a rich body of empirical work that addressed how interactions among fungi and substrates affect fungal activity and organic matter decomposition. In slightly more complex but less controlled conditions than previously reported, we assessed how differences in substrate quality caused by variations in tree species, litter origin and state of decay affect fungal activity within the forest floor. Litter state of decay was the dominant factor affecting fungal biomass, exoenzyme production and C-use efficiency. The study also demonstrates that juxtaposition of fresh and well decomposed substrates also affect fungal activity within individual substrates. Subsidizing exoenzyme production (sensu Schimel and Weintraub (2003)) through energy or N supplied from juxtaposed substrates increases exoenzyme production in C- or N-limited substrates and favored N retention. This dynamic is particularly relevant to situations where large sudden inputs of fresh litter occur such as following stand replacing natural disturbances or to situations where fresh litter inputs are drastically reduced such as after forest harvesting. A better understanding of fine scale mechanisms affecting litter decomposition could improve our ability to forecast ecosystem response to disturbance.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.06.017.

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