Functional attributes of ectomycorrhizal fungi influence isotopic patterns in sporocarps

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Abstract

Carbon and nitrogen stable isotopes in ectomycorrhizal fungi may provide new insights into the functioning of specific taxa in terrestrial C and N cycling. Here, we assembled a global database of isotope measurements from ectomycorrhizal sporocarps to assess whether isotopic patterns vary with sporocarp C:N ratio, mean annual temperature, mean annual precipitation, fungal taxonomy (genus), exploration type, and hydrophobicity of ectomycorrhizae. A regression model with genus nested within exploration type and exploration type nested within hydrophobicity explained 33% and 47% of $\delta^{13}C$ and $\delta^{15}N$ variability, respectively. Of this explained variance for $\delta^{15}N$, the factors C/N, genus, exploration type, and hydrophobicity accounted for 38, 33, 15, and 9%; for $\delta^{13}C$, genus, exploration type, and C/N accounted for 46, 32, and 20% of variance. Climatic parameters did not correlate significantly with $\delta^{15}N$ or $\delta^{13}C$. Because recalcitrant N compounds are higher in $\delta^{15}N$ than labile compounds, we suggest that hydrophobicity of ectomycorrhizae correlates with enzymatic capabilities to access recalcitrant compounds. Climate has little influence on carbon isotopic patterns, perhaps because sporocarp fruiting responds to periods of high soil moisture when the $\delta^{13}C$ of recent photosynthate across different sites may be broadly similar. Our analysis suggests that morphology reflects functional attributes and two fundamental strategies of nitrogen acquisition by ectomycorrhizal fungi, with one strategy focusing on soluble compounds directly available for uptake and a second strategy focused on using enzymatic capabilities to acquire nitrogen from insoluble pools.

Introduction

· C and N isotopes can indicate ectomycorrhizal status and may provide additional insights into the varied role of different ectomycorrhizal taxa in C and N cycling.

· The exploration type concept developed in Agerer (2006) proposed that morphological characteristics of how ECM fungi explore the soil has functional significance for the forms of N accessed by different taxa (Hobbie & Agerer 2010) (Figure 1).

· Spatial extent of hyphae varies from contact, short-distance, and long-distance exploration types, together with three medium-distance exploration subtypes.

· Exploration type generally is conserved at the genus level, particularly for taxa with hydrophobic ectomycorrhizae.

· Hydrophobicity of ectomycorrhizae and rhizomorph presence also may be important functional characteristics for exploitation of patchily distributed resources.

· These characteristics are linked to enzymatic capabilities to access soluble (e.g., ammonium) versus insoluble (e.g., protein) nutrients.

· We tested statistically whether morphological characteristics influenced $\delta^{13}C$ and $\delta^{15}N$ patterns in sporocarps (Table 1).

· Prior work (Mayor et al. 2009) found that climatic parameters (mean annual temperature and precipitation, MAT, and MAP) influenced isotopic patterns;

· We tested this again when morphology and C/N (indicates protein content, higher $\delta^{13}C$ and $\delta^{15}N$ than structural carbohydrates) were independent factors. Data from a range of sites worldwide (Figure 2) were used.

Figure 2. Sites for sporocarp collection are indicated by black dots (Mayor et al. 2009).

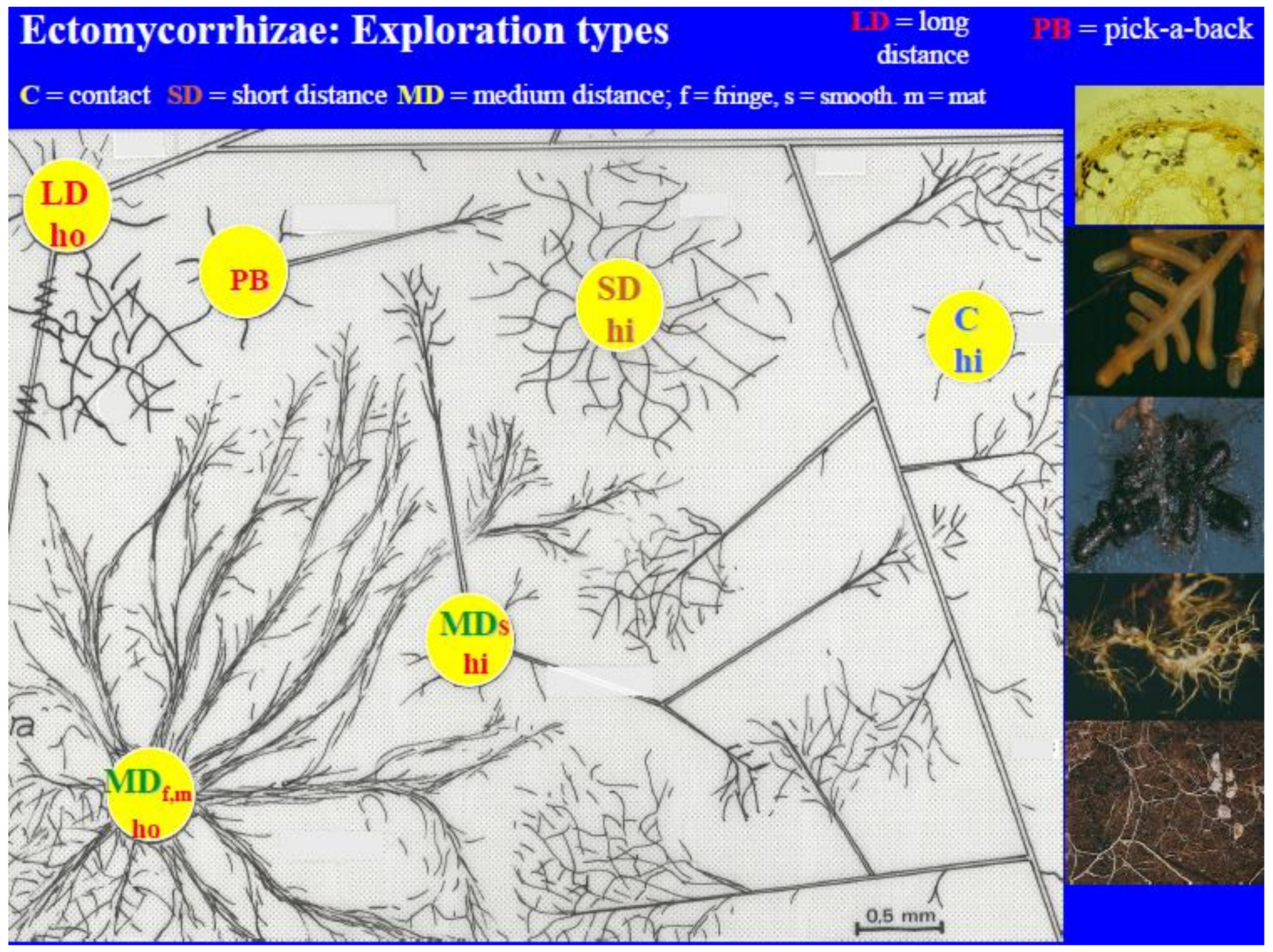

Figure 1. Exploration type schematic showing a cross-sectional view through ectomycorrhizae. Hyphae indicated by fine lines, rhizomorphs (aggregated hyphea for transport) indicated by thicker lines. Photos top to bottom of exploration types: pick-a-back (Chroogomphus), contact, short, medium, and long-distance.

Results and Discussion

· We found no effect of climate in contrast to previous work (Mayor et al. 2009) that did not control for exploration type.

· One possible explanation is that climate itself influences the distribution of exploration types, as suggested in one small study (Hobbie & Agerer 2010).

· C/N negatively influenced $\delta^{13}C$ and $\delta^{15}N$, presumably because C/N is controlled by the balance of protein (high $\delta^{13}C$ and $\delta^{15}N$) and carbohydrates within sporocarps (Hobbie et al. 2012).

· Hydrophobicity influenced $\delta^{15}N$ but not $\delta^{13}C$. We suggest that hydrophobicity in ECM fungi is an adaptation to patchily distributed resources, long-distance transport, high biomass demands, and good enzymatic capabilities to access recalcitrant N forms. These factors can favor retention of ^{15}N-enriched material in sporocarps and also select for ^{15}N-enriched sources (e.g., protein vs ammonium).

· Ongoing work will increase the study size to ~2500 samples (from 245 here), which should increase the study resolution.

Table 1. Mixed regression model of effects of climatic variables (mean annual temperature and precipitation, MAP & MAT), sporocarp C/N, hydrophobicity, exploration type, and genus on sporocarp $\delta^{15}N$ and $\delta^{13}C$. Genus was nested within exploration type, exploration type nested within hydrophobicity. N = 245, P < 0.0001 for both $\delta^{15}N$ and $\delta^{13}C$.

Response	$\delta^{15}N$		$\delta^{13}C$	
Adjusted r^2	0.473		0.333	
Mean	4.8±2.9‰		-25.9±1.1‰	
Source for Effect Tests	%Variance	Prob > F	%Variance	Prob > F
MAT	2.29	0.0547	0.49	0.4199
MAP	2.36	0.0513	1.10	0.2265
C/N	37.75	**<0.0001**	20.32	**<.0001**
HO	9.39	**0.0001**	0.02	0.8823
ET[HO]	15.33	**0.0043**	31.77	**<0.0001**
Genus[HO,ET]	32.87	**<0.0001**	46.30	**<0.0001**

Terms for Parameter Estimates	Value±SE (‰)	Prob>|t|	Value±SE (‰)	Prob>|t|
Intercept	10.5±0.8	<.0001	-24.2±0.3	<.0001
MAT	-0.1±0.0	0.0547	0.0±0.0	0.4199
MAP	0.0±0.0	0.0513	0.0±0.0	0.2265
C/N	-0.4±0.0	**<.0001**	-0.1±0.0	**<.0001**
HO[N]	-1.9±0.5	**0.0001**	0.0±0.2	0.8823
HO[N]:ET[cont/med-sm/sh-sm]	-2.3±0.9	**0.0102**	-1.7±0.3	**<.0001**
HO[N]:ET[contact]	1.8±1.0	0.0785	0.0±0.4	0.9392
HO[N]:ET[contact, short smooth]	-2.5±1.2	**0.0372**	-1.0±0.5	**0.0353**
HO[N]:ET[medium-smooth]	-0.6±0.8	0.4977	-0.4±0.3	0.2332
HO[N]:ET[short]	0.2±0.9	0.7995	-0.2±0.4	0.5805
HO[N]:ET[short-medium-smooth]	-0.6±0.7	0.3416	0.2±0.3	0.4483
HO[Y]:ET[long]	-1.8±0.9	**0.0498**	-0.4±0.4	0.325
HO[Y]:ET[medium-fringe]	0.0±1.0	0.9818	-0.4±0.4	0.2342
HO[Y]:ET[medium-mat]	2.5±1.3	0.0572	1.0±0.5	**0.0494**
HO[N]:ET[contact]:[Chroogomphus]	0.6±1.1	0.5718	0.3±0.4	0.4025
HO[N]:ET[medium-smooth]:[Amanita]	-0.3±1.0	0.7465	-0.1±0.4	0.736
HO[N]:ET[medium-smooth]:[Cantharellus]	0.9±1.0	0.3498	0.5±0.4	0.1672
HO[N]:ET[medium-smooth]:[Craterellus]	5.3±2.5	**0.0351**	1.3±1.0	0.1783
HO[N]:ET[medium-smooth]:[Entoloma]	-6.9±1.8	**0.0002**	-2.3±0.7	**0.0016**
HO[N]:ET[medium-smooth]:[Gomphidius]	3.4±1.6	**0.0271**	0.8±0.6	0.1781
HO[N]:ET[short]:[Inocybe]	-2.2±0.9	**0.017**	0.0±0.4	0.9826
HO[Y]:ET[long]:[Boletus]	0.7±1.2	0.5461	1.0±0.5	**0.0458**
HO[Y]:ET[long]:[Leccinum]	2.3±0.9	**0.0165**	-1.2±0.4	**0.0009**
HO[Y]:ET[long]:[Paxillus]	-2.9±1.8	**0.102**	-1.6±0.7	**0.0205**
HO[Y]:ET[long]:[Suillus]	2.9±1.0	**0.0061**	1.6±0.4	**<.0001**
HO[Y]:ET[long]:[Tylopilus]	-2.3±1.2	**0.047**	0.3±0.4	0.5391
HO[Y]:ET[medium-fringe]:[Cortinarius]	-0.8±0.8	0.2753	-0.2±0.3	0.4451
HO[Y]:ET[medium-fringe]:[Hebeloma]	-2.7±1.4	0.0512	-0.7±0.5	0.1888
HO[Y]:ET[medium-fringe]:[Hydnum]	2.5±1.6	0.1204	0.9±0.6	0.1583
HO[Y]:ET[medium-mat]:[Hydnellum]	-0.7±1.8	0.6927	1.3±0.7	0.0698
HO[Y]:ET[medium-mat]:[Phellodon]	0.1±1.8	0.9431	0.9±0.7	0.2069

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