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REVIEW AND SYNTHESES

Elucidating the nutritional dynamics of fungi using stable isotopes

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Abstract

Mycorrhizal and saprotrophic (SAP) fungi are essential to terrestrial element cycling due to their uptake of mineral nutrients and decomposition of detritus. Linking these ecological roles to specific fungi is necessary to improve our understanding of global nutrient cycling, fungal ecophysiology, and forest ecology. Using discriminant analyses of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope values from 813 fungi across 23 sites, we verified collector-based categorizations as either ectomycorrhizal (ECM) or SAP in > 91% of the fungi, and provided probabilistic assignments for an additional 27 fungi of unknown ecological role. As sites ranged from boreal tundra to tropical rainforest, we were able to show that fungal $\delta^{13}C$ (26 sites) and $\delta^{15}N$ (32 sites) values could be predicted by climate or latitude as previously shown in plant and soil analyses. Fungal $\delta^{13}C$ values are likely reflecting differences in C-source between ECM and SAP fungi, whereas ^{15}N enrichment of ECM fungi relative to SAP fungi suggests that ECM fungi are consistently delivering ^{15}N depleted N to host trees across a range of ecosystem types.

Keywords

^{13}C, ^{15}N, discriminant analysis, ectomycorrhizae, microbial ecology, nutrient cycling, saprotroph.

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INTRODUCTION

Fungi function at two fundamental biogeochemical interfaces between soil and plants. Decomposer fungi mineralize organic carbon (C) compounds in detritus and liberate mineral nutrients in the process, while mycorrhiza-forming fungi, as mutualistic root extensions, enhance mineral, and perhaps organic, nutrient uptake in exchange for plant photosynthate (Leake & Read 1997). Within the large guild of ectomycorrhizal (ECM) fungi there are potential, though rare, 'cheaters' (Egger & Hibbett 2004; Douglas 2008) and species with proteolytic capabilities that may blur these distinctions (Chen *et al.* 2001; Buée *et al.* 2007). However, dividing fungi into saprotrophic (SAP) and ECM functional groups has proven useful to biogeochemical and ecological research despite considerable variation among fungal species (Read & Perez-Moreno 2003; Gadd 2006). Ectomycorrhizal fungi are a diverse assemblage of ~7000–10 000 spp. that mutualisticaly associate with woody plant hosts in a number of families of dominant tree species (e.g., Pinaceae, Fagaceae, Dipterocarpaceae, Myrtaceae subfam. Leptospermoideae, and Fabaceae subfam. Caesalpiniodeae) in boreal, temperate, and to a more limited extent tropical regions of the world (Halling 2001; Read & Perez-Moreno 2003; Taylor & Alexander 2005). The formation of macroscopic sporocarps (mushrooms) by many ECM and SAP fungi allows for experimental tractability unavailable for most microbial organisms. As a result, there are well developed species concepts, a rapidly developing comprehensive phylogeny (Hibbett *et al.* 2007), and unique opportunities for advancing ecological research on forest nutrient cycles, anthropogenic impacts, and fungal interactions with host plants (Wardle *et al.* 2004; Clemmensen *et al.* 2006; Hobbie & Hobbie 2006; Treseder *et al.* 2007; Buée *et al.* 2007).

Assigning ecological roles to individual taxa is necessary to conduct research on the biogeochemical importance of fungi. Assignment has typically been based on the following methods: (i) fruiting on, and presumed decomposition of, dead plant tissue by SAP fungi; (ii) exclusive co-occurrence of sporocarps with ECM forming host plants; (iii)

phylogenetic distance to fungi with previously categorized ecological role; (iv) direct tracing of hyphae from sporocarp to ECM rootlet; (v) molecular comparison of sporocarp to ECM rootlet; and (vi) dual isotope values of C and N to determine nutritional mode. However, each of these methods has limitations: (i) fungal growth on well decomposed wood and/or aerial fruiting habits can confound categorization based solely on fruiting substratum (e.g. Henkel *et al.* 2006); (ii) exclusive co-occurrence of sporocarps with suitable host plants is often unresolved due to inadequate field observation; (iii) the evolutionary 'switching' by many ECM forming basidiomycete fungi makes assignment to ecological role based on phylogeny alone questionable (Hibbett *et al.* 2000; Matheny *et al.* 2006); (iv) direct evidence, such as tracing hyphae from fruiting body to ECM rootlet, is difficult or impossible to obtain in most soil matrices; and (v) while molecular comparisons certainly can link ECM root tips to sporocarps (e.g. Horton & Bruns 2001), their widespread adoption by ecologists remains technologically and financially constrained. In this study, we explored the ability of the isotope based method to assign ecological roles to fungi by quantifying the error associated with the technique.

The $^{15}N : {}^{14}N$ and $^{13}C : {}^{12}C$ stable isotope ratios [expressed as $\delta^{15}N$ and $\delta^{13}C$ in permil (‰) values relative to standards] of fungi provide time integrated biogeochemical information regarding the acquisition, transformation, and export of C and N by fungi under natural conditions (Griffith 2004). Unique C and N cycling pathways in ECM and SAP fungi lead to different isotope fractionation effects (Hobbie & Wallander 2006). The $\delta^{15}N$ enrichment of ECM relative to SAP fungi is thought to result from assimilation and transfer of ^{15}N depleted N to host plants, a process that cumulatively leads to fungal enrichment and host plant depletion (Hobbie *et al.* 1999, 2005; Högberg *et al.* 1999a,b). Thus, the absence of N transfer to plants by SAP fungi causes them to appear ^{15}N depleted relative to ECM fungi within a site. Patterns of $\delta^{13}C$ in fungi are largely attributed to isotope differences in the substrate(s) used as an energy source. Saprotrophic fungi use C from plant tissues and soil organic compounds comprised of diverse C sources each with distinct $\delta^{13}C$ values often 1–6 ‰ different from more commonly measured bulk plant foliage or roots (Gleixner *et al.* 1993; Marshal *et al.* 2007; Bowling *et al.* 2008), whereas ECM fungi receive plant photosynthate C that is isotopically more homogeneous relative to plant tissue (Gebauer & Dietrich 1993; Högberg *et al.* 1999a,b; Henn & Chapela 2001; Baldocchi & Bowling 2003). The resulting differences in either fungal $\delta^{15}N$ or $\delta^{13}C$ have been used to differentiate ECM from SAP fungi (Taylor *et al.* 1997; Gebauer & Taylor 1999; Hobbie *et al.* 1999; Högberg *et al.* 1999a,b). Recently, the simultaneous use of both isotopes has improved this approach (Kohzu *et al.* 1999; Hobbie *et al.* 1999, 2001; Taylor *et al.* 2003; Trudell *et al.* 2004; Hart *et al.* 2006; Clemmensen *et al.* 2006; Zeller *et al.* 2007). However, global variability in C and N isotope values in plants and soils (Amundson *et al.* 2003) suggests that cross site comparisons may only be possible following some form of site normalization to correct for differences in average isotopic baselines among sites (Henn & Chapela 2001; Post 2002). Isotopic baselines are the $\delta^{15}N$ or $\delta^{13}C$ values of basal C and N sources within a trophic system or food web, such as photosynthate or labile mineral nutrient sources (reviewed in Post 2002). If large scale site variability in baseline isotope patterns is partially attributed to climate, such as with plant and soil $\delta^{15}N$ and $\delta^{13}C$ values (Amundson *et al.* 2003; Marshal *et al.* 2007; J. Craine *et al.*, unpublished data), then site normalization of $\delta^{15}N$ or $\delta^{13}C$ values would clarify the influence of physiology and nutrition on ECM and SAP C and N isotope patterns.

The accumulation of multiple datasets from around the northern hemisphere has enabled us to address both the utility and cause of C and N isotope differences in ECM and SAP fungi. In order to determine if fungal $\delta^{15}N$ or $\delta^{13}C$ patterns across ecosystems are similar to plants and soils, we assessed the explanatory capacity of mean annual temperature (MAT), mean annual precipitation (MAP), and latitude (LAT). Cross site comparisons were optimized by use of site based normalization to remove variability associated with changes in background isotope values and uneven ECM and SAP sampling within sites. We then used a large number of published and unpublished fungal $\delta^{15}N$ or $\delta^{13}C$ values to statistically discriminate ecological categorizations (SAP vs. ECM) of fungi with suspected and unknown ecological roles. Isotope values in fungi provide a form of ecological information independent of phylogenetics, soil excavation, or molecular sequencing, and when combined with one or more of these other techniques, provides definitive evidence of the nutritional ecology of specific fungi and can inform biogeochemical and evolutionary research in many of the world's forested ecosystems.

METHODS

Data assembly

To test global predictions of ECM and SAP isotope patterns, we compiled $\delta^{15}N$ and $\delta^{13}C$ values from one novel and ten published data sets. Compiled data included 913 $\delta^{15}N$ and 813 $\delta^{13}C$ values from collector categorized ECM or SAP fungi, and 27 fungi of unknown ecological role, together comprising 148 genera. Of the 32 study sites included, 30 were from temperate, boreal, subarctic, or arctic ecosystems and two from tropical rainforest (site descriptions available from references listed in Table 1). The tropical sites included fungi from a dipterocarp dominated

Malaysian rainforest (Kohzu *et al.* 1999) and a Guyanese rainforest dominated by a leguminous tree. Lowland tropical rainforest sites with ECM trees, while underrepresented in our analysis, are globally uncommon outside of dipterocarp or caesalpinioid dominated rainforests as well (Taylor & Alexander 2005). Fungal $\delta^{15}N$ data from areas with high levels of atmospheric N deposition from anthropogenic sources were excluded to eliminate possible confounding of natural fungal $\delta^{15}N$ patterns (Gebauer & Taylor 1999; Lilleskov *et al.* 2002). For each site, MAT, MAP, and LAT were compiled from original manuscripts, or extrapolated from nearby climate stations when not reported.

Guyana field site and sample processing

Fungal sporocarps were collected during the 2003–2006 June-August rainy seasons in the Upper Potaro River Basin in the Pakaraima Mountains of Guyana (5°18′04.8′N, 59°54′40.4′W; elevation 710 m). The moist evergreen forests in this region receive 3855 mm $year^{-1}$ of rain, and occur on well drained, highly oligotrophic soils that are low in phosphorus (P), calcium, and magnesium, and high in iron and aluminum (Henkel 2003; Mayor & Henkel 2006). The fresh foliar N : P mass ratio of 25.5 ($n = 5$ canopy sun leaves) for *Dicymbe corymbosa* Spruce ex. Benth (Caesalpiniaceae) and the highly weathered parent material are suggestive of P limiting conditions to primary productivity (Güsewell 2004).

Fungal sporocarps were collected from monodominant stands of the ECM forming canopy tree, *D. corymbosa*, morphologically described while fresh, identified to species or morphospecies, and field-dried with desiccants (Henkel *et al.* 2002). Additional herbarium specimens collected from this area within the last 10 years were also analysed for isotope values to target taxonomically unusual fungi of unknown ecological role. In the laboratory, equal portions of pilei and stipes or entire sporocarps from 56 fungi were finely ground, dried at 60 °C for 24 h, and analysed on a ThermoFinnigan continuous flow isotope ratio mass spectrometer coupled to a Costech elemental analyzer at the University of Florida. Stable isotope abundances are reported as: $\delta^{15}N$ or $\delta^{13}C = (R_{sample}/R_{standard} - 1) \bullet 1000$, where $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$ of the sample and reference standard (atmospheric N_2 and PeeDee belemnite-C, respectively). Run error rates were typically ≤ 0.2‰ for $\delta^{15}N$ and ≤ 0.1‰ for $\delta^{13}C$. Voucher specimens for Guyana fungi are maintained at Humboldt State University.

Data analyses

Linear regressions were conducted on site mean $\delta^{15}N$ and $\delta^{13}C$ values to assess the individual ability of MAT, MAP, and LAT to explain ECM and SAP mean isotope patterns. Best fit polynomial equations (linear vs. second order) were chosen based on R^2 comparisons. Combined, nine fungi from Tanigawa and Okinawa, Japan (Kohzu *et al.* 1999) were removed from $\delta^{15}N$ correlations as extreme statistical outliers indicated by box-plots. The presence of significant correlations among site mean fungal $\delta^{13}C$ and predictor variables indicated that accuracy in discriminant categorization of ECM and SAP fungi could indeed be enhanced through a normalization procedure as previously suggested (Henn & Chapela 2001).

To normalize datasets prior to discriminant analysis, we separately calculated the mean $\delta^{15}N$ and $\delta^{13}C$ values of the ECM and SAP groups within each site. Next, we averaged these two group means and subtracted this new unbiased mean from each individual fungal isotope value. This normalization procedure removed the overall sampling bias toward ECM fungi across sites (62% were ECM) and centered site means to zero. Site normalization excluded isotope values from four fungi representing sites with only single ecological categories (Kagoshima, Okinawa, and Oodai, Japan). A comparable, yet more complicated site normalization procedure was also used by Henn & Chapela (2001) on a subset of the data presented here. Their site normalization procedure was designed to examine the contributions of fungal physiology and ecology (e.g. substrate) on ^{13}C fractionation in fungi. Because of this objective, their site corrections involved 'removal of the difference in means between ECM and SAP fungi for C data' (Henn & Chapela 2001) to correct for substrate and highlight remaining isotopic differences caused by physiology. Our relatively more simple transformation procedure was designed to retain the ECM–SAP differences, regardless of cause, while reducing cross site variability indicated by the abiotic proxies of climate and latitude. Therefore, our normalization procedure was applied to both C and N isotope values without the assumptions needed for substrate corrections.

We used standard discriminant multivariate analysis of both site normalized and actual fungal isotope values to: (i) statistically test for a global isotopic difference among ECM and SAP ($\mathbf{\Delta}_{ECM-SAP}$) fungi; (ii) assign collector based categorization error terms for fungi; and (iii) categorize fungi of unknown ecological role from several of the sites using probabilities arising from the entire dataset. In the discriminant analysis, probabilities of categorical assignment were set proportional to occurrence, and because the assumption of equivalent covariance among variables were not met, a pooled variance quadratic function was used instead.

Discriminant analyses have been described as circular processes because they use predefined groups to inform categorization of those same groups (Quinn & Keough 2005). To alleviate this concern we validated categorization with a second discriminant analysis using a 50% random subset of the data to categorize all remaining fungi, and a separate cluster analysis using only fungal isotope values to

Table 1 Summary of data by study, site, geography, climate, and ecological role

		(N)			(°C)	(mm year^{-1})	ECM (‰)		SAP (‰)		$\Delta_{ECM-SAP}$	
Ref.*	Site	ECM	SAP	LAT	MAT	MAP	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
1	Aheden, Sweden	53	0	64	1.0	600	N/A	6.0	N/A	N/A	N/A	N/A
	Betsele, Sweden	5	0	64	1.0	570	N/A	8.1	N/A	N/A	N/A	N/A
	Flakaliden, Sweden	21	0	64	2.3	600	N/A	6.1	N/A	N/A	N/A	N/A
	Kulbacksliden, Sweden	8	0	64	1.2	523	N/A	4.3	N/A	N/A	N/A	N/A
	Norrliden, Sweden	1	0	64	1.6	595	N/A	5.4	N/A	N/A	N/A	N/A
	Svartberget, Sweden	5	0	64	1.6	595	N/A	5.0	N/A	N/A	N/A	N/A
	Vilan, Sweden	7	0	64	5.1	542	N/A	5.3	N/A	N/A	N/A	N/A
						Study mean	–	**5.7**	–	–	–	–
2	Aishu, Japan	19	21	35	11.7	2353	−24.6	5.0	−22.6	1.6	−2.0	3.5
	Chiba, Japan	3	6	35	14.7	1550	−26.2	−0.8	−23.7	−0.6	−2.6	−0.1
	Kagoshima, Japan	0	1	31	17.8	2236	N/A	N/A	−22.9	−7.1	N/A	N/A
	Kyoto, Japan	28	26	35	15.8	1814	−24.6	4.8	−23.1	0.6	−1.5	4.2
	Lambir, Sarawak Malaysia	17	14	4	26.0	2700	−26.8	7.6	−25.0	−0.4	−1.8	7.9
	Miyajima, Japan	1	1	34	17.0	1546	−24.6	9.8	−22.1	1.5	−2.5	8.3
	Norikura, Japan	9	3	36	6.7	2766	−24.3	8.4	−21.6	−0.1	−2.7	8.5
	Okinawa, Japan	1	0	24	23.0	1736	−25.0	21.2	N/A	N/A	N/A	N/A
	Ontake, Japan	9	8	35	6.7	2766	−24.1	2.4	−21.8	−2.7	−2.3	5.1
	Oodai, Japan	0	2	34	15.7	1511	N/A	N/A	−22.2	−0.2	N/A	N/A
	Shirahama, Japan	1	2	33	16.8	1730	−24.5	4.4	−23.1	0.7	−1.5	3.8
	Tanigawa, Japan	2	6	36	5.2	1692	−24.8	19.1	−22.3	−1.0	−2.5	20.0
						Study mean	**−25**	**8.2**	**−22.7**	**−0.7**	**−2.1**	**6.8**
3†	Glacier Bay Alaska, USA	4	4	59	14.9	1830	−25.4	4.5	−22.9	−1.9	−2.5	6.4
4	Mixed conifer California, USA	18	25	N/A	N/A	N/A	−25.8	9.0	−22.5	−0.1	−3.3	9.1
5	Woods Creek Oregon, USA	20	25	45	11.0	1000	−26.2	3.9	−22.8	−1.8	−3.5	5.7
6	Aheden, Sweden	29	4	64	1.0	600	−25.8	7.8	−23.3	0.5	−2.4	8.4
	Stadsskogen, Sweden	110	13	59	5.5	541	−25.7	5.8	−23.1	1.6	−2.6	4.2
						Study mean	**−25.8**	**6.8**	**−23.2**	**0.5**	**−2.6**	**6.3**
7	Deer Park Rd Washington, USA	64	23	47	9.0	1150	−25.4	5.5	−23.3	−1.2	−2.1	6.7
	Hoh River Washington, USA	54	38	47	10.0	3450	−25.2	4.7	−22.9	−2.3	−2.3	7.0
						Study mean	**−25.3**	**5.1**	**−23.1**	**−1.8**	**−2.2**	**6.9**
8	Snowbowl Arizona, USA	9	13	35	4.0	775	−24.0	4.6	−22.0	1.9	−2.0	2.7
	Lamar Haines Arizona, USA	12	13	35	5.0	775	−24.0	3.2	−21.9	2.4	−2.1	0.8
						Study mean	**−24.0**	**3.9**	**−22.0**	**2.2**	**−2.0**	**1.7**
9	Heath tundra, Sweden	10	4	68	−1.0	300	−27.0	1.7	−23.7	0.08	−3.4	1.7
	Tussock tundra Alaska, USA	3	5	68	−8.5	350	−26.4	12.0	−24.7	3.0	1.8	9.0
						Study mean	**−26.7**	**6.9**	**−24.2**	**1.6**	**−2.5**	**5.3**
10	Breuil-Chenue, France	33	14	47	9.0	1280	−26.2	3.1	−22.8	−2.8	−3.5	5.9
	Spruce plantation, France	20	17	47	9.0	1280	−24.1	3.7	−22.6	−0.6	−1.4	4.3
						Study mean	**−25.1**	**3.4**	**−22.7**	**−1.7**	**−2.5**	**5.1**
11	Upper Potaro River, Guyana	29	20	5	24.0	3866	−26.0	5.7	−24.9	1.6	−1.1	4.1
	Sum	**605**	**308**			**Grand mean**	**−25.3**	**6.4**	**−22.9**	**−0.3**	**−2.3**	**5.3‡**

Number (N) of individual ectomycorrhizal (ECM) and saprotrophic (SAP) sporocarps collected from each site. The mean annual temperature (MAT) and precipitation (MAP) values correspond to either published or extrapolated measurements from nearby climate stations. Differences in mean isotope values ($\delta^{13}C$ and $\delta^{15}N$) from each site were subtracted from one another ($\Delta_{ECM-SAP}$) to illustrate variability in ECM–SAP isotope differences among sites.

*References: 1, Taylor *et al.* 1997; 2, Kohzu *et al.* 1999; 3, Hobbie *et al.* 1999; 4, Henn & Chapela 2001; 5; Hobbie *et al.* 1999, 2001; 6, Taylor *et al.* 2003; 7, Trudell *et al.* 2004; 8, Hart *et al.* 2006; 9, Clemmensen *et al.* 2006; 10, Zeller *et al.* 2007; 11, this study.

†Values reported as generic means of 67 ECM and 29 SAP species.

‡Tanigawa, Japan omitted as a statistical outlier.

assign groups (Quinn & Keough 2005). The specific categorical assignments of individual sporocarps were compared among the original and subsequent analyses to test for consistency in sporocarp categorization. The above analyses were conducted using JMP® 7.0.2 (SAS Institute Inc., Cary, NC, USA).

Following site normalization and discriminant analyses we sought to more fully examine the combined ability of MAT, MAP, and LAT, to explain fungal $\delta^{13}C$ and $\delta^{15}N$ patterns using linear mixed effect models. Mixed effect models were necessary because both fungal species and isotope values within sites are non-independent clustered observations, and therefore violate a parametric test requirement of uncorrelated error terms associated with each measurement (Faraway 2006). Modeling non-independent variables as random effects is a well established statistical technique (Crawley 2007; Anderson 2008) that allowed each sample point in our dataset, as opposed to just site means as in linear regression, to inform linear model construction.

Mixed effect models were constructed using the *lme4* (Bates 2008) and *lattice* (Sarkar 2008) packages in R® (version 2.6.2; R Development Core Team 2008). All models had the dependent variables of sporocarp $\delta^{13}C$ and $\delta^{15}N$ described by ecological group (ECM or SAP), site, and either species or genus, both with and without nested interactions. In addition, MAT, MAP, and LAT, were compared in a factorial fashion as additive, multiplicative, or quadratic predictor variables to determine the most informative formulation. These complex forms were justified by the possibility of nonlinear and conditional interactions among predictors as indicated by visual assessment of scatter plot matrices. Centering of MAT, MAP, and LAT (subtraction of each value from overall mean) optimized model fitting procedures and, consequently, reduced multicolinearity among predictor variables. Beginning with the most complex model, mathematical formulations of MAT, MAP, and LAT were sequentially compared using maximum likelihood methods. Following convergence on the optimal fixed effect configuration [e.g. lowest Akaike information criterion (AIC) value], we evaluated random effects, with either species or genera nested within site, using restricted maximum likelihood methods (Crawley 2007). We assessed model quality using the common graphical diagnostics of residuals against fitted values, sample quantile against theoretical quantile plots, and regressions of predictor variables for interpretation of effects (Quinn & Keough 2005). AIC was used to select final models because it is widely regarded as an unbiased estimator that assess relative model fit, penalizes over parameterization, and allows multiple working hypotheses to be simultaneously evaluated (Anderson 2008).

RESULTS

Dual $\delta^{13}C$ and $\delta^{15}N$ graphs of fungal sporocarps illustrate a global divide in isotope values between different ecological categories of fungi (Fig. 1). On average, ECM fungi were significantly ^{15}N enriched and ^{13}C depleted relative to SAP fungi (5.5 ± 0.16 vs. −0.3 ± 0.16 ‰, and −25.5 ± 0.06 vs. −23.0 ± 0.09 ‰ ± SE; $P < 0.001$, *t*-test). Global ranges in sporocarp $\delta^{15}N$ (−7.1 to 21.8 ‰) and $\delta^{13}C$ (−31.7 to −19.0 ‰) values were broad and the range of MAT (−8.5 to 26 °C) and MAP (300–3866 mm year^{-1}) among sites represents much of global climatic variability in terrestrial ecosystems (Table 1). Sorting fungi into either tropical ($n = 2$) or extra-tropical ($n = 30$) sites indicated that fungi from the tropical sites were significantly more ^{13}C depleted and ^{15}N enriched than extra-tropical sites (SAP: $P < 0.05$, two-tailed *t*-test; ECM: $P < 0.05$, Wilcoxon rank sum test). Despite absolute differences among site mean $\delta^{13}C$ and $\delta^{15}N$, the magnitude of isotopic differences between ECM and SAP fungi was consistent across sites (Table 1) and not significantly different from a slope of one (Fig. 2; $\alpha < 0.05$).

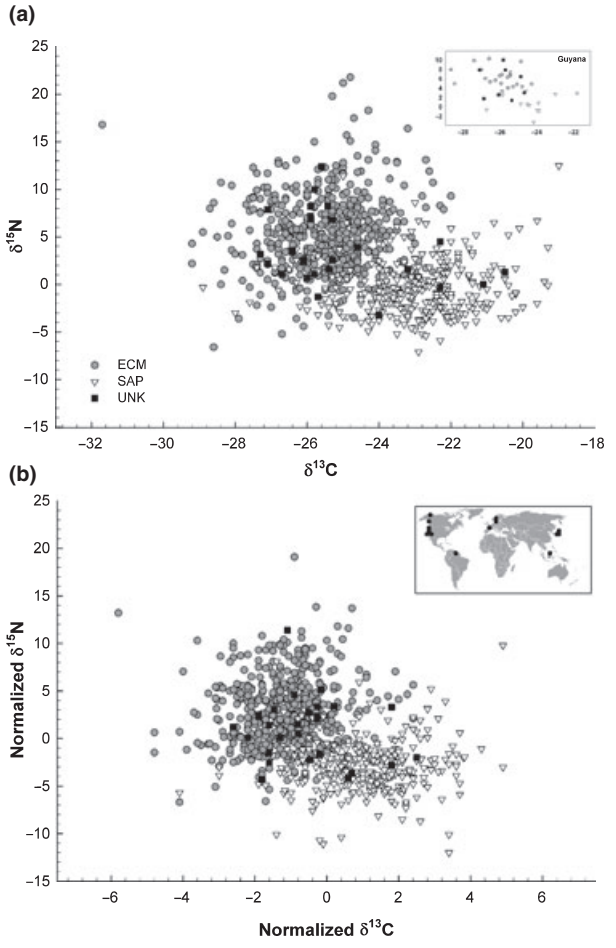

Figure 1 Raw (a) and site-normalized (b) dual isotope graphs of ectomycorrhizal (ECM), saprotrophic (SAP), and fungi of unknown (UNK) ecological role collected from 32 sites around the world. Each symbol represents an individual sporocarp. Inset in (a) illustrates the previously unpublished data set; inset in (b) indicates approximate site locations.

The large number of sporocarp dual isotope measurements used in the discriminant analysis (n = 813) allowed for reliable assignment of collector based categorization error (Appendix S1 in Supporting Information), and consequently, confident categorization of fungi of unknown ecological role based upon > 50% statistical probabilities (Appendix S2). Collector based categorizations using normalized isotope values were deemed valid in 91.2% of sporocarps indicating a high level of agreement with and among categorization methods used by collectors. Site normalization reduced collector categorization error by 0.7% relative to non-normalized isotope values, and retained within site variability. Normalization appears to have only slightly reduced overlap between ECM and SAP groups (Fig. 1b) due to centering of all site means to zero. The increased accuracy of discriminant categorization could be due to increased model efficiency during extraction of eigenvector distances (Quinn & Keough 2005).

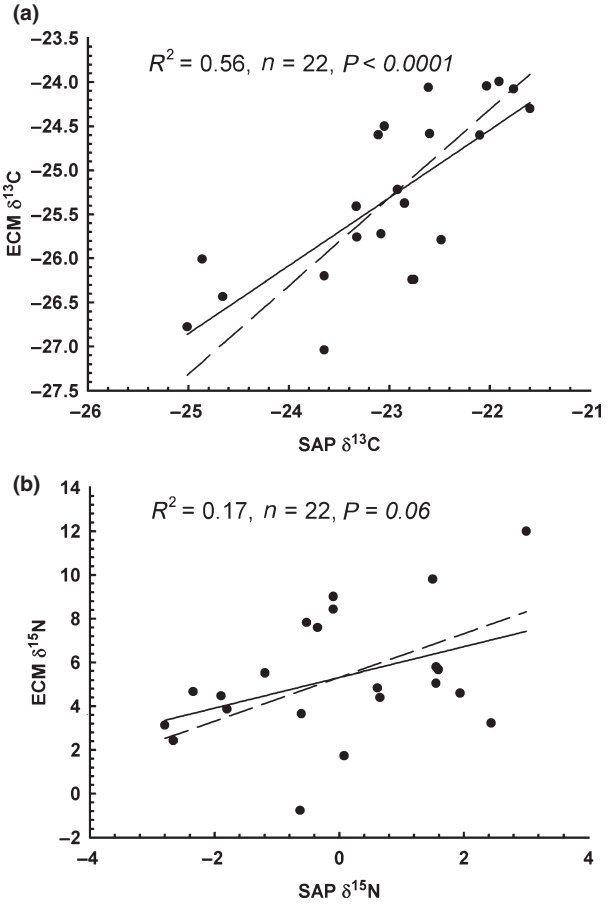

Figure 2 Comparison of the mean δ^{13}C and δ^{15}N of ectomycorrhizal (ECM) and saprotrophic (SAP) fungi from each site. Both A and B linear fits (solid line) are not significantly different from a slope of one (dashed line; $\alpha < 0.05$) indicating a consistent isotopic difference between ECM and SAP fungi across sites. Tanigawa, Japan, was omitted as a statistical outlier. Equations of lines: (a) linear: $f = -7.64 + 0.77(x)$, slope constrained: $f = -2.32 + 1(x)$; (b) linear: $f = -5.32 + 0.7(x)$, slope constrained: $f = 5.32 + 1(x)$.

Discriminant categorization of fungi was further supported by the additional discriminant analysis of a random 50% subset of the data, and cluster analysis of the entire dataset. The random 50% subset increased overall discriminant categorization error by only 0.3% and was in agreement with individual categorizations based on the entire dataset. The cluster analysis also identified ecological categories but increased overall categorization error by an additional 1.5%. These values are similar to error rates derived from the discriminant analysis using the full data set (Appendix S3) and are indicative of low *a priori* categorical-forcing of ecological groups due to assignment by collectors. Combined, these results suggest that sporocarp categorization is robust to a substantial reduction in data input and that collector knowledge, while accurate, is of little statistical importance in predicting fungal ecology relative to δ^{13}C and δ^{15}N values alone.

Climate and latitude serve as reasonably good predictors of fungal isotope values on a global scale. Linear regression of site mean ECM and SAP fungal δ^{13}C indicated significant relationships with MAT (adjusted $R^2 = 0.18$ and 0.63, respectively) and LAT (adjusted $R^2 = 0.49$ and 0.67, respectively), but not with MAP, whereas δ^{15}N was significantly correlated with MAT (adjusted $R^2 = 0.24$) in ECM fungi only (Fig. 3). Singly, MAP and LAT had no predictive power over mean fungal δ^{15}N, but the inclusion of MAT, MAP, and LAT as centered quadratic variables in linear mixed models substantially improved their explanatory power (i.e. $\Delta_I > 2$; Table 2). Removal of LAT from mixed models was deemed useful because it produced comparable model fits as MAT and MAP and reduced obvious correlations among predictor variables. Remaining multicolinearity among centered MAT and MAP was low ($R^2 = 0.13$) and both variables were retained despite potential similarity in information. Linear mixed models indicated that variability in sporocarp δ^{13}C was best explained by MAT, LAT, an interaction between them (MAT*LAT), and sporocarp type (ECM vs. SAP; Table 2). Substituting the random effect of genus for species also substantially increased model fit (Δ_i = 71.2; Table 2). Use of genus allowed for more informative mixed models due to the presence of the same fungal genera across multiple sites, while species were often confined to single locations. Sporocarp δ^{15}N was best described by the fixed effects MAT, MAP, and their quadratic functions (Table 2). Altering random effects to include the interaction of species with site improved model fit, and, as with δ^{13}C, substituting the random effect of genus for species produced a substantially more informative model (Δ_i = 83.0; Table 2).

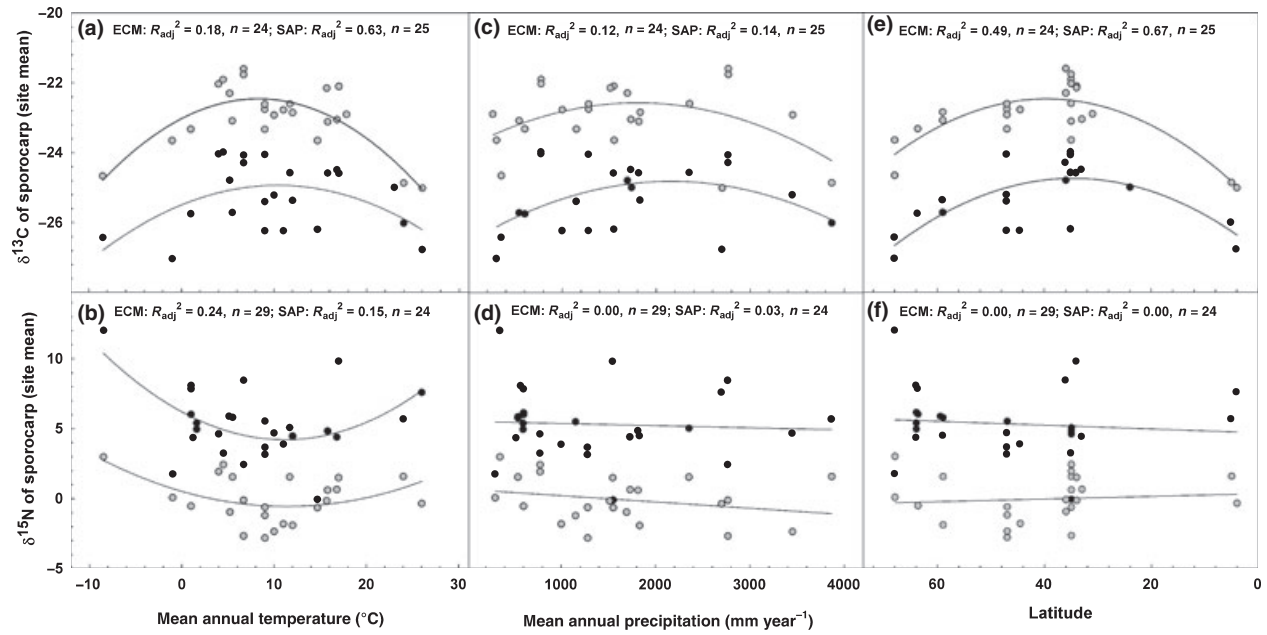

Figure 3 Regressions of site mean fungal $\delta^{13}C$ and $\delta^{15}N$ values with mean annual temperature, mean annual precipitation, and latitude. Black circles are site means of ectomycorrhizal (ECM) fungi; grey circles are site means of saprotrophic (SAP) fungi. Linear vs. quadratic best-fit polynomial equations were compared and equations producing the best R^2 coefficient are presented. Tanigawa and Okinawa, Japan, sites were removed from graphs (b, d, f) as statistical outliers. Equations of lines: (a) $f_{SAP} = -23.02 + 0.14(x) - 0.01(x)^2$, $f_{ECM} = -25.49 + 0.11(x) - 0.005(x)^2$; (b) $f_{SAP} = 0.53 - 0.19(x) + 0.008(x)^2$, $f_{ECM} = 6.22 - 0.36(x) - 0.016(x)^2$; (c) $f_{SAP} = -23.8 + 0.1E^{-2}(x) - 3.9E^{-007}(x)^2$, $f_{ECM} = -26.57 + 0.002(x) - 3.8E^{-007}(x)^2$; (d) $f_{SAP} = 0.69 - 0.5E^{-3}(x)$, $f_{ECM} = 5.55 - 0.2E^{-3}(x)$; (e) $f_{SAP} = -25.56 + 0.16(x) - 0.002(x)^2$, $f_{ECM} = -26.81 + 0.12(x) - 0.002(x)^2$; (f) $f_{SAP} = 0.35 - 0.01(x)$, $f_{ECM} = 4.69 + 0.01(x)$.

DISCUSSION

Implications of the global pattern

The $\delta^{13}C$ and $\delta^{15}N$ based division in ECM and SAP fungi has been described from localized sites and is shown here to exhibit a consistent pattern across the global range of ecosystem types. The magnitude of isotopic differences between ECM and SAP fungi was also similar across sites, as evidenced by a slope similar to one (Fig. 2), and parallel relationships of site mean $\delta^{13}C$ and $\delta^{15}N$ values with climate and latitude (Fig. 3). Site normalization eliminated correlations with climate and LAT but reduced discriminant categorization error by only 0.7%. The similar isotopic differences suggest comparable ecophysiological functioning of these two nutritional groups of fungi across sites that differ widely in climate, plant community, and baseline isotope values. Because of the ubiquity of the pattern, the assembled dataset can be used to confidently (> 90%) categorize the nutritional status of fungi based on $\delta^{13}C$ and $\delta^{15}N$ values of sporocarps or hyphae (Wallander *et al.* 2004). Furthermore, site normalization and collector knowledge increased prediction accuracy, but only by a small margin.

The observed ^{15}N enrichment of ECM relative to SAP sporocarps is attributed to ^{15}N discrimination during the formation and delivery of amino acid N from fungi to plants (e.g., Δ8–10‰, Hobbie & Hobbie 2006). Additional causes of ECM ^{15}N enrichment relative to SAP fungi could include preferential use of ^{15}N enriched forms of N as well as internal processing within the fungus irrespective of transfer to the host plants (Hobbie & Colpaert 2003; Brearley *et al.* 2005; Dijkstra *et al.* 2008). However, field and laboratory observations currently support the N delivery to host plant process as being most influential in terms of isotope fractionation. In addition, frequently observed ^{15}N depletion in ECM associating host plants relative to co-occurring non ECM plants further supports this hypothesis (Hobbie & Hobbie 2008). Lower $\delta^{15}N$ values in non ECM plants are expected because the assimilation and transfer of N by arbuscular mycorrhizal (AM) fungi is thought to impart a smaller ^{15}N fractionation in host plants (e.g. Δ0–2‰, Handley *et al.* 1993). Therefore, a globally similar ECM–SAP divide suggests that ECM fungi are delivering ^{15}N depleted N to host plants under both N limitation to plant productivity, such as might be found in temperate and high latitude ecosystems, as well as in tropical forests that could be limited primarily by P (Mayor & Henkel 2006; Palmiotto *et al.* 2004). In support of these expectations, a recent analysis of foliar $\delta^{15}N$ from 91 studies used the type of root

Table 2 Competing linear mixed model results

Model	*K*	Log-like	AIC	Δ_i*
$\delta^{13}C$				
Species				
Null	3	−1247.2	2504.3	102.4
$MAT_c + LAT_c$	5	−1246.2	2506.4	104.5
$MAT_c + LAT_c + (MAT_c + LAT_c)^2$	4	−1239.9	2491.7	89.8
$MAT_c : LAT_c$	4	−1237.1	2486.2	84.3
$(MAT_c + MAT_c^2) + (LAT_c + LAT_c^2)$	5	−1234.5	2485.0	83.1
$MAT_c + LAT_c + MAT_c : LAT_c$	6	−1228.5	2473.1	71.2
Genus				
Null	3	−1209.3	2428.7	26.8
$MAT_c + LAT_c + MAT_c : LAT_c$	6	−1193.0	2401.9	0.0
$\delta^{15}N$				
Species				
Null	3	−2245.1	4500.1	2088.4
$MAT_c + MAT_c^2 + LAT_c + LAT_c^2$	7	−2241.6	4497.3	2085.7
$MAT_c + MAT_c^2 + MAP_c + MAP_c^2$	7	−2241.1	4496.2	2084.6
Site : Species				
$MAT_c + MAT_c^2 + MAP_c + MAP_c^2$ + SPECIES + SITE : SPECIES	9	−1275.9	2565.7	154.1
$MAT_c + MAT_c^2 + MAP_c + MAP_c^2$ + SITE + SPECIES + SITE : SPECIES	10	−1239.3	2494.6	83.0
Genus				
Null + SITE : GENUS	6	−1204.8	2421.5	9.9
$MAT_c + MAT_c^2 + MAP_c + MAP_c^2$ + SITE + GENUS + SITE : GENUS	10	−1196.4	2411.6	0.0

Models of $\delta^{13}C$ and $\delta^{15}N$ values in ectomycorrhizal (ECM) and saprotrophic (SAP) fungi were based on 913 $\delta^{15}N$ and 813 $\delta^{13}C$ fungal sporocarp measurements. The null mixed model contained the fixed effect of sporocarp type (ECM or SAP) and the random effects of site and either species or genus as indicated. Number of model parameters (*K*), log-likelihood (log-like), Aikake information criteria (AIC) model selection results, centered mean annual temperature (MAT_c), centered mean annual precipitation (MAP_c), centered absolute latitude (LAT_c). *$\Delta_I = AIC_i - AIC_{min}$, where AIC_{min} is the minimum of the different AIC_i values and represents the information lost using other models with higher AIC scores. As a rule of thumb, an $\Delta_I \leq 2$ have substantial support for being more informative over competing models (Anderson 2008).

symbiosis (ECM and ericoid mycorrhizal vs. AM or non-mycorrhizal) to explain 29% of the variation in foliar $\delta^{15}N$ globally (J. Craine *et al.*, unpublished data).

Because ECM and SAP fungi differ fundamentally in C source (i.e. plant photosynthate vs. detritus), sporocarp $\delta^{13}C$ is expected to track the $\delta^{13}C$ values of these two major C pools (Högberg *et al.* 1999a,b; Henn & Chapela 2001). Although the $\delta^{13}C$ values of fungi are thought to reflect patterns found in plant C pools, they are typically 0.3–5‰ more enriched than host tissue (Gleixner *et al.* 1993; Hobbie *et al.* 1999, 2001; Högberg *et al.* 1999a,b; Kohzu *et al.* 1999; Trudell *et al.* 2004; Hart *et al.* 2006). This offset from known (and suspected) substrates may be due to ^{13}C discrimination during decomposition by SAP fungi or during synthesis and translocation of various C pools from host plants to ECM fungi (Högberg *et al.* 2007; Bowling *et al.* 2008). Saprotrophic fungi tend to be relatively more ^{13}C enriched (relative to bulk leaves) than either ECM fungi and their plant sugar C source, or leaf lipids and proteins (Bowling *et al.* 2008). This differential offset from presumed C source suggests additional fractionation pathways may be contributing to SAP ^{13}C enrichment in particular (Kohzu *et al.* 2005). Regardless of physiological contributions to fungal $\delta^{13}C$, differences in the $\delta^{13}C$ values of C pools are considered responsible for the ^{13}C divide among ECM and SAP fungi.

Our analysis illustrates that it is possible to reliably infer ecological roles of fungi over a broad range of conditions using a relatively simple, site based normalization procedure of fungal $\delta^{13}C$ and $\delta^{15}N$ values. It has been suggested that the ECM–SAP divide should become less distinct over large geographical areas and that the isotopic signature of an organism is insufficient evidence to infer ecological role without first standardizing to an appropriate isotopic baseline (Henn & Chapela 2001; Hobbie *et al.* 1999, 2001; Post 2002; Taylor *et al.* 2003). However, our normalization slightly enhanced the resolution of the discriminant analysis through removal of mean isotopic differences among sites without comparison to a measured baseline. Given the small decrease in discriminant categorization error following site normalization (0.7%) it is unlikely that correcting to isotopic baseline measurements (leaves and mineral nutrients) could

reduce categorization error further. For instance, isotopic corrections based on foliar samples could introduce variation based on sample position in the canopy, taxonomic grouping, growth form, or type of root symbiosis (Bustamante *et al.* 2004; J. Craine *et al.*, unpublished data; Pate & Arthur 1998). Additionally, isotopic corrections based on soil $\delta^{15}N$ could introduce variation depending on depth, disturbance, and fertility of the samples (Bustamante *et al.* 2004; Davidson *et al.* 2007; Dijkstra *et al.* 2008).

In addition to the discrimination of fungal ecological roles across multiple ecosystems, our dataset shows an ECM–SAP difference ($\Delta_{ECM-SAP}$) in $\delta^{15}N$ of 5.3‰ and −2.3‰ for $\delta^{13}C$; values that will help constrain the magnitude of isotope fractionations used in modeling efforts. Fractionation attributed to ECM mediated N assimilation and transfer to host plants were recently used to estimate that Alaskan tundra plants received 61–86% of their total N from ECM fungi and reciprocally delivered 8–17% of their photosynthetic C to ECM fungi (Hobbie & Hobbie 2006). These simultaneous mass balance calculations require accurate assessment of the fractionation magnitude associated with ECM delivered N in order to constrain additional estimated, and interdependent, variables (Hobbie & Hobbie 2008). This quantification of elemental cycling is a necessary step to the modeling of N cycles and the partitioning of below ground C allocation. Values in our global analysis can refine this approach because additional fractionation effects, such as during N uptake, have been partially removed through the subtraction of co-occurring SAP $\delta^{15}N$ values. Better parameterization of this key ^{15}N fractionation step is necessary for expansion to, and testing of, C and N mixing models in additional ecosystems (Hobbie & Wallander 2006).

Climatic influence on sporocarp $\delta^{15}N$

Fungal $\delta^{15}N$ patterns were expected to correlate with MAT, MAP, and LAT to the extent that soil and litter $\delta^{15}N$ values correlate with those values. In previous global isotopic analyses, MAT and MAP were found to be good predictors of soil and plant $\delta^{15}N$ due to their influence over soil N cycling and the isotope ratios of ecosystem N inputs and outputs (Amundson *et al.* 2003). This is because warm temperature and high rainfall conditions are conducive to high rates of N mineralization and nitrification leading to a loss of ^{15}N depleted N through denitrification or leaching and consequent ^{15}N enrichment of soil N (Amundson *et al.* 2003; Templer *et al.* 2007). Therefore, the integrated N pool in tropical soils is typically ^{15}N enriched relative to high latitude ecosystems with low soil N availability and conservative N cycles (Högberg 1997; Amundson *et al.* 2003). Significant sporocarp $\delta^{15}N$ enrichment was observed in fungi from tropical relative to temperate sites but the predictive ability of individual variables to explain fungal $\delta^{15}N$ patterns was only weakly correlated with MAT. The curved relationship among ECM $\delta^{15}N$ values and MAT (Fig. 3b) was driven by the coldest tussock tundra site near Toolik, Alaska (Clemmensen *et al.* 2006), the exclusion of which removed the relationship. Causes for the higher than expected $\delta^{15}N$ values in ECM sporocarps at Toolik, AK (12‰) could result from large proportional N transfer by ECM fungi to severely N limited plants (Hobbie & Hobbie 2006) or ECM and SAP access to anomalously ^{15}N enriched N sources as suggested by Lilleskov *et al.* (2002). Whereas individual variables were poor predictors of fungal $\delta^{15}N$, enhanced explanatory power in mixed effect models illustrate that MAT and MAP do hold predictive ability when properly formulated (Δ_i = 9.9; Table 2). In summary, the expectation that fungal $\delta^{15}N$ patterns would respond similarly to that seen for plants and soils was not supported suggesting that fungal physiology exerts primary influence over fungal $\delta^{15}N$ patterns. However, the inclusion of fungal and foliar $\delta^{15}N$ values from more tropical and subtropical sites will undoubtedly improve the predictive ability of discriminant and mixed models and help clarify the secondary influence of climate on sporocarp $\delta^{15}N$.

Climatic influence on sporocarp $\delta^{13}C$

Mean $\delta^{13}C$ values from fungi in the warm/wet sites were most similar to those from the cold/dry sites when viewed in relation to MAT and LAT as single predictor variables. As with $\delta^{15}N$, we determined the influence of the coldest site (Toolik Lake, AK) by removing it, however in this case the relationship between fungal $\delta^{13}C$ and MAT remained (adjusted R^2 = 0.16 ECM, 0.56 SAP). Plant analyses of foliar $\delta^{13}C$ generally provide reliable indices of plant water use efficiency (WUE) owing to ^{13}C discrimination during photosynthesis and its relation to stress induced stomatal closure (Marshal *et al.* 2007). However, the weak relationship between fungal $\delta^{13}C$ and MAP in our analysis indicated that this coarse climatic variable was of little utility in explaining fungal, and presumably plant, C isotope patterns across such diverse sites. For instance, the four dry sites in our meta-analysis differed widely in temperature (−8.5–5 °C), as did the four wettest (9.5–25 °C). Therefore, partitioning individual climatic influences over fungal $\delta^{13}C$ at these extreme conditions is confounded by the joint possibility of greater water stress at dry sites and temperature induced photosynthetic inhibition at the coldest ones similarly altering plant, and indirectly fungal, $\delta^{13}C$ (Allen & Ort 2001). Regional analyses of MAP gradients have strongly correlated with foliar $\delta^{13}C$ in Northern and Southeastern Australia (R^2 = 0.64 and 0.70, respectively; Austin & Sala 1999; Stewart *et al.* 1995), but not in Hawaii (Schuur & Matson 2001). It is more likely that measures of

actual or potential evapotranspiration would be more informative in future studies.

Latitude explained the largest portion of mean fungal $\delta^{13}C$ variability among sites (adjusted $R^2 = 0.49$, ECM; 0.67, SAP) likely due to its integration of multiple effects on plant $\delta^{13}C$ patterns, and combined with MAT and their interaction, substantially increased the explanatory power of mixed models ($\Delta_i = 26.8$; Table 2). The proxies of MAT and LAT likely integrate the timing and form (snow vs. water) of precipitation among sites during growing seasons, as well as other physiological stressors that can modify plant WUE and $\delta^{13}C$ values, such as: (i) within species physiological variability (Pate & Arthur 1998; Schuur & Matson 2001; Kohzu *et al.* 2005); (ii) species replacement, particularly at low soil water contents (Swap *et al.* 2004); (iii) relative N availability and C sink strength of ECM fungi (Hobbie & Colpaert 2003); and (iv) compensatory effects of specific leaf area and leaf N concentration on plant WUE (Schulze *et al.* 2006). Similarly, a global meta-analysis of 1248 plants across 452 sites demonstrated the ability of MAT and LAT to explain patterns in foliar N and P at global scales, suggesting a reflection of both plant physiological adjustments and the relative shifts in nutrient limitations due to changes in the age of soils (Reich & Oleksyn 2004).

Predictions of fungal ecology

Despite the strength of the discriminant analyses, known errors in model categorization have been found in our dataset suggesting other, unknown errors are likely. Known 'SAP categorization errors' are confirmed wood decay fungi that were categorized as ECM by the discriminant model with > 90% probability. These apparent errors included the following individual sporocarp collections: *Pleurotus ostreatus* (Jacq.) P. Kumm. from Chiba, Japan, *Microporus vernicipes* (Berk.) Kunt. from Lambir, Malaysia (Kohzu *et al.* 1999); and *Gymnopilus bellulus* (Peck) Murrill, from Lamar Haines, Arizona (Hart *et al.* 2006). Including other apparent SAP errors with < 90% modeled probabilities increases the SAP errors by six individual samples (Appendix S1). Error in the categorization of decomposer fungi could be partially caused by unique nutritional sources in addition to wood or litter. For instance, access to recent plant photosynthate with relatively low $\delta^{13}C$, or to accruing microbial and insect biomass with relatively high $\delta^{15}N$, could cause SAP fungi to 'appear' as ECM in the discriminant model.

Discriminant model errors regarding ECM fungi are more difficult to discern because of the difficulty in determining the nutrition of terrestrial sporocarps. Several individual sporocarps belonging to genera and species traditionally categorized as ECM were categorized as SAP by the discriminant model with > 90% probability. These presumed 'ECM categorization errors' included: *Cortinarius sp.* (Pers.) Gray Kyoto, Japan, *Tylopilus sp.* P. Karst. from Lambir, Malaysia (Kohzu *et al.* 1999); *Cortinarius variosimilis* M.M. Moser & Ammirati, from Deer Park Rd, WA (Trudell *et al.* 2004); and *Cantherellus pleurotoides* T.W. Henkel, Aime and S.L. Mill. from Guyana (this study; Henkel *et al.* 2006). Including other, typically ECM genera (e.g., *Amanita, Russula, Lactarius, Boletus*) with lower than 90% probabilities would increase this number by 23 individual samples (Appendix S1). Causes for these discrepancies are unknown but warrant caution in accepting predictions based on dual isotope measures of single sporocarp collections in the absence of other evidence (Trudell *et al.* 2004). Triplicate analyses of six species in our Guyana dataset (included in Appendix S3), collected across years, had standard deviations of 0.61–0.71 ($\delta^{13}C$ and $\delta^{15}N$, respectively) and were all assigned to the same ecological group by the discriminant model. When anomalous isotope values do occur for individual sporocarps across sites, particular caution of categorization error is warranted. At low sampling number, site normalized values are likely to be less accurate (e.g. not a true site mean) due to insufficient sporocarp numbers. This is particularly problematic if using archived specimens from distant or poorly documented sites. In contrast, when the same fungal species is categorized 'incorrectly' across multiple sites or collections it may offer insight into unique nutritional, kinetic, or physiological activities that run counter to traditional predictions in groups otherwise considered to have a narrow nutritional mode.

Classification of fungi with unknown ecological roles using site normalized dual isotope measurements produced confident predictions (i.e. probability > 90%) for 19 out of 27 fungi in our dataset, and weak to moderate predictions (50.2–89%) for the remaining eight (Appendix S2). Such predictions could form the basis for research hypotheses about the evolution of the ECM and SAP habit (Hobbie *et al.* 1999, 2001) and be combined in a phylogenetic context (e.g., Wilson *et al.* 2007). For instance, the inclusion of *Phylloporus rhodoxanthus* (Schwein.) Bres., a lamellate genus included within an otherwise poroid hymeniphore forming family (Boletaceae), adds ecological support to the micromorphological, molecular, and observational data categorizing this genus as ECM. In addition, the previously uncertain ecological roles of *Clavulina* Schroet in Cohn, *Helvella* L., *Coltriciella* Murrill, and *Tremellodendron* G.F. Atk., now have strong isotopic evidence for the ECM mode of nutrition; and in conjunction with phylogenetic affinity to other presumed ECM taxa (e.g., Henkel *et al.* 2005), now have additional support (Appendix S2).

In agreement with natural history based methods, accurate identification of fungal genera is useful for predicting the ecological role of many fungi. Consistent generic categorization in the discriminant model and improved linear mixed model predictive capacity using

genus support this. The observation that $\delta^{15}N$ patterns of fungal genera can exhibit either high or low $\delta^{15}N$ (Trudell *et al.* 2004) and $\delta^{13}C$ syndromes (Kohzu *et al.* 1999) suggest that future studies may seek to define a stable isotope based niche space similar to the metrics used to describe tidal food webs (Layman *et al.* 2007). It is intriguing to speculate that specialization of some fungal genera with depth (Lindahl *et al.* 2006; Lee Taylor *pers. comm.*), N source (Lilleskov *et al.* 2002; Hobbie & Hobbie 2008), or other niche dimensions could be represented by an index of sporocarp $\delta^{15}N$ and $\delta^{13}C$ variability. If demonstrated, this could also contribute to how genus increased the ability of mixed models to predict fungal $\delta^{15}N$ and $\delta^{13}C$ patterns globally.

CONCLUSION

The C and N isotopic difference between ECM and SAP fungi provides researchers with a reliable tool for the ecological categorization of fungi regardless of site origin. This time integrated, biogeochemical evidence offers insight into C and N cycles across most forested ecosystems and highlights the global importance of ECM fungi to host plant N nutrition and forest N cycling. The ecophysiology associated with ECM and SAP nutritional roles was shown to exert primary control over fungal $\delta^{13}C$ and $\delta^{15}N$ values, however some of the variability was attributable to climatic and latitudinal proxies. These findings offer a tool by which fungi can be integrated into our understanding of global elemental cycles and illustrates that fungal $\delta^{15}N$ may be partially decoupled from soil and plant isotope patterns. As evidenced here, datasets containing fungi from tropical forests contributed to the reliability of stable isotope analyses to discriminate ecological roles of any sporocarp producing fungus and the future inclusion of fungi from subtropical and southern hemisphere sites will undoubtedly improve our confidence in this approach.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Collector-based misclassifications based on discriminant analysis.
Appendix S2 Categorization of fungi of unknown ecological role based on discriminant analysis.
Appendix S3 Comma-delimited file of the data used in the analyses.

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