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Linking wood-decay fungal communities to decay rates: Using a long-term experimental manipulation of deadwood and canopy gaps

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ABSTRACT

Decomposition transfers carbon (C) from detrital organic matter to soil and atmospheric pools. In forested ecosystems, deadwood accounts for a large proportion of the detrital C pool and is primarily decomposed by wood-inhabiting fungi (WIF). Deadwood reductions linked to forest harvesting may alter WIF richness and composition, thus indirectly influencing the persistence of deadwood and its contribution to C and nutrient cycling. Forest structure was enhanced via canopy gap creation and coarse woody debris (CWD) addition that mimic natural disturbance by windfall within a deciduous northern hardwood forest (Wisconsin, USA) to examine its effect on deadwood-associated biodiversity and function. Experimental sugar maple (*Acer saccharum*) logs were sampled, for DNA extraction, ten years after placement to determine the assembly of fungal community composition and its relationship to wood decay rates.

Our findings suggest that the WIF community responded to gap disturbance by favoring species able to persist under more extreme microclimates caused by gaps. CWD addition under closed canopy tended to favor a different species assemblage from gap creation treatments and the control, where canopy was undisturbed and CWD was not added. This was presumably due to consistent microclimatic conditions and the abundance of CWD substrates for host specialists. Fungal OTU richness was significantly and inversely related to CWD decay rates, likely due to competition for resources. In contrast, fungal OTU composition was not significantly related to CWD decay rates, canopy openness or CWD addition amounts. Our study site represents a diverse fungal community in which complex interactions among wood-inhabiting organisms and abiotic factors are likely to slow CWD decomposition, which suggests that maintaining a biodiverse and microsite-rich ecosystem may enhance the capacity for C storage within temperate forests.

1. Introduction

Understanding the forest carbon cycle has become increasingly urgent in the context of global climate change (Finzi et al., 2020; Hollinger et al., 2021). Decomposition of deadwood arguably remains one of the least understood aspects of the forest carbon cycle, despite the dramatic increase in scientific publications addressing woody debris in recent decades (Russell et al., 2015). Quantifying wood decomposition, and hence the amount of CO_2 released to the atmosphere, poses considerable challenges because of the long time required for complete decomposition, as well as the many biotic and abiotic factors that interact to regulate decomposition rates (Stokland et al., 2012). Further, the predictive power of these factors is strongly scale dependent (Bradford et al., 2014).

In boreal and temperate systems, wood-inhabiting fungi (WIF) are the primary biotic agents of wood decomposition (Harmon and Ferrell, 1990; Boddy and Heilmann-Clausen, 2008). Changes in WIF community composition and diversity in these systems likely affect wood decomposition rates, which has implications for ecosystem carbon and nutrient dynamics (Maynard et al., 2018). Uncovering key functional links between WIF diversity and wood decomposition is necessary to devise management prescriptions that can enhance the provision of important

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ecosystem functions such as carbon storage. However, few studies have linked WIF diversity to ecosystem function, and these have led to inconsistent results (Fukasawa and Matsukura, 2021). In particular, several previous studies have shown decomposition rates to be negatively correlated with fungal richness or abundance (Bradford et al., 2014; Fukami et al., 2010), while others have found the opposite result (van der Wal et al., 2013). These inconsistencies and knowledge gaps confound any attempts to generalize regarding the importance of WIF diversity for ecosystem processes.

Forest harvesting alters woody debris quantity and quality by reducing the amount of woody debris left within a stand and by altering the distribution of species and sizes within the woody debris pool. This reduction in woody debris may alter WIF communities (Blaser et al., 2013; Brazee et al., 2014; Dove and Keeton, 2015). Such alterations indirectly affect the persistence of woody debris and its contribution to forest carbon dynamics (Mackensen et al., 2003; Russell et al., 2015). Historically, complex assemblages of woody debris – and structural complexity in general – in northern hardwood deciduous forests of North America were maintained by frequent, low-to moderate-severity disturbances, typically caused by wind storms that created canopy gaps and added coarse woody debris (CWD) to the forest floor (Hupperts et al., 2018). The structural complexity provided by canopy gaps and CWD is thought to maintain or enhance biodiversity of many taxonomic groups (Bauhus et al., 2009), including WIF (Stokland et al., 2012). However, traditional timber extraction in this region and preferential harvesting of large trees have reduced CWD abundance (Schulte et al., 2007; Hupperts et al., 2018). Further, because WIF community composition typically shifts as deadwood decomposes, abundant and heterogeneous deadwood (i.e., various stages of decay) must be continuously created to maintain WIF diversity, which does not typically occur in intensively managed forests (Abrego and Salcedo, 2013).

In 2004, a large-scale, replicated experiment was initiated to test the effects of enhanced structural complexity on forest ecosystem processes, including wood decomposition, at the Flambeau Experimental site (Wisconsin, USA). The experimental design allowed us to re-inventory sugar maple (*Acer saccharum* Marsh.) logs after 10 y of decay under natural conditions. We used high-throughput DNA sequencing methods to identify WIF species found at a low abundance, which may not form fruit bodies, and to characterize the community more comprehensively when compared to fruit-body inventories alone (Kubartová et al., 2012). This latter consideration is particularly important when assessing the influence of fungal communities on decay rates at the scale of individual logs (Lindner et al., 2011). Profiling the WIF community using high-throughput sequencing methods provides a robust assessment of linkages among forest structure, biodiversity and decay processes (Tomao et al., 2020).

Our aim was to quantify the influence of structural complexity enhancement – canopy gap creation and CWD addition – on WIF community diversity and, in turn, its relationship to CWD decomposition. Thus we ask: (1) does increasing forest structural complexity alter WIF composition and diversity? and (2) to what extent does WIF composition and diversity influence wood decomposition rates?

2. Methods

2.1. Study site

We conducted this study within the Flambeau Experiment, a long-term field experiment initiated in 2004 at the Flambeau River State Forest research area (280 ha), north-central Wisconsin, U.S.A (45°37.4′ N, 90°47.8′ W). The overstory is dominated by sugar maple (*Acer saccharum*) with co-dominant species including white ash (*Fraxinus americana*), American basswood (*Tilia americana*) and bitternut hickory (*Carya cordiformis*) in two co-dominant age cohorts of 80 and 100 y. The soils are loams (Glossudalfs) of the Magnor and Freon series (USDA NRCS, 2012). The climate is characterized by warm summers and cold winters, and the average air temperatures for January and July are −12 and 17 °C, respectively (1980–2010; National Centers for Environmental Information: https://www.ncdc.noaa.gov). Mean annual precipitation is 74 cm, and mean snowfall is 70 cm (1980–2010; National Centers for Environmental Information: https://www.ncdc.noaa.gov).

The Flambeau site was established to study the long-term effects of downed CWD addition and canopy gap creation representative of unmanaged, late-successional forests. Here we focused on four experimental treatments that were randomly applied to five replicates in 80 × 80 m plots, including: control, gap creation, CWD addition and gap + CWD. Three gap sizes were established within whole plots, but we focused on medium (200 m^2) and large (380 m^2) gaps (i.e., subplots) only. In 2007, felled live trees within the Flambeau site but outside the experimental plots were added to plots to obtain an average of 29 Mg ha^{-1} of CWD that represents estimated deadwood volume for old-growth forests in the region (Goodburn and Lorimer, 1998).

2.2. Log selection, measurement and environmental data collection

At the time of treatment implementation, 110 freshly cut maple logs were added to both gap and closed canopy treatments. The logs were approximately 20 cm in diameter and 2 m in length. Logs were placed at selected locations to span the canopy opening. Locations were approximately 4 and 12 m north and south from the center of the canopy opening in the medium subplots and 5.5 and 16.5 m in the large subplots. The inner logs were within the opening (central zone), and the outer logs were beneath closed canopies (transition zone). In the large subplots of gap treatments (gap and gap + CWD), two logs were placed in each of the central gap and transition (under the canopy) areas. In the medium subplot, two logs were placed in the central area and one in the transition zone. In closed canopy (control and CWD addition), logs were randomly placed in two quarters each of the medium and large subplots.

Exact dimensions, including length and diameter at the ends and middle of each log, were recorded at t_0 (2007) and t_{10} (2016). Each log was cored at five equidistant locations along the length, and the cores were dried to a constant weight and weighed. Wood density was calculated from core mass divided by the known core volume and extrapolated to the log. By 10 y, the coring method was problematic because the logs had softened somewhat causing us to shift the methodology to harvesting a subset of the replicates. Thus, three disks were cut from each log, approximately 5 cm wide using a chainsaw. The disks were then dried to constant weight and their volumes recorded to calculate proportion remaining from initial mass. To determine wood decay, the decomposition constant (k) was calculated using the following equation (Olson, 1963):

$$M_t = M_0^{(-kt)}$$

where M_t *and* M_0 are the final and initial wood mass, k is the decomposition constant and t is time of decomposition (10 y). Whole plot CWD volume was inventoried in 2008 (detailed methods in Forrester et al., 2015) and used to calculate the average plot CWD biomass.

For the measurement of soil temperature, iButton Temperature Loggers (Maxim Integrated Products, Inc., Sunnyvale, CA) were buried at 5 cm at the center of each subplot to record bihourly soil temperatures for the duration of the study. Annual maximum soil temperature within each plot was used as a predictor variable of decay rate.

Hemispherical canopy photos were taken at the location of each decomposition log to estimate canopy openness. Photographs were taken in July 2016, using a Nikon Coolpix 5000 camera fitted with an FC-E8 fisheye lens converter mounted on a tripod, 1.3 m above ground surface. The Gap Light Analyzer (Frazer et al., 1999) was used to process the digital images and estimate percent canopy openness.

2.3. Sample collection and processing

A total of 70 experimental logs were selected for WIF sampling (as described in section 2.2). Logs were selected based on their location within gaps: logs placed closest to gap center were preferred in order to maximize the potential gap effect (see Supplementary Material, Figure S1). Additionally, initial log selection during experimental setup favored logs of similar sizes (approximately 20 cm diameter and 2 m length). Samples for fungal metabarcoding analyses were collected by drilling six holes per log and collecting the resulting drill shavings for DNA extraction. Holes were drilled parallel to the ground halfway between the top of the log and ground contact, with two holes drilled on opposite sides of the log at three points: approximately 5 cm from each log end, and at the midpoint of the log lengthwise. Samples were pooled for each log. Holes were drilled with 8 mm diameter lip-and-spur bits to a depth of approximately 10 cm after bark was stripped, similar to the method of Skelton et al. (2019). Bits were washed and DNA-sterilized between each use following Banik et al. (2013). Shavings were collected 50 mL plastic tubes, covered with a 2% CTAB solution and frozen at −20 °C until DNA was extracted. One negative DNA field sample per plot was obtained by manipulating an empty tube and sterile drill bit in a manner similar to that employed for wood sample collection. Extraction of DNA from wood samples was performed according to the CLS/glass milk extraction protocol described in Lindner and Banik (2009). Extracted DNA was PCR-amplified for construction of high-throughput amplicon sequencing (HTAS) libraries using individually barcoded Ion fungal-specific primers fITS7 and ITS4 targeting the ITS2 region (Ihrmark et al., 2012; White et al., 1990) that were modified to be compatible for Ion Torrent sequencing according to manufacturer's recommendations. The forward primer consisted of the Ion A adapter sequence, the Ion key signal sequence, a unique Ion Xpress Barcode sequence (10–12 bp), a single base-pair linker (A), and finally the fITS7 primer (Ihrmark et al., 2012). The reverse primer consisted of the Ion trP1 adapter sequence followed by the conserved ITS4 primer (White et al., 1990). The following reagents were used for each PCR reaction: 7.88 μl DNA-free molecular grade water, 3.0 μl 3 μL Green GoTaq 5x buffer (Promega Corporation, Madison, WI, USA) – final concentration 1x, 0.3 μl of 10 mM dNTPs (Promega Corporation, Madison, WI, USA) for a final concentration 0.2 mM of each dNTP, 0.3 μl of each 10 μM primer for a final concentration of 0.2 μM of each primer, 0.12 μl of 20 mg/ml BSA (New England BioLabs), and 0.1 μL of 5u/μL GoTaq polymerase (Promega) – final concentration of 0.033u/μL of the reaction. Conditions for thermocycling were: initial denaturation of 94 °C for 3 min, then 11 cycles of (94 °C for 30 s, 60 °C for 30 s (drop 0.5 °C per cycle), 68 °C for 1 min), then 26 cycles of (94 °C for 30 s, 55 °C for 30 s, and 68 °C for 1 min), with a final extension of 68 °C for 7 min. Zymo Select-a-size spin columns were used to purify the PCR products (Zymo Research, Irvine, CA, USA). The barcoded libraries were quantified using a Qubit® 2.0 Fluorometer with the high-sensitivity DNA quantification kit (ThermoFisher Scientific, Madison, WI, USA), equilibrated and combined in preparation for sequencing on the Ion Torrent Personal Genome Machine (PGM) semiconductor sequencing platform. An additional barcoded sample containing the known positive control, SYNMO, was included in the sequencing run (Palmer et al., 2018). SYNMO is an equimolar spiked-in mock community control composed of non-biological synthetic ITS sequences. The results from the mock community inform downstream bioinformatics by providing realistic clustering and OTU assignment parameters which is an important and necessary component to any HTAS study of environmental samples (Jusino et al., 2019; Palmer et al., 2018).

2.4. Bioinformatics

We bioinformatically processed our Ion Torrent data using AMPtk version 1.4.1 (Palmer et al., 2018; amptk.readthedocs.io). We pre-processed our merged, individually barcoded reads using USEARCH (version 9.2.64), vsearch (version 2.11.1) and then removed the forward and reverse ITS primers. We discarded any reads shorter than 125 bp. Reads longer than 300 bp were trimmed to 300 and any reads shorter than 300 bp were padded with Ns to help improve the clustering step (Palmer et al., 2018). Sequence reads were then quality-filtered with expected errors less than 1.0 (Edgar and Flyvbjerg 2015), de-replicated, and clustered at 97% similarity to generate operational taxonomic units (OTUs) using UPARSE (Edgar, 2013). Following clustering, any padded Ns were removed, and the processed sequences were mapped to the OTUs. We used SYNMO to account for observed rates of barcode crossover using the filter module in AMPtk. The OTUs were assigned taxonomy using the hybrid taxonomy algorithm in AMPtk and all non-fungal OTUs were removed. OTUs with poor taxonomy assignments were manually identified via BLAST searches of GenBank. Finally, fungal guild assignments were made through FunGuild (version 1.0 Beta; Nguyen et al., 2016), and manual assignments were made for known groups with incomplete ecological assignments via FunGuild.

2.5. Statistical analysis

2.5.1. Description of WIF composition

All analyses were performed in R statistical freeware version 3.5.3 (2019-03-11) (R Core Team, 2019). To visualize the most common OTUs, heat maps were generated using the pheatmap function of the *pheatmap* package (Kolde, 2019). The function uses hierarchical clustering (agglomerative) to arrange the OTU presence/absence matrix into rows corresponding to fungal OTUs with associated taxa and columns into treatments. We used the average agglomeration method and displayed the 50 overall most abundant OTUs and the 30 most abundant OTUs assigned to Basidiomycota. The heatmaps displayed the frequency of occurrence of each fungal OTU (%), which was calculated by dividing the number of samples containing a given fungal OTU by the total number of samples for each treatment and multiplying by 100.

Indicator species analysis was performed to assess the strength of relationship between OTUs and the treatment groups using the function multipatt() from the *indicspecies* package (De Cáceres and Legendre, 2009). The significance ($p < 0.05$) of relationships was assessed using a permutation test computed within the function. Fungal community OTU composition was examined using nonmetric multidimensional scaling (NMDS) implemented by the metaMDS() function from the *vegan* package using a Simpson beta diversity (beta sim) dissimilarity measure (Oksanen et al., 2019). Permutational multivariate analysis of variance (PERMANOVA) was used to test whether the fungal composition differed among treatments and locations (adonis() function in the *vegan* package). Variance in community composition within groups was compared by treatment and location using multivariate homogeneity of groups dispersion (PERMDISP; betadisper() and permutest() functions in the *vegan* package; Anderson, 2006). A linear mixed model with random effect (plot) was used to test whether fungal OTU richness differed among treatments (lme() function in the *nlme* package; n = 70 logs; Pinheiro et al. (2018).

2.5.2. Quantifying of WIF diversity on function

Principal Coordinates analysis (PCoA) axis vectors (PCoA1 and PCoA2) were obtained from the presence/absence of OTUs using the beta sim distance matrix to be used as predictors of log decomposition rates (decay rate, see section 2.2) (function pcoa() in the *ape* package; Paradis and Schliep (2018). Variance partitioning was implemented on the log decomposition rates (k) with the quantification of the structural complexity components (percentage canopy openness, CWD mass), the fungal OTU richness, PCoA axis vectors and maximum soil temperature using partial (linear) regression (function varpart() in the *vegan* package; Peres-Neto et al. (2006). The partition of variance was visualized with a Venn diagram showing the adjusted R^2 of each variable and of their interaction (plot function of varpart()). The relationship between log decomposition rates (k) and fungal OTU richness, fungal OTU

composition (PCoA1 and PCoA2), percent canopy openness, CWD mass and maximum soil temperature was analyzed with multiple linear regression.

3. Results

3.1. Influence of forest structure on WIF composition and richness

A total of 813 OTUs were recovered, of which 317 were assigned to the phylum Basidiomycota and 471 to Ascomycota. A small number of OTUs were assigned to other phyla; ten to Mortierellomycota, two to Glomeromycota, one to Chytridiomycota, and 12 were unassigned. The number of OTUs (or fungal OTU richness) ranged from 10 to 111 per log and averaged 53 ± 3 per log across all structural complexity treatments (Table 1). The most abundant species, OTU8 - *Ascocoryne cylichnium*, was found on 53 out of 70 logs (Fig. 1; Table S1).

Fungal OTU richness did not differ significantly among treatments ($F_{3,10} = 1.40$; $p = 0.30$). However, patterns of fungal occurrence frequencies were more similar between the two gap treatments (gap and gap + CWD) than between gaps and the CWD only treatment (Fig. 1). The two gap treatments shared a high occurrence of *Leptodontidium* spp. (OTU393 and OTU593) and very low occurrence (>10%) of OTU88 – Sordariales sp. compared to CWD addition and the control. Two OTUs corresponding to *Armillaria* spp. (OTU31 and OTU1114) were present in more than 50% of logs placed in gaps but at less than 20% in CWD addition. In contrast, OTU180 – *Lentomitella* sp. and OTU1124 – *Torrentispora* sp. were present in over 80% of logs in the CWD addition but less than 10% in the gap treatment. The most abundant species overall, OTU8 - *Ascocoryne cylichnium*, was present in over 80% of logs sampled in the CWD addition treatment.

There were marked differences in the occurrence of the most abundant Basidiomycota fungal OTUs between the CWD addition and gap + CWD treatments (Fig. 2; Table S2). OTU170 – Atractiellales sp. and OTU2 – *Xeromphalina* sp. were present in more than 60% of logs sampled in the CWD addition but less than 20% of the gap creation treatments. The CWD addition also had the highest occurrence of OTU1148 - *Xeromphalina* sp., OTU158 – Hymenochaetales sp, OTU402 – *Psathyrella obtusata* compared to gaps and the control.

These observations were supported by the indicator species analysis, which revealed that a large number of fungal OTUs were significantly associated with the CWD addition treatment (Table 2). The indicator species included two Basidiomycota taxa, OTU289 - Cantharellales sp. and OTU170 - *Helicogloea* sp., which both had an indicator value greater than 0.57 and were significantly associated with the CWD addition treatment (p = 0.002). In contrast, the OTU31 – *Armillaria gallica* was an indicator species for all treatments except the CWD addition.

We used NMDS ordinations to assess whether WIF composition varied among treatment groups (Fig. 3) and the position of logs within gaps (Figure S1). The ordination plot representing fungal composition from the presence/absence of OTUs revealed differentiation of the CWD addition treatments but a homogeneous distribution of the control and gap treatments sample points (Fig. 3). Additionally, there was a significant relationship between ordination scores and fungal richness ($R^2 = 0.18$; $p = 0.048$) and CWD biomass ($R^2 = 0.23$; $p = 0.024$). However, there were no clear patterns in the ordination showing within-gap CWD placement (Figure S1). These observations were supported by the PERMANOVA analysis (Table 3) which indicated that WIF community composition was significantly affected by gap creation ($F = 1.84$; $R^2 = 0.03$; $p = 0.018$) but only marginally by CWD addition ($F = 1.59$; $R^2 = 0.02$; $p = 0.059$). No significant differences were related to the position of logs within gaps ($F = 1.16$; $R^2 = 0.08$; $p = 0.228$; Table 3).

However, PERMDISP indicated that the structure of variances of fungal species was heterogenous among within-gap log placements ($F = 3.33$; $p = 0.027$).

Table 1
Mean (SE) fungal OTU richness and coefficient of variation (CV) by treatment (control, CWD, gap and gap + CWD).

Treatment	OTU richness	CV (%)	n
Control	61.5 (5.8)	43.6	21
CWD	67.3 (12.9)	47.0	6
Gap	48.2 (5.2)	56.4	27
Gap + CWD	41.7 (4.0)	37.5	15

3.2. Relationship of WIF community to decomposition rates

Decomposition rate *k* was negatively related to fungal OTU richness ($F = 5.82$; $p = 0.02$, $R^2 = 0.12$) and positively related to maximum soil temperature ($F = 5.14$; $p = 0.03$), though the smoothed conditional means showed that the relationships were complex and non-linear (Table 4 and Fig. 4). The fractions used in variance partitioning explained a total of 24% of the variation in decay rates, with maximum soil temperature explaining the most (9.2%), and the combination of soil temperature and richness explaining 8.3% of the variance (Figure S2). Interestingly, the magnitude of the relationship between the decomposition rate and fungal OTU richness of known wood decomposers (178 OTUs) was more strongly negative than with all OTUs ($R^2 = 0.25$; Fig. 5).

4. Discussion

The relationship between WIF diversity and organic matter decomposition in forest ecosystems is important because it regulates C and nutrient cycling, and by doing so contributes to overall site productivity (Duffy et al., 2017) and ecosystem C storage (Yang et al., 2016). We tested whether manipulation of forest structure altered WIF diversity and community composition by profiling the wood-decay fungal community living on moderately decayed CWD at a long-term experimental site manipulated ten years prior. We then tested whether WIF diversity altered CWD decay rates in order to functionally link the WIF community to CWD C persistence within the ecosystem. Our results indicated that structural complexity altered WIF composition but not fungal OTU richness, and we found an inverse relationship between fungal OTU richness and decomposition rates.

4.1. Effect of structural complexity on WIF composition

Ten years after enhancing structural complexity by gap and CWD addition treatments, fungal community composition differed with increased structural complexity, particularly within gaps. Higher temperature and moisture in gaps likely promoted fungal communities adapted to more extreme changes in temperature and moisture compared to the CWD addition treatment (Bässler et al., 2014). Up until 5 y after gap creation, previous research at our study site recorded higher wood temperature and moisture (Forrester et al., 2012, 2015) and higher soil temperature (Stoffel et al., 2010; Lewandowski et al., 2015) in gaps compared to unharvested plots. After a decade, differences in soil temperature between gaps and closed canopy were minimal or reversed (higher under closed canopy) due to the dense understory vegetation that shaded the soil and CWD compared to more open conditions immediately after treatment implementation (Perreault et al., 2020). However, the legacy of microclimatic conditions from gaps on fungal communities may have persisted.

Despite having a marginal effect on overall community structure, CWD addition supported the growth of different fungal species assemblages compared to the gap addition treatments and the control (Figs. 1 and 2). For example, CWD addition enhanced the fungal OTU richness of *Xeromphalina* sp. and *Helicoglolea* sp., but *Armillaria* sp. was nearly absent from these logs and was particularly abundant in the gap treatment and the control. The CWD addition treatment likely provided abundant substrate availability, and more constant temperature and moisture

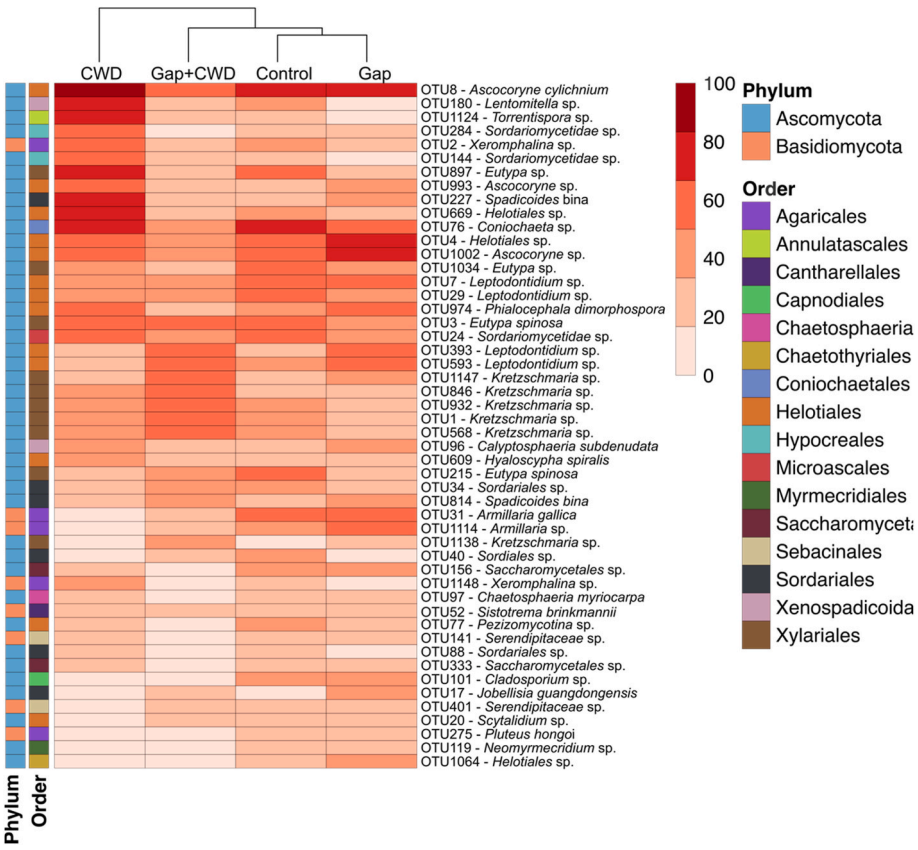

Fig. 1. Frequency of occurrence (%) of the 50 most abundant OTUs shown by Phylum and genus in four treatments: coarse woody debris addition (CWD), gap + CWD addition (Gap + CWD), control (Control) and gaps (Gap). OTUs were ordered by hierarchical agglomeration clustering. N = 70 sugar maple logs.

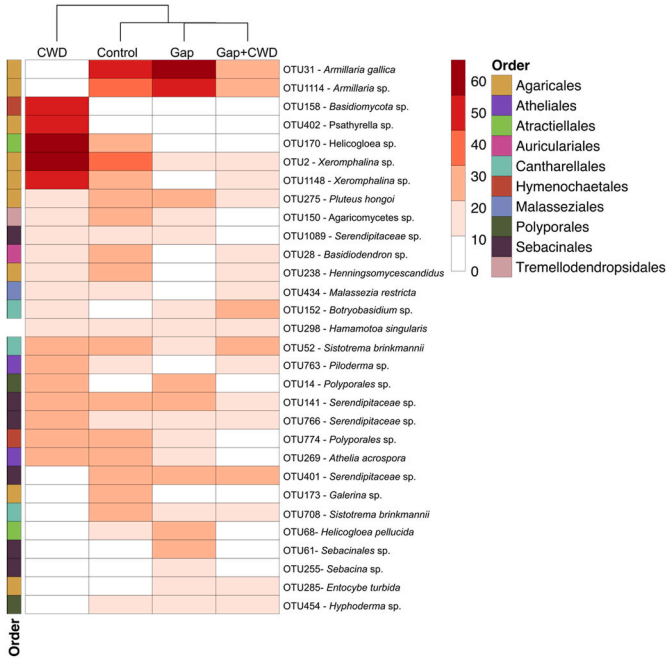

Fig. 2. Frequency of occurrence (%) of the 30 most abundant OTUs associated to Basidiomycota shown by genus in four treatments: coarse woody debris addition (CWD), gap + CWD addition (Gap + CWD), control (Control) and gaps (Gap). N = 70 sugar maple logs.

under closed canopy relative to more extreme microclimatic conditions in gaps. Selection harvest that creates small canopy gaps has been associated with reduced diversity of ectomycorrhizal fungi when compared to closed canopy in temperate forests (Grebenc et al., 2009; de Groot et al., 2016), suggesting that certain fungal species or functional groups may be more sensitive to gap-scale disturbance. Moreover, relatively consistent microclimatic conditions and ample substrate availability in the CWD addition treatment possibly promoted the growth of host specialists, as suggested by a large number of indicator species. This unique fungal community was related to higher mass loss of experimental logs relative to other treatments. A separate study of decomposition rates of multiple substrates, including the experimental logs, found that the CWD addition treatment strongly influenced mass loss patterns (Forrester and Mladenoff, 2013).

4.2. *No effect of structural complexity on WIF richness*

Our results revealed no significant relationship between fungal OTU richness and structural complexity. This is surprising given changes in community composition described above, and a previous study demonstrating that structural complexity increased fungal richness at our study site (Brazee et al., 2014). 5 y after treatment, Brazee et al. (2014) recorded a higher number of fungal observations in CWD addition and gap + CWD, and increased fungal richness in CWD addition. However, their study focused on sporocarp collection only, and the fungal community was sampled before advanced regeneration within the gaps reduced microclimatic differences between gaps and closed canopy. Changes in composition do not necessarily equate to differences in richness, since differing microenvironmental or substrate conditions can select for organisms with certain traits without reducing the total number of species. The species assemblage provides information about community response to disturbance (Bässler et al., 2014), responses that

Table 2
Association among fungal OTUs and treatment groups or combination of treatment groups according to Multilevel Pattern Analysis (indicator species analysis). Treatments groups are control, CWD addition, gap, gap + CWD addition. Phyla are Ascomycota (A) and Basidiomycota (B). n = 70 sugar maple logs.

Treatment group(s)	OTU ID	Indicator value	p-value	Taxa assignment	Phyla
Control	OTU431	0.342	0.05	*Calocera* sp.	B
CWD	OTU289	0.576	0.002	*Cantharellales* sp.	B
	OTU170	0.573	0.002	*Helicogloea* sp.	B
	OTU1124	0.554	0.002	*Torrentispora* sp.	A
	OTU872	0.538	0.004	*Hyaloscypha* sp.	A
	OTU43	0.522	0.01	*Steccherinum fimbriatum*	B
	OTU163	0.522	0.01	Agaricales sp.	B
	OTU479	0.522	0.01	*Togniniella acerosa*	A
	OTU964	0.522	0.01	*Steccherinum fimbriatum*	B
	OTU227	0.488	0.01	*Spadicoides bina*	A
	OTU158	0.487	0.004	*Hymenochaetales* sp.	B
	OTU379	0.48	0.01	*Hyphoderma setigerum*	B
	OTU402	0.468	0.01	*Psathyrella obtusata*	B
	OTU153	0.466	0.02	*Ceriporia reticulata*	B
	OTU310	0.466	0.02	Helotiales sp.	A
	OTU669	0.446	0.02	*Pseudaegerita corticalis*	A
	OTU1135	0.442	0.01	*Tremellodendropsidales*	B
	OTU374	0.404	0.04	*Spadicoides fuscolutea*	A
	OTU26	0.397	0.03	*Pluteus cervinus*	B
	OTU144	0.396	0.05	*Phialemonium* sp.	A
	OTU50	0.387	0.04	*Stereum ostrea*	B
	OTU617	0.387	0.04	*Capronia pilosella*	A
	OTU706	0.387	0.03	*Phaeoacremonium fraxinopennsylvanicum*	A
	OTU176	0.368	0.03	*Anthopsis catenata*	A
	OTU284	0.367	0.05	*Brachysporium nigrum*	A
Gap	OTU146	0.375	0.04	*Xylomelasma* sp.	A
Gap + CWD	OTU123	0.397	0.03	*Chaetosphaeria* sp.	A
Control; CWD	OTU180	0.467	0.01	*Lentomitella obscura*	A
	OTU897	0.41	0.04	*Torrentispora dubia*	A
	OTU229	0.392	0.02	Xylariales sp.	A
Control; Gap	OTU101	0.438	0.01	*Cladosporium* sp.	A
Control; Gap; Gap + CWD	OTU31	0.45	0.03	*Armillaria gallica*	A

may not alter overall richness unless marked differences in substrate availability exist.

Moreover, species exchange can occur between the experimental logs and the naturally present neighboring logs, especially where a large amount of CWD is present (Krah et al., 2018). In our study, CWD naturally present prior to treatment implementation was retained in addition to the whole plot CWD additions and the experimental logs used in the present study. By 10 y, this residual CWD would have been highly decayed, possibly hosting a high diversity of fungal species regardless of treatment. Fungal richness tends to be high in moderate to advanced decay stages (Rajala et al., 2011; Fischer et al., 2012; Kubartová et al., 2012) due the uneven decay process within a log that creates a variety of niches in the well decayed wood (Siitonen, 2001; Heilmann-Clausen and Christensen, 2003). Though we sampled the experimental logs that were only moderately decayed, a legacy effect from other logs in the vicinity could have increased overall fungal richness, even in the control. CWD pieces located close together typically have fungal species assemblages more similar to each other, when compared to pieces more distant, because the likelihood of colonizing another CWD piece declines with distance (Jönsson et al., 2008; Norros et al., 2012). In summary, our finding that WIF richness was unrelated to structural complexity may be explained by the legacy of abundant residual CWD, which was present in all treatments throughout the study.

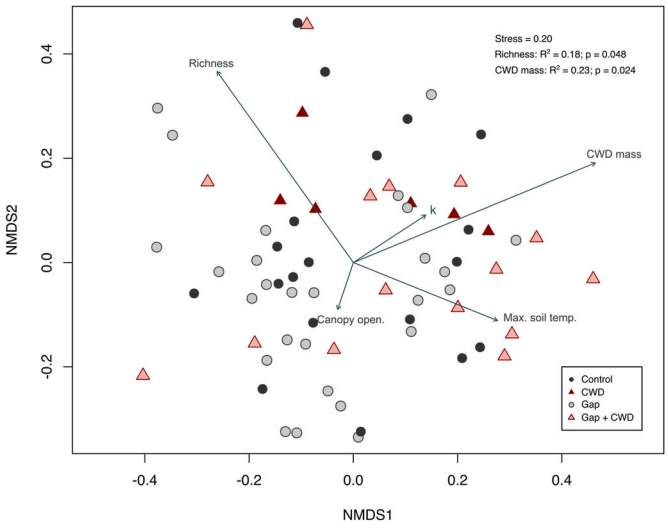

Fig. 3. NMDS ordination of wood-decay fungi community composition (presence/absence) by treatment (control, CWD addition, gap, gap + CWD). Environmental factors (grey vectors; canopy openness, decay rate *k*, maximum soil temperature, fungal richness and CWD mass) were overlaid using the function envfit in the R package *vegan*. Significant variables ($p < 0.05$) are shown in the upper right corner. Fungal richness is the sum of all OTUs present in each sample. N = 67. K = 3.

Table 3
Permutational Multivariate Analysis of Variance (PERMANOVA) and multivariate homogeneity of group dispersions (PERMDISP) for effect of treatment variables (gap, CWD, gap + CWD) and locations (GN, GS, TN, TS, NG (non-gap)) on wood-decay fungal community OTU composition based on 999 permutations. n = 67 sugar maple logs.

	PERMANOVA			PERMDISP	
	F	R^2	p	F	p
Gap	1.84	0.03	**0.018**	0.76	0.401
CWD	1.59	0.02	0.059	0.28	0.605
Gap x CWD	0.84	0.01	0.650	–	–
Location	1.16	0.08	0.228	3.33	**0.027**

Table 4
Statistics for linear model relating coarse woody debris (CWD) decomposition constant k to fungal OTU richness (Richness), maximum soil temperature (Temp.), wood addition biomass (CWD mass; Mg ha^{-1}), percent canopy openness (Can. Openness) and fungal OTU composition (PCoA1 and PCoA2). n = 44 sugar maple logs. Note that richness is negatively related and temperature positively related to *k*. Statistics shown for full and individual model variable.

	F value	p value	R^2
Full model	*1.68*	*0.16*	*0.26*
Richness	5.82	**0.02**	0.02
Temp.	5.14	**0.03**	0.09
CWD mass	1.19	0.28	0.02
Can. Openness	0.07	0.79	0.001
PCoA1	0.10	0.75	0.002
PCoA2	1.24	0.27	0.04

Model statistics: R2 = 0.27, F-statistics = 1.68, p-value = 0.16.

4.3. Relationship of WIF richness to decomposition rates

Our results demonstrated that in sugar maple logs, fungal OTU richness was negatively related to decomposition rates 10 y after tree harvest, consistent with studies in temperate (Fukami et al., 2010) and subtropical (Yang et al., 2016) forests. Importantly, this finding was strengthened when we focused the analysis on just those fungal species

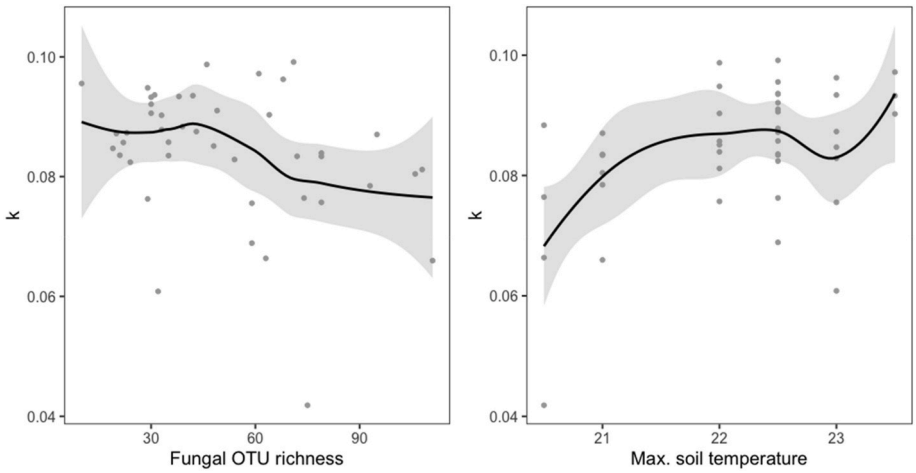

Fig. 4. Coarse woody debris (CWD) decomposition constant k as it relates to fungal OTU richness (left) and maximum soil temperature (right). The curves represent the smoothed conditional means calculated using the LOESS curve-fitting method. 95% confidence intervals shown in light grey. n = 44 sugar maple logs.

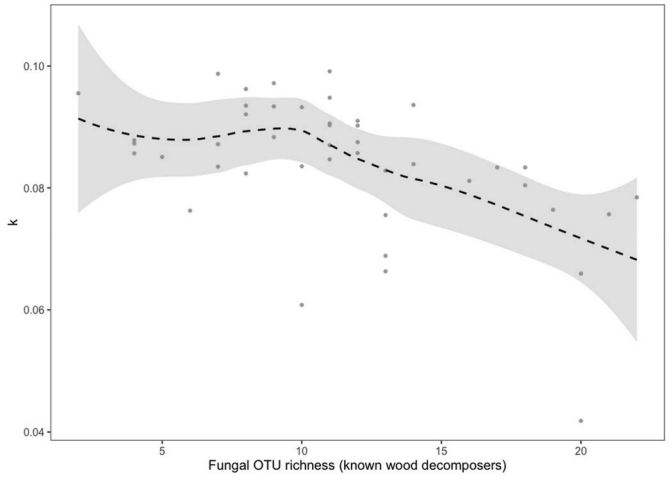

Fig. 5. Coarse woody debris (CWD) decomposition constant *k* as it relates to fungal OTU richness for 178 known wood decomposers ($R^2 = 0.25$). The curve represents the smoothed conditional mean calculated using the LOESS curve-fitting method. The 95% confidence interval is shown in grey. n = 44 sugar maple logs.

known to be wood decomposers. However, other studies in temperate and boreal forests have recorded positive (Hoppe et al., 2015; Kahl et al., 2017) or weak (non-significant; Lindner et al., 2011) relationships between decomposition and fungal richness (Table 5). The variability in physico-chemical characteristics among tree species and decay classes are major drivers of WIF community colonization (Hoppe et al., 2015; Purahong et al., 2018) that may alter this relationship. Freshly fallen wood in early decay stages may exhibit a positive relationship of fungal richness to decomposition because resources may not yet be limiting, while highly decayed wood may become saturated in terms of organisms it can host relative to resource availability. Initial fungal community at the time of mortality and assembly history also play into the development of the relationship of fungal communities to their host (Fukami et al., 2010; Hiscox et al., 2015). The complexity of interactions between biotic and abiotic factors, coupled with the differences in inventory methods, make it difficult to generalize about the functional role of fungal communities in decomposition. Nevertheless, we believe that our study has revealed a highly diverse community in which complex interactions among wood-inhabiting organisms and abiotic factors are likely to inhibit and create variability in decomposition, suggesting that maintaining a biodiverse and microsite-rich ecosystem may enhance C storage capacity.

The inverse relationship between wood decay rates and fungal richness highlights the role of competitive interactions among fungal species in regulating organic matter turnover, due to niche overlap (Yang et al., 2016). At higher diversity, fungi possibly invest more energy in competition and antagonistic interactions than in growth or wood decomposition due to space and resource limitations (Boddy and Heilmann-Clausen, 2008; van der Wal et al., 2013). For example, Purahong et al. (2016) recorded an inverse relationship between fungal richness and the activity of two enzymes involved in litter decomposition (laccase and general peroxidase). They hypothesized that some taxa

Table 5
Comparison of the direction of the relationship between CWD decomposition and fungal richness among different forest types, tree species, inventory method and length of experiment.

Forest type (deciduous/ conifer)	Tree species	Inventory method	Time since placement (years)	Relationship	Reference
Temperate (deciduous)	*Acer saccharum*	Molecular	10	Negative	This study
Temperate (both)	Several (incl. *Acer* spp.)	Molecular, sporocarp	6.5	Positive	1
Temperate (deciduous)	*Nothofagus solandri*	Culture, sporocarp	0.3–1	Negative	2
Temperate (both)	*Fagus sylvatica, Picea abies*	Molecular	–	Positive	3
Subtropical (both)	*Schima superba (Theacaceae), Pinus massoniana (Pinaceae)*	Molecular	1–2	Negative (weak, n. s.)	4
Subtropical (deciduous)	*Lithocarpus chintungensis, L. xylocarpus; Schima noronhae [Theaceae]*	Molecular Molecular, sporocarp	Variable	Negative	5
Boreal (conifer)	*Picea abies*	Molecular, Sporocarp, culture	6	Positive (weak, n. s.)	6
Boreal (conifer)	*Picea abies*	Molecular	12	None	7

[1]Kahl et al., (2017); [2]Fukami et al., (2010); [3]Hoppe et al., (2015); [4]Pietsch et al., (2019); [5]Yang et al., (2016); [6]Lindner et al., (2011); [7]Kubartová et al., (2015).

may be more competitive or efficient at producing these enzymes, or that inter-taxa antagonistic interactions reduced the overall enzyme production efficiency. We would expect these patterns to also play out in wood decomposition, particularly where a high occupancy reduces space availability, and where high fungal diversity leads to high redundancy in metabolic functions (Boddy, 2000). Competition for resources and predation by myxomycetes (molds; Fukasawa et al., 2018) and invertebrates (Crowther et al., 2011) can also contribute to this complex relationship. Multiple interactions take place within decaying wood, among fungal communities but also other organisms (e.g. grazers, bacteria), which may affect wood decomposition rates in complex ways (van der Wal et al., 2013). Further analyses incorporating the functional roles of WIF will be useful to evaluate these findings and to clarify how broadly these patterns apply to other systems.

Variance partitioning showed that a low proportion of variance (24%) in decomposition rates was explained by WIF composition, richness and soil temperature. Yet, maximum soil temperature was important in regulating decomposition at our site, a finding consistent with literature associating high temperature and moisture to increased CWD decomposition rates (e.g. Harmon et al., 1986; Mackensen et al., 2003; Pietsch et al., 2019). Moreover, this finding demonstrates the indirect effect of gaps on the fungal community driving decomposition, via its alteration of microclimatic conditions within and surrounding the logs (Perreault et al., 2020). Our study was designed to focus on linkages between the WIF community and decay rates, thus other factors including wood physico-chemical properties, moisture and understory plant community were not considered. Given that ecological studies often deal with high unexplained variance (Fukasawa et al., 2018; Pioli et al., 2018), we acknowledge that incorporating additional factors, particularly wood physicochemical properties, would likely have increased the variance explained for the sugar maple log decay rates. However, using WIF composition alone is inadequate for predicting the complex of factors involved in shaping the community (Purahong et al., 2018), including assembly history (Fukami et al., 2010). Therefore, we believe that our results point to forest structural diversity as a major factor driving wood decomposition rates, and they corroborate previous studies suggesting that the WIF community driving decomposition is directly influenced by disturbances such as canopy gaps (Brazee et al., 2014; Li et al., 2019). Our results also suggest that CWD addition (or retention) within a stand alters fungal species assemblages, possibly by promoting colonization and growth of host specialists.

5. Conclusions

We tested the relationship of fungal diversity to structural complexity and organic matter decomposition in a northern hardwood forest in which CWD was added and canopy gaps were experimentally created. Gaps significantly altered overall fungal community composition, likely due to microclimatic extremes earlier in the study that promoted fungal species adapted to temperature and moisture fluctuations. The addition of downed CWD was also important in shaping fungal species assemblages, and this treatment hosted a number of indicator species. Decomposition rates decreased with increasing fungal richness, pointing to antagonistic and competitive interactions among organisms taking place within decomposing CWD. A more detailed investigation of complex interactions of wood-inhabiting organisms (including bacteria, molds, invertebrates and other grazers) is needed to understand how changes in abiotic and biotic conditions can have a cascading effect through the community, thereby influencing decomposition rates and other functions. Moreover, since organic matter decay regulates C cycling in forested ecosystems, maintaining high fungal diversity could increase the capacity for C storage (Yang et al., 2016). Increasing forest structural complexity has been promoted as a climate change adaptation strategy (D'Amato et al., 2011); here we suggest that it could also serve as a mitigation strategy, given that greater complexity leads to greater fungal diversity, which in turn may enhance C storage in slowly decaying wood.

Data accessibility

Data and code of this paper will be archived at figshare depository within 2 years of publication.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jodi Forrester reports financial support was provided by National Institute of Food and Agriculture.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2022.101220.

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