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Strategies of carbon and nitrogen acquisition by saprotrophic and ectomycorrhizal fungi in Finnish boreal *Picea abies*-dominated forests

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ABSTRACT

We compared the $\delta^{13}C$ and $\delta^{15}N$ of forest material with an extensive sporocarp collection to elucidate the role of litter, wood and soil as fungal carbon and nitrogen sources in Finnish boreal *Picea abies*-dominated forests. Ectomycorrhizal *Hydnum* and *Cortinarius* had higher $\delta^{15}N$ than other ectomycorrhizal fungi, suggesting use of ^{15}N-enriched, deeper nitrogen. *Russula* had lower $\delta^{15}N$ than other ectomycorrhizal fungi and resembled some litter decay genera, suggesting use of litter-derived nitrogen. There was little variation in $\delta^{15}N$ among other genera of ectomycorrhizal fungi, indicating limited functional diversity in nitrogen use. Saprotrophic *Leotia*, *Gymnopus*, *Hypholoma*, *Pholiota*, *Rhodocollybia* and *Calocera* had $\delta^{15}N$ values similar to ectomycorrhizal fungi, indicating overlap in use of older nitrogen from soil or roots or use of newly fixed nitrogen. Genera of litter and wood decay fungi varied up to 6‰ in $\delta^{13}C$ and 10‰ in $\delta^{15}N$, suggesting large differences in carbon and nitrogen sources and processing. Similar $\delta^{13}C$ between white and brown rot wood decay fungi also suggest that white rot fungi do not use lignin-derived carbon. Together, these $\delta^{13}C$ and $\delta^{15}N$ patterns of fungi from Finnish boreal forests enhance our knowledge of fungal functional diversity and indicate broad use of litter, wood and soil resources.

1. Introduction

Fungi play an important role in decomposing organic matter, cycling nutrients and moving carbon and nitrogen within the forest soil profile. In forests, wood is an essential component of carbon and nitrogen cycling (Harmon et al., 1986; Smith and Read, 2008) and serves as a habitat and source of resources (nutrients and water) for many fungi (Jonsson et al., 2005). Understanding the role of wood and other substrates as a carbon and nitrogen source for fungi in forests will be important for determining how carbon cycling, nitrogen cycling, and fungal communities are affected by intensive forest management in Fennoscandia that significantly reduces the presence of decaying trees.

The 50 million hectares of boreal coniferous forest in Fennoscandia are dominated by Norway spruce (Picea abies) and Scots pine (Pinus sylvestris) (Esseen et al., 1997). Finnish boreal forests have roughly 1500 species of saprotrophic fungi (Siitonen, 2001) (i.e., fungi that get their carbon from the decay of litter, cones and wood (Rayner and Boddy, 1998)) in it alone). Over 2000 taxa of fungi were identified in a Norway spruce-dominated stand used in this study, with ~700 taxa specialized on dead wood (Mäkipää et al., 2017). These fungi are probably already affected by intensive forest management, as 19 % of assessed fungal polypore species that are important wood decomposers in Fennoscandia are already red-listed, with 12 % declining because of a lack of dead wood (Hyvärinen et al., 2019). Still, this rather well-studied group represents only a small portion of wood-inhabiting fungi and much is unknown about fungal decomposers (Rajala et al., 2010; Mäkipää et al., 2017).

One main conduit for belowground carbon transport, ectomycorrhizal fungi, relies on plant-transferred sugars as their carbon source and in return supply plants with essential nutrients such as nitrogen and phosphorus. They can compose 47–84 % of fungal biomass in boreal forest soil (Bååth et al., 2004) and are responsible for storing most soil carbon and making boreal forest soil a carbon sink (Clemmensen et al., 2013). Fungi further contribute to carbon

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cycling by decomposing organic matter such as wood as well as mediating root-associated respiration and carbon allocation belowground (Clark et al., 2002). Within wood, soft-rot fungi first colonize and degrade more labile carbon, such as cellulose and hemicellulose (Daniel and Nilsson, 1998). Then brown- and white-rot fungi colonize and further degrade wood compounds, such as lignin (Baldrian and Lindahl, 2011). Because wood is low in nitrogen, a necessary nutrient for fungal growth for extracellular enzyme activity required to colonize wood, fungi may both import nitrogen to overcome nitrogen limitation in wood (Boberg et al., 2008; Rinne-Garmston et al., 2016) and recycle nitrogen from old to new mycelium (Lilly et al., 1991). In addition, asymbiotic nitrogen fixation contributes to nitrogen stocks in decaying wood. For example, in Finnish boreal forests over 60 % of accumulated nitrogen in highly decayed wood is attributed to nitrogen fixation (Rinne-Garmston et al., 2016). These nitrogen additions increase total nitrogen content in wood with decay stage due to increased fungal mycelia, fungal nitrogen transfer, and colonization by bacterial nitrogen fixers (Boddy and Watkinson, 1995; Clausen, 1996; Rinne-Garmston et al., 2016).

Diversity of fungi in function and niche is necessary to maintain ecosystem processes, such as decomposing organic matter and transferring nitrogen to plants (Fukami et al., 2010; Valentín et al., 2014). This functional role of fungi is reflected in their stable isotope ratios. In general, the ratio of stable carbon isotopes (^{13}C:^{12}C, expressed as $\delta^{13}C$) is lower for fungi that use newer assimilated plant carbon than fungi that use older litter or wood and the ratio of stable nitrogen (^{15}N:^{14}N, expressed as $\delta^{15}N$) is lower for fungi that use plant litter nitrogen compared to those that use older organic nitrogen in soils (Kohzu et al., 1999; Hobbie and Ouimette, 2009). Comparing the $\delta^{13}C$ and $\delta^{15}N$ of fungi and of potential carbon and nitrogen sources can reveal the importance of these sources for different taxa.

Ectomycorrhizal fungi dominate the organic horizons below the litter layer and further decompose soil organic matter and transfer nutrients to plants (Lindahl et al., 2007). Ectomycorrhizal fungi can further be separated into those that prefer labile or insoluble carbon and nitrogen sources and can be grouped as ectomycorrhizae that are hydrophilic or hydrophobic, respectively (Lilleskov et al., 2011). Hydrophilic ectomycorrhizae use labile carbon and nitrogen sources from newer organic material in shallower soils while hydrophobic ectomycorrhizae have higher proteolytic capabilities and use insoluble carbon and nitrogen sources from older organic material in shallow and deeper soils, but the degree to which fungi utilize these deep and shallow soil carbon and nitrogen sources can still vary by species (Chen et al., 2016). When characterizing fungi in boreal forests, it is helpful to group them by such functional classifications as litter and wood decay saprotrophic fungi and hydrophilic and hydrophobic ectomycorrhizal fungi, as this can indicate whether fungi decay wood, litter, or organic matter within the soil profile and whether they transfer nitrogen to plants to facilitate plant growth.

The objective of this study was to determine the role of forest litter, wood and soil as fungal carbon and nitrogen sources by comparing the $\delta^{13}C$ and $\delta^{15}N$ of potential carbon and nitrogen sources for fungi with an extensive collection of sporocarps from Norway spruce-dominated forests. We predicted, as is generally the case, that the $\delta^{15}N$ of ectomycorrhizal fungi that use older organic nitrogen in the soil should be higher than that of saprotrophic fungi that use newer nitrogen sources from decaying plant matter (Högberg et al., 1999; Hobbie et al., 1999) and that $\delta^{13}C$ of ectomycorrhizal fungi that use recent photosynthates will be lower than those that use older carbon from wood or the forest floor (Hobbie et al., 1999; Hobbie, 2005). Furthermore, we hypothesized that wood-associated fungi have unique carbon and nitrogen sources associated with the decay stage of wood and would therefore have distinct $\delta^{13}C$ and $\delta^{15}N$ patterns among one another dependent on the decay stage of wood. By clarifying the carbon and nitrogen sources of an extensive collection of fungi from several Finnish boreal forests using stable isotope analysis, we could identify the role of these fungi in decomposing different types of carbon and nitrogen sources including wood.

2. Methods

Three study sites in Sipoo (60°28′N, 25°12′E), Lapinjärvi (60°39′N, 26°7′E), and Loppi (60°48′N, 24°10′E), all within 70–130 km of one another, are composed of unmanaged forests dominated by Norway spruce (*Picea abies* [L.] Karst) on relatively fertile soil (representing mesic and herb-rich heath forests according to Cajander's site type classification; Hotanen et al., 2008). The volume of living trees was over 400 m^3 ha^{-1} (with Norway spruce over 65 % of the total volume) and the volume of dead trees was over 120 m^3 ha^{-1} in Sipoo and Lapinjärvi sites and 67 m^3 ha^{-1} in Loppi site (for details see Rajala et al., 2012). Sampling was done within an area of 5625 m^2 (75 × 75 m).

We sampled sporocarps of macrofungi growing on the forest floor and on decaying logs in Sep-Oct in 2014 and 2015. For a subset of data collected from Sipoo, the decay stage of the tree that each wood decay fungi was collected from was also recorded. For another subset of data from all three sites, the matter that fungi were collected from (moss, wood, etc.) was also noted. The collected fungi were categorized as saprotrophic wood decay and litter decay fungi and fungi with hydrophobic and hydrophilic ectomycorrhizae (Agerer, 2006; www.mycokey.com). Tree foliage, litter and stemwood were also sampled from nine mature Norway spruce trees for each site. The average distance between sample trees within a site was 25 m. From each tree, we took 2–3 sample branches at a height of 15 m and one stemwood sample using an increment borer (5 mm diameter) at a tree height of 1.3 m. From the collected branches, we took samples of 40–50 current-year needles and woody material (ranging in diameter by 2–3 cm and length by 5–10 cm). Litter samples of needles and twigs were collected from the soil surface from the vicinity of the sample trees. Nine soil cores to a depth of 15 cm were sampled per site for both organic and mineral soil layers. Soil samples were also taken close to the sample trees. Fine root samples from each soil core were collected and separated by soil horizon (mineral and organic) layers.

Dead wood samples (n = 105–126 decay logs per site) representing different decay stages were collected in 2008 (Rajala et al., 2012). Stage of decay was estimated according to stem hardness and general constitution (Harmon and Sexton, 1996). We used a five-class decay scale: I = recently dead tree, II = weakly decayed, III = medium decayed, IV = very decayed, V = almost decomposed (see Mäkinen et al., 2006 for more detailed description of each stage). From each site we also collected nine bryophyte samples. A 7 cm × 7 cm sample of *Pleurozium schreberi* excluding litter and dead parts of the bryophyte was collected by hand.

All samples were transferred to a cool room (+4 to −20 °C) until further processing in the laboratory. Thereafter samples were stored at − 20 °C before freeze drying (sporocarps, fine roots) or drying at 60 °C (other samples) and homogenization. Ground sporocarps, soil, wood and other plant material were analyzed for %C, %N, $\delta^{13}C$, and $\delta^{15}N$ using a Costech 4010 Elemental Analyzer coupled to a Thermo Delta Plus XP IRMS at the University of New Hampshire. Wood samples with high C/N were run twice with different masses to analyze both C and N content separately. Isotopic values are reported as $\delta^{13}C$ and $\delta^{15}N$ (‰) relative to standards VPDB for carbon and atmospheric N_2 for nitrogen with the delta notation $\delta^{13}C$ or $\delta^{15}N$ that is equivalent to $(R_{sample}/R_{standard} - 1)$ x 1000 (‰). Standard deviations

of laboratory standards (tuna, Underhill Oa, Underhill Bs, NIST 1515 apple leaves, and NIST 1575a pine needles) for $\delta^{15}N$ and $\delta^{13}C$ averaged less than 0.3‰.

Variance components in fungal $\delta^{15}N$ and $\delta^{13}C$ were first analyzed with three-level linear mixed-effects models with functional type, genus, and species as nested random effects. On the basis of this preliminary analysis, genus was included as a random effect in the subsequent models when analyzing the effects of functional type, % nitrogen (%N), and % carbon (%C) of fungi on fungal $\delta^{13}C$ and $\delta^{15}N$. Pair-wise comparisons between functional types were conducted using Tukey's post hoc test that takes into account multiple comparisons (Bretz et al., 2011). Due to obvious inequality of genus-level variances, nonparametric Mann-Whitney U tests were used to compare $\delta^{13}C$ and $\delta^{15}N$ of individual genera of fungi. Effects of wood decay stage on wood decay fungi were explored with a linear regression model including genus as a fixed effect to allow for interactions of genus and tree decay stage. A categorical variable constructed from all available combinations of site and year of collection was used as a covariate in all models to filter out site and year effects. Statistical analyses were carried out in R environment statistical computing (R Core Team, 2014).

3. Results

3.1. Source carbon and nitrogen content

Among the carbon and nitrogen sources sampled, mineral soil was the most ^{15}N-enriched with an average $\delta^{15}N$ value of 2.5 ± 0.2‰ across all three sites. The soil organic layer and roots from the mineral soil layer had the next highest $\delta^{15}N$ of all nitrogen sources, with an average value of −2.1 ± 0.2‰ and −1.9 ± 0.2‰, respectively. Roots from the soil organic layer, needles, needle litter, twig litter, branches, moss and wood had $\delta^{15}N$ values ranging from −3.7‰ to −5.6‰ (Fig. 1). The $\delta^{15}N$ of wood in decay stages I-IV ranged from −5.3 to −5.6‰ whereas that of wood in decay stage V was somewhat higher at −4.7 ± 0.1‰. $\delta^{15}N$ of roots in mineral soil and in organic soil were more negative than their associated soil layers by −4.4‰ and −2‰, respectively. Aboveground plant material including needles, needle litter, twig litter, branches and wood were more depleted in ^{15}N than root material.

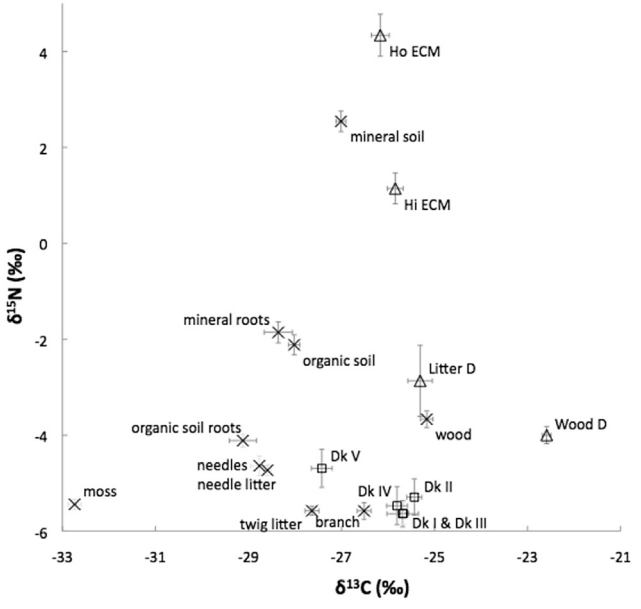

Fig. 1. Mean $\delta^{13}C$ and $\delta^{15}N$ of carbon and nitrogen sources (X), including wood in decay stages I-V (□ Dk I-V) and 4 fungal functional groups (Δ), including fungi with hydrophilic (Hi ECM) and hydrophobic (Ho ECM) ectomycorrhizae, litter decay fungi (Litter D) and wood decay fungi (Wood D) across all three sites. Bars represent SE.

Decaying wood from stages I-IV had the highest $\delta^{13}C$ values, ranging from −25.2‰ to −25.8‰. Branches, mineral soil, decaying wood in stage V and twig litter $\delta^{13}C$ followed from −26.5‰ to −27.6‰. The soil organic layer, roots from the mineral soil layer, needles, needle litter and roots from the soil organic layer ranged from −28 to −29.1‰. Moss had a substantially lower $\delta^{13}C$ than other carbon sources, averaging −32.7 ± 0.1‰ across the different sites. Wood in decay stages I-II had the lowest average %N, ranging from 0.06–0.07 %, then increased to 0.12 % in decay stage III, 0.23 % in decay stage IV and 0.29 % in decay stage V. The %N of branches followed wood with an average of 0.26 % and significantly increased again to 0.70 % for twig litter and 0.73–0.88 % in roots in mineral and organic soil. Needles, moss and needle litter had %N ranging from 1.01-–1.57 % and organic and mineral soil layers had %N of 0.16–1.40 %, respectively (Table 1).

3.2. $\delta^{15}N$ and $\delta^{13}C$ of fungal functional groups

While most of the variation in $\delta^{13}C$ between fungal species can be explained by differences between functional types, genus is also an important factor in the variation of fungal $\delta^{15}N$ (Table S1). However, since between-species variation within genus was smaller than residual variation for both response variables, species was not included as a factor in any of the subsequent models. Overall, $\delta^{15}N$ of saprotrophic fungi was lower than that of ectomycorrhizal species (Table S2). In $\delta^{13}C$, wood decay fungi was clearly and significantly different from other groups. The apparent differences in $\delta^{15}N$ between fungal species with hydrophobic and hydrophilic ectomycorrhizae and between litter decay and wood decay fungi (Table S2, Fig. 1) were not statistically significant when controlling for site and year of collection and within-genus correlation. Both brown and white rot species were grouped together as wood decay fungi, as the difference in average $\delta^{13}C$ and $\delta^{15}N$ between the two types of fungi was less than 0.5‰ for both isotopes ($p = 0.3768$ and 0.7998, respectively).

Carbon and nitrogen content of fungi with hydrophilic and hydrophobic ectomycorrhizae and litter decay fungi were similar and %N ranged from 3.6 % to 5.3 % while %C ranged from 43 % to 43.9 %. Wood decay fungi, however, had a much higher carbon to nitrogen ratio due both to a higher carbon content (45 % carbon) and lower nitrogen content (3.0 % nitrogen) (Table S2).

3.3. Variation within fungal functional groups by genera

Among ectomycorrhizal fungi, only *Russula* was significantly depleted in ^{15}N and only *Cortinarius* and *Hydnum* were significantly enriched in ^{15}N (Table 2) compared to other ectomycorrhizal fungi. Only *Hydnum* was significantly enriched in ^{13}C and only *Cortinarius* significantly depleted. All other genera of hydrophilic and hydrophobic ectomycorrhizal fungi were isotopically similar to each other (Fig. 2). Both saprotrophic wood decay and litter decay fungal groups varied more in $\delta^{13}C$ and $\delta^{15}N$ among genera than ectomycorrhizal fungi (Fig. 3) with both saprotrophic functional groups including genera that were significantly depleted in ^{15}N (litter decay *Micromphale* and wood decay *Fomitopsis*) and genera that were significantly enriched in ^{15}N (litter decay *Leotia* and *Rhodocollybia* and wood decay *Calocera, Galerina, Gymnopus*, and *Hypholoma*; Table 3). Meanwhile, genus-level values of $\delta^{13}C$ were tightly associated with the functional group with litter decay genera generally lower in $\delta^{13}C$ than wood decay genera, except for *Asterodon* (Table 3).

Table 1
Mean ± SE of $\delta^{13}C$, $\delta^{15}N$, %C and %N of carbon and nitrogen sources. Difference among materials for $\delta^{13}C$, $\delta^{15}N$, %C and %N are reported for $\alpha = 0.050$ and $t = 1.968$ (Student's t test).

Material (*n*)	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Branch (27)	-26.5 ± 0.1^{b}	-5.6 ± 0.2^{de}	51.3 ± 0.1^{cd}	0.26 ± 0.01^{f}
Wood Decay I (6)	-25.7 ± 0.3^{a}	-5.6 ± 0.3^{def}	49.5 ± 0.4^{de}	0.06 ± 0.01^{ij}
Wood Decay II (19)	-25.4 ± 0.2^{a}	-5.3 ± 0.4^{cde}	49.5 ± 0.2^{e}	0.07 ± 0.01^{i}
Wood Decay III (24)	-25.7 ± 0.1^{a}	-5.6 ± 0.2^{e}	50.5 ± 0.3^{de}	0.12 ± 0.01^{hij}
Wood Decay IV (26)	-25.8 ± 0.2^{a}	-5.5 ± 0.4^{f}	52.2 ± 0.5^{bc}	0.23 ± 0.02^{fg}
Wood Decay V (3)	-27.4 ± 0.4^{bcde}	-4.7 ± 0.1^{cde}	55.7 ± 1.2^{a}	0.29 ± 0.04^{fgh}
Organic soil (26)	-28.0 ± 0.1^{de}	-2.1 ± 0.2^{b}	46.1 ± 1.1^{g}	1.40 ± 0.05^{b}
Organic soil roots (27)	-29.1 ± 0.3^{g}	-4.1 ± 0.1^{c}	51.2 ± 0.2^{cd}	0.88 ± 0.04^{d}
Mineral soil (22)	-27.0 ± 0.1^{c}	2.5 ± 0.2^{a}	5.0 ± 0.5^{h}	0.16 ± 0.02^{ghj}
Mineral soil roots (23)	-28.4 ± 0.3^{ef}	-1.9 ± 0.2^{b}	47.5 ± 0.5^{f}	0.73 ± 0.04^{e}
Moss (15)	-32.7 ± 0.1^{h}	-5.4 ± 0.1^{de}	46.8 ± 0.1^{f}	1.01 ± 0.04^{c}
Needles (27)	-28.8 ± 0.2^{fg}	-4.6 ± 0.2^{cd}	51.2 ± 0.2^{cd}	1.03 ± 0.02^{c}
Needle litter (27)	-28.6 ± 0.1^{fg}	-4.7 ± 0.1^{cde}	50.3 ± 0.2^{de}	1.57 ± 0.04^{a}
Twig litter (26)	-27.6 ± 0.1^{d}	-5.6 ± 0.1^{de}	52.8 ± 0.2^{ab}	0.70 ± 0.03^{e}

Table 2
Mean ± SE of $\delta^{15}N$ and $\delta^{13}C$ for each genus of fungi with hydrophilic (Hi ECM) and hydrophobic (Ho ECM) ectomycorrhizae, difference from the mean of all other ECM fungi (*d* ($\delta^{15}N$), *d* ($\delta^{13}C$)) and the *p*-value of a nonparametric Mann-Whitney U test for statistically significant difference (*p* ($\delta^{15}N$), *p* ($\delta^{13}C$)); *p* of less than 0.05 marked in bold.

Genus (*n*)	Functional Type	$\delta^{15}N$ (‰)	*d* ($\delta^{15}N$)	*p* ($\delta^{15}N$)	$\delta^{13}C$ (‰)	*d* ($\delta^{13}C$)	*p* ($\delta^{13}C$)
Amanita (9)	Hi ECM	2.8 ± 0.8	−0.2	0.900	−25.7 ± 0.4	0.3	0.428
Cantharellus (5)	Hi ECM	1.2 ± 0.6	−1.8	0.204	−25.9 ± 0.5	0.1	0.869
Lactarius (17)	Hi ECM	1.6 ± 0.4	−1.6	0.061	−25.9 ± 0.3	0.1	0.985
Russula (12)	**Hi ECM**	**−0.8 ± 0.4**	**−4.2**	**<0.001**	−25.9 ± 0.3	0.0	0.969
Cortinarius (25)	**Ho ECM**	**5.4 ± 0.4**	**3.4**	**<0.001**	**−26.4 ± 0.2**	**−0.6**	**0.017**
Hydnum (3)	**Ho ECM**	**12.3 ± 0.9**	**9.7**	**0.003**	**−24.6 ± 0.1**	**1.4**	**0.012**
Leccinum (2)	Ho ECM	2.0 ± 1.8	−0.9	0.635	−26.2 ± 0.0	−0.3	0.691
Paxillus (4)	Ho ECM	2.8 ± 0.7	−0.2	0.748	−26.0 ± 0.1	−0.1	0.847
Piloderma (14)	Ho ECM	2.3 ± 0.7	−0.7	0.439	−25.9 ± 0.2	0.1	0.697
Suillus (3)	Ho ECM	3.7 ± 1.4	0.8	0.487	−25.6 ± 0.7	0.3	0.599
Tylopilus (2)	Ho ECM	0.4 ± 0.1	−2.6	0.213	−25.4 ± 0.7	0.5	0.404

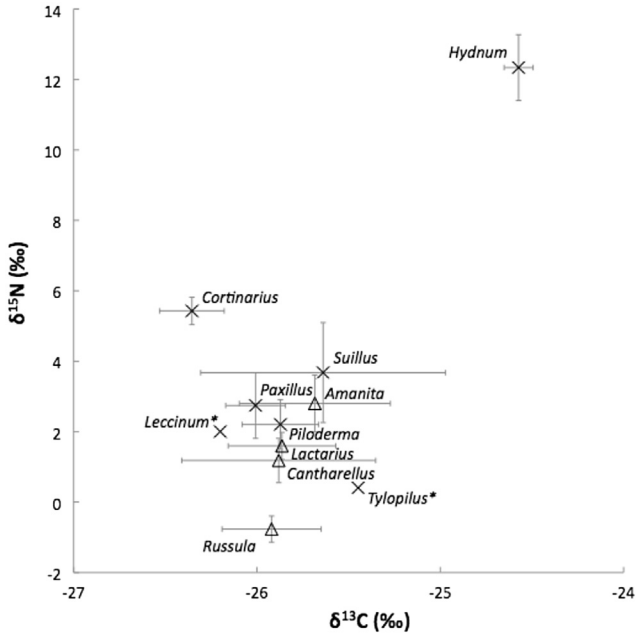

Fig. 2. Mean $\delta^{13}C$ and $\delta^{15}N$ of different genera of fungi with hydrophobic (X) and hydrophilic (Δ) ectomycorrhizae. Bars represent SE. * Indicates $n = 2$.

Ectomycorrhizal fungi with hydrophobic and hydrophilic hyphae varied in %N from 2.82 % to 4.98 % (Table 4). %N of saprotrophic fungi, however, varied widely (1–9 %), whereas %C varied from 39 % to 50 %. Both %N and %C were significant predictors in the linear mixed-effects model of fungal $\delta^{15}N$, but neither was significant in the model of $\delta^{13}C$ (Table 5). The model of $\delta^{15}N$ that allowed for interaction of %N and functional group revealed that %N only had a significant effect on $\delta^{15}N$ of Ho ECM (Table 6).

3.4. Effect of carbon and nitrogen sources on fungal isotopic patterns

Only $\delta^{15}N$ patterns of the polypore genus *Heterobasidion* were significantly correlated with decay stage of wood, specifically when decay stage I was compared to further decay stages (Table 7). Other types of carbon and nitrogen sources from which additional sporocarps were collected from were also noted within a subset of data from the Sipoo site in 2013 and 2014. Similar linear regression models on this subset of data, including genus and carbon and nitrogen sources as factors of fungal $\delta^{13}C$ and $\delta^{15}N$, did not show significant effect of carbon and nitrogen sources.

4. Discussion

Analysis of carbon and nitrogen content and isotope patterns of an extensive collection of fungi and their associated carbon and nitrogen sources in Norway spruce-dominated forest sites in Finland revealed that different fungal genera have distinct niches of carbon and nitrogen sources, even within their respective functional groups, and builds upon a growing body of knowledge about fungal functional diversity. Although ectomycorrhizal and saprotrophic fungi differed in their $\delta^{15}N$ and $\delta^{13}C$ patterns as hypothesized based on earlier studies, genera within each fungal group

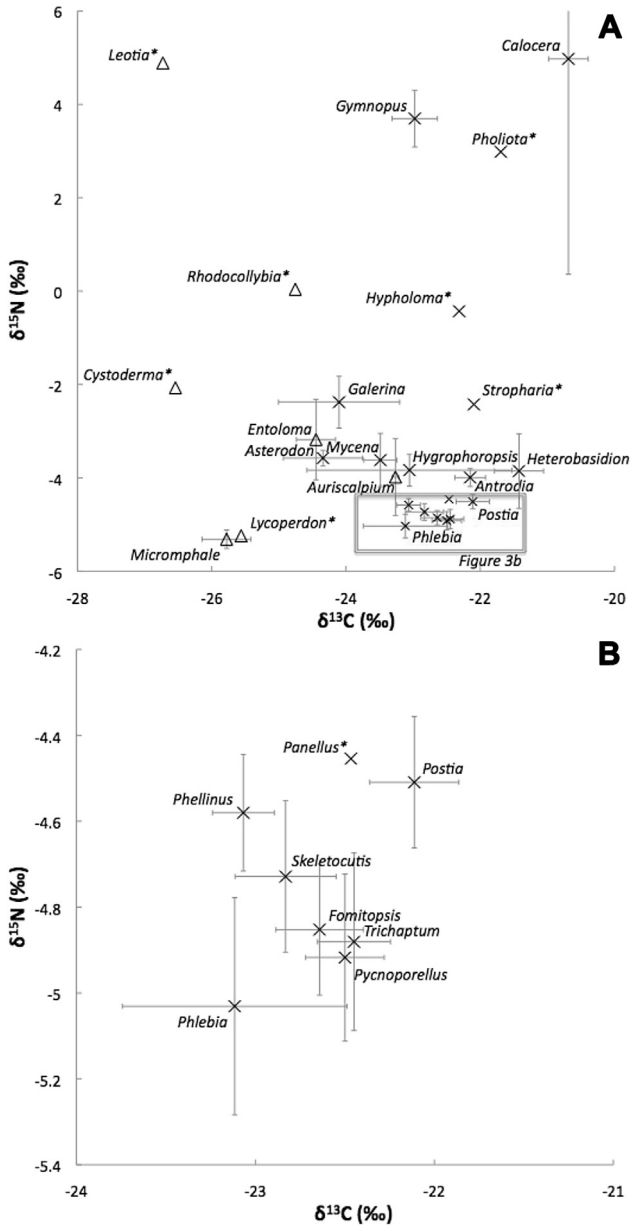

Fig. 3. (A). Mean $\delta^{13}C$ and $\delta^{15}N$ of different genera of saprotrophic wood decay (X) and litter decay (Δ) fungi. Bars represent SE. * Indicates $n \leq 2$ (B). Magnification of boxed in area from Fig. 3A with *Panellus, Postia, Phellinus, Skeletocutis, Fomitopsis, Trichaptum, Pycnoporellus, and Phelbia.*

varied more in isotope patterns than predicted (Tables 2 and 3). Furthermore, although carbon and nitrogen sources typically differed in isotopic signatures, $\delta^{13}C$ and $\delta^{15}N$ of decaying wood varied little and therefore little insight was gained into the sources of fungal carbon and nitrogen from wood of various decay stages. Only wood in decay stage V was isotopically distinct from wood in other decay stages and only one wood-associated fungus differed in $\delta^{15}N$ with wood decay stage (Fig. 4). However, similar $\delta^{13}C$ between brown and white rot fungi provided unique indication that both do not use lignin-derived carbon.

4.1. $\delta^{15}N$ and $\delta^{13}C$ patterns of fungal carbon and nitrogen sources

Within the Norway spruce-dominated forests wood in decay stages I-IV was similar in $\delta^{15}N$ whereas wood in decay stage V was up to 0.9‰ enriched in ^{15}N relative to wood in other decay stages (Fig. 4, Table 1). This is likely due to increased colonization by mycorrhizal communities in wood of decay stage V and increased abundance of nitrogen-fixing bacteria (Rajala et al., 2012; Rinne-Garmston et al., 2016) and their contribution of enriched ^{15}N to wood in decay stage V. $\delta^{15}N$ of all fungal nitrogen sources ranged from −5.6‰ to 2.5‰ with lowest $\delta^{15}N$ in decaying wood in stages I-IV, branches and twig litter and highest $\delta^{15}N$ in mineral soil (Table 1). In a similar pattern compared to a Norway spruce forest in Germany (Gebauer and Schulze, 1991), $\delta^{15}N$ decreased from soils and roots to needle litter, needles and moss to twig litter and branches and likely reflected transfer of ^{15}N-depleted nitrogen from soils to roots and to leaves by plant as well as microbial processes (Hobbie and Ouimette, 2009). Furthermore, the close resemblance of $\delta^{15}N$ in shallow organic soil and roots to above ground plant material suggests that *P. abies* preferentially utilized nitrogen from the shallow soil layer as opposed to the deeper mineral soil layer with higher $\delta^{15}N$ (Gebauer and Schulze, 1991). However, fractionation by mycorrhizae during transfer of nitrogen to *P. abies* could confound plant $\delta^{15}N$ patterns. Roots from the organic horizon were 2‰ lower in $\delta^{15}N$ than the surrounding soil while roots from the deeper mineral horizon were 4.4‰ lower in $\delta^{15}N$ than the surrounding mineral soil. It is possible that the larger difference between root $\delta^{15}N$ and soil $\delta^{15}N$ in the mineral horizon is due to plant reallocation of ^{15}N-depleted shoot nitrogen back down to roots to facilitate growth and maintenance and that this translocation causes the difference in $\delta^{15}N$ roots and soil in the mineral layer (Gebauer and Schulze, 1991).

$\delta^{13}C$ values of potential fungal carbon sources ranged from −25.2‰ for wood to −32.7‰ for moss (*P. schreberi*). Moss was 3.6‰ depleted in ^{13}C relative to other potential carbon sources (Table 1) due to the leaf morphology of mosses (low internal conductance of CO_2), the higher proportion of CO_2 intake derived from soil respiration (Gebauer and Meyer, 2003) and the limited diffusion of CO_2 due to the thin water film on moss surface (Hanson et al., 2014; Royles et al., 2014). This $\delta^{13}C$ pattern was also previously observed in a Canadian black spruce (*Picea mariana*) forest in which moss (*P. schreberi)* $\delta^{13}C$ was 5‰ lower than black spruce needles and both were lower than $\delta^{13}C$ released in soil respiration (Flanagan et al., 1999). $\delta^{13}C$ of wood was quite similar from decay stages I-IV but $\delta^{13}C$ of wood in decay stage V was 2‰ lower than wood in the other decay stages. This decrease in $\delta^{13}C$ at decay stage V is likely due to increased proportions of ^{13}C-depleted lignin (Kohzu et al., 2005; Rajala et al., 2012). The higher %C in decay stage V (56 %) than in earlier decay stages (49–52 %) also suggested higher lignin content and lower carbohydrate content, since lignin is higher in %C than carbohydrates (Lamlom and Savidge, 2003). Twig litter was also ^{13}C-depleted relative to branches and the higher proportion of %N relative to %C indicates that progressive decay of carbohydrates in these woody materials has increased the proportion of ^{13}C-depleted lignin (Kohzu et al., 2005; Rajala et al., 2012). Conversely, needles and needle litter had similar $\delta^{13}C$ values of approximately −29‰ despite an increase in the proportion of %N to %C in litter, possibly because of the lower amount of ^{13}C-depleted lignin in needles compared to woody material. Both roots from the organic layer and from mineral soil were roughly 1‰ lower in $\delta^{13}C$ relative to their surrounding soil. Thus, the $\delta^{13}C$ values of the roots were closer to the $\delta^{13}C$ values of needles from where they obtain recently photosynthesized carbon.

4.2. $\delta^{15}N$ and $\delta^{13}C$ of fungal functional groups

Isotopic patterns of ectomycorrhizal and saprotrophic fungi were consistent with studies of Scots pine and Norway spruce forests in Sweden as well as for a temperate forest in Alaska and

Table 3
Mean ± SE of $\delta^{15}N$ and $\delta^{13}C$ for each genus of litter and wood decay fungi, difference from the mean of all other saprotrophic fungi (d ($\delta^{15}N$), d ($\delta^{13}C$)) and the p value of a nonparametric Mann-Whitney U test for statistically significant difference (p ($\delta^{15}N$), p ($\delta^{13}C$)); p of less than 0.05 marked in bold.

Genus (n)	$\delta^{15}N$ (‰)	d ($\delta^{15}N$)	p ($\delta^{15}N$)	$\delta^{13}C$ (‰)	d ($\delta^{13}C$)	p ($\delta^{13}C$)	Function	Hardness	Morphology
Auriscalpium (2)	−4.0 ± 0.8	0.0	0.598	−23.3 ± 0.0	−0.4	0.541	Litter D	Soft	Toothed
Cystoderma (2)	−2.1 ± 1.5	1.9	0.085	**−26.5 ± 0.3**	**−3.7**	**0.018**	Litter D	Soft	Gilled cap
Entoloma (4)	−3.2 ± 0.9	0.8	0.125	**−24.4 ± 0.3**	**−1.6**	**0.014**	Litter D	Soft	Gilled cap
Leotia (2)	**4.9 ± 1.4**	**9.0**	**0.017**	**−26.7 ± 0.0**	**−3.9**	**0.017**	Litter D	Soft	Jelly fungi
Lycoperdon (2)	−5.2 ± 0.5	−1.3	0.205	**−25.6 ± 0.3**	**−2.7**	**0.029**	Litter D	Soft	Puffball
Micromphale (6)	**−5.3 ± 0.2**	**−1.4**	**0.012**	**−25.8 ± 0.4**	**−3.0**	**0.000**	Litter D	Soft	Gilled cap/Miniature
Rhodocollybia (2)	**0.0 ± 0.3**	**4.1**	**0.029**	−24.8 ± 0.2	−1.9	0.056	Litter D	Soft	Gilled cap
Antrodia (15)	−4.0 ± 0.2	0.0	0.082	−22.5 ± 0.4	0.4	0.161	Wood D	Hard	Polypore
Asterodon (4)	−3.6 ± 0.2	0.4	0.067	**−24.3 ± 0.6**	**−1.5**	**0.042**	Wood D	Soft	Toothed corticioid
Calocera (3)	**0.6 ± 2.2**	**4.7**	**0.011**	**−20.4 ± 0.2**	**2.5**	**0.006**	Wood D	Soft	Jelly fungi
Fomitopsis (21)	**−4.9 ± 0.2**	**−1.0**	**0.025**	−22.6 ± 0.2	0.2	0.589	Wood D	Hard	Polypore
Galerina (3)	**−2.4 ± 0.6**	**1.6**	**0.021**	−24.1 ± 0.9	−1.3	0.149	Wood D	Soft	Gilled cap
Gymnopus (4)	**3.7 ± 0.6**	**7.8**	**0.001**	−23.0 ± 0.3	−0.1	0.676	Wood D	Soft	Gilled cap
Heterobasidion (10)	−3.9 ± 0.8	0.2	0.765	**−21.4 ± 0.4**	**1.5**	**0.002**	Wood D	Hard	Polypore
Hygrophoropsis (3)	−3.8 ± 0.3	0.2	0.263	−23.1 ± 1.5	−0.2	0.757	Wood D	Soft	Gilled cap
Hypholoma (2)	**−0.4 ± 0.5**	**3.6**	**0.034**	−22.3 ± 0.3	0.5	0.572	Wood D	Soft	Gilled cap
Mycena (5)	−3.6 ± 0.6	0.4	0.215	−23.5 ± 0.2	−0.7	0.131	Wood D	Soft	Gilled cap
Panellus (1)	−4.5	−0.5	0.922	−22.5	0.4	0.812	Wood D	Soft	Gilled cap/Miniature
Phellinus (41)	−4.6 ± 0.1	−0.7	0.116	−23.1 ± 0.2	−0.3	0.094	Wood D	Hard	Polypore
Phlebia (5)	−5.0 ± 0.3	−1.1	0.125	−23.1 ± 0.6	−0.3	0.747	Wood D	Soft	Corticioid
Pholiota (1)	3.0	7.0	0.106	−21.7	1.2	0.321	Wood D	Soft	Gilled cap
Postia (31)	−4.5 ± 0.2	−0.6	0.526	**−22.0 ± 0.3**	**1.0**	**0.002**	Wood D	Soft	Polypore
Pycnoporellus (8)	−4.9 ± 0.2	−1.0	0.127	−22.5 ± 0.2	0.4	0.526	Wood D	Soft	Polypore
Skeletocutis (14)	−4.7 ± 0.2	−0.8	0.213	−22.8 ± 0.3	0.0	0.746	Wood D	Soft	Polypore
Stropharia (2)	−2.4 ± 0.5	1.6	0.060	−22.1 ± 0.1	0.8	0.337	Wood D	Soft	Gilled cap
Trichaptum (19)	−4.7 ± 0.2	−0.8	0.101	−22.4 ± 0.2	0.4	0.159	Wood D	Soft	Polypore

Table 4
Mean ± SE of %N and %C of different genera of fungi according to their functional type, including hydrophilic (Hi ECM) and hydrophobic (Ho ECM) ectomycorrhizae, litter decay fungi (Litter D), wood decay fungi (Wood D).

Genus (n)	N%	C%	Functional Type
Amanita (9)	4.56 ± 0.38	43.88 ± 0.33	Hi ECM
Cantharellus (5)	2.82 ± 0.23	43.35 ± 0.57	Hi ECM
Lactarius (17)	3.74 ± 0.20	44.16 ± 0.18	Hi ECM
Russula (12)	3.05 ± 0.14	43.87 ± 0.42	Hi ECM
Cortinarius (25)	3.52 ± 0.14	42.18 ± 0.19	Ho ECM
Hydnum (3)	3.57 ± 0.23	43.37 ± 0.54	Ho ECM
Leccinum (2)	3.54 ± 0.04	43.32 ± 1.27	Ho ECM
Paxillus (3)	4.25 ± 0.39	41.88 ± 0.58	Ho ECM
Piloderma (14)	3.14 ± 0.14	46.04 ± 0.57	Ho ECM
Suillus (3)	4.92 ± 0.92	41.90 ± 0.54	Ho ECM
Tylopilus (2)	4.98 ± 0.51	45.41 ± 0.09	Ho ECM
Auriscalpium (2)	1.44 ± 0.05	42.23 ± 0.57	Litter D
Cystoderma (2)	5.65 ± 0.32	42.27 ± 1.04	Litter D
Entoloma (4)	8.90 ± 0.59	39.04 ± 0.66	Litter D
Leotia (2)	4.07 ± 0.52	42.17 ± 0.81	Litter D
Lycoperdon (2)	6.43 ± 0.03	46.88 ± 0.86	Litter D
Micromphale (6)	3.61 ± 0.17	44.08 ± 0.14	Litter D
Rhodocollybia (2)	6.61 ± 0.30	43.43 ± 0.18	Litter D
Antrodia (15)	1.48 ± 0.17	47.30 ± 0.44	Wood D
Asterodon (4)	3.32 ± 0.40	47.75 ± 0.20	Wood D
Calocera (4)	2.31 ± 0.28	43.76 ± 0.16	Wood D
Fomitopsis (21)	0.80 ± 0.04	50.22 ± 0.60	Wood D
Galerina (3)	3.13 ± 0.35	40.87 ± 0.85	Wood D
Gymnopus (4)	5.89 ± 0.40	41.83 ± 0.71	Wood D
Heterobasidion (10)	2.75 ± 0.09	42.82 ± 0.19	Wood D
Hygrophoropsis (3)	5.68 ± 1.36	41.34 ± 0.98	Wood D
Hypholoma (2)	4.20 ± 0.50	42.35 ± 0.26	Wood D
Mycena (5)	5.06 ± 0.71	42.89 ± 0.70	Wood D
Panellus (1)	3.82	42.21	Wood D
Phellinus (41)	1.03 ± 0.04	48.51 ± 0.21	Wood D
Phlebia (5)	1.36 ± 0.18	50.21 ± 1.43	Wood D
Pholiota (1)	5.48	44.24	Wood D
Postia (31)	2.32 ± 0.20	44.32 ± 0.27	Wood D
Pycnoporellus (8)	1.57 ± 0.19	49.38 ± 0.47	Wood D
Skeletocutis (14)	1.22 ± 0.06	47.80 ± 0.32	Wood D
Stropharia (2)	4.61 ± 0.05	42.39 ± 0.15	Wood D
Trichaptum (19)	1.20 ± 0.04	43.12 ± 0.22	Wood D

old-growth conifer forests (Högberg et al., 1999; Hobbie et al., 1999; Trudell et al., 2003) and suggested that higher $\delta^{15}N$ of ectomycorrhizal fungi compared to saprotrophic fungi was likely due to the ^{15}N enrichment of ectomycorrhizal fungi during transfer of ^{15}N-depleted nitrogen to plants and the use of relatively ^{15}N-enriched older nitrogen in the soil compared to the ^{15}N-depleted nitrogen at the surface of forest soils that is used by saprotrophic fungi (Hobbie, 2005; Lindahl et al., 2007) (Fig. 1). Furthermore, $\delta^{15}N$ of fungi with hydrophobic ectomycorrhizae was 3.0‰ higher than fungi with hydrophilic ectomycorrhizae, 6.3‰ higher than litter decay fungi and 7.1‰ higher than wood decay fungi (Table S2), indicating that fungi with hydrophobic ectomycorrhizae used nitrogen deeper in soils that is more processed and has a long residence time. Our result is consistent with a previous study that indicated that fungi with hydrophobic ectomycorrhizae prefer less soluble carbon and nitrogen sources and have higher proteolytic capabilities than fungi with hydrophilic ectomycorrhizae (Lilleskov et al., 2011). Deeper mineral soils contain the largest proportion of old nitrogen and had the highest average $\delta^{15}N$ (2.5‰) of all nitrogen sources, but was still lower than that of fungi with hydrophobic ectomycorrhizae. Although Taylor and Fransson (2007) proposed that $\delta^{15}N$ of litter decay fungi should be higher than wood decay fungi due to the higher proportion of ^{15}N-enriched protein of litter decay fungi and the use of relatively older nitrogen sources, we did not find significant difference between these two functional types.

$\delta^{13}C$ of ectomycorrhizal fungi was more than 3‰ lower than wood decay fungi, indicating use of recent ^{13}C-depleted sugars by ectomycorrhizal fungi relative to use of ^{13}C-enriched cellulose from the forest floor by wood decay fungi, such as that derived from relatively ^{13}C-enriched twig litter and wood that is high in cellulose content (Kohzu et al., 1999; Hobbie, 2005) (Fig. 1). Similar patterns of lower $\delta^{13}C$ of litter relative to wood and $\delta^{13}C$ of litter decay fungi relative to wood decay fungi suggest that litter decay fungi and wood decay fungi use separate carbon pools. White and brown rot wood decay fungi did not differ in $\delta^{13}C$ indicating that proposed incorporation of lignin carbon in white rot fungi (Kohzu et al., 2005) is minimal compared to the incorporation of carbon from other

Table 5
ANOVA tables analyzing effects of %N and %C on $\delta^{15}N$ and $\delta^{13}C$ of all fungi including genus as a random effect. Numerator and denominator degrees of freedom (numDF and denDF, respectively), the *F* statistic and *p* values are reported; *p* of less than 0.05 marked in bold.

	$\delta^{15}N$				$\delta^{13}C$			
	numDF	denDF	*F*	*p*	numDF	denDF	*F*	*p*
SiteYear	4	264	3.477	**0.009**	4	264	15.369	**<0.001**
Functional Type	3	33	12.593	**<0.001**	3	33	44.19	**<0.001**
%N	1	264	11.015	**0.001**	1	264	0.04	0.841
%C	1	264	6.738	**0.010**	1	264	1.769	0.185

Table 6
Parameter estimates ± SE and Wald tests for the significance (*p* of less than 0.05 marked in bold) of difference from 0 for linear mixed-effects model investigating effects on fungal $\delta^{15}N$ of %N, functional type (hydrophilic (Hi ECM) and hydrophobic (Ho ECM) ectomycorrhizae, litter decay fungi (Litter D), wood decay fungi (Wood D)), the interaction of %N and functional type, and site, with genus included as a random effect. The intercept is the modeled mean $\delta^{15}N$ for the Hi fungi at Lapinjärvi site on year 2015 when %N = 0, and the parameters for the other levels of categorical predictors (site and functional type) are differences from these reference levels. Correspondingly, the parameter in the "plain N" row is the estimated linear regression coefficient of $\delta^{15}N$ vs. %N for the Hi ECM fungi and those in the "x N" rows are the differences of the coefficients for other functional groups from this reference group.

Model term	Parameter estimate±SE	DF	*t*	*p*
intercept	0.1 ± 1.8	262		
Loppi 2015	**0.8 ± 0.3**	**262**	**2.84**	**0.005**
Sipoo 2013	0.2 ± 0.4	262	0.45	0.651
Sipoo 2014	−0.1 ± 0.4	262	−0.25	0.803
Sipoo 2015	0.0 ± 0.3	262	0.19	0.850
Ho ECM	−1.1 ± 2.3	33	−0.46	0.645
Litter D	0.0 ± 2.9	33	−0.01	0.992
Wood D	**−4.0 ± 1.9**	**33**	**−2.08**	**0.045**
N	0.2 ± 0.3	262	0.88	0.377
Ho ECM × N	**1.0 ± 0.4**	**262**	**2.64**	**0.009**
Litter D × N	−0.7 ± 0.5	262	−1.52	0.130
Wood D × N	0.0 ± 0.3	262	0.11	0.912

Table 7
Parameter estimates ± SE and Wald tests for the significance (*p* of less than 0.05 marked in bold) of their difference from 0 for linear regression model investigating the effect of decay stage on $\delta^{15}N$ of wood decay fungi by genus. The intercept is the modeled mean $\delta^{15}N$ for the baseline genus *Antrodia* on baseline decay stage I at Lapinjärvi site on year 2015, the parameter in the 'decay stage > I' line is the modeled mean between decay stages > I and decay stage I for *Antrodia*, parameters for the genera refer to their difference from *Antrodia* at decay stage I, and the interaction parameters to the genus-specific differences between decay stages > I and decay stage I.

Model term	Parameter estimate ± SE	*t*	*p*
intercept	−3.4 ± 0.8		
Loppi 2015	0.2 ± 0.2	0.72	0.474
Sipoo 2015	−0.1 ± 0.2	−0.44	0.663
decay stage > I	−0.6 ± 0.7	−0.84	0.400
Asterodon	0.5 ± 0.5	0.97	0.335
Fomitopsis	−1.3 ± 1.2	−1.11	0.269
Heterobasidion	**2.8 ± 1.0**	**2.73**	**0.007**
Phellinus	−0.5 ± 0.3	−1.81	0.073
Phlebia	−1.0 ± 1.2	−0.84	0.404
Postia	−1.1 ± 1.0	−1.08	0.282
Pycnoporellus	**−2.4 ± 1.2**	**−1.98**	**0.049**
Skeletocutis	**−0.7 ± 0.3**	**−2.14**	**0.034**
Trichaptum	**−1.0 ± 0.4**	**−2.79**	**0.006**
(decay stage > I) × *Fomitopsis*	0.5 ± 1.2	0.38	0.708
(decay stage > I) × *Heterobasidion*	**−3.3 ± 1.0**	**−3.24**	**0.001**
(decay stage > I) × *Phlebia*	−0.1 + 1.3	−0.05	0.964
(decay stage > I) × *Postia*	0.6 + 1.0	0.59	0.553
(decay stage > I) × *Pycnoporellus*	1.7 + 1.2	1.35	0.180

wood components. The similar $\delta^{13}C$ between white rot and brown rot wood decay fungi and the similar $\delta^{13}C$ of wood decay fungi

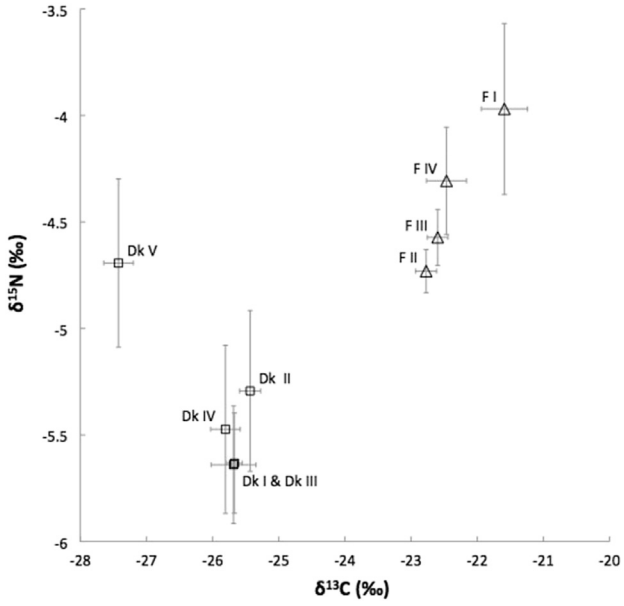

Fig. 4. Mean $\delta^{13}C$ and $\delta^{15}N$ of wood decay fungi (Δ) collected from wood (□) in different decay stages. Bars represent SE.

(−22.7‰) and of estimated wood cellulose (−23.2‰, as calculated according to Livingston and Spittlehouse, 1996) also support the hypothesis that white rot fungi do not degrade lignin to incorporate lignin-derived carbon but rather use their ligninolytic capabilities to improve access to cellulose and other carbohydrates (Hobbie, 2005).

4.3. Variation within fungal functional groups by genera

Fungal taxon was the second most important factor, next to functional type, in explaining $\delta^{13}C$ and $\delta^{15}N$ patterns in fungi. Three genera of ectomycorrhizal fungi had distinct $\delta^{15}N$ patterns relative to other ectomycorrhizal fungi (Fig. 2, Table 2). *Russula* averaged 4.2‰ lower in $\delta^{15}N$ than other ectomycorrhizal fungi, suggesting use of relatively newer ^{15}N-depleted nitrogen from shallow soil, such as decaying wood and twigs, as also indicated from ^{15}N tracer studies in a temperate pine forest (Hobbie et al., 2014). *Cortinarius* and *Hydnum* were significantly higher in $\delta^{15}N$ relative to other ectomycorrhizal fungi by 3.4‰ and 9.7‰, respectively, suggesting use of relatively ^{15}N-enriched nitrogen from deeper in mineral soil. These patterns are similar to a previous study in two old-growth conifer forests (Trudell et al., 2003) and Swedish boreal forests (Taylor et al., 1997) that also surveyed $\delta^{15}N$ patterns in Russula and Cortinarius and observed higher $\delta^{15}N$ in Cortinarius relative to Russula. Although Hobbie et al. (2014) proposed that different proportions of distinct ^{15}N-enriched biochemical fractions could also be responsible for the distinct $\delta^{15}N$ of bulk fungi, such as greater proportions of ^{15}N-enriched protein or ^{15}N-depleted chitin,

carbon and nitrogen contents of *Russula*, *Cortinarius* and *Hydnum* resemble other ectomycorrhizal fungi and their $\delta^{15}N$ differences cannot be attributed to this mechanism.

Only *Hydnum* was significantly enriched in ^{13}C relative to other ectomycorrhizal fungi, by 1.4‰ (Fig. 2, Table 2), possibly indicating use of a relatively ^{13}C-enriched source of carbon, such as old organic matter in soils, rather than photosynthates supplied by plant association. Although this is uncharacteristic of ectomycorrhizal fungi, the %C and %N of *Hydnum* is similar to that of other ectomycorrhizal fungi and the higher $\delta^{13}C$ of this taxon cannot be explained by a decrease in the proportion of relatively ^{13}C-depleted material, such as lipids (Hayes, 2002). Still, *Hydnum* sample size was small ($n = 3$) and more sporocarps should be sampled to confirm this ^{13}C trend. *Paxillus*, *Piloderma*, and *Suillus* fungi with hydrophobic ectomycorrhizae and *Amanita*, *Cantharellus* and *Lactarius* fungi with hydrophilic ectomycorrhizae were isotopically similar (Table 2), indicating use of similar pools of carbon and nitrogen, such as roots.

The larger variation in $\delta^{13}C$ of −26.7‰ to −20.4‰ in saprotrophic wood and litter decay fungi (Fig. 3, Table 3) compared to ectomycorrhizal fungi that ranged from −26.4‰ to −25.4‰ (Fig. 2, Table 2) indicates greater niche diversity in saprotrophic fungi than in ectomycorrhizal fungi for carbon. Wood decay polypore fungi varied substantially less in $\delta^{13}C$ and $\delta^{15}N$ than soft-bodied wood decay fungi and litter decay fungi, indicating that carbon and nitrogen acquisition was restricted to wood in long-lived polypore species. Although sample sizes were small, litter decay fungi *Leotia* and *Rhodocollybia* and wood decay fungi *Calocera*, *Gymnopus*, *Hypholoma* and *Pholiota* were higher in $\delta^{15}N$ by over 3‰ compared to other saprotrophic fungi and over 6‰ higher than wood and litter nitrogen sources, suggesting that these genera of fungi use older pools of nitrogen from the soil, like ectomycorrhizal fungi, or use wood that has been enriched with nitrogen by nitrogen fixers (Hoppe et al., 2014; Mäkipää et al., 2018). However, more replicates and species should be analyzed to confirm differences among genera. These results are similar to a previous study in a Central European mixed forest stand by Gebauer and Taylor (1999) that found overlap in ^{15}N patterns between ectomycorrhizal and saprotrophic fungi and greater dependence of fungal $\delta^{15}N$ on the type of substrate that it was growing on and nitrogen availability. Litter decay fungi *Micromphale* and wood decay fungi *Fomitopsis* had significantly lower $\delta^{15}N$ values compared to the mean of other saprotrophic fungi, indicating use of woody material and leaves for nitrogen. All other genera of saprotrophic fungi varied widely in $\delta^{13}C$ and indicated diverse niches of carbon sources. The morphology and softness of fruiting bodies, which may be indicative of different proportions of protein and structural compounds, did not affect isotopic patterns of sporocarps.

In addition to fungal functional type and taxon, fungal %N and %C were also significant controlling factors of fungal $\delta^{15}N$ but not of $\delta^{13}C$ (Table 5). This difference in response is likely due to the typical fungal acquisition of carbon from carbohydrates as opposed to organic nitrogen sources. Interestingly, a more in depth analysis of these data revealed that %N specifically affected $\delta^{15}N$ of fungi with hydrophobic ectomycorrhizae (Table 6) as opposed to other fungi with different functional types. This supports a previous study that linked nitrogen concentration and, ultimately, the proportion of protein to chitin to $\delta^{15}N$ in ectomycorrhizal fungi (Hobbie et al., 2012).

4.4. Effect of carbon and nitrogen sources on fungal isotopic patterns

In wood-associated polypores, only $\delta^{15}N$ of *Heterobasidion* was significantly affected by wood decay stage. *Heterobasidion* $\delta^{15}N$ decreased after initial stages of wood decay along with $\delta^{15}N$ of wood (Table 7), suggesting that decaying wood is an essential nitrogen source. The unique $\delta^{15}N$ pattern of *Heterobasidion* and similar pattern of decreasing $\delta^{15}N$ after further decay of wood in decay stage I may be attributed to its function as a saproparasite, capable of also acquiring nutrients from live wood before it dies and decays. Although we hypothesized that wood decay fungi depend on the wood they grow on for carbon and nitrogen and would therefore have isotopic patterns associated with the decay stage of wood, wood decay stage only weakly correlated with a decrease in fungal $\delta^{15}N$. Rather, the lack of correlation between polypore isotope patterns and decaying wood suggests that carbon and nitrogen are stored for long periods of time within polypores. Similarly, the type of carbon and nitrogen sources other kinds of ectomycorrhizal and saprotrophic fungi grew on, such as moss, litter or wood, did not significantly correlate with fungal $\delta^{13}C$ and $\delta^{15}N$. The lack of a correlation between the type of carbon and nitrogen sources that sporocarps were collected from and fungal $\delta^{13}C$ and $\delta^{15}N$ suggests that fungal hyphae integrated carbon and nitrogen that was assimilated from beyond the immediate surface that sporocarps were collected from.

5. Conclusions

a) Observed isotopic $\delta^{13}C$ and $\delta^{15}N$ patterns indicate that ectomycorrhizal fungi use older nitrogen and younger carbon sources than saprotrophic fungi.
b) Only *Hydnum* and *Cortinarius* fungi with hydrophobic ectomycorrhizae and *Russula* with hydrophilic ectomycorrhizae had higher $\delta^{15}N$ than fungi with hydrophilic ectomycorrhizae, indicating that these particular fungi utilize nitrogen from deeper in the soil.
c) Litter decay fungi and wood decay fungi have the largest range of $\delta^{13}C$ values, indicating a diverse range of carbon sources and greater niche diversity. Saprotrophic fungi *Leotia*, *Gymnopus*, *Hypholoma*, *Pholiota*, *Rhodocollybia* and *Calocera* have $\delta^{15}N$ values similar to ectomycorrhizal fungi indicating use of older N from soil or roots.
d) The similar $\delta^{13}C$ between white rot and brown rot wood decay fungi and the similar $\delta^{13}C$ of wood decay fungi and of estimated wood cellulose suggest that white rot fungi do not use lignin-derived carbon but rather use ligninolytic capabilities to improve access to cellulose and other carbohydrates.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2019.03.005.

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