

REVIEW AND SYNTHESIS

Ectomycorrhizal impacts on plant nitrogen nutrition: emerging isotopic patterns, latitudinal variation and hidden mechanisms

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Abstract

Ectomycorrhizal (EcM)-mediated nitrogen (N) acquisition is one main strategy used by terrestrial plants to facilitate growth. Measurements of natural abundance nitrogen isotope ratios (denoted as $\delta^{15}\text{N}$ relative to a standard) increasingly serve as integrative proxies for mycorrhiza-mediated N acquisition due to biological fractionation processes that alter $^{15}\text{N}:^{14}\text{N}$ ratios. Current understanding of these processes is based on studies from high-latitude ecosystems where plant productivity is largely limited by N availability. Much less is known about the cause and utility of ecosystem $\delta^{15}\text{N}$ patterns in the tropics. Using structural equation models, model selection and isotope mass balance we assessed relationships among co-occurring soil, mycorrhizal plants and fungal N pools measured from 40 high- and 9 low-latitude ecosystems. At low latitudes ^{15}N -enrichment caused ecosystem components to significantly deviate from those in higher latitudes. Collectively, $\delta^{15}\text{N}$ patterns suggested reduced N-dependency and unique sources of EcM ^{15}N -enrichment under conditions of high N availability typical of the tropics. Understanding the role of mycorrhizae in global N cycles will require reevaluation of high-latitude perspectives on fractionation sources that structure ecosystem $\delta^{15}\text{N}$ patterns, as well as better integration of EcM function with biogeochemical theories pertaining to climate-nutrient cycling relationships.

Keywords

Above- and below-ground interactions, nutrient cycling, nutrient limitation, plant–soil interactions, tropical ecology, structural equation modelling, ^{15}N .

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INTRODUCTION

Soil N availability limits plant growth in many high-latitude ecosystems due to the slow accumulation of biologically fixed N during ecosystem development (Chapin *et al.* 1986). In low-latitude forests, phosphorus (P) is generally more limiting due to higher rates of biological N fixation and losses of P to soil weathering processes (Hedin *et al.* 2003; Menge *et al.* 2012). Arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) associations are two main types of mycorrhizae that play integral roles in helping plants meet mineral nutrient demands (Smith & Read 2008; Smith & Smith 2011). In general, most plants associate with AM fungi in the ancient, monophyletic phylum Glomeromycota, particularly tropical forest trees and herbaceous species. Ectomycorrhizal plants, while taxonomically more rare, are common within boreal and temperate forests (e.g. Pinaceae, Fagaceae, Betulaceae, Nothofagaceae and others) (Tedersoo & Smith 2013), but also in several ecologically important tropical trees from the Amherstieae and Mirbelieae of Fabaceae, Dipterocarpaceae, Leptospermoideae of

Myrtaceae and others (Brundrett 2009). Ectomycorrhizal fungi include a diverse assemblage of families and genera of the Basidiomycota, and to a lesser extent Ascomycota (Smith & Read 2008).

Although both of these mycorrhizal types confer nutritive and other benefits to their host plants, they are functionally distinct due to differences in mode of interaction, hyphal morphology, cellular biochemistry, enzymatic capacity and carbon costs to host plants (Taylor & Alexander 2005; Smith & Read 2008). For instance, EcM fungi are thought to provide plants with greater access to organic N bound in chitin, proteins and tannins (Lucas & Casper 2008; Talbot *et al.* 2008; Wurzburger & Hendrick 2009), whereas AM fungi predominantly access mineral or amino acid N due to very limited hydrolytic and oxidative capacity (Courty *et al.* 2010; Smith & Smith 2011). Because EcM plant litter and fungal residues are generally more refractory or gradually accumulate in soil, these two mycorrhizal types also differ in their influence on carbon and mineral nutrient cycling (Cornelissen *et al.* 2001; Langley & Hungate 2003; Read & Perez-Moreno 2003; Phillips & Fahey

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2006; Orwin *et al.* 2011; Clemmensen *et al.* 2013; Phillips *et al.* 2013; Averill *et al.* 2014).

There are few tools to evaluate mycorrhizal roles in N cycling *in situ*. Analyses of natural abundance N isotope ratios ($^{15}\text{N}:$ ^{14}N expressed as $\delta^{15}\text{N}$ relative to standard), as an integrator of N cycling, can provide a glimpse into mycorrhizal functional ecology within soil profiles and across biomes (Lindahl *et al.* 2007; Courty *et al.* 2011; Tedersoo *et al.* 2012b; Nave *et al.* 2013). This is possible because the isotopic imprint of the EcM symbiosis is manifest in both plant and fungal associates. Ectomycorrhizal plants are generally ^{15}N -depleted relative to AM or non-mycorrhizal plants (Schulze *et al.* 1994; Michelsen *et al.* 1998; Craine *et al.* 2009) and EcM fungi typically are ^{15}N -enriched relative to co-occurring saprotrophic fungi (reviewed in Mayor *et al.* 2009). Such observations suggest that relative (i.e. plant and fungal) $\delta^{15}\text{N}$ values provide a time-integrated, non-destructive tracer of not only soil N sources, but also the relative demand for EcM-derived N (reviewed in Hobbie & Högberg 2012). This is because the relative N isotope concentrations in EcM plant and fungal symbionts are currently understood to result from the delivery of ^{15}N -depleted N transfer compounds to host plants and subsequent retention of ^{15}N -enriched N by fungi (Hobbie & Colpaert 2003). As a result of these apparently linked sources of ^{15}N -fractionation, one can estimate the proportion of plant N derived from EcM fungi across successional chronosequences, natural gradients and under fertilisation or N deposition regimes (Hobbie *et al.* 2005; Averill & Finzi 2011; Högberg *et al.* 2011; Mayor *et al.* 2012; Nave *et al.* 2013).

These ^{15}N mass balance frameworks were developed from a few intensively studied high-latitude tundra and boreal ecosystems where plant productivity is predominantly N-limited (Hobbie & Hobbie 2008). It remains unknown if the same plant and fungal $\delta^{15}\text{N}$ patterns are present in lower latitude subtropical and tropical (hereafter sub/tropical) ecosystems where EcM trees are growing under conditions of more rapid N cycling (Kuyper 2012). Weathered soils, humid conditions and low available P often result in high N losses and concomitantly ^{15}N -enriched soils (Houlton *et al.* 2006; Brookshire *et al.* 2012). Thus, conditions of high N availability, combined with potentially enriched background $\delta^{15}\text{N}$, may obscure the formation of distinct $\delta^{15}\text{N}$ patterns and their subsequent utility in studying tropical EcM associations. This gap in understanding is particularly acute as 80% of EcM ecology literature occurred in only two predominantly high-latitude plant groups (i.e. Pinaceae and Fagales; Dickie & Moyersoen 2008; Alexander & Selosse 2009).

Evidence from EcM plant species in the tropics has suggested that relative $^{15}\text{N}:$ ^{14}N ratios among EcM and AM trees are inconsistent with those described from high-latitude forests. For instance, data from the Afro-tropics suggested that $\delta^{15}\text{N}$ values in some EcM trees are equivalent to or even higher than those of co-occurring AM trees (Högberg 1990; Högberg & Alexander 1995; Cerling *et al.* 2004; Tedersoo *et al.* 2012b). In addition, EcM plants in temperate forests subjected to high N deposition are occasionally ^{15}N -enriched relative to co-occurring AM plants, suggesting that N saturation can obscure the EcM signal (Schulze *et al.* 1994; Pardo

et al. 2006). Such increases in EcM plant $\delta^{15}\text{N}$ values following N additions have been attributed to functional variation in associated EcM fungal taxa or to the bypassing of EcM-mediated N uptake (Lilleskov *et al.* 2002, 2011; Högberg *et al.* 2011).

Evidence also suggests that some tropical trees may rely on EcM-mediated N acquisition, particularly in monodominant forests with high soil organic matter and low N availability (Torti *et al.* 2001; Henkel *et al.* 2002; Brearley *et al.* 2003; Mayor & Henkel 2006; Newbery *et al.* 2006). In addition, $\delta^{15}\text{N}$ values from some tropical fungi were consistently ^{15}N -enriched relative to sympatric saprotrophic fungi independent of climate, geography or substratum (Mayor *et al.* 2009). Thus, the observation of consistent ^{15}N -enrichment of tropical EcM fungi, but not necessarily corresponding ^{15}N -depletion of tropical EcM plants, calls into question the current paradigm explicitly linking the two patterns.

Until now, the paucity of data sets that included co-occurring soil, fungal and plant $\delta^{15}\text{N}$ values from low-latitude ecosystems prevented full assessment of how changes to host-symbiont nutrient limitations influence $\delta^{15}\text{N}$ patterns across biomes. Here, we seek to overcome this limitation by assessing if there are globally unifying or deviating trends in EcM plant N dynamics. To do this, we assembled several published and original data sets containing $\delta^{15}\text{N}$ values representing the major co-occurring ecosystem components involved in N cycling: soils, sporocarps of EcM and saprotrophic fungi, and foliage from both EcM and AM plants. To address both direct and indirect causes of ecosystem $\delta^{15}\text{N}$ patterns at large scales, we compared structural equation models (SEM) to examine hypothetical causal pathways among ecosystem components (Grace *et al.* 2010; Lam & Maguire 2012). For instance, incorporation of indirect climatic influences over soil $\delta^{15}\text{N}$ and N concentrations, and the possibility of distinctive patterns in N cycling among AM and EcM systems, is made possible by comparing competing path diagrams in SEM. These data permit a balanced examination of relative ecosystem $\delta^{15}\text{N}$ patterns so that: (1) the influence of EcM fungi over plant $\delta^{15}\text{N}$ patterns may be assessed in an inclusive global context, (2) any alternative pathways of causality can potentially be elucidated and (3) estimates of the importance of EcM fungi for the N nutrition of host plants may be placed within a context of biogeochemical predictions regarding plant nutrient limitations. Linking plant nutrient demands with the functional role of distinct mycorrhizal types has been highlighted as a research priority in ecosystem science (Phillips *et al.* 2013) and examining latitudinal variation in ecosystem $\delta^{15}\text{N}$ patterns offers a unique opportunity to assess the role of EcM in N cycling (Courty *et al.* 2010).

MATERIALS AND METHODS

We compiled data from studies published up through July 2013 that included soil, plant and fungal $\delta^{15}\text{N}$ values with original data. Samples collected by the authors were silica dried in the field prior to transporting to one of several laboratories for isotopic analyses. To evaluate general trends across disparate studies, data were aggregated for sites < 100 km distant. Compiling site-based variability in this manner permitted

comparison at the global scale without potentially confounding effects of spatial autocorrelation. In some cases this meant averaging among sites that differed slightly in underlying parent material, elevation or plant taxa (i.e. Fortuna Reserve, Panama, this study; Oregon, USA in Hobbie *et al.* 2012; New Hampshire, USA in Averill & Finzi, 2011). Due to floristic heterogeneity and/or sampling limitations, some sites contained only one dominant EcM plant species, whereas others contained $\delta^{15}\text{N}$ values from many EcM and AM species (> 8; see Tables S1 and S2). The number of sampled fungal taxa representing different trophic groups also varied by site (e.g. 2 to > 50). In total, we averaged data from 47 sites taken from 22 published and 7 original studies (Fig. 1).

Mean annual temperature (MAT) and precipitation (MAP), along with geographical positions (lat./long.), were taken from the published studies, studies referred to therein, obtained on site, or extracted from a global climate database (New *et al.* 2002). Statistical analyses and graphical representations used absolute values of latitude. Stand age, elevation and soil N concentrations ($[\text{N}] \text{ mg g}^{-1}$) were also extracted if available. Owing to the varying methods across studies, soil $[\text{N}]$ was measured from samples of varying layers or depths (0–5, and 5–10, 12 or 15 cm). When separate organic and mineral $[\text{N}]$ values were reported, we averaged their concentrations on a volumetric basis and hereafter refer to them as surface soil $[\text{N}]$. Similarly, ‘organic’ and ‘mineral’ layers may not necessarily coincide with strict definitions of C content but such divisions were retained for $\delta^{15}\text{N}$ values to address presumed ^{15}N -enrichment with depth. Soil C content was infrequently reported, preventing use of C/N ratios in subsequent analyses. Several studies had missing values for one or more ecosystem components (e.g. saprotrophic fungi or AM plant $\delta^{15}\text{N}$ values). In such instances, the original authors were asked for additional metadata and to assess if serially published studies contained duplicated sample values. Site metadata and references are given in Table S1. Taxonomic identities of organisms and geographical locations of original soil, fungal and plant $\delta^{15}\text{N}$ values are included in Table S2. Overall, sites varied widely in latitude (–13 to 74 °N), altitude (5 to 2780 m a.s.l.), mean annual precipitation (183–7032 mm year $^{-1}$) and mean annual temperature (–9.8 to 26 °C). Surface soil N

concentrations ($[\text{N}]$) ranged from 0.6 to 35.7 mg g $^{-1}$ and soil $\delta^{15}\text{N}$ values ranged from –4.6 to 8.7 ‰.

The included data sets have certain limitations. First, most studies involving ^{15}N analyses of both plants and fungi have been undertaken in arctic, boreal and temperate ecosystems of the Northern Hemisphere, whereas studies in tropical regions are rare, and data from temperate forests of the Southern Hemisphere nearly non-existent. Second, data for other potentially important factors influencing the pathways of causality put forth in SEM, such as N and P availability, mineral N $\delta^{15}\text{N}$ values or soil clay content, were lacking for most sites and therefore not included.

Statistical analyses

Graphical assessments and univariate linear regressions were performed in JMP[®] Pro 10.0.0 (SAS Institute Inc., Cary, NC, USA). Generalised least-squared model selections were conducted using the *nlme* package version 3.1-104 (Pinheiro *et al.* 2011) in the R statistical environment (R Development Core Team 2012). Structural equation modelling was performed using Amos Version 7.0 (SPSS, Chicago, IL, USA). Explanatory variables were compared to normal distributions and outliers were assessed using goodness-of-fit tests. Two extreme outliers were subsequently removed from the *soil [N]* data that were heavily influenced by anthropogenic N deposition (*soil [N]* = 35.7, mg/g) and a single high $\delta^{15}\text{N}$ value in mineral soil from Gabon.

Structural equation models are similar to many widely accepted statistical methods such as regression and path analysis, but are better suited to test assumptions regarding pathways (both direct and indirect) of causality among multiple ecosystem components in a theoretical context (Grace *et al.* 2010). Unlike regression and ANOVA analyses, SEM enable us to examine whether preconceived model structures (i.e. strength and direction of causality) match with theoretical frameworks based on *a priori* knowledge (Grace *et al.* 2010; Lam & Maguire 2012). Use of SEM has been gaining traction in the biological and ecological literature (Shipley 2000; Grace 2006; Lavorel & Grigulis 2013). Our SEM included $\delta^{15}\text{N}$ values taken from the main co-occurring pools of N: AM plants,



Figure 1 Approximate geographical locations of the published (filled circle) and original (open triangle) sites used in this study.

EcM plants, saprotrophic fungi, EcM fungi and surface soils. We also included available variables that were perceived to have direct (i.e. soil [N]) and indirect (i.e. climate, elevation, forest age) influence over the N cycling in these forested ecosystems. The SEM were analysed using an exploratory approach owing to initial uncertainty in the strength and direction of climatic influences over N cycling pathways. Initially, a full model including all available variables that may influence the demand for and pathways of N cycling were constructed using: soil [N] (mg/g), stand age (year), elevation (m), MAT, MAP, high vs. low latitude and lat./long. Climate (MAT and MAP) and the absolute value of latitude were also assessed as square root transformations to account for non-linearity. Variables were subsequently removed using backward elimination stepwise regression until only the minimum significant non-redundant variables remained. Model outputs supported the supplementation of climatic data with the strongly correlated (see Fig. S3), yet putatively more encompassing, latitudinal proxy ($R = 0.77$ and 0.61 for latitude vs. MAT and MAP, respectively) to best account for observed trends in isotopic gradients. The relatively small number of low-latitude data sets prevented separate SEM constructions for high-latitude and low-latitude ecosystems to specifically contrast these ecosystem types; instead we included these categories as potentially exogenous model parameters. Categorical groupings of high- vs. low-latitude sites were made at $\pm 27^\circ$ latitude to allow for statistical contrasts. This break point was defined by the furthest site from the equator that retained a subtropical climate (e.g. Hou *et al.* 2012) but does not correspond to a globally universal latitudinal 'break point' for sub/tropical conditions due to regional climatic variability.

During the process of model construction, separate models for the $\delta^{15}\text{N}$ values of saprotrophic fungi and EcM and AM plants were explored to ascertain distinct pathways and correlations of error terms among these components individually. Soil $\delta^{15}\text{N}$ values were initially modelled as exogenous with no causal agents in the exploratory models. Next, plausible relationships between all ecosystem $\delta^{15}\text{N}$ components were explored by assessing path diagrams and model fit parameters [i.e. chi square, root mean square error of approximation [RMSEA] and probability of a close fit [PClose]]. A non-significant P value indicates that the model structure does not differ significantly and that the model is a feasible representation of the data (see goodness-of-fit tests below). Competing SEM were compared with the most parsimonious models based on corrected Akaike Information Criteria (AICc) output (see Table S4) for comparison of statistical methods. In addition, because of strong correlation among ecosystem $\delta^{15}\text{N}$ values, latent variables representing shared variation among observed variables were defined and incorporated when significant, but omitted from the final path diagram to simplify visual presentation and to prevent overly abstract construction of the role such latent variables might have in structuring ecosystem $\delta^{15}\text{N}$ values (see Grace *et al.* 2010 for a discussion of such theoretical constructs in ecosystem ecology). We used best-fit regression functions (linear, quadratic, cubic) and correlation analysis (Pearson-product moment) to examine relationships

among ecosystem $\delta^{15}\text{N}$ values, or their relative differences, with absolute values of latitude to more thoroughly examine relationships that emerged from the SEM and hypothetical predictions.

We also examined if the following system of ^{15}N mass balance models developed in high-latitude forests (equations from Hobbie & Högberg 2012) were able to provide reasonable estimates for N transferred in the EcM symbiosis using averaged $\delta^{15}\text{N}$ values from the high- and low-latitude sites.

$$\delta^{15}\text{N}_{\text{EcM plant}} = \delta^{15}\text{N}_{\text{available N}} + \Delta \times (\log_e \times f) / (1 - f) \quad (1)$$

$$\delta^{15}\text{N}_{\text{EcM fungi}} = \delta^{15}\text{N}_{\text{available N}} - \Delta \times \log_e \times (1 - f) \quad (2)$$

$$\Delta = (\delta^{15}\text{N}_{\text{available N}} - \delta^{15}\text{N}_{\text{EcM plant}}) / (1 + \delta^{15}\text{N}_{\text{EcM plant}}) \quad (3)$$

where $\delta^{15}\text{N}_{\text{available N}}$ represents the combined value of all available soil N sources used, Δ represents the effective discrimination against ^{15}N during the production of N transfer compounds from available N by EcM fungi and f represents the proportion of total tree N comprised of those compounds. Assignment of three of the parameters used in each of these simultaneous equations permits solving for the fourth unknown parameter of interest. For instance, using plant and fungal $\delta^{15}\text{N}$ values reported in data sets, Δ values estimated from laboratory studies, and a range of $\delta^{15}\text{N}_{\text{available N}}$ values approximating actual soil measurements, estimates are possible of the upper and lower proportional bounds of N transferred by EcM (f).

RESULTS

Patterns among soil, plant and fungal $\delta^{15}\text{N}$ values

The $\delta^{15}\text{N}$ values of mineral and organic soil horizons were positively correlated across sites ($R = 0.70$, $n = 27$), and mineral soils were on average $3.0 \pm 1.6\text{‰}$ (mean \pm SD) more ^{15}N -enriched than those of organic soils (matched pairs t -test: $P < 0.001$, $n = 27$). Soil $\delta^{15}\text{N}$ values from surface organic layers were negatively correlated with latitude (quadratic polynomial: $R^2 = 0.298$, $P = 0.001$, $n = 42$; $\gamma = 0.36 - 0.029 \times \chi + 0.0021 \times (\chi - 42.02)^2$; Fig. 2a), leading to significant ^{15}N -enrichment of sub/tropical forest soils compared to those of higher latitude ecosystems ($P = 0.036$, unequal variance t -test; Fig. 2a). In pursuit of inherent biases in our data set, we examined the possibility that the highest latitude soil $\delta^{15}\text{N}$ values were driving the relationships by removing all sites above 51° , refitting regression models and comparing test statistics (see Fig. S5). Removal of sites $>51^\circ$ did not decrease the variance explained or the significance of model formulations seen in Fig. 2a, but statistical support for ^{15}N -enrichment of sub/tropical mineral soils compared to higher latitude ecosystems increased ($P = 0.046$ with $>51^\circ$ removed vs. $P = 0.07$ with all sites; Fig. S5).

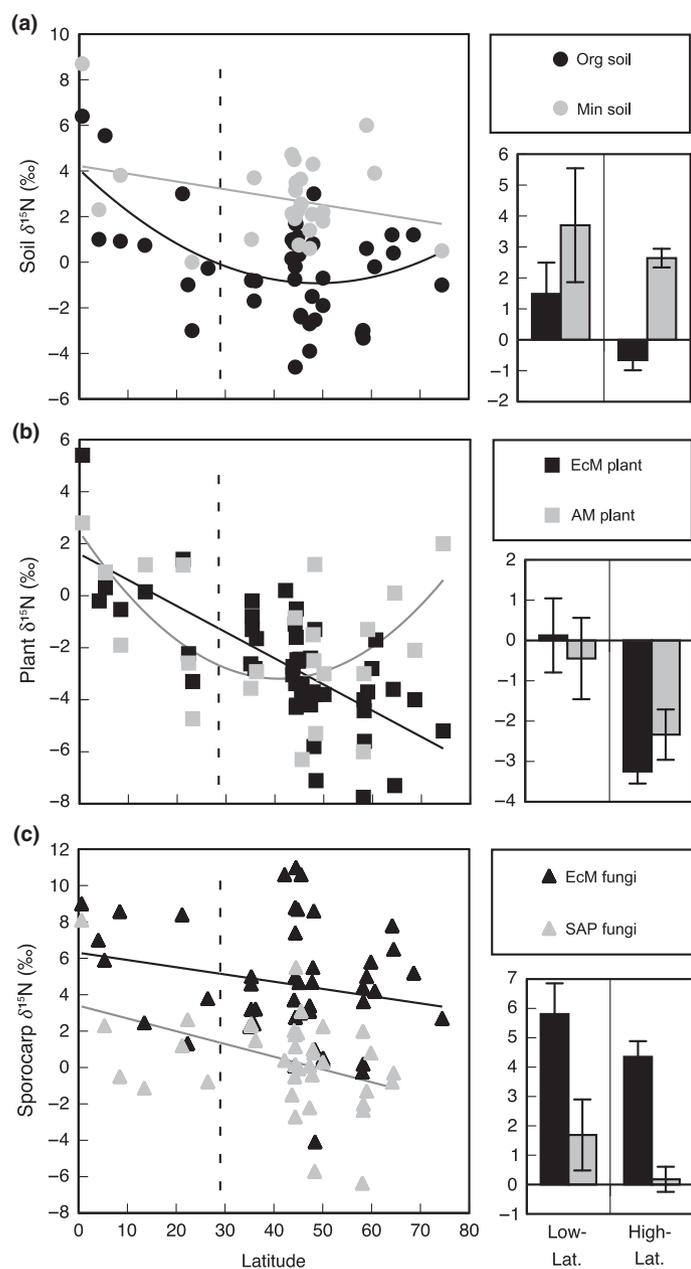


Figure 2 Average values for high-latitude and low-latitude components (\pm SE), divided by dotted lines, are presented to the right of main figures and categorised based on site descriptions of sub/tropical climatic influences. (a) Organic soil vs. |latitude|: quadratic polynomial, $R^2 = 0.30$, $P = 0.001$, $n = 42$; Mineral soil vs. |latitude|: $R^2 = 0.09$, $P = 0.14$, $n = 27$; Low-latitude organic soil $\delta^{15}\text{N}$ values were significantly enriched ($P = 0.036$); (b) Ectomycorrhizal plant vs. |latitude|: $R^2 = 0.52$, $P < 0.0001$, $n = 47$; AM plant: $R^2 = 0.38$, $P = 0.012$, $n = 22$; Low-latitude EcM and AM plant $\delta^{15}\text{N}$ values were significantly enriched ($P = 0.0036$, $P = 0.070$, respectively); (c) Saprotrophic fungal sporocarps vs. |latitude|: $R^2 = 0.18$, $P = 0.007$, $n = 39$; EcM fungal sporocarps vs. |latitude|: $R^2 = 0.03$, $P = 0.26$, $n = 46$.

Foliar $\delta^{15}\text{N}$ values of EcM plants were negatively correlated with latitude ($R^2 = 0.52$, $P < 0.0001$, $n = 47$; $\gamma = 1.6 - 0.10 \times \chi$) and foliar $\delta^{15}\text{N}$ values from AM plants exhibited a comparable but non-linear relationship with latitude (quadratic polynomial, $R^2 = 0.375$, $P = 0.012$, $n = 22$; $\gamma = -2.95 -$

$0.0059 \times \chi + 0.0033 \times (\chi - 40.04)^2$; Fig. 2b). The fits for AM and EcM plant $\delta^{15}\text{N}$ values were largely insensitive to removal of the high-latitude sites but removal of sites greater than 51° increased the significance of the regression fitting AM plant $\delta^{15}\text{N}$ values with latitude and also increased statistical support (e.g. from 0.07 to 0.046) for ^{15}N -enrichment of AM plant $\delta^{15}\text{N}$ values in the sub/tropical group owing to removal of the relatively ^{15}N -enriched sites at the highest latitudes (Fig. S5). Mean annual temperature and precipitation generally explained less variance than latitude for EcM plants (i.e. $R^2 = 0.25$, $P < 0.001$ and $R^2 = 0.26$, $P = 0.001$ respectively) and AM plants ($R^2 = 0.39$, $P = 0.009$ and $R^2 = 0.05$, $P = 0.60$, for quadratic polynomials respectively (Fig. S3). Foliar $\delta^{15}\text{N}$ values of sub/tropical EcM plants were 3.4‰ greater than EcM plants from higher latitudes (unequal variance t -test: $t = 3.49$; $P = 0.004$; Fig. 2b) and those for AM plants were 1.9‰ greater in sub/tropical forests ($t = 1.59$, $P = 0.070$; Fig. 2b). Accordingly the $\delta^{15}\text{N}$ differences between co-occurring EcM and AM plants were negatively correlated with latitude (cubic polynomial fit: $R^2 = 0.58$, $P = 0.001$, $n = 22$; Fig. 3a) and sub/tropical differences were greater than in high latitudes (i.e. $\Delta\delta^{15}\text{N}_{\text{EcM-AM}} = 0.6 \pm 0.5\text{‰}$ vs. $-1.6 \pm 0.7\text{‰}$ in low and high latitudes, respectively; unequal variance t -test: $t = 2.51$, $P = 0.010$). Large $\delta^{15}\text{N}$ differences between

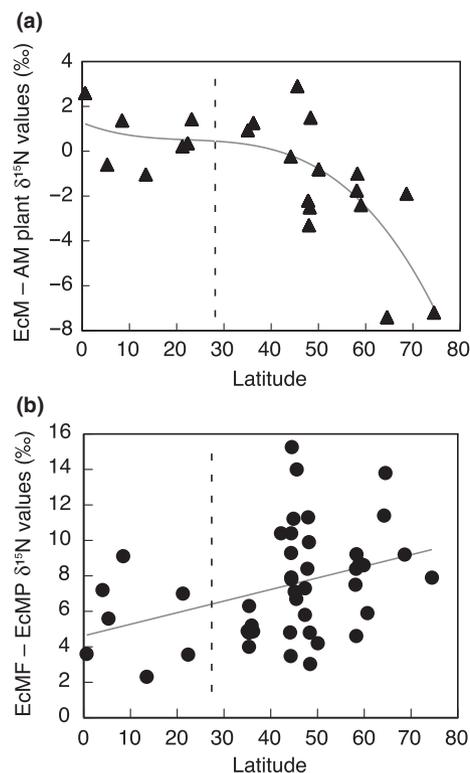


Figure 3 Relative differences among plant $\delta^{15}\text{N}$ values in relation to absolute values of latitudinal origin. (a) The $\delta^{15}\text{N}$ difference between EcM and AM plants, a metric of the relative influence of the EcM habit, was negatively related to latitude ($\gamma = 2.3 - 0.055 \times \chi - 0.0026 \times (\chi - 40.04)^2 - 5.016 \times 10^{-5} \times (\chi - 40.041)^3$; $R^2 = 0.59$, $P = 0.0003$, $n = 22$), with the 'typical' differences present only in higher latitude ecosystems. (b) The difference between the $\delta^{15}\text{N}$ values of EcM fungi and plants, a metric of fractionation associated with EcM N delivery, was positively correlated with |latitude| ($\gamma = 4.62 + 0.065 \times \chi$; $R^2 = 0.13$, $P = 0.01$, $n = 44$).

co-occurring EcM and AM plants were greatest in only the highest latitude sites, however, making this metric particularly sensitive to site removal. For instance removal of just the extreme high-latitude sites (e.g. $> 61^\circ$) weakened statistical support for $\Delta\delta^{15}\text{N}_{\text{EcMP-AMP}}$ correlations with latitude and categorical differences among sub/tropical sites compared to the high-latitude sites, and removal of all high-latitude sites (e.g. $> 51^\circ$) eliminated all statistical support ($R^2 = 0.129$, $n = 16$, $P = 0.171$; Fig. S5).

Isotopic fractionation ($\Delta = \delta^{15}\text{N}_{\text{plant}} - \delta^{15}\text{N}_{\text{organic soil}}$) during the uptake and/or translocation of soil N was compared as a metric to assess latitudinal differences among fractionation in EcM and AM plants. The average fractionation of EcM plants was greater than that of AM plants (avg. $\Delta\delta^{15}\text{N}_{\text{EcM plants}} = -2.6\text{‰}$ vs. $\Delta\delta^{15}\text{N}_{\text{AM plants}} = -1.7\text{‰}$) and comparison of the slopes of fitted lines indicated minimal fractionation of relatively ^{15}N -depleted soil N and increasing fractionation of relatively ^{15}N -enriched soil N ($\delta^{15}\text{N}_{\text{EcM plant}}$ slope = 0.63 and $\delta^{15}\text{N}_{\text{AM plant}}$ slope = 0.76) with similar intercepts of *c.* -7‰ where fractionation from source N is expected to no longer occur (Fig. 4a,b).

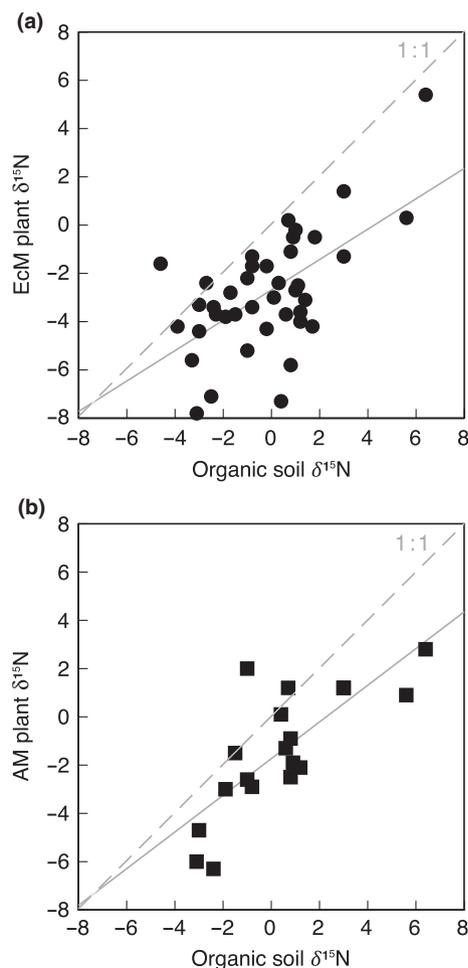


Figure 4 $\delta^{15}\text{N}$ offsets of EcM and AM plants and underlying organic soils. Deviation of fitted models from the 1:1 line (dashed) is a metric of the isotopic fractionation ($\Delta = \delta^{15}\text{N}_{\text{plant}} - \delta^{15}\text{N}_{\text{organic soil}}$) of plants during uptake, transfer and translocation of this metric of soil N. (a) EcM: $R^2 = 0.37$, $P < 0.0001$, $n = 40$. (b) AM: $R^2 = 0.59$, $P = 0.0001$, $n = 19$.

Sporocarp $\delta^{15}\text{N}$ values from saprotrophic fungi were negatively correlated with latitude ($R^2 = 0.18$, $P = 0.007$, $n = 39$; $\gamma = 3.396 - 0.070 \times \chi$), whereas those from EcM fungi showed no significant relationship (Fig. 2c). Removal of the high-latitude sites did not alter the non-significant latitudinal regression of EcM sporocarp $\delta^{15}\text{N}$ values but did weaken the support for saprotrophic $\delta^{15}\text{N}$ patterns ($R^2 = 0.11$, $P = 0.079$; Fig. S5). On average EcM sporocarp $\delta^{15}\text{N}$ values were 4.3 ‰ more enriched than saprotrophic fungi (Fig. 2c; matched pairs test: $t = -8.99$, $P < 0.001$, $n = 31$). These enrichment differences were slightly, but not significantly, smaller in low-latitude forests owing to overall trends in both trophic groups ($\Delta\delta^{15}\text{N}_{\text{ECMF-SAPF}} = 4.6$ vs. 3.9‰ respectively). Similarly, the differential ^{15}N -enrichments of tropical EcM systems caused the differences between EcM plants and fungi, ranging from 2.3 to 15.3 ‰, to be smallest in low-latitude forests (average difference = 5.5 vs. 7.8 ‰ respectively; unequal variance *t*-test: $t = -2.20$, $P = 0.026$) and to be positively correlated with latitude ($R^2 = 0.13$, $P = 0.01$, $n = 44$; Fig. 3b). Although both EcM sporocarp $\delta^{15}\text{N}$ and surface soil total [N] were unrelated to latitude, EcM sporocarp $\delta^{15}\text{N}$ was negatively correlated with soil [N] ($R^2 = 0.34$, $P < 0.001$; Fig. 5a) and positively correlated with EcM plant $\delta^{15}\text{N}$ across all sites ($R^2 = 0.23$, $P = 0.001$, $n = 44$; Fig. 5b).

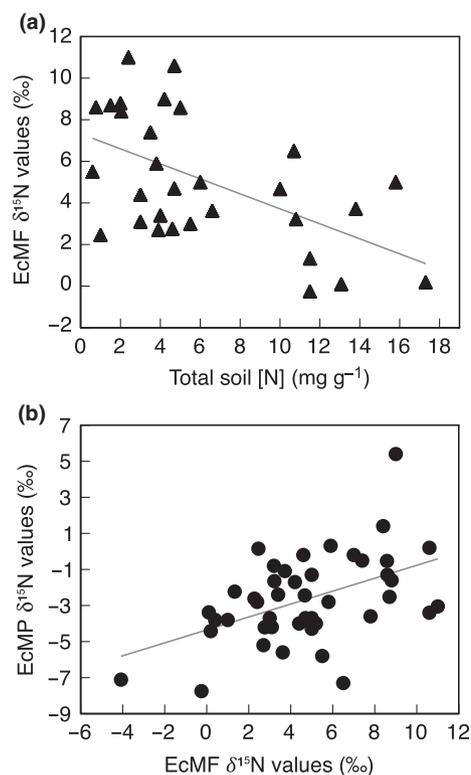


Figure 5 Significant relationships of $\delta^{15}\text{N}$ values from EcM fungi, plants and surface soil [N]. (a) The $\delta^{15}\text{N}$ values of EcM sporocarps were negatively related to surface soil [N] across sites ($R^2 = 0.34$, $P < 0.001$) suggesting their ^{15}N -enrichment is partially a function of growth under low N conditions. (b) The $\delta^{15}\text{N}$ values of EcM plants and fungi were positively correlated with one another across the broad range of sites ($R^2 = 0.23$, $P < 0.001$, $n = 43$) illustrating the N cycling dependency of the relationship.

Mass balance mixing models

Using averaged values from high-latitude data sets, mass balance solutions for EcM plant $\delta^{15}\text{N}$ values were only possible with several parameter modifications. First, the effective discrimination (Δ in eqns 1–3) magnitude was reduced below that derived for *Pinus* EcM forests, from 9 to 7 ‰ (Hobbie & Colpaert 2003), and ^{15}N -enriched soil N sources ($\delta^{15}\text{N}_{\text{available N}}$) were assigned above the available bulk surface soil $\delta^{15}\text{N}$ values. Both assumptions are reasonable given the likelihood that non-*Pinus* EcM systems may vary in effective discrimination magnitudes and that bulk soils may not approximate EcM access to ^{15}N -enriched soil N sources either at greater soil depths or in dissolved organic forms (Mayor *et al.* 2012; Hobbie *et al.* 2013). The solution space resulting from the simultaneous equations required $\delta^{15}\text{N}_{\text{available N}}$ values from 3.8 to 6.5 ‰ based on trees receiving 50–100% of their N from EcM respectively. These high proportional dependencies and enriched $\delta^{15}\text{N}_{\text{available N}}$ sources agreed with field studies in arctic, alpine, boreal and temperate ecosystems (Hobbie & Hobbie 2006; Averill & Finzi 2011; Mayor *et al.* 2012; Nave *et al.* 2013). However, solving for sub/tropical EcM plant $\delta^{15}\text{N}$ values required even more ^{15}N -enriched soil N sources, ranging from 2.9 to 9.4 ‰ despite being coupled with a reduced proportion of EcM-derived N from 10 to 50% respectively. Such ^{15}N -enriched N sources appear to encompass mineral and organic N forms based on detailed soil $\delta^{15}\text{N}$ measurements made from one of our tropical sites ($\delta^{15}\text{N}_{\text{NH}_4} = 1.0$ ‰, $\delta^{15}\text{N}_{\text{NO}_3} = -2.9$ ‰, $\delta^{15}\text{N}_{\text{DON}} = 7.6$ ‰; Fortuna, Panama; J. Mayor, unpublished data). Furthermore, solution spaces for sub/tropical EcM forests required us to nearly eliminate EcM discrimination to $\Delta = 2$ ‰. Following estimation of possible solutions for the simultaneous parameters that matched observed plant $\delta^{15}\text{N}$, we unsuccessfully attempted to further constrain these estimates with inclusion of observed $\delta^{15}\text{N}_{\text{EcM fungi}}$ values in eqn 2. For instance, in high-latitude ecosystems, estimated parameters could not approximate $\delta^{15}\text{N}_{\text{EcM fungi}}$ values within even 5 ‰ of those observed. Furthermore, the proportional dependencies on EcM N became highly sensitive to small increases in assigned $\delta^{15}\text{N}_{\text{available N}}$ (e.g. small shifts in $\delta^{15}\text{N}_{\text{available N}}$ from 4.5 to 5.5 ‰ produced f values ranging from 10 to 50% of total tree N supply, respectively). In conclusion, the mass balance models derived from high-latitude N-limited ecosystems failed to approximate observed EcM $\delta^{15}\text{N}$ values, particularly in sub/tropical forests, despite various concessions begin made in assignment of model parameters.

Structural equation modelling

The *a priori* model fit for $\delta^{15}\text{N}$ values of EcM plant foliage ($\chi^2 = 7.73$, $df = 5$, $P = 0.172$) had a RMSEA of 0.11 and a PClose of 0.23. This model suggests that the $\delta^{15}\text{N}$ values of EcM plant foliage were directly effected by latitude associated processes (coefficient estimate = -0.65) and indirectly by the competing influences of soil [N] and $\delta^{15}\text{N}$ values as mediated by the $\delta^{15}\text{N}$ values of co-occurring EcM sporocarps (coefficient estimate = 0.38). The *a priori* model fit for $\delta^{15}\text{N}$ values of AM plant foliage ($\chi^2 = 0.01$, $df = 1$, $P = 0.919$) had a RMSEA of 0.00 and PClose of 0.92. This model suggests that the $\delta^{15}\text{N}$

values of AM plant foliage were directly effected by organic soil $\delta^{15}\text{N}$ values (coefficient estimate = 0.73) as mediated by the indirect affect of latitude (coefficient estimate = -0.35). The *a priori* model fit for $\delta^{15}\text{N}$ values of EcM sporocarps ($\chi^2 = 4.04$, $df = 2$, $P = 0.133$) had a RMSEA of 0.15 and PClose of 0.17. This model suggests that the $\delta^{15}\text{N}$ values of EcM sporocarps were directly effected by latitude (coefficient estimate = 0.341) and soil [N] (coefficient estimate = -0.48). The *a priori* model fit for $\delta^{15}\text{N}$ values of saprotrophic sporocarps ($\chi^2 = 0.92$, $df = 1$, $P = 0.337$) had a RMSEA of 0.00 and PClose of 0.37, suggesting that saprotrophic fungal $\delta^{15}\text{N}$ values were directly effected by surface soil $\delta^{15}\text{N}$ values (coefficient estimate = 0.54) as mediated by the indirect affect of latitude associated variation (coefficient estimate = -0.36).

A final unified path diagram depicting relationship among all observed variables had a RMSEA of 0.10 and PClose of 0.18 ($\chi^2 = 19.59$, $df = 13$, $P = 0.106$). The final fitted model shows the distinctive relationships of ecosystem $\delta^{15}\text{N}$ in both fungal and plant components and the complexity of causes influencing $\delta^{15}\text{N}$ values of EcM symbioses at broad scales (Fig. 6). A correlated error term co-influencing EcM and saprotrophic sporocarp $\delta^{15}\text{N}$ (coefficient estimate = -1.86 , $P = 0.043$) produced fits that were marginally better (ΔAICc reduction of 2.36), but was omitted from graphical presentations for clarity. The model pathways previously identified in *a priori* SEM were retained in the fitted diagram for the $\delta^{15}\text{N}$ value of both EcM and AM plants (SEM $R^2 = 0.63$ and 0.55 respectively). The two fungal trophic groups also retained distinctive pathways influencing sporocarp $\delta^{15}\text{N}$ values; the $\delta^{15}\text{N}$ values of both EcM (SEM $R^2 = 0.39$) and saprotrophic (SEM $R^2 = 0.34$) fungi were positively effected by the $\delta^{15}\text{N}$ of organic soils, as mediated by latitude associated processes. However, in contrast to *a priori* model specifications, EcM fungal $\delta^{15}\text{N}$ values were also effected by surface soil [N]. Therefore, the net effect of both soil [N] and $\delta^{15}\text{N}$ values affects the $\delta^{15}\text{N}$ values of EcM sporocarps directly and EcM plants indirectly. The SEM variables retained as influencing

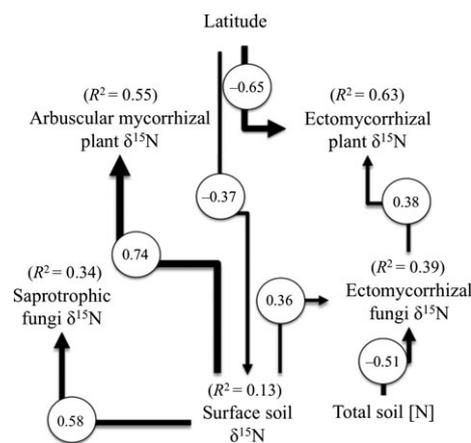


Figure 6 Final path diagram fit from competing SEM relating ecosystem $\delta^{15}\text{N}$ values, soil N concentrations [N] and latitudinal position of sites. The weights of pathway arrows correspond to the size of coefficient estimates (direct effects) within circles. Squared multiple correlations (R^2) are included alongside each endogenous latent variable.

ecosystem component $\delta^{15}\text{N}$ values were also retained in all high AICc-ranked models, lending additional support to the interpretation of the SEM (See Table S3). The provisioning of indirect and direct pathways in the SEM is an advantage over multiple regression models.

DISCUSSION

Despite large variation in soils, plants and fungi at the global scale, ecosystem components in lower latitudes exhibited ^{15}N -enrichment indicative of more rapid N cycling. In the context of this background variation in ecosystem $\delta^{15}\text{N}$, we explicitly sought to determine if latitudinal variation in *relative* $\delta^{15}\text{N}$ patterns correspond to theoretical shifts in mycorrhizal mediation of plant N demands. Below, we evaluate biome-scale differences in the pattern and function of EcM systems to critically evaluate mechanisms structuring ecosystem $\delta^{15}\text{N}$ patterns.

Soil $\delta^{15}\text{N}$ patterns

Consistent with previous meta-analyses, soil $\delta^{15}\text{N}$ values were significantly more ^{15}N -enriched in sub/tropical forests (Martinelli *et al.* 1999; Amundson *et al.* 2003). Similarly, deeper soil layers were more ^{15}N -enriched and comparable in value to that seen in previous analyses of soil $\delta^{15}\text{N}$ profiles (Hobbie & Ouimette 2009). As site dominance by EcM trees could potentially confound latitudinal patterns, we checked for significant differences in soil $\delta^{15}\text{N}$ values among sites with only EcM plants relative to those where both EcM and AM plant $\delta^{15}\text{N}$ values were reported. Organic soil $\delta^{15}\text{N}$ values from 21 sites containing only EcM plants were only marginally more ^{15}N -depleted (-0.8‰) than soils from 19 sites containing both AM and EcM plants (0.5‰ ; $P = 0.099$, one-way *t*-test assuming equal variances), suggesting that sites containing only EcM plants did not strongly bias our latitudinal interpretations. Based on surveys of temperate forests, lower $\delta^{15}\text{N}$ values in EcM-associated soils might result from greater nitrate retention in EcM-dominated stands relative to AM-dominated stands (Phillips *et al.* 2013; Midgley & Phillips 2014). The $\delta^{15}\text{N}$ differences of organic relative to mineral soils were also marginally reduced in sites containing only EcM trees (-2.7‰ vs. -3.8‰ , respectively; $P = 0.08$, one-way *t*-test assuming equal variance, $n = 23$), a trend opposite to previous predictions (Hobbie & Ouimette 2009). Whereas soil ^{15}N -profiles in high-latitude forests are largely due to the accumulation of EcM mycelial residues (Hobbie & Ouimette 2009; Clemmensen *et al.* 2013), fractionating gaseous losses also influence soil ^{15}N -enrichment in the tropics. Soil anoxia induced by high precipitation, combined with rapid rates of N cycling, leads to increased ratios of gaseous-to-hydrological N losses during nitrification and denitrification (Schelsinger & Bernhardt 2013). Such fractionating losses leave behind ^{15}N -rich N that can adhere to weathered clays, and ultimately contribute to soil and plant ^{15}N -enrichment over time (Kramer *et al.* 2003; Houlton *et al.* 2006; Hietz *et al.* 2011; Mayor *et al.* 2014). It is therefore apparent that the drivers of soil $\delta^{15}\text{N}$ profiles in high- and low-latitude ecosystems may be caused by fundamentally different mechanisms.

Plant $\delta^{15}\text{N}$ patterns

Previous meta-analyses have shown that EcM plants are typically ^{15}N -depleted relative to AM and non-mycorrhizal plants at the global scale, irrespective of co-occurrence of both mycorrhizal types within individual sites (e.g. Craine *et al.* 2009). In this study, this distinction was absent from sub/tropical forests containing both AM and EcM trees and evidence for it at even mid-latitudes (e.g. $30\text{--}45^\circ$) appears weak. As the mechanism commonly evoked to explain ^{15}N -depletion of EcM plants relative to AM plants requires EcM-mediated delivery of ^{15}N -depleted N to host plants (reviewed in Hobbie & Högberg 2012), our results suggest a distinct functional role of EcM associations in sub/tropical forest N cycles. One hypothetical mechanism is that EcM trees in sub/tropical forests take up the majority of their N directly from soils, without mediation by mycorrhizae. This is unlikely given the high degree of root colonisation in most EcM genera (personal observations), the dominance of the same EcM fungal lineages along the latitudinal gradient (Tedersoo *et al.* 2012a), and the SEM results. Alternatively, sub/tropical EcM fungi deliver comparable amounts of N to host plants but without strong ^{15}N -depletion of source N during transfer. Such reductions in the magnitude of effective isotopic fractionation are supported by the mass balance exercises requiring smaller Δ values and the divergent $\delta^{15}\text{N}$ patterns among EcM and AM plants at high latitudes.

In this study pre-existing ^{15}N mass balance models were unable to match observed EcM plant and fungal $\delta^{15}\text{N}$ values and therefore could not quantitatively estimate presumed changes in the proportion of plant N derived from EcM fungi across the broad array of ecosystems. This shortcoming could not be avoided despite flexibility assigned to several of the parameters used in the system of mass balance equations. Of those changes, the reduction in effective fractionation magnitudes (Δ), an adjustment requiring a particularly large reduction in sub/tropical forest solutions, highlights a potential uncertainty regarding the physiological function of EcM in the tropics. It is therefore apparent that universal application of these mass balance equations will not only require better assessment of soil N $\delta^{15}\text{N}$ values (a parameter we also permitted to vary widely from bulk soil $\delta^{15}\text{N}$ measurements based on data from one tropical site included here), but also the elucidation of additional mechanisms by which low-latitude EcM sporocarps become ^{15}N -enriched independent of presumably lower host plant N demands (discussed below).

The SEM path analysis suggests that despite any latitudinally distinct processing of N by EcM, fungal activity remains an important direct influence on host plant $\delta^{15}\text{N}$ variability. Latitude (a crude proxy for climate, soil weathering, etc.) negatively affected EcM plant $\delta^{15}\text{N}$ in the path diagram, but EcM fungal $\delta^{15}\text{N}$ values positively affected EcM plant $\delta^{15}\text{N}$ with no significant interaction between them (coefficient estimate = 0.05, $P = 0.626$). However, the SEM path diagram highlighted competing indirect soil variables that appear to affect EcM plant $\delta^{15}\text{N}$ by differentially affecting EcM fungal $\delta^{15}\text{N}$. This indirect influence could result from access to and demand for soil N being inversely related to one another. In other words, high soil N availability leads to lower ^{15}N

retained in EcM fungi, as shown in Fig. 5a, when either N demand by host plants is low (Hobbie & Högberg 2012; but see Näsholm *et al.* 2013) or when high mineral N availability makes accessing ^{15}N -enriched organically bound N an unnecessary enzymatic expenditure (Bödeker *et al.* 2014). Under this scenario fungal sporocarp $\delta^{15}\text{N}$ values closely match the $\delta^{15}\text{N}$ values of soil N sources when ^{15}N -fractionating (i.e. high Δ) N delivery to host plants is reduced.

In contrast to EcM plants, the relationship between AM plant $\delta^{15}\text{N}$ values and latitude-dependent processes were indirect. While AM plants were more ^{15}N -enriched in low latitudes, there was enrichment in the highest latitude sites as well. As mentioned, removal of the high-latitude sites and refitting models did not change overall conclusions, however (Fig. S5). Based on Fig. 2b and the SEM path diagram, the non-linear AM plant $\delta^{15}\text{N}$ relationship appears due to the close tracing of soil $\delta^{15}\text{N}$ values by AM plants independently of soil [N]. This relationship and the fractionation magnitude observed in Fig. 4b, suggests that surface soil $\delta^{15}\text{N}$ values at least approximate the N forms available to AM plants over a broad range of ecosystems, particularly where soil $\delta^{15}\text{N}$ values are between -2 and 2 ‰ and plant $\delta^{15}\text{N}$ values are between -3 and 2 ‰. This indicates that using average ^{15}N -offsets (c. 2 ‰ or less; Pate *et al.* 1993; Michelsen *et al.* 1998) in mass balance modelling exercises incorporating fractionation associated with uptake and translocation of N in AM plants may not be the best choice for all ecosystems because plant $\delta^{15}\text{N}$ values appear to diverge most from soil $\delta^{15}\text{N}$ at mid-latitudes where AM $\delta^{15}\text{N}$ values are less than -4 ‰ (Fig. 2b and Fig. 4b). It should also be noted, that the signal of EcM N delivery, represented by divergence between EcM and AM plant $\delta^{15}\text{N}$ values in Fig. 3a, appears to begin only above 45° , a pattern that suggests this signal of EcM fractionation may be driven by AM plant $\delta^{15}\text{N}$ values in high latitude ecosystems where they are markedly more ^{15}N -enriched relative to co-occurring EcM plants. Thus, the 'typical' EcM signal derived from a comparison of EcM and AM plant $\delta^{15}\text{N}$ differences appears to largely be a phenomenon present in sites above 45° and to be completely absent from the tropics. The inclusion of more data from sites at between 0° and 45° would precisely resolve where an EcM ^{15}N signal becomes apparent in plants.

Fungal $\delta^{15}\text{N}$ patterns

Unlike soils, plants and saprotrophic fungi, EcM fungi were not significantly ^{15}N -enriched at low latitudes – a pattern comparable to previous and ongoing meta-analyses of EcM fungal $\delta^{15}\text{N}$ patterns (Mayor *et al.* 2009; Erik Hobbie, personal communication). The question then becomes what could maintain uniformity in EcM fungal $\delta^{15}\text{N}$ values across these diverse biomes that vary in plant and soil $\delta^{15}\text{N}$ values, soil nutrient availabilities and climate?

The SEM path diagram suggests that both EcM and saprotrophic fungi are positively effected by soil $\delta^{15}\text{N}$, yet EcM $\delta^{15}\text{N}$ values are also strongly effected by the competing influences of soil [N] and the presumed demand of N by host plants (discussed in the preceding section). Based on the framework of mass balance equations mathematically linking

fungal and plant $\delta^{15}\text{N}$, we anticipated the $\delta^{15}\text{N}$ differences between EcM plants and sporocarps would become smaller in sub/tropical forests because of an expected reduction in overall N demands by sub/tropical plants growing under conditions of greater relative P limitation (Vitousek *et al.* 2010). The regression in Fig. 3b indicates that ^{15}N -differences between co-occurring EcM sporocarps and plants were indeed diminished in lower latitude ecosystems as expected. Yet, the regressions and SEM path diagram indicate that the relative trend seen in Fig. 3b was driven largely by latitude associated variation in EcM plant $\delta^{15}\text{N}$ values. If the relative ^{15}N -enrichment of tropical EcM plants is caused by a reduced reliance on EcM fungi for soil N and possibly use of ^{15}N -enriched soil N sources, then there must be physiological mechanisms that account for the consistent ^{15}N -enrichment of EcM fungi relative to co-occurring saprotrophic fungi in sub/tropical forests irrespective of N delivery to host plants.

Alternative hypotheses for EcM function in sub/tropical forests

As mentioned, we have assumed, based on relative $\delta^{15}\text{N}$ patterns, that EcM fungi deliver N to associated tropical trees without a high degree of ^{15}N -fractionation during synthesis of N transfer compounds. In the absence of this typical high-latitude fractionating outlet, there are several non-exclusive mechanisms that we speculate could lead to consistent ^{15}N -enrichment of EcM fungi in the tropics. For instance, sub/tropical EcM fungi may: (1) acquire N from sources that are uniquely ^{15}N -enriched (e.g. proteins; Emmerton *et al.* 2001; Hobbie & Högberg 2012), (2) forage at greater soil depths (Hobbie & Ouimette 2009), (3) be disproportionately dominated by taxa that are characteristically ^{15}N -enriched (e.g. *Cortinarius*; Hobbie & Agerer 2009; Cox *et al.* 2010), (4) undergo accelerated hyphal turnover times with concomitantly greater internal ^{15}N -recycling (Hobbie *et al.* 2012; Ekblad *et al.* 2013; Pena *et al.* 2013); or, (5) have additional unrecognised N outlets by which the fungal mycelium loses disproportionately more ^{14}N to surrounding soil during processes such as acquisition of P or other limiting mineral nutrients from weathered tropical forest soils (Lambers *et al.* 2008; Lucas & Casper 2008; Marklein & Houlton 2011; Pritsch & Garbaye 2011; Tedersoo *et al.* 2012b). We suggest that any combination of these non-exclusive processes could contribute to relative ^{15}N -enrichment of EcM fungi in the tropics and that such differences in EcM mediation of plant-soil N cycling might also contribute to sporocarp $\delta^{15}\text{N}$ variability in higher latitude ecosystems as well (Lilleskov *et al.* 2011). Evaluation of these largely physiological mechanisms within lower latitude, and particularly sub/tropical, forests is necessary to produce globally consistent frameworks relating N cycling process with the $\delta^{15}\text{N}$ values of EcM components (Alexander & Selosse 2009).

As a theoretical exercise, we diagrammed how mechanisms (1) and (5), the use of ^{15}N -enriched proteins and an additional enzymatic N loss pathway, could modify mass balance models to account for the patterns observed in this study. This exercise illustrates how the relative importance of two different N loss pathways from fungal mycelium, combined by the resulting usage of ^{15}N -enriched N sources, could result in the

observed $\delta^{15}\text{N}$ values in both high and low-latitude EcM systems respectively (see Fig. S6). Continued research in low-latitude EcM forests could expand mechanistic and biogeographical understanding of mycorrhizal functional roles in ecosystem nutrient economies (e.g. Phillips *et al.* 2013), as well as the functional relevance of differences among fungal lineages (Buée *et al.* 2007). Our study, using simultaneous analyses of the major ecosystem $\delta^{15}\text{N}$ components across broad latitudinal gradients, has identified latitudinal discrepancies and distinct avenues for continued research.

CONCLUSIONS

In previous syntheses, mycorrhizal types were implicated in having global influences on plant $\delta^{15}\text{N}$ values (Amundson *et al.* 2003; Craine *et al.* 2009). Our study places these findings into a more nuanced context by including original data sets from the tropics with more exhaustive measurements from co-occurring soil, fungi and plants. The presence of an EcM isotopic 'signal' in typically N-limited higher latitude ecosystems (tundra, boreal and temperate forests) appears absent from plants, but not fungi, in lower latitude EcM forests. This deviation could result from differential processes related to N availability in excess of plant demand, access to ^{15}N -enriched soil N sources, and/or unique, as yet undetermined, ^{15}N -fractionation outlets in tropical EcM fungi. Therefore, ^{15}N -based mixing models derived from high-latitude EcM associations lack utility when applied to the high N conditions typical of many tropical and extra-tropical low-latitude forests as well. Understanding EcM symbioses in the context of global N cycles will allow better integration of mycorrhizal functional processes with theories pertaining to climate–nutrient cycling relationships.

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AUTHORSHIP

JM and LT designed the study; JM, MB, TH and LT collected data; MB performed the SEM and model selection

analyses; FB and KP contributed new methods and materials. JM wrote the first draft of the manuscript and all authors contributed to revisions.

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