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

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A B S T R A C T
Sporocarps of wood decay fungi contain functional information about how different taxon 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.

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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...
$\delta^{13}$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 (Tece 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 13C-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 (Slezak 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 in 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 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 $\delta(^{15}N) = (R_{sample}/R_{standard} - 1) \times 1000$, where $R = ^{13}C/^12C$ 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 acetonitrile. Standard deviations for acetonitrile 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 trees, 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 oc- cidentalis (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. menziesii. 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 14C 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 δ 14C values, as parts per thousand (%δ) deviation of the 14C/12C ratio from the standard oxalic acid and corrected for mass-dependent isotopic fractionation using the concurrently measured δ13C value of the sample (Hobbie et al., 2002). Sample δ14C values could then be corrected for 14C decay back to any arbitrary year using the equation: N = N0 × exp (−kt), where N0 = δ14Cmeasured + 1000‰, k = 1/8267, the decay constant for 14C, and t is the value in years.

Patterns in δ15N and δ13C 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 δ15N and δ13C included: species, wood type, year, species × wood type, species × year, wood type × year, and year × wood type × year. Independent variables for sporocarp δ15N and δ13C included C/N, log C/N, %N, log %N, %C, species, and year, with the ash-free values for %C and %N used. In addition, we also compared δ15N and δ13C among species using ANOVA and post hoc Tukey tests. For cellulose δ13C, there was only one relevant variable, tissue type, with log number as a random effect. Experimental data and statistical analyses are given as a Mendeleev 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 δ13C 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 δ15N values in Abies and Tsuga (−4.6‰) were lower than in Pseudotsuga and Thuja (−3.2‰) but did not differ significantly among species (Appendix 2; Hobbie et al., 2019). Nitrogen concentrations were lower in heartwood than in 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 δ13C (adjusted r2 = 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 δ15N, 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 δ13C or δ15N. However, in the regression, Pseudotsuga was 1.4‰ depleted and Pseudotsuga heartwood 1.8‰ depleted in 15N in 1985 relative to 1995. Across all species, sapwood was 0.6‰ depleted in 15N in 1985 relative to 1995.

Cellulose from wood and bark was enriched in 13C relative to bulk wood and bark by 1.6 ± 0.1‰ and 2.7 ± 0.1‰, respectively. The δ13C in bulk wood and the corresponding wood cellulose were strongly correlated (adjusted r2 = 0.967); δ13C in bulk bark and bark cellulose were also strongly correlated (adjusted r2 = 0.984) (Fig. 1). Multiple regression for δ13C 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 δ13C of atmospheric CO2 (McCarroll and Loader, 2004) and the ages of sapwood and heartwood for the four species to calculate the average δ13C of atmospheric CO2 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 δ13C enrichment of source CO2 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 δ13C, δ15N, %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 δ15N and δ13C, as indicated in Fig. 2. Oxyporus, Mycena, Hypholoma, and Trametes were high in δ13C whereas Fomitopsis, and Hericium were low. Mycena and Hericium were highest in δ13C and Fomitopsis and Trametes were lowest.

In regression models of sporocarp δ15N and δ13C, taxon was the
Table 1

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<th>Species</th>
<th>%N_{se}</th>
<th>%C_{se}</th>
<th>% ash</th>
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<td>Fomitopsis pinicola³</td>
<td>0.56 ± 0.06</td>
<td>46.15 ± 0.90</td>
<td>0.70 ± 0.24</td>
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<td>Hericium abietis³</td>
<td>1.44 ± 0.23</td>
<td>44.78 ± 1.48</td>
<td>7.26 ± 2.07</td>
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<tr>
<td>Hypholoma capnoides³</td>
<td>3.72 ± 0.12</td>
<td>46.68 ± 0.58</td>
<td>10.57 ± 0.91</td>
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<tr>
<td>Mycena occidentalis⁴</td>
<td>5.15 ± 0.07</td>
<td>49.72 ± 1.51</td>
<td>13.33 ± 0.46</td>
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<tr>
<td>Oxyporus sp.⁵</td>
<td>2.31 ± 0.09</td>
<td>43.24 ± 0.28</td>
<td>2.71 ± 0.08</td>
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<tr>
<td>Trametes versicolor⁶</td>
<td>0.89 ± 0.09</td>
<td>40.44 ± 0.30</td>
<td>1.60 ± 0.11</td>
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Table 2

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<td>–21.03 ± 0.17</td>
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<tr>
<td>C/N</td>
<td>15.4</td>
<td>–0.015 ± 0.005</td>
<td>0.004</td>
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<tr>
<td>Fp&amp;Hc&amp;Os&amp;Tv – Ha&amp;Mo</td>
<td>84.6</td>
<td>–0.79 ± 0.11</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3

Stepwise multiple regression on sporocarp δ¹³C. Adjusted r² = 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). -0.97 ± 0.11‰, Hericium abietis (Ha) and Mycena occidentalis (Mo), 0.79 ± 0.11‰.

4. Discussion

4.1. δ¹³C patterns

4.1.1. Patterns in tree tissues

The δ¹²C enrichment of cellulose relative to bulk tissue (Fig. 1) reflected the proportion of cellulose carbon in the tissue and its δ¹³C enrichment relative to other compounds, such as the general 3–4‰ enrichment in δ¹³C of cellulose relative to lignin (Benner et al., 1987). The higher δ¹³C enrichment of cellulose in bark than in wood arose because of the greater proportion of δ¹³C-depleted compounds in

Fig. 2. δ¹⁵N and δ¹³C in wood decay fungi ± standard error. Taxon is indicated next to symbol. Tukey test results are indicated next to taxon, with capital letters for δ¹⁵N and lower-case letters for δ¹³C. Taxa without the same letter differ at p < 0.05. Average wood cellulose δ¹³C (n = 12) and bulk wood δ¹³C (n = 17) across all species are also given ± standard error, with values averaged across sapwood and heartwood (filled circle).
that outer bark represents twice the number of years of inner bark, then the ~0.2‰ enrichment in cellulose 13C 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 δ13C of atmospheric CO2 for these periods between ~7.3‰ (1970—1985) and ~7.0‰ (1938—1985). Given the average estimated δ13C of atmospheric CO2 during the time of sapwood formation and heartwood formation of ~7.20‰ and ~6.9‰, bark cellulose is lower in δ13C than can be accounted for by shifts in the δ13C of atmospheric CO2. Thus, about 1‰ of the depletion in δ13C of bark cellulose relative to wood cellulose may reflect other factors, such as the contribution of bark photosynthesis to bark carbon (Gartner, 1996), since 13C-depleted, tree-respired CO2 would be the dominant source for such photosynthesis (Cernusak et al., 2001).

### 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 toulene, 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 r2) 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, δ13C patterns in cellulose are useful for understanding fungal carbon sources and 13C enrichment from cellulose to fungi. Wood primarily consists of 13C-enriched carbohydrates and 13C-depleted lignin (Benner et al., 1987). The 13C enrichment of wood decay fungi relative to wood reflected several factors, including the loss of 13C-depleted CO2 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 $^{13}$C of Pseudotsuga and Tsuga of −24.91 ± 0.24 (n = 24) and average sporocarp $^{13}$C of −21.80 ± 0.15‰ (n = 40).

A second factor likely influencing $^{13}$C enrichment in fungi was the isotopic patterns of the main classes of compounds comprising the sporocarps. For example, the high $^{13}$C of protein, intermediate $^{13}$C of carbohydrates, and low $^{13}$C of lipids were used by Hobbie et al., 2012 to account for differences in sporocarp $^{13}$C. Here, the coefficient of the protein fraction with $^{13}$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 $^{13}$C of the sporocarps while increasing the carbon concentration. The high $^{13}$C, low $^{15}$N, and low $^{13}$C of Fomitopsis suggested a high proportion of $^{13}$C-depleted lipids such as pinicolic acid and agoric 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 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 $^{15}$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 $^{13}$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 $^{13}$C. Its $^{13}$C was high at about −20.6‰. However, cellulose $^{13}$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 $^{13}$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 ± 0.1% (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 $^{13}$C patterns. In living wood, sucrose, the transport form of carbon in trees, is higher in $^{13}$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 $^{13}$C values and were older, suggesting they assimilated little sucrose. Both T. versicolor and H. capnoides have numerous enzymes adapted to wood degradation (Grams 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 $^{13}$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 $^{13}$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 (Jalc et al., 1997). In contrast, cellulose was preferentially removed during 16 weeks of kenaf decomposition by Oxyopus 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 Oxyopus may contribute to its low $^{13}$C signature in our study. Hypholoma fasciculare is a nonselective white-rot fungus (Snajdr et al., 2010). We assume that H. capnoides should also be nonselective in its decay mode, which should contribute to its low $^{13}$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 $^{13}$C of Hericium, given that the $^{13}$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. $^{15}$N patterns

With information on the proportion of total N-containing compounds that were proteinaceous in origin (frac$_{protein}$), we could estimate the $^{15}$N enrichment of protein relative to other N-containing compounds. Here, the coefficient of the protein fraction with $^{15}$N was 4.62 ± 1.19, indicating that sporocarp protein was
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 $\%N_{\text{ash-corrected}} = 0.71 - 2.44 \times \text{frac}_{\text{pro}} + 11.74 \times \text{frac}_{\text{pro}}^2$ (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, Oxyergus) 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 $-0.058\%/\text{year}$, 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 $0.4 \pm 1.2\%$ higher than the corresponding decomposed wood (Kohzu et al., 1999). Unfortunately, the wood sampled here was not directly linked to the sporocarps collected, so
we relied on the wood sampled in 1985 and 1995 for comparison. Here, the fungi colonizing heartwood were actually lower in δ15N 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 δ15N of available N in heartwood and sapwood in early decomposition, with higher rates of N turnover in sapwood leading to δ15N enrichment of the available N, which is then assimilated by sapwood-colonizing fungi (Fig. 5).

This scenario is consistent with the increased δ15N of many wood components between 1985 and 1995 (Appendix 4) and consistent with the increased sporocarp δ15N 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 δ13C because of the Susa effect, these differences were too small to translate clearly into the hypothesized higher δ13C of fungi colonizing heartwood compared to those colonizing sapwood (Hypothesis 2). However, one of the heartwood colonizers, Hericium, was quite high in δ13C, which was attributed to preferential assimilation of δ13C-enriched hemicellulose (Hypothesis 3) rather than simultaneous assimilation of hemicellulose and cellulose, as inferred from literature studies. In contrast, low δ13C of Oxyporus may derive from preferential assimilation of cellulose. Based on correlations between C:N, δ3N, protein fraction, and stable isotope ratios, sporocarp composition influenced δ13C and δ15N patterns (Hypothesis 5), although the detailed analyses from pyrolysis measurements indicated that differences in composition only caused small shifts in sporocarp δ13C (Table 4). Finally, we suggested that the lower δ15N of the two species colonizing heartwood (compared to those colonizing sapwood) reflected both 15N partitioning between chitin (low δ15N) and protein (high δ15N) in sporocarp formation and the additional factor of increased δ15N 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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