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Isotopic and compositional evidence for carbon and nitrogen dynamics during wood decomposition by saprotrophic fungi

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ABSTRACT

Sporocarps of wood decay fungi contain functional information about how different taxa partition carbon and nitrogen resources from wood. We combined carbon and nitrogen concentrations, isotopic ratios (^{13}C:^{12}C, ^{15}N:^{14}N, and ^{14}C:^{12}C, expressed as $\delta^{13}C$, $\delta^{15}N$, and $\Delta^{14}C$ values), and compositional patterns in wood, cellulose, and sporocarps to investigate functional and isotopic differences in six taxa of decay fungi during log decomposition. Radiocarbon ($\Delta^{14}C$) measurements separated fungi into heartwood colonizers (*Fomitopsis* and *Hericium*, ~30+-year-old carbon) and sapwood colonizers (*Mycena*, *Hypholoma*, and *Trametes*, 1-12-year-old carbon). Decay modes influenced $\delta^{13}C$, with *Hericium*, a selective white-rot fungus, higher in $\delta^{13}C$ than nonselective white-rot fungi because *Hericium* preferentially assimilated ^{13}C-enriched hemicellulose rather than cellulose. Fungal $\delta^{15}N$ was lower in heartwood colonizers than in sapwood colonizers, presumably reflecting greater N turnover and ^{15}N enrichment in sapwood than in heartwood. Sporocarp $\delta^{15}N$ correlated with sporocarp %N and with the relative proportion of protein in N-containing pyrolysis products because fungal protein was 4–5‰ higher in $\delta^{15}N$ (and 3–4‰ higher in $\delta^{13}C$) than non-protein. From these measurements, we improved the quantitative and conceptual understanding of how sources, composition and metabolic processing determined isotopic composition of fungi.

1. Introduction

Coarse woody debris in forests is important as a carbon source and nutrient sink (Harmon et al., 1986; Stevens, 1997; Siitonen, 2001). Coarse woody debris is primarily decomposed by saprotrophic fungi that have developed specialized enzyme systems to degrade the recalcitrant compounds common in wood, especially lignin. These fungi derive their energy and carbon from the decay of relatively labile substances in wood, such as cellulose and hemicellulose, and are efficient in scavenging nitrogen from decomposing wood (Rayner and Boddy, 1988). However, the difficulty of investigating fungal processes in the field, particularly in complex, impermeable substrates such as wood, has hindered efforts to determine the exact carbon and nitrogen sources assimilated by such fungi.

One approach is to use carbon and nitrogen isotope ratios (^{13}C:^{12}C, ^{15}N:^{14}N, and ^{14}C:^{12}C; expressed as $\delta^{13}C$, $\delta^{15}N$, and $\Delta^{14}C$) as natural tracers of fungal C and N dynamics in wood decay fungi. Such measurements in fungi have proven useful in investigating the cycling of these elements by saprotrophic fungi in field studies (Kohzu et al., 1999, 2005; 2007; Hobbie et al., 2001; Taylor and Fransson, 2007). For example, atmospheric radiocarbon (^{14}C) increased from 1955 to 1963 because of ^{14}C generated during thermonuclear testing and has declined since then after the signing of the Nuclear Test Ban treaty; radiocarbon in fungi has been used to distinguish among ectomycorrhizal, litter decay, and wood decay fungi (Hobbie et al., 2002) because of the different ages of carbon assimilated by these three functional types. This approach could presumably be used to distinguish among colonization strategies of different taxa of wood decay fungi, such as heartwood (older) versus sapwood (younger) colonization. Similarly, the decline in $\delta^{13}C$ of atmospheric CO_2 because of anthropogenic addition of ^{13}C-depleted fossil fuels to the atmosphere (the Suess effect, McCarroll and Loader, 2004) could lead to lower

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$\delta^{13}C$ in fungi colonizing sapwood rather than heartwood. And finally, differences in $\delta^{15}N$ between N sources have been used to distinguish among ectomycorrhizal fungi, wood decay fungi, and litter decay fungi (Kohzu et al., 1999). This approach may also apply to fungi colonizing different log components, such as heartwood, sapwood, or bark, since field studies indicate little or no difference in $\delta^{15}N$ between wood decay fungi and their wood substrates (Kohzu et al., 2007).

Sources of carbon and their isotopic fractionation during biosynthesis can influence fungal isotopic patterns (Hobbie et al., 2012). For example, lignin is 3–4‰ depleted in ^{13}C relative to cellulose (Benner et al., 1987). Hemicellulose is somewhat higher in $\delta^{13}C$ than cellulose (Deines, 1980) and the pentose monomers of hemicellulose, xylose and arabinose, are higher in $\delta^{13}C$ than hexose monomers such as the glucose of cellulose (Teece and Fogel, 2007; Dungait et al., 2008, 2011). The dominant wood decomposers in forests, white-rot fungi, have good abilities to degrade lignin but do not incorporate lignin-derived carbon (Hobbie, 2005). Since wood decay fungi selectively assimilate wood carbohydrates and lose ^{13}C-depleted CO_2 during metabolism (Kohzu et al., 2005), sporocarps of wood decay fungi are generally enriched in ^{13}C by 3–4‰ relative to bulk wood and by ~2‰ relative to wood cellulose (Gleixner et al., 1993; Kohzu et al., 1999; Hobbie et al., 2001). White-rot fungi differ in their preferences for the two primary wood carbohydrates, hemicellulose and cellulose (Blanchette, 1991), with fungi preferentially attacking hemicellulose and lignin known as selective white-rot fungi and those attacking hemicellulose, cellulose, and lignin simultaneously known as nonselective white-rot fungi. These decay modes have not yet been linked to $\delta^{13}C$ patterns in wood decay fungi.

The chemical composition of fungi can also influence their isotopic patterns (Hobbie et al., 2012). For example, compounds are enriched in ^{13}C in the order protein > carbohydrates > chitin > lipids and enriched in ^{15}N in the order protein > chitin (Taylor et al., 1997). Thus, compositional information that provides the relative abundance of different compound classes may help to interpret isotopic patterns. One common technique to assess composition in environmental samples is pyrolysis coupled to gas chromatography-mass spectrometry (pyr-GC-MS) (Grandy et al., 2009; Wickings et al., 2012; Haddix et al., 2016). This technique, suitable for solid samples such as fungal and wood biomass, can be used to quantify the relative abundance of hundreds of individual compounds which can be then grouped into broad compound classes (e.g. proteins, carbohydrates, lipids), thus providing a 'fingerprint' of the chemical composition.

One opportunity to study resource use of wood decay fungi during decomposition began in 1985 at the H.J. Andrews Experimental Forest of Oregon, USA (Harmon et al., 1994). Experimental logs were of four dominant tree species of the Pacific Northwest, *Tsuga heterophylla*, *Pseudotsuga menziesii*, *Abies amabilis*, and *Thuja plicata*. Wood samples were collected from Year 0 (1985) and Year 10 (1995) and sporocarp samples of different fungal species were collected between Year 3 and Year 7. This provided a multi-year opportunity to study wood decay fungi and carbon and nitrogen dynamics during log decomposition by comparing isotopic patterns in wood and different fungal taxa.

Our hypotheses included:

(1) Radiocarbon can distinguish between sapwood (young) and heartwood (old) colonizers.
(2) Because atmospheric CO_2 has declined over time in $\delta^{13}C$ (the Suess effect), heartwood colonizers will have higher $\delta^{13}C$ signatures than sapwood colonizers.
(3) ^{13}C partitioning among different source compounds can alter $\delta^{13}C$ patterns. Fungi selectively targeting hemicellulose will be higher in $\delta^{13}C$ than those targeting cellulose or targeting both hemicellulose and cellulose.
(4) Fungal $\delta^{15}N$ will reflect the $\delta^{15}N$ of the colonized wood.
(5) Because different chemical classes differ in their isotopic values (e.g., protein is higher in $\delta^{13}C$ and $\delta^{15}N$ than chitin), sporocarp chemical composition will also influence $\delta^{13}C$ and $\delta^{15}N$ patterns.

To assess these hypotheses, we: (1) measured isotopic patterns in wood, bark, and wood decay fungi from the study; (2) assessed how fungi differed in chemical composition (from pyrolysis) and in age (from radiocarbon) of assimilated carbon; (3) tested how fungal composition, age of assimilated carbon, or fungal life history characteristics, such as colonization patterns (e.g., sapwood versus heartwood colonizers) or carbohydrate preference (e.g., hemicellulose versus cellulose), affected fungal $\delta^{13}C$ or $\delta^{15}N$.

2. Methods

Initial log characteristics are described in detail in Harmon et al. (1994). In September 1985, healthy trees of four conifer species were felled at the H.J. Andrews Experimental Forest on the west slope of the Cascade Range (44°10′N, 122°25′W) in Oregon, USA. Logs from the trees were placed at six sites on the forest floor. The species included *Tsuga heterophylla*, *Pseudotsuga menziesii*, *Abies amabilis*, and *Thuja plicata*. Logs averaged 52 cm in diameter (range 45–55 cm) and 5.5 m in length. An 8–10 cm thick cross section was removed from each end for nutrient analysis and divided into heartwood, sapwood, inner bark and outer bark. *Abies* bark was not separated between inner bark and outer bark. In 1995, additional cross sections were sampled and divided similarly. The number of annual rings in sapwood for *Abies*, *Pseudotsuga*, *Thuja*, and *Tsuga* were 12–40, 16–30, 7–20, and 35–50, respectively, whereas the annual ring numbers for these four species in heartwood were 60–77, 45–79, 80–200, and 33–60 (Appendix 1). We note that inner bark and outer bark tissues are created independently.

Sporocarps from wood decay fungi were harvested from the logs between 1988 and 1992. All sporocarps began formation after experimental establishment, as shown by the consistent orientation of the hymenium towards the ground. The host log species was recorded and a unique log number was assigned. Tissues of wood, bark, and sporocarps were coarsely ground, then finely ground with a Wiley mill to pass a 40-mesh screen. In total, 90 wood samples and 40 fungal samples were analyzed for %C, %N, $\delta^{13}C$, and $\delta^{15}N$. From 19 of the wood and bark samples, alpha-cellulose was extracted using standard procedures at Oregon State University (Roden et al., 2005). %C, %N, $\delta^{13}C$, and $\delta^{15}N$ were measured at the isotope laboratory at the U.S. Environmental Protection Agency in Corvallis, Oregon, USA. Stable isotope measurements ($\delta^{15}N$ and $\delta^{13}C$) are reported as $\delta^{n}X = (R_{sample}/R_{standard} - 1) \times 1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, n = 13 or 15, and X = C or N. The standard for $\delta^{15}N$ is atmospheric N_2, the standard for $\delta^{13}C$ is Pee Dee belemnite (limestone). The working standard was acetanilide. Standard deviations for acetanilide across all runs were 0.09‰ for $\delta^{15}N$ and 0.15‰ for $\delta^{13}C$. Ash content for different tree tissues in *P. menziesii* were taken from Schowalter and Morrell (2002) and used for all tree species, with those values calculated as 0.61 ± 0.13%, 0.88 ± 0.12%, 0.21 ± 0.07%, and 0.11 ± 0.05% for outer bark, inner bark, sapwood, and heartwood, respectively. Ash contents of sporocarps were subsequently estimated from elemental concentrations given in Harmon et al. (1994) and from the ash residue after combustion at 550 °C. Concentrations of carbon, nitrogen, and ash were used to estimate the ash-free concentrations of carbon and nitrogen.

Fourteen sporocarps were qualitatively examined for their chemical characteristics using pyrolysis-GC-MS. Two samples each of *Fomitopsis pinicola* (1990), *Hericium abietis* (1992), *Mycena occidentalis* (1992), *Oxyporus* sp. (1992), and *Trametes versicolor* (1990) were selected so that both samples of a species were from the same year, together with two samples of *Hypholoma capnoides* from the first collection year (1988) and two samples from the final collection year (1992). All samples were from two logs of *P. mensziesii*. We used a filament pyrolyzer (CDS Pyroprobe 5150) connected to a gas chromatograph (Thermo Fisher Scientific, Austin, Texas, USA) in line with a ITQ 900 mass spectrometer. Peaks corresponding to pyrolysis products were compared to reference spectra after deconvolution and extraction using AMDIS software and National Institute of Standards and Technology mass spectral libraries and published literature (Pouwels et al., 1989; Schulten and Schnitzer, 1997; Grandy et al., 2007; Kallenbach et al., 2016).

Twelve sporocarp samples were analyzed for ^{14}C content in 2016. The same samples as for pyrolysis GC-MS were selected except *Oxyporus* was not analyzed. Sporocarp material was converted to graphite targets at the Woods Hole Oceanographic Institution accelerator mass spectrometry facility. Results are expressed as $\Delta^{14}C$ values, as parts per thousand (‰) deviation of the ^{14}C:^{12}C ratio from the standard oxalic acid and corrected for mass-dependent isotopic fractionation using the concurrently measured $\delta^{13}C$ value of the sample (Hobbie et al., 2002). Sample $\Delta^{14}C$ values could then be corrected for ^{14}C decay back to any arbitrary year using the equation: $N = N_0 \times \exp(-kt)$, where $N_0 = \Delta^{14}C_{measured} + 1000‰$, $k = 1/8267$, the decay constant for ^{14}C, and t is the value in years.

Patterns in $\delta^{15}N$ and $\delta^{13}C$ signatures in wood or sporocarps were analyzed using stepwise multiple regression in JMP (JMP 13 Pro, SAS Institute, Middleton, Massachusetts, USA). Models that minimized the values of AICc (Akaike Information Criteria with a correction for sample size) were selected (Carleton et al., 2008). Independent variables for wood $\delta^{15}N$ and $\delta^{13}C$ included: species, wood type, year, species × wood type, species × year, wood type × year, and year × wood type × year. Independent variables for sporocarp $\delta^{15}N$ and $\delta^{13}C$ included C/N, $\log_e$ C/N, %N, $\log_e$ %N, %C, species, and year, with the ash-free values for %C and %N used. In addition, we also compared $\delta^{15}N$ and $\delta^{13}C$ among species using ANOVA and *post hoc* Tukey tests. For cellulose $\delta^{13}C$, there was only one relevant variable, tissue type, with log number as a random effect. Experimental data and statistical analyses are given as a Mendeley data set in Hobbie et al. (2019). To examine how closely patterns of pyrolysis among individual samples were related, hierarchical clustering was used with Ward's minimum variance method, as detailed in JMP.

3. Results

3.1. Wood

Wood carbon and nitrogen are sources for wood decay fungi, so comparing fungal and wood isotopic patterns can provide insight into fungal processing of this wood. Wood $\delta^{13}C$ was lowest in *Tsuga* (−26.2‰) and highest in *Thuja* (−25.0‰), and lower in outer bark (−26.7‰) than in sapwood (−24.6‰), with inner bark and heartwood intermediate (Appendix 2). The $\delta^{15}N$ values in *Abies* and *Tsuga* (~-4.6‰) were lower than in *Pseudotsuga* and *Thuja* (~-3.2‰) but did not differ significantly among tissues (Appendix 2; Hobbie et al., 2019). Nitrogen concentrations were lower in heartwood and sapwood (~0.05%) than in bark (~0.23%). C:N ratios therefore ranged from about 200 in inner bark to 1200 in heartwood (Appendix 2). In a multiple regression for $\delta^{13}C$ (adjusted $r^2 = 0.53$), wood type and the interaction of wood type and species accounted for 75% of explained variance, with year × wood type and year × species explaining the remainder (Appendix 3; Hobbie et al., 2019). In a multiple regression for wood $\delta^{15}N$, species, wood type, year × wood type, year × species, and year × species × wood type were significant factors (Appendix 4; Hobbie et al., 2019). Year alone did not significantly affect $\delta^{13}C$ or $\delta^{15}N$. However, in the regression, *Pseudotsuga* was 1.4‰ depleted and *Pseudotsuga* heartwood 1.8‰ depleted in ^{15}N in 1985 relative to 1995. Across all species, sapwood was 0.6‰ depleted in ^{15}N in 1985 relative to 1995.

Cellulose from wood and bark was enriched in ^{13}C relative to bulk wood and bark by 1.6 ± 0.1‰ and 2.7 ± 0.1‰, respectively. The $\delta^{13}C$ in bulk wood and the corresponding wood cellulose were strongly correlated (adjusted $r^2 = 0.967$); $\delta^{13}C$ in bulk bark and bark cellulose were also strongly correlated (adjusted $r^2 = 0.984$) (Fig. 1). Multiple regression for $\delta^{13}C$ of cellulose samples with the fixed factor of tissue type (sapwood, heartwood, inner bark, and outer bark) and the random factor of log number indicated that heartwood cellulose was 0.4 ± 0.3‰, 1.4 ± 0.3‰, and 1.6 ± 0.3‰ higher than cellulose of sapwood, outer bark, and inner bark, respectively (Appendix 5). We used the yearly $\delta^{13}C$ of atmospheric CO_2 (McCarroll and Loader, 2004) and the ages of sapwood and heartwood for the four species to calculate the average $\delta^{13}C$ of atmospheric CO_2 during the time of heartwood and sapwood formation. For *Abies*, *Pseudotsuga*, *Thuja*, and *Tsuga* respectively, values were −6.75‰, −6.77‰, −6.61‰, and −6.72‰ for heartwood and −7.22‰, −7.26‰, −7.25‰, and −7.08‰ for sapwood, for a ^{13}C enrichment of source CO_2 of heartwood relative to sapwood of 0.47‰, 0.49‰, 0.64‰, and 0.35‰.

3.2. Fungi

A total of 40 sporocarps were analyzed for $\delta^{13}C$, $\delta^{15}N$, %C, and %N from 10 different logs, with most (33) coming from two logs of *Pseudotsuga* and two logs of *Tsuga*. Ash-corrected fungal %N varied from 0.6% for *Fomitopsis* to 5.1% for *Mycena* and ash-corrected fungal %C varied from 40.4% for *Trametes* to 49.7% for *Mycena* (Table 1). Fungi differed by species in $\delta^{15}N$ and $\delta^{13}C$, as indicated in Fig. 2. *Oxyporus*, *Mycena*, *Hypholoma*, and *Trametes* were high in $\delta^{15}N$ whereas *Fomitopsis*, and *Hericium* were low. *Mycena* and *Hericium* were highest in $\delta^{13}C$ and *Fomitopsis* and *Trametes* were lowest.

In regression models of sporocarp $\delta^{15}N$ and $\delta^{13}C$, taxon was the

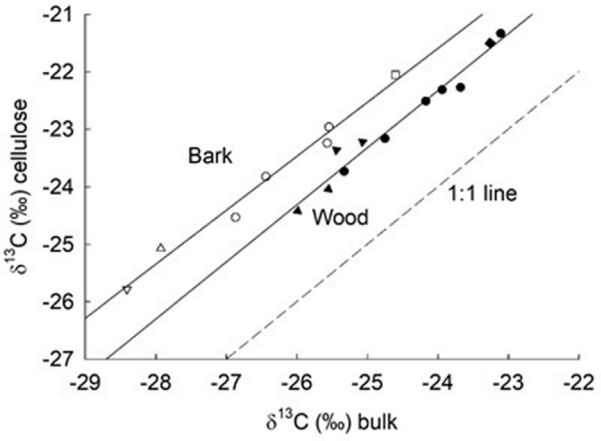

Fig. 1. Regression of wood alpha-cellulose versus bulk wood and bark cellulose versus bulk bark. Equations: Wood: $\delta^{13}C_{cellulose} = 0.993 \times \delta^{13}C_{wood} + 1.51‰$, $r^2 = 0.967$, $n = 11$; Bark: $\delta^{13}C_{cellulose} = 0.939 \times \delta^{13}C_{bark} + 0.94‰$, $r^2 = 0.984$, $n = 7$. Symbols are clear for bark and filled for wood, with species designated by circles (*Pseudotsuga*), upright triangles (*Tsuga*), downward triangles (*Abies*), and squares (*Thuja*).

Table 1
Ash content and ash-free %N and %C in fungal taxa. Ash content was calculated from Harmon et al. (1994).[1]Brown-rot, Mallams et al. (2010).[2]White-rot, Mallams et al. (2010).[3]White-rot, Kuhar et al. (2007).[4]White-rot, Hintikka (1970).[5]White-rot, Fackler et al. (2006).

Species	%N±se	%C±se	% ash	*n*
Fomitopsis pinicola[1]	0.56 ± 0.06	46.15 ± 0.90	0.70 ± 0.24	3
Hericium abietis[2]	1.44 ± 0.23	44.78 ± 1.48	7.26 ± 2.07	6
Hypholoma capnoides[3]	3.72 ± 0.12	46.68 ± 0.58	10.57 ± 0.91	20
Mycena occidentalis[4]	5.15 ± 0.07	49.72 ± 1.51	13.33 ± 0.46	4
Oxyporus sp.[5]	2.31 ± 0.09	43.24 ± 0.28	2.71 ± 0.08	4
Trametes versicolor[3]	0.89 ± 0.09	40.44 ± 0.30	1.60 ± 0.11	4

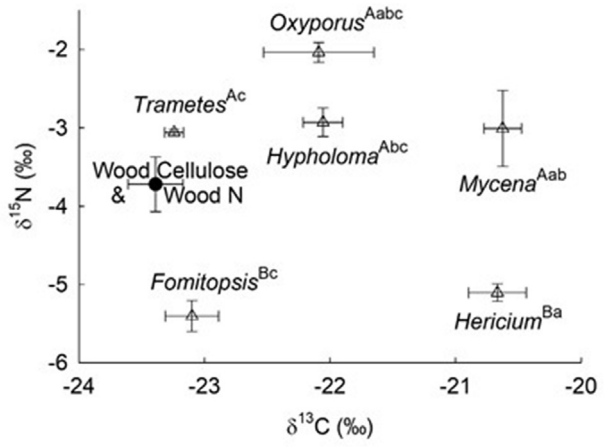

Fig. 2. $\delta^{15}N$ and $\delta^{13}C$ in wood decay fungi ± standard error. Taxon is indicated next to symbol. Tukey test results are indicated next to taxon, with capital letters for $\delta^{15}N$ and lower-case letters for $\delta^{13}C$. Taxa without the same letter differ at p = 0.05. Average wood cellulose $\delta^{13}C$ (n = 12) and bulk wood $\delta^{15}N$ (n = 17) across all species are also given ± standard error, with values averaged across sapwood and heartwood (filled circle).

most important factor. In regression models of sporocarps, fungal $\delta^{15}N$ was primarily affected by taxa and secondarily affected by year, with an overall adjusted r^2 of 0.744 (Table 2). For $\delta^{15}N$, *Trametes* was intermediate, *Fomitopsis* and *Hericium* were low, and the other three taxa were high. The $\delta^{15}N$ increased with time, by 0.27 ± 0.07‰ $year^{-1}$, and was primarily driven by multi-year patterns in *Hypholoma.* Fungal $\delta^{13}C$ was significantly affected by taxa and C/N (adjusted r^2 = 0.589), with *Hericium* and *Mycena* high in $\delta^{13}C$ relative to the other four taxa. C/N negatively affecting $\delta^{13}C$, with a coefficient of −0.015‰ C/N^{-1} (Table 3).

Fourteen sporocarps were analyzed by pyrolysis GC-MS. Pyrolysis products were assigned to different functional groups by origin (Table 4). Polysaccharides were the largest pool (37.5 ± 16.2% (sd), n = 14), followed by unknown origin (21.4 ± 3.0%), protein

Table 2
Stepwise multiple regression on sporocarp $\delta^{15}N$. Adjusted r^2 = 0.744,P < 0.001, n = 40. Factors included species and year. Species was 83% and year 17% of explained variance, respectively. Species coefficients were: *Fomitopsis pinicola* (*Fp*) and *Hericium abietis* (*Ha*), −1.49 ± 0.24‰; *Hypholoma capnoides* (*Hc*), *Mycena occidentalis* (*Mo*), and *Oxyporus sp.* (*Os*), 0.91 ± 0.12‰; and *Trametes versicolor* (*Tv*), −0.33 ± 0.24‰. Year of collection is a continuous variable, with 1985 corresponding to sporocarp year 0.

Term	%Variance	Estimate±se	P
Intercept	–	−5.15 ± 0.41	<0.001
Year	17.0	0.27 ± 0.07	<0.001
Fp&Ha&Tv − Mo&Hc&Os	73.2	−0.91 ± 0.12	<0.001
Fp&Ha − Tv	9.8	−0.58 ± 0.21	0.009

Table 3
Stepwise multiple regression on sporocarp $\delta^{13}C$. Adjusted r^2 = 0.589, n = 40. Variables included C/N and species. Species coefficients were: *Fomitopsis pinicola* (*Fp*), *Hypholoma capnoides* (*Hc*), *Oxyporus sp.* (*Os*), and *Trametes versicolor* (*Tv*), −0.79 ± 0.11‰; *Hericium abietis* (*Ha*) and *Mycena occidentalis* (*Mo*), 0.79 ± 0.11‰.

Term	%Variance	Estimate±se	P
Intercept	–	−21.03 ± 0.17	<0.001
C/N	15.4	−0.015 ± 0.005	0.004
Fp&Hc&Os&Tv − Ha&Mo	84.6	−0.79 ± 0.11	<0.001

(11.8 ± 8.6%), aromatic (11.5 ± 3.1%), N-bearing (10.8 ± 3.0%), and lipids (7.8 ± 8.4%). As confirmed by Tukey *post hoc* tests, *Mycena* and *Hypholoma* were high in protein and lipids while low in polysaccharides relative to the other four taxa. Compositional variability was primarily in the polysaccharide, lipid, and protein pools. In cluster analysis, the fleshy fungi (*Hericium*, *Mycena*, and *Hypholoma*) separated from the woody (*Fomitopsis* and *Oxyporus*) or leathery (*Trametes*) fungi, with *Hericium* and *Oxyporus* then separating from the initial two species clusters (Appendix 7). Ash-free % N correlated positively with protein (adjusted r^2 = 0.795, p < 0.001) and lipid (adjusted r^2 = 0.682, p < 0.001), and correlated negatively with polysaccharides (adjusted r^2 = 0.752, p < 0.001). Ash-free %N was uncorrelated with the N-bearing pool (adjusted r^2 = −0.064, p = 0.651), but strongly correlated with the proportion of protein ($frac_{pro}$) in the total N pool [protein/(protein + N-bearing)] (adjusted r^2 = 0.906, p < 0.001). Ash-free %C correlated strongest with the proportion of lipids in the combined lipids and carbohydrates pool [lipids/(lipids + carbohydrates)] (adjusted r^2 = 0.662, p < 0.001) and with the lipid pool itself (adjusted r^2 = 0.628), and was negatively correlated with the carbohydrate pool (adjusted r^2 = 0.490, p = 0.003). Fungal $\delta^{15}N$ and $\delta^{13}C$ correlated with the proportion of total N-containing compounds that were proteinaceous in origin ($frac_{pro}$), according to the equations $\delta^{15}N_{fungi}$ = 4.62 ± 1.19 × $frac_{pro}$ − 5.68 ± 0.58‰ (adjusted r^2 = 0.520, p = 0.001, n = 14) and $\delta^{13}C_{fungi}$ = 3.58 ± 0.89 × $frac_{pro}$ − 23.19 ± 0.43‰ + species effect, where the species effect was 0 for *Fomitopsis* and *Trametes*, -0.49 ± 0.21‰ for *Hypholoma* and *Oxyporus*, and 0.49 ± 0.21‰ for *Hericium* and *Mycena* (adjusted r^2 = 0.571, p = 0.004, n = 14; Appendix 8).

The 12 radiocarbon measurements clearly separated fungi by a Tukey test into two groups. *Fomitopsis* and *Hericium* had pre-1955 values lower than 0‰, and *Hypholoma*, *Mycena*, and *Trametes* had higher values, reflecting incorporation of ^{14}C derived from thermonuclear testing (Fig. 3). We took 1985, the year of log harvest, as Year 0. Estimated ages in years pre-1985 for the different taxa were then 30+ for *Hericium* and *Fomitopsis*, 12 for *Trametes*, 6 for *Hypholoma*, and one for *Mycena*. For these samples, we analyzed whether $\delta^{15}N$ correlated with radiocarbon age. In a stepwise regression on $\delta^{15}N$, lowest AICc was with %N and sample age as independent variables (adjusted r^2 = 0.922, n = 12, p < 0.0001, Table 5).

4. Discussion

4.1. $\delta^{13}C$ patterns

4.1.1. Patterns in tree tissues

The ^{13}C enrichment of cellulose relative to bulk tissue (Fig. 1) reflected the proportion of cellulose carbon in the tissue and its ^{13}C enrichment relative to other compounds, such as the general 3–4‰ enrichment in ^{13}C of cellulose relative to lignin (Benner et al., 1987). The higher ^{13}C enrichment of cellulose in bark than in wood arose because of the greater proportion of ^{13}C-depleted compounds in

Table 4
Compound classes in sporocarps from pyrolysis GC-MS analyses. Two samples per taxa except n = 4 for *Hypholoma*. The protein fraction was calculated as protein/(protein + N-bearing). [1]The shifts in $\delta^{13}C$ from compositional differences were calculated from the proportions of each class of pyrolysis products and an estimated ^{13}C offset from bulk from the literature of each of those classes (Appendix 6; Hobbie et al., 2019).

Parameter	*Fomitopsis* ±se (%)	*Hericium* ±se (%)	*Hypholoma* ±se (%)	*Mycena* ±se (%)	*Oxyporus* ±se (%)	*Trametes* ±se (%)
Aromatic	11.5 ± 2.5	15.7 ± 0.1	12.1 ± 1.7	8.2 ± 0.2	8.3 ± 0.6	12.8 ± 0.7
Lipid	2.3 ± 0.9^{c}	5.9 ± 1.0^{bc}	9.7 ± 1.1^{b}	25.2 ± 3.5^{a}	1.3 ± 0.2^{c}	0.6 ± 0.6^{c}
Polysaccharide	54.9 ± 0.8^{a}	40.7 ± 0.5^{a}	21.0 ± 2.4^{b}	19.8 ± 6.0^{b}	52.5 ± 0.3^{a}	52.6 ± 3.1^{a}
N-Bearing	10.9 ± 2.4	13.3 ± 0.3	12.7 ± 0.6	7.4 ± 0.3	8.6 ± 0.4	9.6 ± 4.5
Protein	2.0 ± 0.3^{c}	8.4 ± 0.5^{bc}	21.1 ± 2.7^{a}	19.6 ± 0.6^{ab}	7.7 ± 0.8^{bc}	2.7 ± 0.1^{c}
Unknown	18.9 ± 0.8^{b}	16.9 ± 0.1^{b}	24.6 ± 0.9^{a}	21.5 ± 1.7^{ab}	21.5 ± 0.3^{ab}	21.7 ± 1.5^{ab}
Protein fraction	0.16 ± 0.05^{e}	0.39 ± 0.02^{cd}	0.62 ± 0.02^{ab}	0.72 ± 0.01^{a}	0.47 ± 0.04^{bc}	0.25 ± 0.08^{de}
$\delta^{13}C$ shift[1]	0.06	−0.15	0.11	−0.45	0.45	0.13

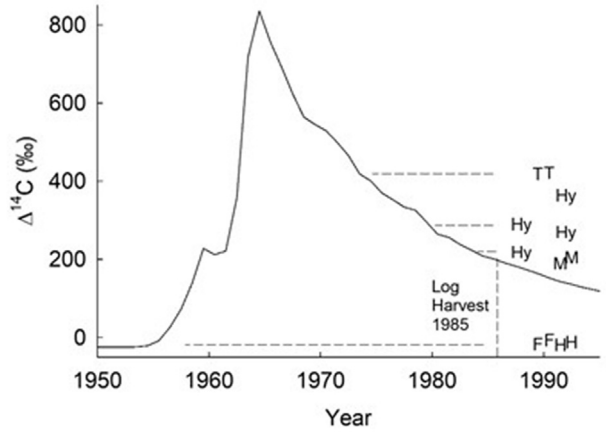

Fig. 3. Yearly radiocarbon ($\Delta^{14}C$) values of Northern Hemisphere atmospheric carbon dioxide (solid line), together with sporocarp radiocarbon versus year of sampling. Samples are abbreviated as *Trametes*, T; *Hypholoma*, Hy; *Mycena*, M; *Hericium*, H; *Fomitopsis*, F. Log harvest in September 1985 is indicated by a vertical dashed line; age of assimilated carbon (years before 1985) is proportional to length of horizontal dashed lines.

bark than in wood. For example, *Tsuga* bark is about 40% lignin (using proximate methods) and 25% cellulose, whereas *Tsuga* wood may be 40% cellulose (Harmon, 1992).

Several factors contributed to bark cellulose being 1–1.6‰ lower in $\delta^{13}C$ than wood cellulose. One potential factor is the addition of anthropogenic, ^{13}C-depleted CO_2 to the atmosphere since the Industrial Revolution (the Suess effect), which decreased the $\delta^{13}C$ of atmospheric CO_2 by 1.17‰ by 1985 relative to pre-industrial levels, with 0.67‰ of that decline just since 1961 (McCarroll and Loader, 2004). As a result, the $\delta^{13}C$ of fixed sugars used for tissue construction should be lower in bark than in sapwood, and lower in sapwood than in heartwood. Although we did not have age estimates for inner bark and outer bark, the volume of outer bark was about twice that of inner bark (Harmon et al., 1994). If we assume that outer bark represents twice the number of years of inner bark, then the ~0.2‰ enrichment in cellulose ^{13}C of outer bark relative to inner bark suggests that inner bark may consist of 16–24 years of growth (1985–~1962–1970) and outer bark 32–48 years of growth (1985–~1938–1954), with an average $\delta^{13}C$ of atmospheric CO_2 for these periods between −7.36‰ (1970–1985) and −7.05‰ (1938–1985). Given the average estimated $\delta^{13}C$ of atmospheric CO_2 during the time of sapwood formation and heartwood formation of −7.20‰ and −6.69‰, bark cellulose is lower in $\delta^{13}C$ than can be accounted for by shifts in the $\delta^{13}C$ of atmospheric CO_2. Thus, about 1‰ of the depletion in ^{13}C of bark cellulose relative to wood cellulose may reflect other factors, such as the contribution of bark photosynthesis to bark carbon (Gartner, 1996), since ^{13}C-depleted, tree-respired CO_2 would be the dominant source for such photosynthesis (Cernusak et al., 2001).

Table 5
Fungal $\delta^{15}N$ correlates with age (in years) and %N. Adjusted $r^2 = 0.922$, n = 12, $p < 0.001$. Ash-free %N explained 31.9% of variance, age explained the remaining 68.1% (defined as years pre-1985 that carbon assimilated by fungi was fixed by trees). $\delta^{13}C$, C/N, and log C/N, and the fraction of N-containing pyrolysis products that were protein were included in stepwise but were unimportant. Age was set to 30 years for the four samples with $\Delta^{14}C$ less than 0‰.

Term	%Variance	Estimate±se	P
Intercept	–	−3.76 ± 0.43	<0.001
Ash-free %N	31.9	0.275 ± 0.096	0.019
Age	68.1	−0.058 ± 0.014	0.002

4.1.2. Patterns in fungi

Fungal pyrolysis GC-MS of fungi provided a novel way to examine fungal chemical composition. Patterns of pyrolysis products were species-specific (Table 4), and accordingly could be used in future studies to investigate links between patterns of wood decay and the transformation of wood into soil organic matter via specific fungi. Pyrolysis GC-MS has primarily been used on plant and soil samples, and it would be particularly helpful for studies of fungal composition to optimize identification of pyrolysis products that could be traced to fungal melanin, lipids, chitin, and nucleic acids, as these compounds play key roles in fungal protection, decomposition rates, structural support, and reproduction. Because several amino acids contain sulfur but the above four compound classes do not, identifying pyrolysis products of sulfur-containing amino acids could also be a priority, as it would aid in partitioning fungal protein from other N-containing compounds in fungi. In addition, future work should elucidate the specific origin of the pyrolysis products currently labeled as of unknown origin, which were 17–27% of all pyrolysis products. For example, the most common of the 184 identified pyrolysis products was toluene, at 6.3% of pyrolysis products, and it has been classified as of unknown origin. However, it has been identified as derived in some instances from protein (Bracewell and Robertson, 1984), and including it in the protein pool increased the correlation (adjusted r^2) between ash-free %N and protein from 0.764 to 0.795 (n = 14).

Because polymeric carbohydrates within wood such as cellulose, rather than wood itself, are the dominant carbon sources for most decay fungi, $\delta^{13}C$ patterns in cellulose are useful for understanding fungal carbon sources and ^{13}C enrichment from cellulose to fungi. Wood primarily consists of ^{13}C-enriched carbohydrates and ^{13}C-depleted lignin (Benner et al., 1987). The ^{13}C enrichment of wood decay fungi relative to wood reflected several factors, including the loss of ^{13}C-depleted CO_2 during respiration (Kohzu et al., 2005), a

1.5–2‰ metabolic enrichment in ^{13}C in fungi relative to assimilated substrates, the lack of incorporation of lignin-derived carbon (^{13}C-depleted), and the preferential assimilation of cellulose or carbohydrate-derived carbon in wood (^{13}C-enriched) (Gleixner et al., 1993; Hobbie, 2005). Given the 1.6‰ enrichment in ^{13}C of wood cellulose relative to bulk wood and fungal metabolic enrichment in ^{13}C, we expected a 3–4‰ enrichment in ^{13}C of fungi relative to bulk wood, as seen in prior studies. Our average ^{13}C enrichment was similar at 3.11 ± 0.28‰, based on an average wood $\delta^{13}C$ in *Pseudotsuga* and *Tsuga* of −24.91 ± 0.24 (n = 24) and average sporocarp $\delta^{13}C$ of −21.80 ± 0.15‰ (n = 40).

A second factor likely influencing $\delta^{13}C$ in fungi was the isotopic patterns of the main classes of compounds comprising the sporocarps. For example, the high $\delta^{13}C$ of protein, intermediate $\delta^{13}C$ of carbohydrates, and low $\delta^{13}C$ of lipids were used by Hobbie et al., 2012 to account for differences in sporocarp $\delta^{13}C$. Here, the coefficient of the protein fraction with $\delta^{13}C$ was 3.58 ± 0.89, indicating that sporocarp protein was 3–4‰ higher in ^{13}C than non-protein components. Gleixner et al. (1993) further suggested that chitin should be depleted in ^{13}C relative to fungal carbohydrates because the chitin monomer N-acetyl glucosamine is synthesized from a glucose molecule plus a ^{13}C-depleted, two-carbon acetyl-coA molecule. The presence of ^{13}C-depleted, carbon-rich waxes in sporocarps (Jelsma and Kreger, 1978) should also decrease the $\delta^{13}C$ of the sporocarps while increasing the carbon concentration. The high %C, low %N, and low $\delta^{13}C$ of *Fomitopsis* suggested a high proportion of ^{13}C-depleted lipids such as pinicolic acid and agaric acid (Laver and Fang, 1986; Petrova et al., 2007); however, pyrolysis indicated that lipid relative abundance was quite low, and calculations suggest that composition itself is unlikely to account for the observed differences. We estimated that compositional differences accounted for a 0.4‰ enrichment in *Oxyporus* and 0.4‰ depletion in *Mycena* relative to *Fomitopsis*, *Hericium*, *Hypholoma* and *Trametes* (Table 4 and Appendix 6). The high %N of *Mycena* (and *Hypholoma*) suggested a high proportion of ^{13}C-enriched protein, as confirmed by pyrolysis, but the high proportion of ^{13}C-depleted lipid content of *Mycena* counteracted this factor.

A third factor that could influence $\delta^{13}C$ patterns is the site of wood decay. For example, the white-rot fungus *H. abietis* colonizes heartwood (Mallams et al., 2010) and may accordingly assimilate older wood carbohydrates that are higher in $\delta^{13}C$. Its $\delta^{13}C$ was high at about −20.6‰. However, cellulose $\delta^{13}C$ in heartwood is only 0.4‰ higher than sapwood, which will be difficult to detect given natural variability. Radiocarbon results confirmed that *Hericium* colonized heartwood, since it averaged over 30 years in age of assimilated carbon. Based on the radiocarbon age of 30+ years for the brown-rot fungus *F. pinicola*, it also primarily assimilated heartwood carbon, although it is reported as colonizing sapwood initially and then heartwood (Mallams et al., 2010). Despite colonizing heartwood it was lower in $\delta^{13}C$ than *Hericium*. One plausible explanation is simply that *Fomitopsis* fractionates less against ^{13}C in its respiration and metabolism than other fungi.

Such differences in ^{13}C enrichment relative to supplied carbohydrates among fungal taxa under controlled growth conditions are common, with reported ^{13}C enrichments in different taxa of 1.4‰ and −0.7‰ relative to assimilated glucose/agar, −0.1‰ and −0.6‰ relative to assimilated glucose, and 0.3 ± 1.4‰ (sd, 12 taxa) relative to assimilated sucrose (summarized in Hobbie, 2005). Here, compositional differences among species can only account for a maximum shift of 0.9‰, with a standard deviation of only 0.3‰ (Table 4). In contrast, actual differences among species varied up to 2.6‰ (Fig. 2), with a standard deviation of the species means of 1.1‰.

Assimilation of non-cellulosic carbon in trees, such as sucrose or hemicellulose, could also drive $\delta^{13}C$ patterns. In living wood, sucrose, the transport form of carbon in trees, is higher in $\delta^{13}C$ than the tree ring cellulose formed from this sucrose (Rinne et al., 2015). This is attributed to a ^{13}C depletion by invertase during the cleavage of sucrose into glucose and fructose, with glucose the ultimate precursor for cellulose formation. In the experimental system here, living trees were cut into logs, and the sapwood of those logs should presumably be quite high in sucrose. This sucrose should be quickly assimilated by decay organisms and could then plausibly serve as a ^{13}C-enriched source for saprotrophic fungi colonizing the sapwood. From radiocarbon measurements, *Mycena* assimilated carbon only a year or two old, so is the most likely candidate for using sucrose, and it was also ^{13}C-enriched (along with *Hericium*) relative to the other taxa in Fig. 2 and in the statistical analysis (Table 3). The other taxa colonizing sapwood, *Hypholoma* (six years old) and *Trametes* (12 years old) had lower $\delta^{13}C$ values and were older, suggesting they assimilated little sucrose. Both *T. versicolor* and *H. capnoides* have numerous enzymes adapted to wood degradation (Gramss et al., 1998; Tanaka et al., 1999).

Wood hemicellulose will persist for decades or centuries, since its assimilation requires specialized enzymes (Doria et al., 2014). In Table 3, *Hericium* is significantly higher in $\delta^{13}C$ than other taxa except *Mycena*. We suggest that one contributing factor may be the strong ability of *H. abietis* to access hemicellulose relative to cellulose in wood, as hemicellulose and its constituent pentose sugars appear higher in $\delta^{13}C$ than cellulose (Deines, 1980; Dungait et al., 2008). In the related species *Hericium erinaceus*, xylanase activity was high relative to cellulase (Schimpf and Schutz, 2016) and hemicellulose was preferentially removed during wood decomposition (Jalč et al., 1997). In contrast, cellulose was preferentially removed during 16 weeks of kenaf decomposition by *Oxyporus latemarginatus,* with cellulose content declining from 55% to 28% whereas hemicellulose content remained constant at 27–28% (Halis et al., 2012). Thus, preferential assimilation of cellulose-derived carbon by *Oxyporus* may contribute to its low $\delta^{13}C$ signature in our study. *Hypholoma fasciculare* is a nonselective white-rot fungus (Šnajdr et al., 2010). We assume that *H. capnoides* should also be nonselective in its decay mode, which should contribute to its low $\delta^{13}C$ values relative to *Hericium*. The different ages pre-1985 of the carbon of *Hypholoma* (six years) and *Hericium* (30+ years) will also contribute to the higher $\delta^{13}C$ of *Hericium*, given that the $\delta^{13}C$ of atmospheric CO_2 in 1979 was −7.4‰ and in 1955 was −6.9‰. This difference is about a third of the 1.6‰ ^{13}C enrichment in the regression analysis of *Hericium* relative to *Hypholoma* (Table 3).

In Fig. 4, we show the possible patterns of ^{13}C partitioning during sucrose transport, cellulose synthesis, assimilation of sucrose-derived, hemicellulose-derived, and cellulose-derived carbon, and fungal synthesis of different compounds. Hemicellulose is composed of both hexoses and pentoses (primarily xylose and arabinose), with the pentoses arising by decarboxylation of the C_6 atom of glucose. Since this C_6 atom is about 5‰ depleted in ^{13}C relative to the other glucose atoms (Hobbie and Werner, 2004), its removal should lead to pentoses being approximately 1‰ enriched in ^{13}C relative to the source glucose (Dungait et al., 2008). Hemicellulose should accordingly also be enriched in ^{13}C relative to cellulose, depending on the relative proportion of pentose carbon versus hexose carbon (see Fig. 4).

4.2. $\delta^{15}N$ patterns

With information on the proportion of total N-containing compounds that were proteinaceous in origin ($frac_{pro}$), we could estimate the ^{15}N enrichment of protein relative to other N-containing compounds. Here, the coefficient of the protein fraction with $\delta^{15}N$ was 4.62 ± 1.19, indicating that sporocarp protein was

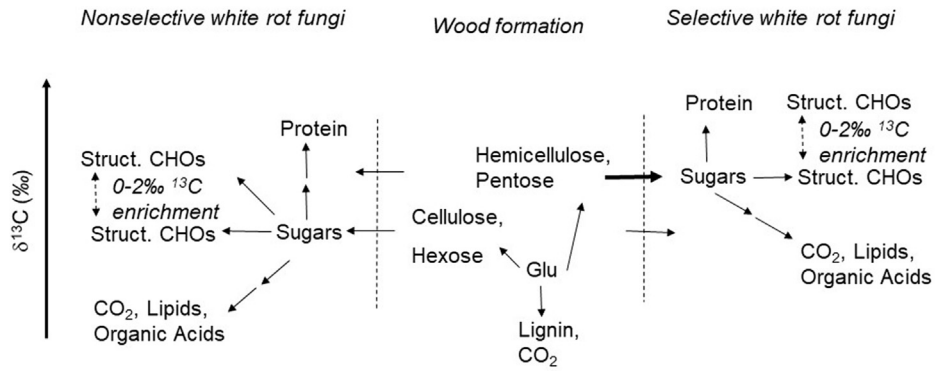

Fig. 4. Movement and isotopic fractionation of carbon isotopes between wood and saprotrophic fungi, illustrating how fungi consuming cellulose and fungi consuming sucrose or hemicellulose could differ isotopically. Isotopic fractionation along non-horizontal arrows is 1–2‰. As indicated on the figure, variable ^{13}C enrichment of structural carbohydrates relative to assimilated sugars can produce a range of $\delta^{13}C$ values for fungal carbohydrates. Patterns derived from Gleixner et al. (1993), Benner et al. (1987), Hobbie (2005), and Rinne et al. (2015). Struct. CHOs = structural carbohydrates, Glu = glucose, Suc = sucrose, Hemi = hemicellulose.

4–5‰ higher in ^{15}N than non-protein components, although this assumes that protein and N-bearing compounds contain similar quantities of N. Pyrolysis (Py-GC-MS) provides the relative abundance of individual compounds and compound classes (Grandy et al., 2007; Haddix et al., 2016), rather than the relative abundances of the N within these compounds, so quantifying pyrolysis products into proteinaceous and non-proteinaceous components may not reflect accurately the true proportions of these two nitrogen pools in sporocarps. Indirect evidence for this is shown in Appendix 8, where nitrogen concentrations are a non-linear function of increases in the proportion of pyrolysis products of protein origin, with toluene included as one of these proteinaceous products, according to the equation $\%N_{ash\text{-}corrected} = 0.71 - 2.44 \times frac_{pro} + 11.74 \times frac^2_{pro}$ (adjusted $r^2 = 0.972$). This result suggests that proteinaceous pyrolysis products contain more N on average than pyrolysis products classified as 'N-bearing'.

The significant correlation of $\delta^{15}N$ with year (Table 2) could derive from several factors. One possibility is that it reflected the increasing proportion of ^{15}N-enriched microbially derived nitrogen accessed by fungi over several years of sporocarp production, with ^{15}N-depleted N lost as gaseous N. The small increases in $\delta^{15}N$ in sapwood between 1985 and 1995 are consistent with this, as are the somewhat larger ^{15}N increases calculated in the regression model for *Pseudotsuga* (Appendix 4). A second possibility is that N_2 fixation by microbes in the decomposing wood (Larsen et al., 1978; Griffiths et al., 1993) has added N somewhat higher in $\delta^{15}N$ than the original wood. Nitrogen fixation in decaying wood would also increase the N content and $\delta^{15}N$, since wood $\delta^{15}N$ averaged −3 to −4‰, lower than the 0‰ to −2‰ range commonly cited for N fixation. Nitrogen fixation in well-decayed logs contributed sufficient N to increase the $\delta^{15}N$ of *P. abies* logs in a Finnish study (Rinne et al., 2017); although nitrogen budgets were not quantified, N fixation also contributed to decaying logs at all decay stages in the Pacific Northwest, with higher fixation in sapwood than in heartwood (Hicks et al., 2003). Although the rates at which N were added to logs in the Finnish study were rather low, particularly in the early stages of wood decay, at less than 10 mg N yr^{-1} kg^{-1} wood. It therefore seems unlikely that fixed nitrogen was added at sufficient levels to alter the $\delta^{15}N$ of sapwood nitrogen in just a few years.

The fungal taxa separated rather clearly in regression analysis into two groups of either high (*Hypholoma*, *Mycena*, *Oxyporus*) or low (*Fomitopsis*, *Hericium*) $\delta^{15}N$, with *Trametes* intermediate (Table 2). Species varied consistently in composition. If species information was removed, then sporocarp %N became a significant factor (Table 5), presumably reflecting the relative proportion of N that was ^{15}N-enriched protein rather than ^{15}N-depleted chitin. However, the age of assimilated carbon was the most important factor in this analysis. It varied from one year (*Mycena*) to 30 years (*Hericium* and *Fomitopsis*), which given the age coefficient of −0.058‰/year, would correspond to a 1.7‰ decline in $\delta^{15}N$ from *Mycena* to the two heartwood colonizers.

In prior studies, many wood decay fungi have $\delta^{15}N$ values resembling the decomposed wood on which they were found, with sporocarps only 0.4 ± 1.2‰ higher than the corresponding decomposed wood (Kohzu et al., 1999). Unfortunately, the wood sampled here was not directly linked to the sporocarps collected, so

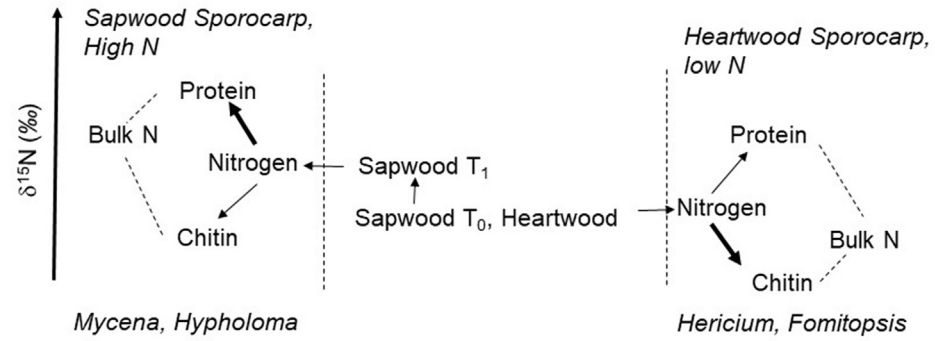

Fig. 5. Movement and isotopic fractionation of nitrogen isotopes in sapwood and in heartwood, showing ^{15}N increases in sapwood from T_0 to T_1 and acquisition by heartwood fungi of N from primary decomposition. Compositional effects (e.g, protein content) are also shown. Sporocarps under high N conditions derive more N from ^{15}N-enriched protein, sporocarps under low N conditions derive more N from ^{15}N-depleted chitin.

we relied on the wood sampled in 1985 and 1995 for comparison. Here, the fungi colonizing heartwood were actually lower in $\delta^{15}N$ than wood by about 1.5‰ (Fig. 2) and ~2‰ lower than sapwood colonizers (Table 2), with little influence of composition (N content) on within-species variability. We suggest that this reflected differences in the $\delta^{15}N$ of available N in heartwood and sapwood in early decomposition, with higher rates of N turnover in sapwood leading to ^{15}N enrichment of the available N, which is then assimilated by sapwood-colonizing fungi (Fig. 5).

This scenario is consistent with the increased $\delta^{15}N$ of many wood components between 1985 and 1995 (Appendix 4) and consistent with the increased sporocarp $\delta^{15}N$ with year of collection (Table 2) because of the rapid turnover of sapwood N. This distinguishing between sapwood-derived N and heartwood-derived N was perhaps only possible because of the unique conditions of this wood decay experiment, in which sporocarps were collected from fresh logs, allowing the N dynamics of early decomposition in sapwood and heartwood to be studied in fungi of different strategies.

5. Conclusions

The suite of measurements provided several new insights into interpreting carbon and nitrogen isotope signatures in wood decay fungi. Radiocarbon clearly could distinguish between fungi colonizing primarily sapwood (young) or primarily heartwood (old) (Hypothesis 1). Although cellulose of sapwood and heartwood should differ by ~0.4‰ in $\delta^{13}C$ because of the Suess effect, these differences were too small to translate clearly into the hypothesized higher $\delta^{13}C$ of fungi colonizing heartwood compared to those colonizing sapwood (Hypothesis 2). However, one of the heartwood colonizers, *Hericium*, was quite high in $\delta^{13}C$, which was attributed to preferential assimilation of ^{13}C-enriched hemicellulose (Hypothesis 3) rather than simultaneous assimilation of hemicellulose and cellulose, as inferred from literature studies. In contrast, low $\delta^{13}C$ of *Oxyporus* may derive from preferential assimilation of cellulose. Based on correlations between C/N, %N, protein fraction, and stable isotope ratios, sporocarp composition influenced $\delta^{13}C$ and $\delta^{15}N$ patterns (Hypothesis 5), although the detailed analyses from pyrolysis measurements indicated that differences in composition only caused small shifts in sporocarp $\delta^{13}C$ (Table 4). Finally, we suggested that the lower $\delta^{15}N$ of the two species colonizing heartwood (compared to those colonizing sapwood) reflected both ^{15}N partitioning between chitin (low $\delta^{15}N$) and protein (high $\delta^{15}N$) in sporocarp formation and the additional factor of increased $\delta^{15}N$ of available N in sapwood because of microbial processing, despite bulk measurements in wood not showing this clearly (Hypothesis 4).

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Supplementary data

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