

Mycorrhizal vs saprotrophic status of fungi: the isotopic evidence

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Summary

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- Relative abundances of carbon and nitrogen isotopes in fungal sporocarps are useful in assessing mycorrhizal or saprotrophic status, and might provide insights into the evolutionary history of these traits.
- Sporocarps of known mycorrhizal or saprotrophic genera were collected at Woods Creek, OR, USA, and isotopically compared with foliage, litter, soils and wood collected from the same site. Possible trophic strategies were then isotopically assessed in archived specimens of the Pezizales of known molecular phylogeny from the western United States.
- At Woods Creek, mycorrhizal fungi were $3.5\% \pm 0.6\%$ depleted in $\delta^{13}\text{C}$ and $5.7\% \pm 0.4\%$ enriched in $\delta^{15}\text{N}$ compared with saprotrophic fungi. By contrast, fungi from four genera of uncertain mycorrhizal status (*Clavulina*, *Helvella*, *Otidia*, and *Ramaria*) were only $0.4\% \pm 0.4\%$ enriched in $\delta^{13}\text{C}$ and $1.2\% \pm 1.1\%$ depleted in $\delta^{15}\text{N}$ relative to mycorrhizal fungi.
- In archived samples, the $\delta^{13}\text{C}$ measurements appeared to be a better indicator of trophic strategy than $\delta^{15}\text{N}$ measurements. The $\delta^{13}\text{C}$ measurements suggested that mycorrhizal or saprotrophic status was conserved within families of the Pezizales (as determined by molecular phylogeny), with the Helvellaceae and Tuberaceae mycorrhizal and Discinaceae and Morchellaceae being largely saprotrophic.

Key words: mycorrhizal fungi, saprotrophic fungi, stable isotopes, carbon dynamics, nitrogen dynamics, molecular phylogeny, fungal evolution.

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Introduction

Advances in scientific understanding often proceed from the application of established techniques to new areas. In the last 10 years we have seen a rapid increase in the use of stable isotope ratios to address questions in ecosystem ecology, paleoecology, physiological ecology, and food web studies (Ehleringer *et al.*, 1993; Lajtha & Michener, 1994; Griffiths, 1998).

Differences in carbon and nitrogen isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) among sporocarps of mycorrhizal and saprotrophic fungi (Hobbie, 1997; Högberg *et al.*, 1999; Kohzu *et al.*, 1999) may provide a way of determining trophic strategies for a broad spectrum of fungi whose strategies have been a matter of speculation only. Many putatively mycorrhizal fungi have proven

impossible to culture, and therefore their mycorrhizal status cannot be directly proven according to the currently accepted methods of Koch's postulates. In addition, growth experiments on different media and enzymatic analyzes have shown that many mycorrhizal taxa have some saprotrophic capabilities (Cromack & Caldwell, 1992). Because of the difficulty in determining fungal trophic status in natural ecosystems, the study of stable isotopes may be useful in determining the probable role of specific fungi in ecosystems and may also provide data for studies on whether trophic strategies are evolutionarily conserved.

In general, mycorrhizal fungi are enriched in ^{15}N and depleted in ^{13}C relative to saprotrophic fungi (Hobbie *et al.*, 1999a; Kohzu *et al.*, 1999, but see Gebauer & Taylor, 1999). Kohzu *et al.* (1999) furthermore demonstrated that saprotrophic

fungi living on litter were usually depleted in ^{13}C relative to wood decay fungi but enriched relative to mycorrhizal fungi. Mycorrhizal fungi are also enriched in ^{15}N relative to plants in both field studies (Taylor *et al.*, 1997; Hobbie *et al.*, 1999a) and culture studies (Kohzu *et al.*, 2000). One plausible explanation for this last observation is that some mycorrhizal fungi preferentially transfer isotopically depleted compounds to plants. As a result, mycorrhizal fungi become enriched and plants become depleted in ^{15}N (Högberg, 1997; Hobbie *et al.*, 1999a,b; Kohzu *et al.*, 2000). An enrichment in ^{13}C of mycorrhizal fungi relative to plants was attributed to the transfer of isotopically enriched sugars from plants to fungi (Hobbie *et al.*, 1999a), whereas a ^{13}C enrichment of saprotrophic fungi relative to their substrate was attributed to preferential use of ^{13}C -enriched glucose during chitin formation (Gleixner *et al.*, 1993). To summarize, the pattern for $\delta^{15}\text{N}$ is plants < saprotrophic fungi < mycorrhizal fungi, and the pattern for $\delta^{13}\text{C}$ is plants < mycorrhizal fungi < litter decay fungi < wood decay fungi. However, litter decay fungi in particular may often overlap isotopically for both nitrogen and carbon with mycorrhizal fungi. We hypothesized that given the isotopic differences between saprotrophic and mycorrhizal fungi in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, a combined index such as $\Delta_{\text{CN}} = \delta^{13}\text{C} - \delta^{15}\text{N}$ may better resolve the fungal trophic strategy than $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ measurements alone.

We tested the potential of isotopic measurements to provide useful information on trophic strategies of macrofungi in three ways: (1) through analysis of isotopic ratios and elemental compositions of putatively mycorrhizal and saprotrophic (both litter and wood decay) fungi collected in a single locality (Woods Creek, OR, USA); (2) through comparison of $\delta^{13}\text{C}$ values of wood decay fungi and their substrates; and (3) through isotopic analysis of specimens of related fungi collected in different areas.

In order to understand some of the mechanisms of isotopic fractionation in mycorrhizal and saprotrophic fungi, we compared $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ on soils, litter, and foliage from Woods Creek with isotopic measurements of fungi and with $\delta^{13}\text{C}$ measurements of wood. Where possible, fungi were classified as to mycorrhizal or saprotrophic status, and saprotrophic fungi were further classified into either litter or wood decay fungi. In addition, saprotrophic fungi were also classified (where possible) into brown and white rot fungi and the saprotrophic sporocarps were also classified as either fleshy or persistent, with the persistent fungi either woody or leathery in texture. We report on patterns between $\delta^{13}\text{C}$ values of wood decay fungi and the wood from which they grew from a separate set of specimens.

In the third part of the study, we measured isotopic abundances of archived specimens of Pezizales (Ascomycota) collected in the western USA, in order to study whether a correspondence existed between trophic strategies (as deduced from isotopic abundances), and phylogenetic relationships, as previously deduced from DNA sequence analyzes and other methods

(Trappe, 1971; Maia *et al.*, 1996; O'Donnell *et al.*, 1997). We hypothesized that the isotope ratios in sporocarps might provide insights into the evolutionary plasticity of life-history strategies in this group and that significant information on isotopic ratios can be determined from archived collections. The results of these studies were also used to test the general applicability of the index (Δ_{CN}) derived from isotopic data on Woods Creek fungi.

Materials and Methods

The field site is in the Woods Creek drainage on the north slope of Mary's Peak, Benton County, OR, USA, (44.5259° N, 123.5428° W, altitude 500 m) and is dominated by Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Franklin & Dyrness, 1984). Sporocarps were collected in September 1997 (fleshy specimens), May 1998 (only wood decay specimens), and December 1998 (both kinds); only apparently healthy sporocarps were gathered. Sporocarps from the December 1998 collection were separated into caps and stipes. Sporocarps were identified at least to genus and in some cases species before being dried at 50°C (Table 1). Soil, litter, foliage, and wood samples of Douglas-fir were collected in December 1998 ($n = 5$). The $\delta^{13}\text{C}$ signatures of wood decay fungi and their substrates were provided by Steve Macko at the University of Virginia, USA, from unpublished data. For the study on comparing trophic strategies with phylogenetic relationships, samples of specimens of selected morels, truffles, and related cup-fungi belonging to the Pezizales were furnished by Weber and Trappe from their research collections deposited in the herbarium at Oregon State University (OSC), USA. All samples were ground in a ball-mill to a fine powder. The caps and stipes from the December 1998 collection were analyzed separately; here we have averaged the cap and stipe values to give one value per sporocarp. Samples were analyzed for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, and %N on a Finnigan Delta-Plus isotope ratio mass spectrometer linked to a Carlo Erba NC2500 elemental analyzer (Finnigan MAT GmbH, Bremen, Germany), and located at the US Environmental Protection Agency, Corvallis, OR, USA. The internal standards for isotopic and concentration measurements were acetanilide and pine needles (NIST 1575). Stable isotope abundances are reported as:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C}\text{‰} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000,$$

(R , $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ of either the sample or the reference standard (atmospheric N_2 for nitrogen, PeeDee belemnite for carbon).) The standard deviation of isotopic measurements of the standards used was $\pm 0.1\text{‰}$ for $\delta^{15}\text{N}$ and $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$. Samples with more of the heavy isotope are referred to as heavier, or enriched; samples with more of the light isotope are lighter, or depleted.

The statistical package Statview (Abacus Concepts, Inc., Berkeley, CA, USA) was used to test for relationships among

Table 1 Taxa sampled at Woods Creek

Species (<i>n</i>)	Decay				
	Type ^a	Mode ^b	Form ^c	Substrate ^d	Symbol ^e
<i>Amanita vaginata</i> (Bull. Fr.) Vittad.	m				a +
<i>Amanita</i> sp.	m				b +
<i>Boletus zelleri</i> Murrill (2)	m				c +
<i>Cantharellus cibarius</i> Fr. Fr. (2)	m				d +
<i>Cortinarius cinnamomeoluteus</i> Orton	m				e +
<i>Gomphidius oregonensis</i> Peck	m				f +
<i>Inocybe geophylla</i> (Sowerby: Fr.) P. Kumm.	m				g +
<i>Inocybe leiocephala</i> G.F. Atk.	m				h +
<i>Inocybe</i> sp.	m				i +
<i>Laccaria amethysteo-occidentalis</i> Cooke	m				j +
<i>Lactarius</i> sp.	m				k +
<i>Lactarius uvidus</i> (Fr. Fr.) Fr.	m				l +
<i>Russula albonigra</i> Krombh. Fr.	m				m +
<i>Russula cremericolor</i> Earle	m				n +
<i>Russula</i> gr. <i>fragilis</i>	m				o +
<i>Russula</i> sp. (2)	m				P +
<i>Tricholoma imbricatum</i> (Fr.) P. Kumm.	m				q +
<i>Agaricus</i> sp.	s		f	l	a =
<i>Collybia</i> sp.	s		f	w	b =
<i>Fomitopsis cajanderi</i> P. Karst.	s	b	p	w	c =
<i>Fomitopsis pinicola</i> (Sw. Fr.) P. Karst. (4)	s	b	p	w	d =
<i>Galerina heterocystis</i> (Atk.) A.H. Sm. & Singer	s		f	l	e =
<i>Hirschioparus</i> sp.	s	b	w	w	f =
<i>Hygrophoropsis aurantiaca</i> (Wulfen: Fr.) R. Maire	s	w	f	w	g =
<i>Hypholoma fasciculare</i> (Huds. Fr.) Kumm. (2)	s	w	f	w	h =
<i>Lepiota</i> sp.	s	w	f	unk	i =
<i>Mycena</i> gr. <i>murina</i>	s		f	l	j =
<i>Phellinus pini</i> (Brot. Fr.) A. Ames	s	w	p	w	k =
<i>Polyporus badius</i> (Pers.) Schwein.	s		p	w	l =
<i>Pleurocybella porrigens</i> (Pers. Fr.) Singer	s		f	w	m =
<i>Psathyrella</i> sp. (3)	s		f	unk	n =
<i>Psathyrella</i> gr. <i>tephrophylla</i>	s		f	l	o =
<i>Pseudohydnum gelatinosum</i> (Scop. Fr.) P. Karst.	s		f	w	p =
<i>Stereum</i> sp.	s	w	p	w	q =
<i>Stropharia ambigua</i> (Peck) Zeller	s		f	w	r =
<i>Trichaptum abietinum</i> (Dicks. Fr.) Ryvarden	s	w	p	w	s =
<i>Tricholomopsis sulfureoides</i> (Peck) Singer	s		f	w	t =
<i>Clavulina cristata</i> (Holmsk. Fr.) J. Schröt. (2)	unk				a*
<i>Clavulina rugosa</i> (Bull. Fr.) J. Schröt.	unk				b*
<i>Helvella crispa</i> Scop. Fr.	unk				c*
<i>Helvella lacunosa</i> Afzel. Fr.	unk				d*
<i>Helvella</i> sp.	unk				e*
<i>Otidia onotica</i>	unk				f*
<i>Otidia</i> sp.	unk				g*
<i>Ramaria</i> sp. (2)	unk				h*

^am, mycorrhizal fungus; s, saprotrophic fungus; unk, unknown type; ^bbrown rot; w, white rot; ^cf, fleshy; p, persistent; ^dl, litter; w, woody; unk, unknown substrate. ^eSymbol used in Figs 1 and 2.

the data. Isotopic results for the different pools sampled were compared by a one-way, two-tailed ANOVA. Separation of means was performed by a Tukey-Kramer posthoc test at the 0.05 significance level. Means are reported ± one standard error (SE). Correlation coefficients between variables were analyzed as to whether different from zero at the 0.05 significance level using Fisher's *r* to *z* transformation.

Results

Of the 55 sporocarps collected from Woods Creek, 20 were from mycorrhizal genera, 25 were from saprotrophic genera, and 10 were from genera of unknown or mixed status (*Clavulina*, *Helvella*, *Otidia*, and *Ramaria*). Mycorrhizal and saprotrophic fungi were statistically distinct in both $\delta^{15}\text{N}$ and

Averages by class (<i>n</i>)	%N	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Foliage (5)		-3.67 ± 0.19^a	-28.60 ± 0.36^a
Litter (5)		-2.90 ± 0.09^b	-27.05 ± 0.05^b
A horizon soil (5)		-0.21 ± 0.38^c	-26.22 ± 0.05^{bc}
Wood (4)		not detected	-25.27 ± 0.71^c
Fungi			
mycorrhizal (20)	3.43 ± 0.23	3.87 ± 0.50^A	-26.23 ± 0.29^A
unknown (10)	3.69 ± 0.21	2.70 ± 1.23^A	-25.89 ± 0.28^A
saprotrophic (25)	3.11 ± 0.43	-1.80 ± 0.43^B	-22.77 ± 0.32^B
brown rots (6)	1.16 ± 0.07^a	-2.89 ± 0.46	-22.12 ± 0.36
white rots (8)	2.92 ± 0.75^{ab}	-2.47 ± 0.64	-22.31 ± 0.50
other (11)	4.32 ± 0.61^b	-0.72 ± 0.73	-23.45 ± 0.56
saprotrophic			
fleshy (15)	4.36 ± 0.50^A	-1.00 ± 0.57^A	-23.18 ± 0.49
persistent (10)	1.25 ± 0.13^B	-3.01 ± 0.45^B	-22.14 ± 0.25
saprotrophic			
litter (4)	5.40 ± 1.30^a	-0.06 ± 1.67	-23.81 ± 1.36
unknown (3)	5.33 ± 0.83^a	-0.01 ± 0.58	-24.26 ± 0.61
wood (18)	2.23 ± 0.34^b	-2.49 ± 0.38	-22.29 ± 0.27

Table 2 Average %N, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ at Woods Creek for selected ecosystem pools, means \pm standard error (SE) are given. *n* is given in parentheses after class. Five separate groups are compared: (1) foliage, litter, soil, and wood; (2) mycorrhizal fungi, saprotrophic fungi, and fungi of unknown type; (3) brown rot, white rot, and 'other' saprotrophic fungi; (4) fleshy and woody saprotrophic fungi; and (5) litter vs woody decay saprotrophic fungi. Values within a group followed by the same letter are not statistically different, based on Tukey's posthoc comparison at $P = 0.05$

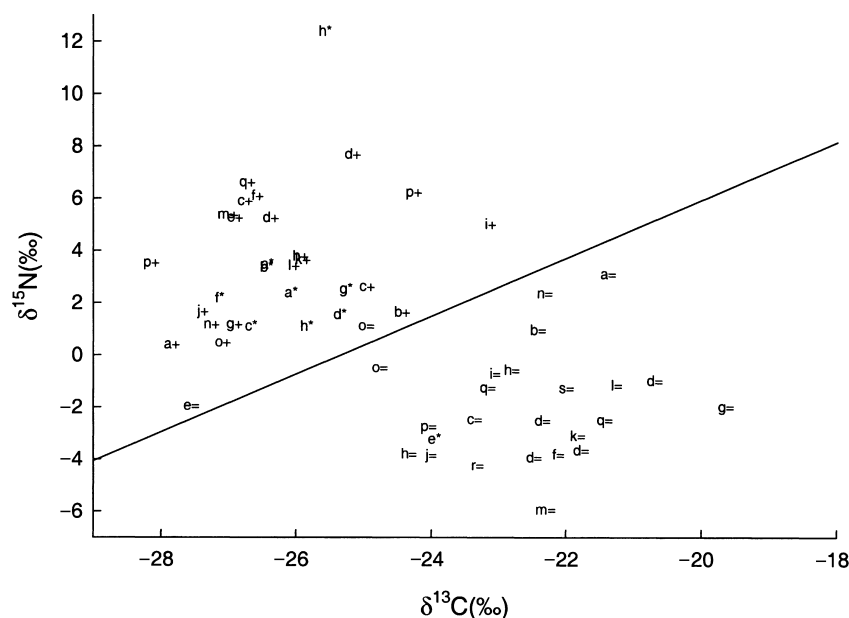


Fig. 1 Woods Creek $\delta^{15}\text{N}$ vs $\delta^{13}\text{C}$ by type. Key to specimens is in Table 1. A line is drawn to separate mycorrhizal from saprotrophic fungi at $\delta^{15}\text{N} = \delta^{13}\text{C} + 25\%$.

$\delta^{13}\text{C}$, with mycorrhizal fungi $5.7 \pm 0.6\%$ enriched and $3.5 \pm 0.4\%$ depleted in ^{15}N and ^{13}C , respectively, relative to saprotrophic fungi (Table 2, $df = 43$, $P < 0.0001$ for both). In contrast, the four genera of unknown status were not isotopically distinct for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ from mycorrhizal fungi (Table 2). The small numbers ($n = 4$ and $n = 3$, respectively) of saprotrophic fungi fruiting on either litter or on unknown substrate were significantly lower than mycorrhizal fungi in $\delta^{15}\text{N}$ ($P = 0.007$ and $P = 0.008$, respectively) and significantly higher than mycorrhizal fungi in $\delta^{13}\text{C}$ ($P = 0.01$ and $P = 0.02$, respectively). Wood decay fungi followed a similar pattern, being about 6‰ depleted in $\delta^{15}\text{N}$ and 4‰ enriched in $\delta^{13}\text{C}$ relative to mycorrhizal fungi (Table 2). In general, $\delta^{13}\text{C}$ values

less than -24% indicated a mycorrhizal strategy, whereas $\delta^{13}\text{C}$ values greater than -24% indicated saprotrophy.

In comparing isotopic ratios of sporocarps of all fungi collected at Woods Creek, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were negatively correlated overall ($r^2 = 0.29$, $n = 55$, $P = 0.0002$) but were not correlated within mycorrhizal or saprotrophic nutritional modes (Fig. 1). The $\delta^{15}\text{N}$ and %N of saprotrophic fungi were positively correlated ($r^2 = 0.45$, $n = 25$, $P = 0.0001$), whereas those of mycorrhizal fungi were not ($r^2 = 0.01$) (Fig. 2). A combined index of $\Delta_{\text{CN}} = \delta^{13}\text{C} - \delta^{15}\text{N}$ successfully separated mycorrhizal from saprotrophic fungi in all cases except for the saprotrophic fungi *Psathyrella* and *Galerina*, both of which had similar $\delta^{13}\text{C}$ signatures to mycorrhizal fungi.

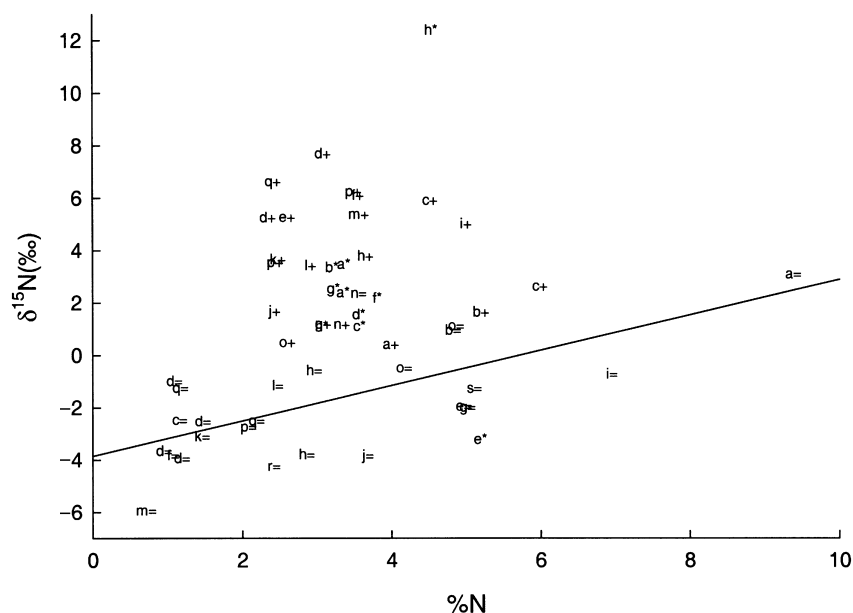


Fig. 2 Woods Creek $\delta^{15}\text{N}$ vs %N in fungi. Key to specimens in Table 1. Regression line drawn for saprotrophic fungi at $\delta^{15}\text{N} = 0.67 \times \%N - 3.89$.

Table 3 Carbon isotopic compositions of wood decay fungi, their substrate, and the isotopic difference between them. Average difference is \pm SE

Taxa	$\delta^{13}\text{C}$ (‰)	Tree	$\delta^{13}\text{C}$ (‰)	Difference (‰)	Location
<i>Polyporus</i> sp.	-22.99	<i>Pinus</i> sp.	-26.62	3.63	Port Aransas, Texas
<i>Polyporus</i> sp.	-17.36	<i>Pinus</i> sp.	-20.79	3.42	Port Aransas, Texas
<i>Trichaptum</i> sp.	-20.72	<i>Quercus</i> sp.	-24.67	3.95	Port Aransas, Texas
<i>Clitocybe subilludens</i> (Murrill) Sacc.	-23.91	<i>Quercus</i> sp.	-27.52	3.61	Port Aransas, Texas
<i>Poria</i> sp.	-21.43	<i>Juniperus</i> sp.	-24.79	3.36	Ingleside, Texas
<i>Irpex lacteus</i> (Fr. : Fr.) Fr.	-22.09	<i>Fagus</i> sp.	-25.30	3.21	Virginia
Average difference				$3.5 \pm 0.3\%$	

The dividing line was calculated as $\delta^{15}\text{N} = \delta^{13}\text{C} + 25\%$ (Fig. 1).

The $\delta^{15}\text{N}$ of foliage was not significantly depleted relative to that of persistent saprotrophic fungi, somewhat depleted (2.7‰, $P = 0.02$) relative to fleshy saprotrophic fungi, and highly depleted (7.5‰, $P < 0.0001$) relative to mycorrhizal fungi (Table 2). In contrast, foliage was significantly lower in $\delta^{13}\text{C}$ relative to both mycorrhizal and saprotrophic fungi. A progressive enrichment in ^{13}C and ^{15}N was seen going from foliage to litter to soil, with wood enriched in ^{13}C relative to other pools (Table 2).

The average enrichment of 3.5‰ in $\delta^{13}\text{C}$ between saprotrophic fungi and their woody substrate (Table 3) was similar to the 3‰ enrichment previously observed by Gleixner *et al.* (1993) in both soft-rot and white-rot fungi, and was identical to the enrichment reported between saprotrophic fungi and wood by Kohzu *et al.* (1999). For Woods Creek samples, fungi classified as producing brown-rot (decomposing cellulose primarily) did not differ isotopically from fungi classified as producing white rot (decomposing cellulose and lignin) (Table 2).

Isotopic patterns for the archived samples were overall less clear-cut than for the Woods Creek samples, although some interesting patterns did emerge. Although the archived samples for this study were taken from a wide geographical range, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were still weakly correlated ($r^2 = 0.11$, $n = 49$, $P = 0.02$) (Table 4). The confirmed mycorrhizal genus *Tuber* was similar isotopically in $\delta^{13}\text{C}$ to mycorrhizal genera from the Woods Creek study. Based on $\delta^{13}\text{C}$, the genera *Barsisia*, *Dingleya*, *Helvella*, and *Labyrinthomyces* appeared mycorrhizal. All of these genera have suspected mycorrhizal species. *Aleuria* and *Paurocotylis* also appeared mycorrhizal, whereas *Gyromitra*, *Morchella elata*, *Pseudorhizina californica*, and *Verpa bohemica* appeared saprotrophic. With the exception of *Morchella elata*, these 'saprotrophic' fungi had quite low N isotopic ratios ($\delta^{15}\text{N} = 0.2\text{‰} \pm 0.6\text{‰}$) compared with the overall average for archived specimens ($3.1\text{‰} \pm 0.5\text{‰}$). The truffle *Tuber gibbosum* and the cup fungus *Sowerbyella rhenana* were particularly high in $\delta^{15}\text{N}$ (+12‰ to +19‰). If we define mycorrhizal fungi as those with $\delta^{13}\text{C} \leq -24\text{‰}$, several taxa (e.g. *Hydnotrya*) are classified as having both mycorrhizal and saprotrophic specimens (Fig. 3).

Table 4 Average isotopic composition of archived specimens, including collection #

Species	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	Type ^a	Voucher #	Species	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	Type ^a	Voucher #
<i>Aleuria aurantia</i>	-0.73	-29.43	M/C	JMT 23188	<i>Hydnотrya variiformis</i>	1.99	-24.97	T	JMT 5035
<i>Aleuria aurantia</i>	3.52	-26.34	M/C	NSW 6727	<i>Hydnотrya variiformis</i>	8.29	-24.00	T	JMT 5060
<i>Aleuria aurantia</i>	2.06	-27.26	M/C	NSW 8140	<i>Labyrinthomyces varius</i>	11.59	-25.30	T	JMT 22587
<i>Balsamia magnata</i>	3.37	-24.26	T	JMT 13020	<i>Leucangium carthusianum</i>	5.21	-24.92	T	JMT 17223
<i>Barssia oregonensis</i>	8.67	-24.03	T	JMT 17202	<i>Leucangium carthusianum</i>	11.29	-23.30	T	JMT 17310
<i>Barssia oregonensis</i>	3.22	-25.07	T	JMT 5871	<i>Leucangium carthusianum</i>	5.71	-24.09	T	JMT 19453
<i>Caloscypha fulgens</i>	2.64	-21.19	M/C	NSW 6495	<i>Leucangium carthusianum</i>	8.08	-24.25	T	JMT 7205
<i>Caloscypha fulgens</i>	0.61	-23.32	M/C	NSW 6583	<i>Morchella</i> sp.	6.66	-25.79	M/C	NSW 7703
<i>Caloscypha fulgens</i>	3.85	-21.96	M/C	NSW 6597	<i>Morchella</i> sp.	4.46	-25.57	M/C	NSW 7702
<i>Choiromyces venosus</i>	7.22	-25.37	T	JMT 7014	<i>Morchella elata</i>	4.11	-22.47	M/C	NSW 6605
<i>Dingleya verrucosa</i>	7.18	-25.31	T	JMT 12617	<i>Morchella elata</i>	6.16	-22.56	M/C	NSW 7717
<i>Discina perlata</i> ss. lato	5.06	-21.68	M/C	NSW 6776	<i>Morchella elata</i>	0.48	-22.82	M/C	NSW 7724
<i>Discina perlata</i> ss. lato	5.99	-22.58	M/C	NSW 6775	<i>Paurocotylis pila</i>	-0.04	-27.19	T	JMT 15517
<i>Disciotis venosa</i>	4.03	-23.07	M/C	NSW 6543	<i>Paurocotylis pila</i>	3.57	-27.50	T	JMT 17593
<i>Disciotis venosa</i>	3.78	-24.76	M/C	NSW 6220	<i>Paurocotylis pila</i>	9.61	-25.41	T	JMT 4005
<i>Fischerula subcaulis</i>	6.81	-25.09	T	JMT 1899	<i>Paurocotylis pila</i>	1.13	-27.52	T	JMT 9874
<i>Gyromitra esculenta</i>	-0.64	-21.20	M/C	NSW 6195	<i>Pseudorhizina californica</i>	2.14	-21.72	M/C	NSW 6661
<i>Gyromitra esculenta</i>	5.14	-22.65	M/C	NSW 6436	<i>Pseudorhizina californica</i>	0.18	-22.91	M/C	NSW 7300
<i>Gyromitra esculenta</i>	-0.26	-21.34	M/C	NSW 7706	<i>Pseudorhizina californica</i>	-2.38	-22.96	M/C	NSW 7301
<i>Gyromitra melaleucooides</i>	0.55	-21.82	M/C	NSW 6115	<i>Rhizina undulata</i>	8.23	-22.60	M/C	NSW 6651
<i>Gyromitra melaleucooides</i>	0.83	-22.11	M/C	NSW 7709	<i>Rhizina undulata</i>	5.26	-23.19	M/C	NSW 7889
<i>Gyromitra melaleucooides</i>	-0.21	-21.43	M/C	NSW 7726	<i>Rhizina undulata</i>	8.96	-26.91	M/C	NSW 8125
<i>Gyromitra montana</i>	2.45	-21.84	M/C	NSW 6113	<i>Sowerbyella rhenana</i>	19.22	-25.46	M/C	NSW 7624
<i>Gyromitra montana</i>	2.95	-20.82	M/C	NSW 7113	<i>Sowerbyella rhenana</i>	15.13	-23.55	M/C	NSW 7909
<i>Gyromitra montana</i>	0.98	-21.92	M/C	NSW 7699	<i>Tuber gibbosum</i>	16.56	-25.36	T	JMT 7033
<i>Helvella compressa</i>	4.43	-24.06	M/C	NSW 7211	<i>Tuber gibbosum</i>	15.24	-25.32	T	JMT 7789
<i>Helvella compressa</i>	8.40	-24.44	M/C	NSW 7248	<i>Tuber gibbosum</i>	12.03	-25.57	T	JMT 8805
<i>Helvella compressa</i>	4.08	-25.52	M/C	NSW 7358	<i>Verpa bohemica</i>	-3.08	-22.83	M/C	NSW 6771
<i>Helvella compressa</i>	4.23	-25.81	M/C	NSW 7688	<i>Verpa bohemica</i>	-2.75	-22.88	M/C	NSW 7078
<i>Helvella maculata</i>	3.15	-25.33	M/C	NSW 6379	<i>Verpa bohemica</i>	-3.30	-22.60	M/C	NSW 7080
<i>Helvella maculata</i>	3.68	-25.54	M/C	NSW 6709	<i>Verpa conica</i>	2.88	-23.65	M/C	NSW 7090
<i>Helvella maculata</i>	2.76	-26.90	M/C	NSW 7636	<i>Verpa conica</i>	-0.06	-22.31	M/C	NSW 7086
<i>Hydnотrya cerebriiformis</i>	9.51	-23.04	T	JMT 1208	<i>Wynnella silvicola</i>	6.37	-24.62	M/C	NSW 7496
<i>Hydnотrya cerebriiformis</i>	2.25	-23.99	T	JMT 12458	<i>Wynnella silvicola</i>	2.41	-25.98	M/C	NSW 6219
<i>Hydnотrya cerebriiformis</i>	2.29	-25.89	T	JMT 7571					

^aM/C, morel/cup fungi; T, truffle fungi.

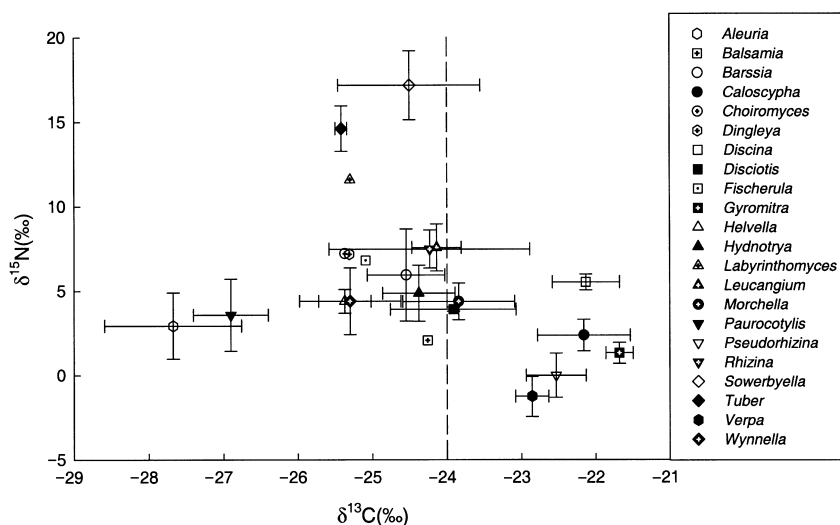


Fig. 3 Isotopic values in archived sporocarps by genus. $\delta^{15}\text{N}$ plotted vs $\delta^{13}\text{C}$, \pm standard error (SE). Line to tentatively separate mycorrhizal from saprotrophic fungi at $\delta^{13}\text{C} = -24\text{‰}$.

Discussion

The results from the Woods Creek study clearly show that both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values differed for mycorrhizal and wood decay fungi. Carbon isotopes should be a more reliable indicator of the fungal trophic strategy when comparing across sites than nitrogen isotopes, because fungal carbon is ultimately derived from the well-mixed atmospheric pool of carbon dioxide. However, the isotopic discrimination during photosynthesis against ^{13}C will vary as a function primarily of local humidity, so differences across sites in mycorrhizal and saprotrophic $\delta^{13}\text{C}$ should ultimately be traced to patterns of discrimination during photosynthesis at large scales. In contrast, the $\delta^{15}\text{N}$ of the available nitrogen in the soil varies greatly depending on the local history of N dynamics at a site. In addition, the $\delta^{15}\text{N}$ of mycorrhizal fungi may differ depending on whether the fungi prefer to take up mineral N or organic N (Gebauer & Taylor, 1999), although these isotopic differences in fungi have yet to be related to actual differences in $\delta^{15}\text{N}$ between these two possible N sources. Hobbie *et al.* (2000) suggested that if fractionation occurs during the creation of amino acids that are subsequently transferred from mycorrhizal fungi to plants, then mycorrhizal $\delta^{15}\text{N}$ should also vary as a function of the fraction of fungal N that is transferred to plants. The somewhat different results reported in Gebauer & Taylor (1999), in which mycorrhizal fungi were in general not enriched in $\delta^{15}\text{N}$ relative to saprotrophic fungi, may result from the very different site conditions, particularly with regard to the level of N deposition. Gebauer & Taylor (1999) reported that total mineral N deposition in precipitation was $19 \text{ kg ha}^{-1} \text{ yr}^{-1}$, whereas inputs at Woods Creek are presumably much lower. At a nearby coastal site annual N inputs in precipitation were less than $2 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Bormann *et al.*, 1989). High levels of N input can have dramatic effects on the species composition of mycorrhizal fungi (Wallenda & Kottke, 1998, Taylor *et al.*, 2000), and may therefore influence average $\delta^{15}\text{N}$ for mycorrhizal fungi if genera of high $\delta^{15}\text{N}$ are particularly affected. In addition, the two stands are of very different ages, only 11 yr for Gebauer & Taylor (1999) vs approximately 50–65 yr for our study (Hunt & Trappe, 1987). Stand age has also been linked to changing patterns of mycorrhizal sporocarp fruiting (Allen, 1991).

Overall, the results from Woods Creek suggest that high $\delta^{13}\text{C}$ signatures indicate saprotrophic status and a combination of low $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ signatures indicates mycorrhizal status. However, depleted $\delta^{13}\text{C}$ signatures (-24‰ to -28‰) combined with relatively low $\delta^{15}\text{N}$ signatures (enriched relative to soil 0‰ to 4‰) also occurred. Some of these fungi are clearly mycorrhizal (e.g. *Russula*), some are clearly saprotrophic (e.g. *Psathyrella*), and mycorrhizal status is unknown or variable in others (e.g. *Ramaria*). Based on the very high $\delta^{15}\text{N}$ signature observed in one *Ramaria* sporocarp, this specimen is probably mycorrhizal. The other genera of unknown mycorrhizal status (*Clavulina*, *Helvella*, and *Otidia*) also appeared potentially mycorrhizal, but the uncertainties are too large for conclusive interpretation.

Our current mechanistic understanding of how sporocarp signatures are produced is limited, and we therefore at present rely on various correlative approaches such as have been used in this and prior studies. Two recent culture studies with plant-mycorrhizal systems indicate a promising direction for improved interpretation of mycorrhizal and plant $\delta^{15}\text{N}$ signatures (Högberg *et al.*, 1999; Kohzu *et al.*, 2000). Similar culture studies are needed for $\delta^{13}\text{C}$ patterns in order to improve the interpretation of mycorrhizal and saprotrophic $\delta^{13}\text{C}$ signatures.

The similarity in $\delta^{13}\text{C}$ between saprotrophic fungi classified as brown-rotting or white-rotting, coupled with the well-known isotopic depletion of lignin relative to cellulose of 4‰ – 6‰ (Benner *et al.*, 1987), suggests that white-rotting fungi primarily incorporate cellulytic breakdown products, with lignin-derived compounds either metabolized to carbon dioxide or only degraded extracellularly. In support of this proposal, Gleixner *et al.* (1993) found both no difference in $\delta^{13}\text{C}$ between soft-rot fungi (without lignolytic capabilities) and white-rot fungi, and no difference in isotopic fractionation among woody substrate and these two fungal types. The elevated $\delta^{13}\text{C}$ of saprotrophic fungi relative to their substrate (Table 3) was attributed by Gleixner *et al.* (1993) to a 2‰ enrichment in ^{13}C during the synthesis of fungal chitin. The rather wide range of $\delta^{13}\text{C}$ values recorded for litter decay fungi may be due to different $\delta^{13}\text{C}$ of their substrate, but could also arise from either selective incorporation of isotopically distinct substrate components or differences in fractionation during metabolism and fruit body formation. In this context, it would be worthwhile to measure substrate use patterns (Worrall *et al.*, 1997) or enzyme activities of different saprotrophic genera to observe whether correlations between these parameters and $\delta^{13}\text{C}$ values exist.

The lower $\delta^{13}\text{C}$ of foliage relative to wood (3.3‰ difference) may in part explain the lower $\delta^{13}\text{C}$ seen at Woods Creek in litter decay fungi relative to wood decay fungi. Similarly, Leavitt & Long (1982) recorded that foliar cellulose was 2.0‰ to 3.5‰ depleted relative to wood cellulose. If we assume that foliar cellulose should be isotopically similar to foliar-created sugars exported to mycorrhizal fungi, these patterns may also explain the depletion of mycorrhizal fungi in $\delta^{13}\text{C}$ relative to saprotrophic fungi, as the majority (18/25) of the saprotrophic fungi from Woods Creek fed on woody tissues.

We speculate that the correlation between saprotrophic $\delta^{15}\text{N}$ and $\%N$ in this study ($r^2 = 0.45$) reflects the increasing availability of N from undecayed wood to litter to humus, and a parallel increase in $\delta^{15}\text{N}$ as a consequence of isotopic fractionation during decomposition. The observed differences in $\%N$ and $\delta^{15}\text{N}$ between fleshy and persistent saprotrophic fungi may also reflect this pattern. An interesting possibility is that increases in the $\delta^{15}\text{N}$ of available N (as measured by saprotrophic fungi) also reflect the degree to which the available N has been isotopically enriched as a result of the transfer of isotopically depleted N by mycorrhizal fungi. Therefore, low saprotrophic $\delta^{15}\text{N}$ signatures may indicate species that

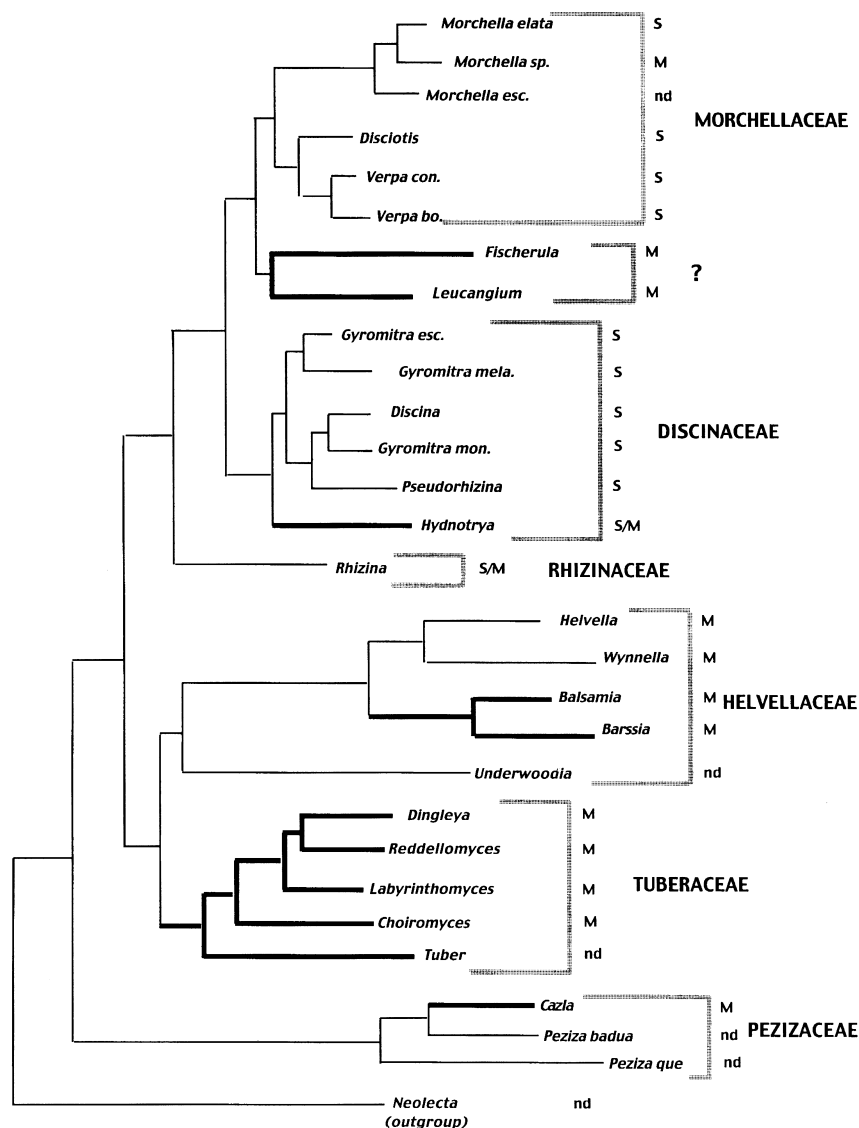


Fig. 4 Phylogenetic analysis of the Pezizales from molecular evidence, with mycorrhizal (M) or saprotrophic (S) status as tentatively inferred from isotopic measurements. 'nd' indicates no isotopic data. Bold lines for taxa indicate hypogeous fruiting strategy. Modified from O'Donnell *et al.* (1997), with permission.

obtain their N from sources that are in general enzymatically unavailable to mycorrhizal fungi, such as N from wood. Interestingly, a reanalysis of data presented by Gebauer & Taylor (1999) shows a similar pattern, with %N and $\delta^{15}\text{N}$ of sporocarps positively correlated in saprotrophic fungi ($r^2 = 0.56$, $P = 0.0007$, $n = 15$), but not in mycorrhizal fungi ($r^2 = 0.09$, $n = 9$).

After isotopic analyzes on the Woods Creek and archived samples we assigned the individual taxa of the archived samples to mycorrhizal or saprotrophic trophic strategies based on (1) whether the isotopic index $\Delta_{\text{CN}} = \delta^{13}\text{C} - \delta^{15}\text{N}$ was less than or greater than -25‰ , or (2) whether the $\delta^{13}\text{C}$ signature was less than or greater than -24‰ , with lesser values indicating mycorrhizal and greater values indicating saprotrophic trophic strategies. The isotopic index derived at Woods Creek of ($\delta^{13}\text{C} - \delta^{15}\text{N}$) did not prove useful in predicting mycorrhizal status on archived samples (see Fig. 3), presumably because

$\delta^{15}\text{N}$ could vary across sites depending on N cycling history of a stand, climate, or plant–mycorrhizal interactions (Nadelhoffer & Fry, 1994). However, in almost all cases the isotopic predictions from the second rule agreed with the limited literature available (Fig. 4). Although little is actually known about the mycorrhizal status of many of the genera measured, several of the genera are known ectomycorrhizal fungi, including *Tuber*, *Labyrinthomyces*, and *Dingleya* (O'Donnell *et al.*, 1997). Although the results on archived samples where minimal information was available about site conditions were suggestive of trophic patterns, our current understanding indicates that more site-specific information on isotopic patterns in substrate would assist in the interpretation of isotopic patterns.

Several of the results from the isotopic analyzes on archived specimens may shed some light on topics of current interest to mycologists. In the clade corresponding to the Morchellaceae, *Verpa* and *Disciotis* appear to be saprotrophic whereas

Morchella includes both apparently saprotrophic and mycorrhizal species. The mycorrhizal status of *Morchella* has been long-debated (Buscot, 1989, 1992; Wipf *et al.*, 1997; Harbin and Volk, 1999; Dahlstrom *et al.*, 2000), so it is perhaps not surprising that isotopic information suggests that both trophic strategies are probable.

Trappe (1971) cited reports suggesting that *Barssia oregonensis*, *Choiromyces venosus*, *Hydnotrya variformis*, *Leucangium canthusianum*, *Tuber gibbosum*, and *G. esculenta* might be mycorrhizal and Maia *et al.* (1996) cited additional reports suggesting *Balsamia magnata*, *Fischerula subcaulis*, *Hydnotrya cerebriformis*, and *Labyrinthomyces varius* might be mycorrhizal as well as some species of *Helvella* and *Morchella* not included in our study. Within the clade of the Pezizales that we focused on, almost all of the fungi classified as mycorrhizal are hypogeous in their fruiting habit (Fig. 4). This pattern therefore agrees with previous suggestions that nearly all hypogeous fungi are mycorrhizal (O'Donnell *et al.*, 1997).

Caloscypha fulgens presents an interesting situation. Research on the molecular phylogeny of the Pezizales led Landvik *et al.* (1997) to suggest that *Caloscypha* is aligned not with the Otidiaceae but rather with the Helvellaceae and Tuberales, occupying a phylogenetic position between *Tuber* and *Helvella*. *Caloscypha fulgens* produces a cup-shaped telomorph where sexual spores (ascospores) are formed, and an anamorph, *Geniculodendron pyriforme*, on which asexual spores (conidiospores) are formed (Paden *et al.*, 1978). As *Geniculodendron pyriforme* in earlier years but now as *Caloscypha fulgens* (Paden *et al.*, 1978) this organism has been recognized as a conifer seed pathogen that mummifies conifer seeds in seed caches. *Caloscypha fulgens* appears clearly saprotrophic in our studies, the first member of the clade composed of the Helvellaceae and Tuberales to have such an isotopic profile.

Two additional members of the Pezizales on which little prior information on mycorrhizal status was available were tested. The cup fungus *Aleuria aurantia* fruits in disturbed areas whereas *Sowerbyella rhenana*, also a cup fungus, is most often found in rotation-age to old-growth forests in the Pacific north-west. Neither Trappe (1971) nor Maia *et al.* (1996) list either genus as having been reported as possibly forming mycorrhizae. However, based on isotope analysis, both *Aleuria aurantia* and *Sowerbyella rhenana* may be mycorrhizal. Although one of the *Sowerbyella* samples was equivocal for $\delta^{13}\text{C}$, the very high $\delta^{15}\text{N}$ of both *Sowerbyella* samples (+15‰ and +19‰) strongly suggests it is mycorrhizal, as such high $\delta^{15}\text{N}$ signatures have only so far been observed in mycorrhizal genera. The very depleted $\delta^{13}\text{C}$ signatures of two genera, *Aleuria* and *Paurocotylis*, from the archived samples relative to confirmed mycorrhizal genera (e.g. *Tuber*) is striking. However, without more site- or substrate-specific information, we are unwilling to conclude that these genera are mycorrhizal, given that in the Woods Creek study similarly depleted values were seen in one litter decay fungus, *Galerina*. Studies using pure culture synthesis, molecular techniques, or perhaps isotopic tracer

studies are needed to test the mycorrhizal nature of these three genera.

The variable results based on $\delta^{13}\text{C}$ for archived specimens of *Hydnotrya* spp., *Leucangium carthusianum*, and *Rhizina undulata* illustrate the need for information about the isotopic signature of fixed carbon in the sample locations. Such site-specific information would allow determination of the probable $\delta^{13}\text{C}$ of mycorrhizal and saprotrophic fungi at those sites. Based on the relatively high $\delta^{15}\text{N}$ signatures of *Hydnotrya* and *Leucangium*, coupled with the $\delta^{13}\text{C}$ measurements, these two genera are probably mycorrhizal. *Rhizina undulata* is pathogenic on young conifers to varying degrees in different areas.

Conclusions

Difficulties in culturing many putatively mycorrhizal fungi have limited our ability to determine their trophic status. We suggest that isotopic measurements on sporocarps may be profitably and quickly employed as indicators of mycorrhizal status if other ecosystem C and N pools are adequately characterized isotopically. Although archived samples appear to be a promising data source for isotopic studies of fungal functioning, a better mechanistic understanding of how isotopic patterns are created in fungi will allow us to apply these measurements with greater confidence.

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