ECOLOGY LETTERS

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

Journal:	*Ecology Letters*
Manuscript ID:	Draft
Manuscript Type:	Reviews and Syntheses
Date Submitted by the Author:	n/a
Complete List of Authors:	Mayor, Jordan; Swedish University of Agricultural Sciences, Forest Ecology & Management; Smithsonian Tropical Research Institute, Bahram, Mohammad; University of Tartu, Institute of Ecology and Earth Sciences Henkel, Terry; Humboldt State University, Department of Biological Sciences Beugger, Franz; Institute of Soil Ecology, Helmholtz Zentrum München, German Research Center for Environmental Health Pritsch, Karin; Institute of Soil Ecology, Helmholtz Zentrum München, German Research Center for Environmental Health Tedersoo, Leho; Natural History Museum of Tartu University, Institute of Ecology and Earth Sciences; University of Tartu, Institute of Ecology and Earth Sciences
Key Words:	Above- and below-ground interactions, nutrient cycling, nutrient limitation, plant-soil interactions, tropical ecology, structural equation modeling, 15N

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

Jordan Mayor[1,2]*, Mohammad Bahram[3], Terry Henkel[4], Franz Buegger[5], Karin Pritsch[5], Leho Tedersoo[3,6],

[1] Smithsonian Tropical Research Institute, P.O. Box 0843-03092, Balboa, Ancon, Republic of Panama

[2] *Current address:* Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå 90183, Sweden

[3] Institute of Ecology and Earth Sciences, University of Tartu, 14a Ravila, 50411 Tartu, Estonia

[4] Department of Biological Sciences, Humboldt State University, 1 Harpst St., Arcata, CA 95521, USA

[5] Institute of Soil Ecology, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstaedter Landstraße 1, D - 85764 Neuherberg, Germany

[6] Natural History Museum of Tartu University, 46 Vanemuise, 51005 Tartu, Estonia

* Author for correspondence: clavulina@gmail.com, (+1) 352.283.1731

Running title: Ectomycorrhizal impacts on ecosystem ^{15}N

Keywords: Above- and below-ground interactions, nutrient cycling, nutrient limitation, plant-soil interactions, tropical ecology, structural equation modeling, ^{15}N

Type of Article: Reviews and Syntheses

Word count: Abstract: *195*; Main Text (excluding Abstract and Acknowledgements): *6,549*

Number of Tables: 0

Number of figures: 6

Number of citations: 87

Supporting information: One *Excel* file containing four tables (Table S1-S3) and one figure (Fig. S4) and two *PDF* files (Fig. S5-S6)

Statement of 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, in particular LT and TH.

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 δ^{15}N relative to a standard) increasingly serve as integrative proxies for mycorrhiza-mediated N acquisition due to biological fractionation processes that alter ^{15}N:^{14}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 δ^{15}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, δ^{15}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 δ^{15}N patterns, as well as better integration of EcM function with biogeochemical theories pertaining to climate-nutrient cycling relationships.

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 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}N:^{14}N expressed as $\delta^{15}N$ 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) $\delta^{15}N$ 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 fertilization 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 $\delta^{15}N$ 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 δ^{15}N, may obscure the formation of distinct δ^{15}N patterns and their subsequent utility in studying tropical EcM associations. This gap in understanding is particularly acute since 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}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 δ^{15}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 δ^{15}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). Additionally, δ^{15}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 datasets that included co-occurring soil, fungal, and plant δ^{15}N values from low latitude ecosystems prevented full assessment of how changes to host-symbiont nutrient limitations influence δ^{15}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 datasets containing δ^{15}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 δ^{15}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 δ^{15}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 δ^{15}N patterns so that: (1) the influence of EcM fungi over plant δ^{15}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 δ^{15}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 δ^{15}N along with similar original data obtained by the authors. Original samples were collected by the authors and 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 Colin & Averill, 2011). Due to floristic heterogeneity and/or sampling limitations, some sites contained only one dominant EcM plant species whereas others contained δ^{15}N values from many species (>8; see Table S1, S2 in Supporting Information). 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 ([N] mg g^{-1}) were also extracted if available. Owing to the varying methods across studies, soil [N] was measured from samples of varying layers or depths (0 to 5, and 5 to 10, 12, or 15 cm). When separate organic and mineral [N] were reported, these values were averaged over total core depths and are hereafter referred to as surface soil [N]. Similarly, "organic" and "mineral" layers may not necessarily coincide with strict definitions of C content

but such divisions were retained for δ^{15}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 δ^{15}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 geographic locations of original soil, fungal, and plant δ^{15}N values are included in Table S2. Overall, sites varied widely in latitude (-13 to 74 ºN), altitude (5 to 2780 m a.s.l.), mean annual precipitation (183 to 7032 mm yr^{-1}), and mean annual temperature (-9.8 to 26 ºC). Surface soil N concentrations ([N]) ranged from 0.6 to 35.7 mg g^{-1} and soil δ^{15}N values ranged from -4.6 to 8.7 ‰.

The included datasets 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, while 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 δ^{15}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). Generalized 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 modeling was performed using Amos Version 7.0 (SPSS, Chicago, IL). 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 δ^{15}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 δ^{15}N values taken from the main co-occurring pools of N: AM plants, 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 influence over the N cycling in these forested ecosystems (i.e. climate, elevation, forest age). The SEM were analyzed 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 (yr), 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. S4 in Supporting Information), 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 datasets 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 ±27º 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 δ^{15}N values of saprotrophic fungi and EcM and AM plants were explored in order to ascertain distinct pathways and correlations of error terms among these components individually. Soil δ^{15}N values were initially modeled as exogenous with no causal agents in the exploratory models. Next, plausible relationships between all ecosystem δ^{15}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 Aikake Information Criteria (AICc) output (see Table S3 in Supporting Information) for comparison of statistical methods. In addition, because of strong correlation among ecosystem δ^{15}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 δ^{15}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 δ^{15}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 $\delta^{15}N$ values from the high and low latitude sites.

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

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

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

Where $\delta^{15}N_{available\ N}$ 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 $\delta^{15}N$ values reported in datasets, Δ values estimated from laboratory studies, and a range of $\delta^{15}N_{available\ N}$ 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 $\delta^{15}N$ values

The $\delta^{15}N$ values of mineral and organic soil horizons were positively correlated across sites (R = 0.70, n = 27), and mineral soils were on average 3 ±1.6 ‰ (mean ± s.d.) more ^{15}N-enriched than those of organic soils (matched pairs t-test: $P < 0.001$, n = 27). Soil $\delta^{15}N$ values from

surface organic layers were negatively correlated with latitude (quadratic polynomial: R^2 = 0.30, 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 dataset, we examined the possibility that the highest latitude soil δ^{15}N values were driving the relationships by removing all sites above 51° and refitting the same regression models. Removal of these high-latitude sites did not decrease the variance explained or the significance of model formulations seen in Fig. 2 (see Figure S5 in Supporting Information).

Foliar δ^{15}N values of EcM plants were negatively correlated with latitude (R^2 = 0.52, P < 0.001, n = 47; $\gamma = 1.6 - 0.10 \times \chi$) and foliar δ^{15}N values from AM plants exhibited a comparable but non-linear relationship with latitude (quadratic polynomial, R^2 = 0.37, P = 0.012, n = 22; $y = -2.95 - 0.0059 \times \chi + 0.0033 \times (\chi - 40.04)^2$; Fig. 2b). 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 see Figure S4 in Supporting Information). Foliar δ^{15}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 = 0.07; Fig. 2b). Accordingly the δ^{15}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), and these average differences statistically compared according to high and low latitude groupings (Fig. 3a). Significant differences were present only in higher latitude groupings (i.e. $\Delta\delta^{15}N_{EcMP\text{-}AMP}$ = 0.6 ±0.5 ‰ vs. -1.6 ±0.7 ‰ in low and high latitudes, respectively; unequal variance t-test: t = 2.51, P = 0.01). Removal of the two highest latitude sites required a quadratic (vs. cubic) polynomial to achieve statistical significance of the fitted relationship (P = 0.05, n = 20; data not shown). Isotopic fractionation ($\Delta = \delta^{15}N_{plant} - \delta^{15}N_{organic}$

$_{soil}$) 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. $\Delta\delta^{15}N_{EcM\ plants}$ = -2.6 ‰ vs. $\Delta\delta^{15}N_{AM\ plants}$ -1.7 ‰) 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 ($\delta^{15}N_{EcM\ plant}$ slope = 0.63 and $\delta^{15}N_{AM\ plant}$ slope = 0.76) with similar intercepts of *c.* -7 ‰ where fractionation from source N is expected to no longer occur (Fig. 4a,b).

Sporocarp δ^{15}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). On average EcM sporocarp δ^{15}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 ($\Delta\delta^{15}N_{ECMF\text{-}SAPF}$ = 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 δ^{15}N and surface soil total [N] were unrelated to latitude, EcM sporocarp δ^{15}N was negatively correlated with soil [N] (R^2 = 0.34, P < 0.001; Fig. 5a) and positively correlated with EcM plant δ^{15}N across all sites (R^2 = 0.23, p = 0.001, n = 44; Fig. 5b).

Mass balance mixing models

Using averaged values from high-latitude datasets, mass balance solutions for EcM plant δ^{15}N values were only possible with several parameter modifications. First, the effective discrimination (Δ in Eqn.'s 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 ($\delta^{15}N_{available\ N}$) were assigned above the available bulk surface soil δ^{15}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 $\delta^{15}N_{available\ N}$ 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 $\delta^{15}N_{available\ N}$ 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 δ^{15}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 10–50 %, respectively. Such ^{15}N-enriched N sources appear to encompass mineral and organic N forms based on detailed soil δ^{15}N measurements made from one of our tropical sites ($\delta^{15}N_{NH4}$ = 1.0 ‰, $\delta^{15}N_{NO3}$ = -2.9 ‰, $\delta^{15}N_{DON}$ = 7.6 ‰; Fortuna, Panama; J. Mayor, unpublished data). Furthermore, solution spaces for sub/tropical EcM forests required us to nearly eliminate EcM discrimination to Δ = 2 ‰. Following estimation of possible solutions for the simultaneous parameters that matched observed plant δ^{15}N, we unsuccessfully attempted to further constrain these estimates with inclusion of observed $\delta^{15}N_{EcM\ fungi}$ values in Eqn. 2. For instance, in high latitude ecosystems, estimated parameters could not approximate $\delta^{15}N_{EcM\ fungi}$ values within even 5 ‰ of those observed. Further, the proportional dependencies on EcM N became highly sensitive to small increases in assigned $\delta^{15}N_{available\ N}$ (e.g. small shifts in $\delta^{15}N_{available\ N}$ 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 δ^{15}N values, particularly in sub/tropical forests, despite various concessions begin made in assignment of model parameters.

Structural equation modeling

The *a priori* model fit for δ^{15}N values of EcM plant foliage (χ^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 δ^{15}N values of EcM plant foliage were directly effected by latitude (coefficient estimate = -0.65) and indirectly by the competing influences of soil [N] and δ^{15}N values as mediated by the δ^{15}N values of co-occurring EcM sporocarps (coefficient estimate = 0.38). The *a priori* model fit for δ^{15}N values of AM plant foliage (χ^2 = 0.01, *df* = 1, *P* = 0.919) had a RMSEA of 0.00 and PClose of 0.92. This model suggests that the δ^{15}N values of AM plant foliage were directly effected by organic soil δ^{15}N values (coefficient estimate = 0.73) as mediated by the indirect affect of latitude (coefficient estimate = -0.35). The *a priori* model fit for δ^{15}N values of EcM sporocarps (χ^2 = 4.04, *df* = 2, *P* = 0.133) had a RMSEA of 0.15 and PClose of 0.17. This model suggests that the δ^{15}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 δ^{15}N values of saprotrophic sporocarps (χ^2 = 0.92, *df* = 1, *P* = 0.337) had a RMSEA of 0.00 and PClose of 0.37, suggesting that saprotrophic fungal δ^{15}N values were directly effected by surface soil δ^{15}N values (coefficient estimate = 0.54) as mediated by the indirect affect of latitude (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 (χ^2 = 19.59, *df* = 13, *P* = 0.106). The final fitted model shows the distinctive relationships of ecosystem δ^{15}N in both fungal and plant components and the complexity of causes influencing δ^{15}N values of EcM symbioses at broad scales (Fig. 6). A correlated error term co-influencing EcM and saprotrophic sporocarp δ^{15}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 δ^{15}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 δ^{15}N values; the δ^{15}N values of both EcM (SEM R^2 = 0.39) and saprotrophic (SEM R^2 = 0.34) fungi were positively effected by the δ^{15}N of organic soils, as mediated by latitude-dependent processes. However, in contrast to *a priori* model specifications, EcM fungal δ^{15}N values were also effected by surface soil [N]. Therefore, the net effect of both soil [N] and δ^{15}N values affect the δ^{15}N values of EcM sporocarps directly and EcM plants indirectly. The SEM variables retained as influencing ecosystem component δ^{15}N values were also retained in all high AICc-ranked models, lending additional support to the interpretation of the SEM (See Table S3 in Supporting Information). 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 δ^{15}N, we explicitly sought to determine if latitudinal variation in *relative* δ^{15}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 in order to critically evaluate mechanisms structuring ecosystem δ^{15}N patterns.

Soil δ^{15}N patterns

Consistent with previous meta-analyses, soil δ^{15}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 δ^{15}N profiles (Hobbie & Ouimette 2009). Organic soil δ^{15}N values from the 21 sites containing only EcM plants were marginally more ^{15}N-depleted (-0.8 ‰) than soils from the 19 sites containing both AM and EcM plants (0.5 ‰; P = 0.099, one-way t-test assuming equal variances). Based on surveys of temperate forests, EcM-associated soil ^{15}N-depletion might result from greater nitrate retention in EcM-dominated stands relative to AM-dominated stands (Phillips *et al.* 2013; Midgley & Phillips 2014). As expected, the ^{15}N-enrichment of organic- relative to mineral soils was slightly smaller in sites containing only EcM trees (-2.7 ‰ vs. -3.8 ‰, respectively; P = 0.08, one-way t-test assuming equal variance, n = 23) in contrast to the opposite prediction in a previous analysis (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 (Schlesinger & 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 δ^{15}N profiles from high- and low-latitude ecosystems may be caused by fundamentally different mechanisms.

Plant δ^{15}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 the present study this distinction was absent from sub/tropical forests containing both AM and EcM trees. 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 colonization 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 ^{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.

In the present study pre-existing ^{15}N mass balance models were unable to match observed EcM plant and fungal δ^{15}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 δ^{15}N values (a parameter we also permitted to vary widely from bulk soil δ^{15}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 affect over host plant δ^{15}N variability. Latitude (a crude proxy for climate, soil weathering, etc.) negatively affected EcM plant δ^{15}N in the path diagram, but EcM fungal δ^{15}N values positively affected EcM plant δ^{15}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 δ^{15}N by differentially affecting EcM fungal δ^{15}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 uneccessary enzymatic expenditure (Bödeker *et al*. 2014). Under this scenario fungal sporocarp δ^{15}N values closely match the δ^{15}N values of soil N sources when ^{15}N-fractionating (i.e. high Δ) N delivery to host plants is reduced. Evidence for this interpretation are seen in the eight sites containing the most ^{15}N-enriched EcM fungal values also being among those with the lowest soil [N] (average $\delta^{15}N_{EcM\ fungi}$ = 9.2 ‰ in sites with average [N] = 2.83 mg g^{-1}, representing the upper and lower quartiles, respectively; sites: 8, 16, 17, 21, 29, 32, 38, 44 in Table S1).

In contrast to EcM plants, the relationship between AM plant δ^{15}N values and latitude-dependent processes were indirect. While AM plants were more ^{15}N-enriched in low latitudes, there was evidence for enrichment in some higher latitude sites as well (although these did not drive the resulting regression once sites above 51° were removed; Figure S5). Based on Fig. 2b and the SEM path diagram, the non-linear AM plant δ^{15}N relationship appears due to the close tracing of soil δ^{15}N values by AM plants independently of soil [N]. This relationship, and the fractionation magnitude observed in Fig. 4b, suggests that surface soil δ^{15}N values at least approximate the N forms available to AM plants over a broad range of ecosystems. The

average ^{15}N-fractionation magnitude was comparable to previous estimates (*c.* 2 ‰ or less) of fractionation associated with uptake and translocation of N in AM plants in Australian woodland and the subarctic (Pate *et al.* 1993; Michelsen *et al.* 1998).

Fungal δ^{15}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 δ^{15}N patterns (Mayor *et al.* 2009; Erik Hobbie, personal communication). The question then becomes what could maintain uniformity in EcM fungal δ^{15}N values across these diverse biomes that vary in plant and soil δ^{15}N values, soil nutrient availabilities, and climate?

The SEM path diagram suggests that both EcM and saprotrophic fungi are positively effected by soil δ^{15}N, yet EcM δ^{15}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 δ^{15}N, we anticipated the δ^{15}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 δ^{15}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 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 δ^{15}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 disproportionally 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 unrecognized 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 δ^{15}N variability in high latitude ecosystems as well (Lilleskov *et al.* 2011). Evaluation of these largely physiological mechanisms within sub/tropical forests is necessary to produce globally consistent frameworks relating N cycling process with the δ^{15}N values of EcM components (Alexander & Selosse 2009).

As a theoretical exercise, we diagramed 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 δ^{15}N values in both high and low latitude EcM systems, respectively (see Fig. S6 in Supporting Information). Continued research in low latitude EcM forests could expand mechanistic 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 δ^{15}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 δ^{15}N values (Amundson *et al.* 2003; Craine *et al.* 2009). Our study places these findings into a more nuanced context by including original datasets 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 sub/tropical EcM forests. This deviation in the tropics 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 tropical ecosystems. 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.

ACKNOWLEDGMENTS

Support for J.M. was provided by the National Science Foundation's International Research Fellowship Program (OISE-1012703) and a Wallenberg Scholar Award to D. Wardle. Support for L.T. and M.B. was provided by the Estonian Science Foundation grants 9286, PUT171, FIBIR, and EMP265. Support for T. Henkel was provided by NSF DEB 0918591 and the National Geographic Society. We thank: B. Turner for facilitating laboratory analyses in Panama; J. Dalling for providing access to plot census data in Fortuna, Panama; the Guyana Environmental Protection Agency and STRI-ANAM in Panama for research permits; K. Hosaka, and P.Q. Thu and T.T. Dang for facilitating collecting trips to Japan and Vietnam; G. Crummer, D. Agudo, H. Harend, M. Haugas, and S. Anslan for lab assistance with some of the original stable isotope analyses; and, M. Dunthorn and three anonymous reviewers for improving earlier versions of the manuscript.

FIGURE CAPTIONS

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

Fig. 2. Average values for high latitude and low latitude components (± *s.e.*), divided by dotted lines, are presented to the right of main figures and categorized 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 and mineral soil δ^{15}N values were significantly enriched (P = 0.0085 and 0.075, respectively); (**b**) Ectomycorrhizal plant vs. |latitude|: R^2 = 0.52, P < 0.001, n = 47; AM plant: R^2 = 0.37, P = 0.012, n = 22; Low latitude EcM and AM plant δ^{15}N values were significantly enriched (P = 0.019, P = 0.07, 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).

Fig. 3. Relative differences among plant δ^{15}N values in relation to absolute values of latitudinal origin. (**a**) The δ^{15}N difference between EcM and AM plants, a metric of the relative influence of the EcM habit, was negatively related to latitude ($\gamma = 2.3 - 0.055 \times \chi - 0.0026 \times (\chi - 40.04)^2 - 5.016e\text{-}5 \times (\chi - 40.041)^3$; R^2 = 0.59, P = 0.0003, n = 22), with the "typical" differences present only in higher latitude ecosystems. (**b**) The difference between the δ^{15}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).

Fig. 4. δ^{15}N fractionation values of EcM and AM plants and underlying organic soils. Deviation of fitted models from the 1:1 line (dashed) are a metric of the isotopic fractionation ($\Delta = \delta^{15}N_{plant} - \delta^{15}N_{organic\ soil}$) 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.

Fig. 5. Significant relationships of δ^{15}N values from EcM fungi, plants, and surface soil [N]. (**a**) The δ^{15}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 δ^{15}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.

Fig. 6. Final path diagram fit from competing SEM relating ecosystem δ^{15}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.

SUPPORTING INFORMATION

TO BE MADE AVAILABLE ON-LINE ONLY

Table S1. List of site averaged metrics extracted from published and original datasets, global climate data, and geographical position of sites.

Table S2. δ^{15}N, δ^{13}C, %N, and %C of all plants, fungi, and soil from original data sets used in this study.

Table S3. List of AICc model weightings derived from generalized least squares model selection; an independent statistical examination of predictions from the SEM.

Figure S4. Scatterplot matrix regressing ecosystem δ^{15}N values with climate (MAT, MAP) and latitude.

Figure S5. Comparison of ecosystem component regressions used in Figure 2 of main text with and without all sites > 51° removed to demonstrate the robustness of the δ^{15}N patterns.

Figure S6. Hypothetical mass balance mixing model relationships for high latitude and low latitude EcM systems highlighting the potential of extracellular enzyme outlets and N sources to influence δ^{15}N values of EcM plants and fungi.

REFERENCES

1. Alexander I.J. & Selosse M.-A. (2009). Mycorrhizas in tropical forests: a neglected research imperitive. *New Phytol.*, 182, 14-16.
2. Amundson R., Austin A.T., Schuur E.A.G., Yoo K., Matzek V., Kendall C., Uebersax A., Brenner D. & Baisden W.T. (2003). Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochem. Cy.*, 17, 31.
3. Averill C. & Finzi A. (2011). Increasing plant use of organic nitrogen with elevation is reflected in nitrogen uptake rates and ecosystem δ^{15}N. *Ecology*, 92, 883-891.
4. Averill C., Turner B.L. & Finzi A.C. (2014). Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, 505, 543-545.
5. Bödeker I.T.M., Clemmensen K.E., de Boer W., Martin F., Oson Å. & Lindahl B.D. (2014) Ectomycorrhizal *Cortinarius* species participate in enzymatic oxidation of humus in northern forest ecosystems. *New Phytol.*, 203, 245-256.
6. Brearley F.Q., Press M.C. & Scholes J.D. (2003). Nutrients obtained from leaf litter can improve the growth of dipterocarp seedlings. *New Phytol.*, 160, 101-110.
7. Brookshire E.N.J., Gerber S., Menge D.N.L. & Hedin L.O. (2012). Large losses of inorganic nitrogen from tropical rainforests suggest a lack of nitrogen limitation. *Ecol. Lett.*, 15, 9-16.
8. Brundrett M.C. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil*, 320, 37-77.
9. Buée M., Courty P.E., Mignot D, Garbaye J. 2007. Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. *Soil Biol. Biochem.,* 39, 1947-1955.
10. Cairney J.W.G. (2011). Ectomycorrhizal fungi: the symbiotic route to the root for phosphorus in forest soils. *Plant Soil*, 344, 51-71.
11. Cerling T., Hart J. & Hart T. (2004). Stable isotope ecology in the Ituri forest. *Oecologia*, 138, 5-12.
12. Chapin F.S., Vitousek P.M. & Vancleve K. (1986). The nature of nutrient limitation in plant-communities. *Am. Nat.*, 127, 48-58.
13. Clemmensen K.E., Bahr A., Ovaskainen O., Dahlberg A., Ekblad A., Wallander H., Stenlid J., Finlay R.D., Wardle D.A. & Lindahl B.D. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, 339, 1615-1618.
14. Cornelissen J.H.C., Aerts R., B. C., A. W.M.J. & van der Heijden M.G.A. (2001). Carbon cycling traits of plant species are linked with mycorrhizal strategy. *Oecologia*, 129, 611-619.

15.
Courty P.-E., Walder F., Boller T., Ineichen K., Wiemken A., Rousteau A. & Selosse M.-A. (2011). Carbon and nitrogen metabolism in mycorrhizal networks and mycoheterotrophic plants of tropical forests: A stable isotope analysis. *Plant Physiol.*, 156, 952-961.
16.
Courty P.E., Buee M., Diedhiou A.G., Frey-Klett P., Le Tacon F., Rineau F., Turpault M.P., Uroz S. & Garbaye J. (2010). The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. *Soil Biol. Biochem.*, 42, 679-698.
17.
Cox F., Barsoum N., Lilleskov E.A. & Bidartondo M.I. (2010). Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. *Ecol. Lett.*, 13, 1103-1113.
18.
Craine J.M., Elmore A.J., Aidar M.P.M., Bustamante M., Dawson T.E., Hobbie E.A., *et al.* (2009). Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol.*, 183, 980-992.
19.
Dickie I.A. & Moyersoen B. (2008). Towards a global view of ectomycorrhizal ecology. *New Phytol.*, 151, 257-264.
20.
Ekblad A., Wallander H., Godbold D.L., Cruz C., Johnson D., Baldrian P., *et al.* (2013). The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. *Plant Soil*, 366, 1-27.
21.
Emmerton K.S., Callaghan T.V., Jones H.E., Leake J.R., Michelsen A. & Read D.J. (2001). Assimilation and isotopic fractionation of nitrogen by mycorrhizal fungi. *New Phytol.*, 151, 503-511.
22.
Grace J.B. (2006). *Structural Equation Modeling and Natural Systems*. Cambridge University Press, New York, USA.
23.
Grace J.B., Anderson T.M., Olff H. & Scheiner S.M. (2010). On the specification of structural equation models for ecological systems. *Ecol. Monogr.*, 80, 67-87.
24.
Hedin L.O., Brookshire E.N.J., Menge D.N.L. & Barron A.R. (2009). The nitrogen paradox in tropical forest ecosystems. *Annu. Rev. Ecol. Syst.*, 40, 613-635.
25.
Hedin L.O., Vitousek P.M. & Matson P.A. (2003). Nutrient losses over four million years of tropical forest development. *Ecology*, 84, 2231-2255.
26.
Henkel T.W., Terborgh J. & Vilgalys R.J. (2002). Ectomycorrhizal fungi and their leguminous hosts in the Pakaraima Mountains of Guyana. *Mycol. Res.*, 106, 515-531.
27.
Hietz P., Turner B.L., Wanek W., Richter A., Nock C.A. & Wright S.J. (2011). Long-term change in the nitrogen cycle of tropical forests. *Science*, 334, 664-666.
28.
Hobbie E., van Diepen L., Lilleskov E., Ouimette A., Finzi A. & Hofmockel K. (2013). Fungal functioning in a pine forest: evidence from a ^{15}N-labeled global change experiment. *New Phytol.*, 201, 1431-1439.

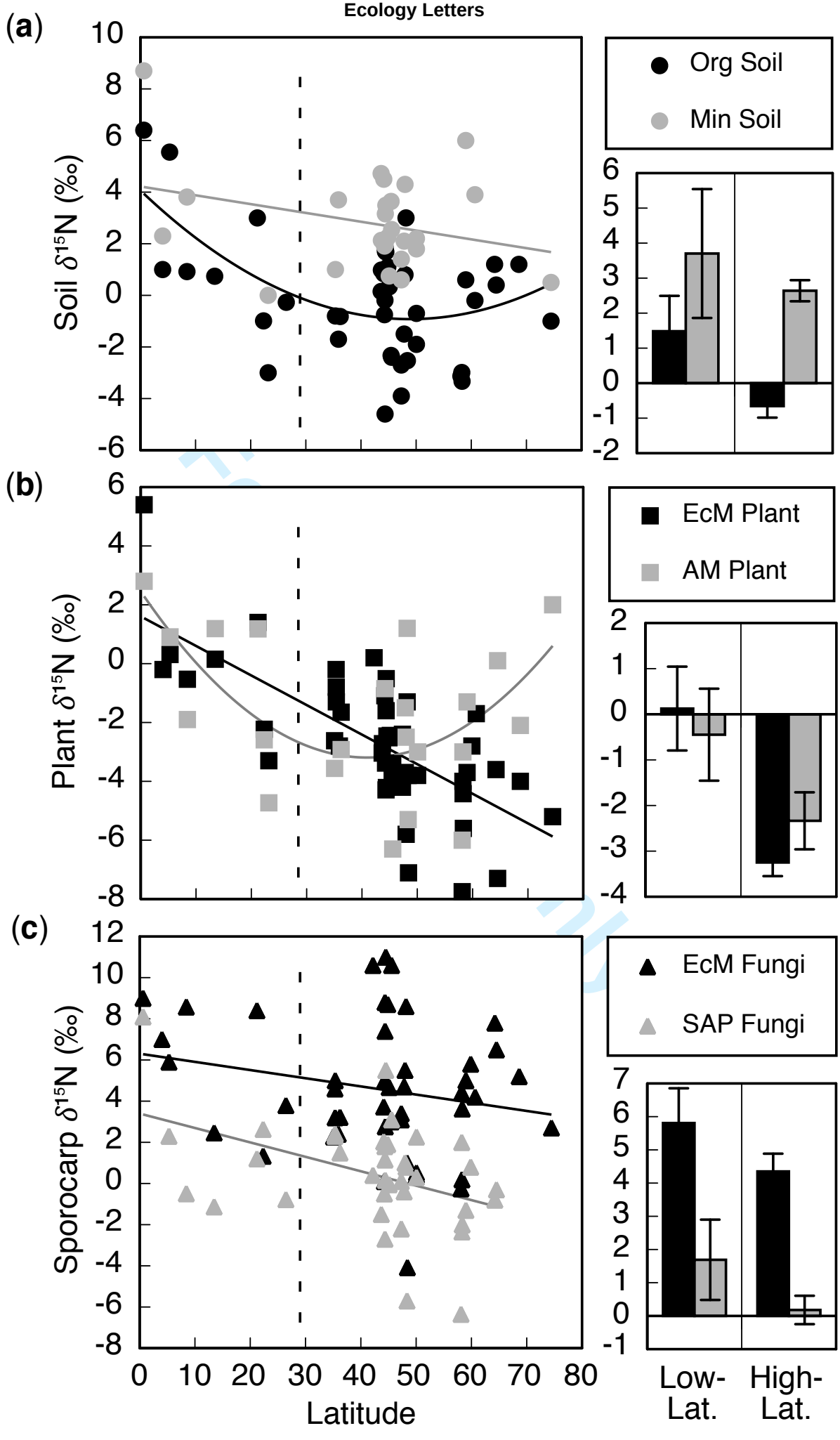
29.
Hobbie E.A. & Agerer R. (2009). Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. *Plant Soil*, 327, 71-83.
30.
Hobbie E.A. & Colpaert J.V. (2003). Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. *New Phytol.*, 157, 115-126.
31.
Hobbie E.A. & Hobbie J.E. (2008). Natural abundance of ^{15}N in nitrogen-limited forests and tundra can estimate nitrogen cycling through mycorrhizal fungi: A review. *Ecosystems*, 11, 815-830.
32.
Hobbie E.A. & Högberg P. (2012). Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. *New Phytol.*, 196, 367-382.
33.
Hobbie E.A., Jumpponen A. & Trappe J. (2005). Foliar and fungal ^{15}N:^{14}N ratios reflect development of mycorrhizae and nitrogen supply during primary succession: testing analytical models. *Oecologia*, 146, 258-268.
34.
Hobbie E.A. & Ouimette A.P. (2009). Controls of nitrogen isotope patterns in soil profiles. *Biogeochemistry*, 95, 355-371.
35.
Hobbie E.A., Sánchez F.S. & Rygiewicz P.T. (2012). Controls of isotopic patterns in saprotrophic and ectomycorrhizal fungi. *Soil Biol. Biochem.*, 48, 60-68.
36.
Hobbie J.E. & Hobbie E.A. (2006). ^{15}N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. *Ecology*, 87, 816-822.
37.
Högberg P. (1990). ^{15}N natural abundance as a possible marker of the ectomycorrhizal habit of trees in mixed African woodlands. *New Phytol.*, 115, 483-486.
38.
Högberg P. & Alexander I. (1995). Roles of root symbioses in African woodland and forest: Evidence from ^{15}N abundance and foliar analysis. *J. Ecol.*, 83, 217-224.
39.
Högberg P., Johannison C., Yarwood S., Callesen I., Nasholm T., Myrold D.D. & Högberg M.N. (2011). Recovery of ectomycorrhiza after 'nitrogen saturation' of a conifer forest. *New Phytol.*, 189, 515-525.
40.
Hou W., Lian B., Dong H., Jiang H. & Wu X.B. (2012). Distinguishing ectomycorrhizal and saprophytic fungi using carbon and nitrogen isotopic compositions. *Geosci. Front.*, 3, 351-356.
41.
Houlton B.Z., Sigman D.M. & Hedin L.O. (2006). Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. *Proc. Nat. Acad. Sci. USA*, 103, 8745-8750.
42.
Kramer M.G., Sollins P., Sletten R.S. & Swart P.K. (2003). N isotope fractionation and measures of organic matter alteration during decomposition. *Ecology*, 84, 2021-2025.
43.
Kuyper T.W. (2013) Ectomycorrhiza and the open nitrogen cycle in an afrotropical rainforest. *New Phytol.,* 195, 728-729.

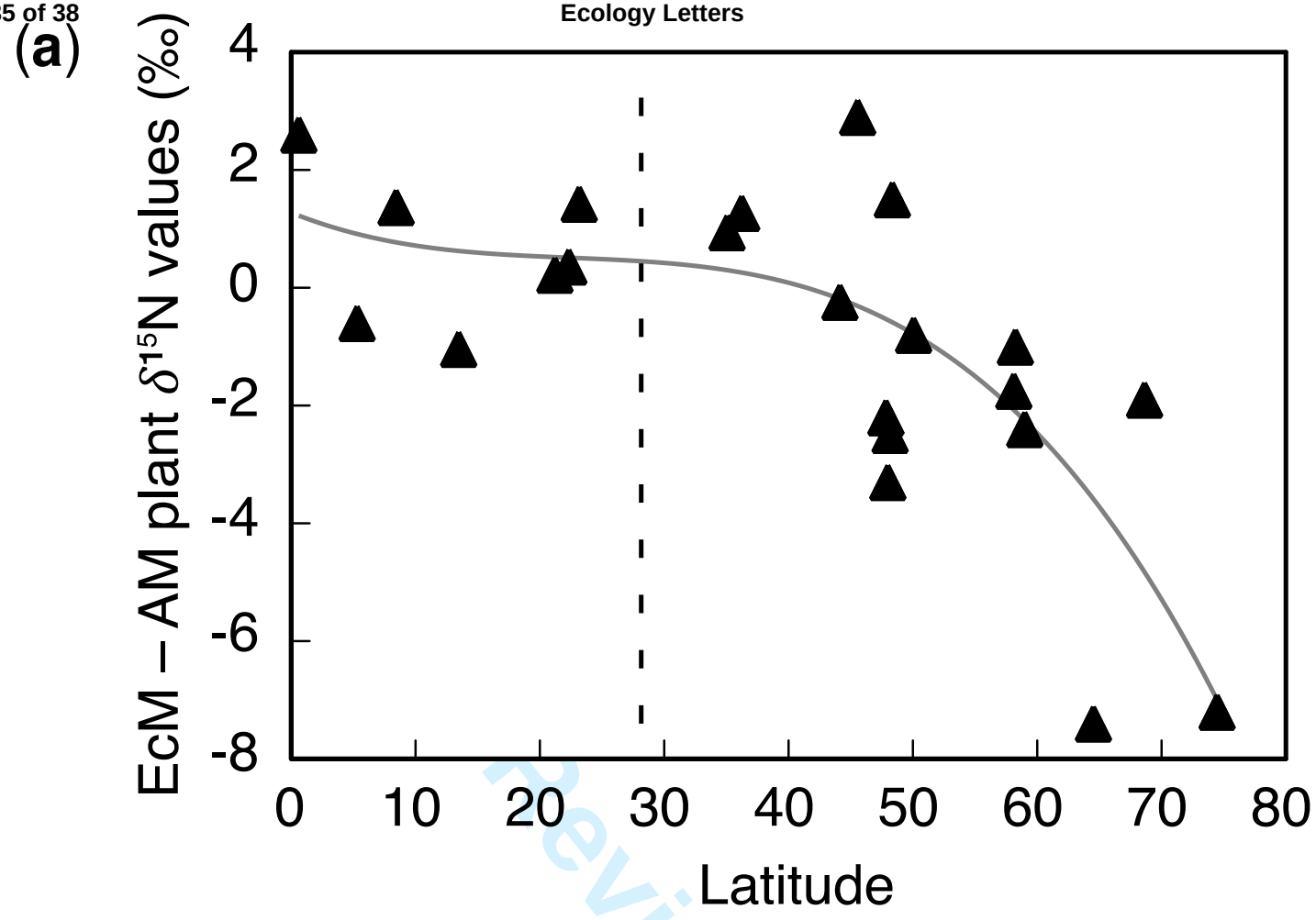
44.
Lam T.Y. & Maguire D.A. (2012). Structural Equation Modeling: Theory and applications in forest management. *Int. J. For. Res.*, doi:10.1155/2012/263953.
45.
Lambers H., Raven J.A., Shaver G.R. & Smith S.E. (2008). Plant nutrient-acquisition strategies change with soil age. *Trends Ecol. Evol.*, 23, 95-103.
46.
Langley J.A. & Hungate B.A. (2003). Mycorrhizal controls on belowground litter quality. *Ecology*, 84, 2302-2312.
47.
Lavorel S. & Grigulis K. (2013). How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. *J. Ecol.*, 100, 128-140.
48.
Lilleskov E.A., Hobbie E.A. & Fahey T.J. (2002). Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytol.*, 154, 219-231.
49.
Lilleskov E.A., Hobbie E.A. & Horton T.R. (2011). Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology*, 2, 174-183.
50.
Lindahl B.D., Ihrmark K., Boberg J., Trumbore S.E., Högberg P., Stenlid J., *et al.* (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol.*, 173, 611-620.
51.
Lucas R.W. & Casper B.B. (2008). Ectomycorrhizal community and extracellular enzyme activity following simulated atmospheric N deposition. *Soil Biol. Biochem.*, 40, 1662-1669.
52.
Marklein A.R. & Houlton B.Z. (2011). Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol.*, 193, 696-704.
53.
Martinelli L.A., Piccolo M.C., Townsend A.R., Vitousek P.M., Cuevas E., McDowell W., *et al.* (1999). Nitrogen stable isotopic composition of leaves and soil: Tropical versus temperate forests. *Biogeochemistry*, 46, 45-65.
54.
Mayor J.R. & Henkel T.W. (2006). Do ectomycorrhizas alter leaf-litter decomposition in monodominant tropical forests of Guyana? *New Phytol.*, 169, 579-588.
55.
Mayor J.R., Schuur E.A.G. & Henkel T.W. (2009). Elucidating the nutritional status of fungi. *Ecol. Lett.*, 12, 171-183.
56.
Mayor J.R., Schuur E.A.G., Mack M.C., Hollingsworth T.N. & Bååth E. (2012). Nitrogen isotope patterns in Alaskan black spruce reflect organic nitrogen sources and the activity of ectomycorrhizal fungi. *Ecosystems*, 15, 819-831.
57.
Mayor J.R., Wright S.J., Schuur E.A.G., Brooks M.E. & Turner B.L. (2014). Stable nitrogen isotope patterns of trees and soils altered by long-term nitrogen and phosphorus addition to a lowland tropical rainforest. *Biogeochemistry*, 119, 293-306.

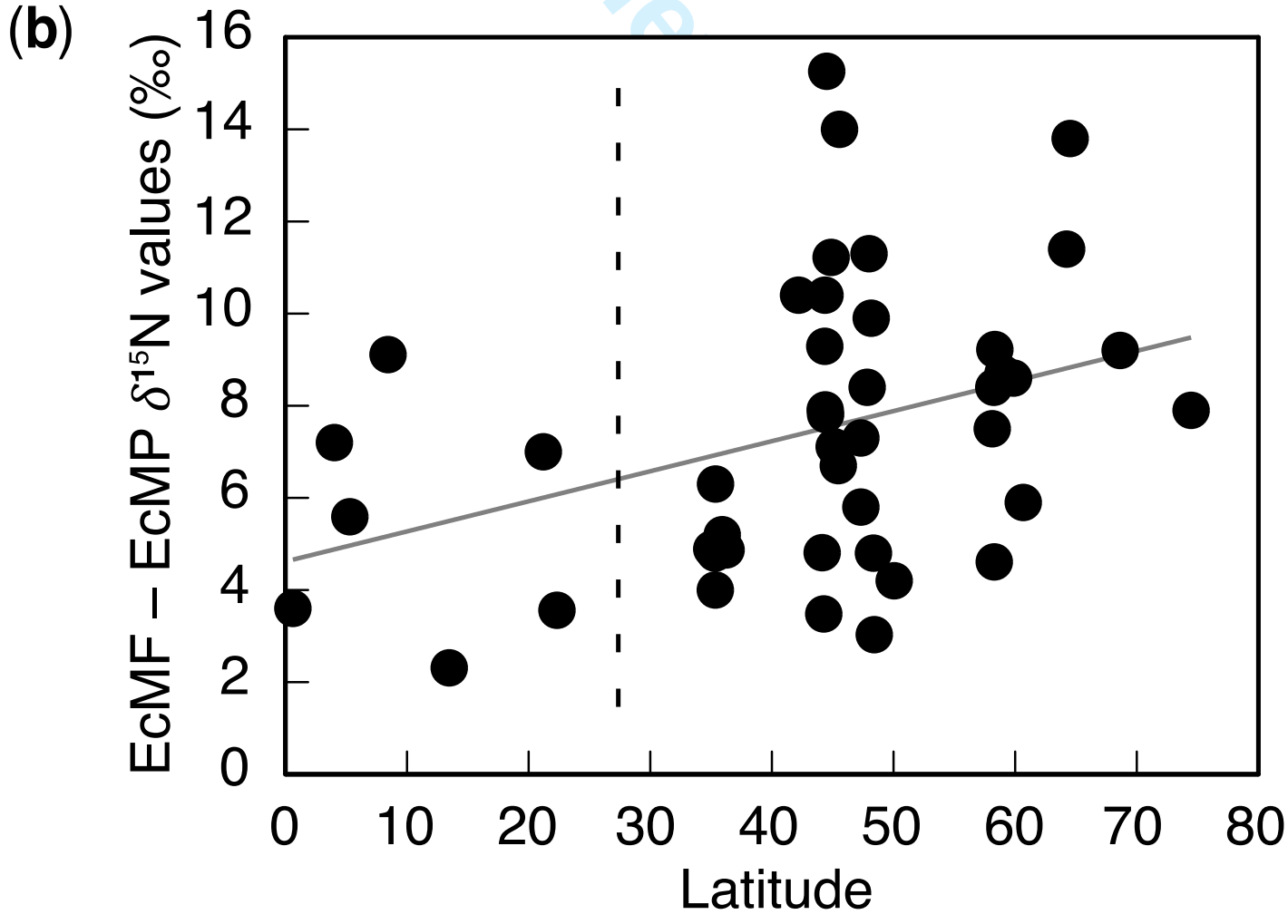
58.
Menge D.N.L., Hedin L.O. & Pacala S.W. (2012). Nitrogen and phosphorus limitation over long-term ecosystem development in terrestrial ecosystems. *PLoS ONE*, 7, doi:10.1371/journal.pone.0042045.
59.
Michelsen A., Quarmby C., Sleep D. & Jonasson S. (1998). Vascular plant ^{15}N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. *Oecologia*, 115, 406-418.
60.
Midgley M.G. & Phillips R.P. (2014). Mycorrhizal associations of dominant trees influence nitrate leaching responses to N deposition. *Biogeochemistry*, 117, 241-253.
61.
Näsholm T., Högberg P., Franklin O., Metcalfe D., Keel S.G., Campbell C., *et al.* (2013). Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *New Phytol.*, 198, 214-221.
62.
Nave L., Nadelhoffer K., Le Moine J., van Diepen L., Cooch J. & Van Dyke N. (2013). Nitrogen uptake by trees and mycorrhizal fungi in a successional northern temperate forest: insights from multiple isotopic methods. *Ecosystems*, 16, 590-603.
63.
New M., Lister D., Hulme M. & Makin I. (2002). A high-resolution data set of surface climate over global land areas. *Climatic Research*, 21, 1-25.
64.
Newbery D.M., Chuyong G.B. & Zimmermann L. (2006). Mast fruiting of large ectomycorrhizal African rain forest trees: importance of dry season intensity, and the resource-limitation hypothesis. *New Phytol.*, 170, 561-579.
65.
Orwin K.H., Kirschbaum M.U.F., St John M.G. & Dickie I.A. (2011). Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. *Ecol. Lett.*, 14, 493-502.
66.
Pardo L.H., Templer P.H., Goodale C.L., Duke S., Groffman P.M., Adams M.B., *et al.* (2006). Regional assessment of N saturation using foliar and root delta ^{15}N. *Biogeochemistry*, 80, 143-171.
67.
Pate J.S., Stewart G.R. & Unkovich M. (1993) ^{15}N natural abundance of plant and soil components of a *Banksia* woodland ecosystem in relation to nitrate utilization, life form, mycorrhizal status and N_2-fixing abilities of component species. *Plant Cell Env., 16, 365-373.*
68.
Pena R., Tejedor J., Zeller B., Dannenmann M. & Polle A. (2013). Interspecific temporal and spatial differences in the acquisition of litter-derived nitrogen by ectomycorrhizal fungal assemblages. *New Phytol.*, 199, 520-528.
69.
Phillips R.P., Brzostek E. & Midgley M.G. (2013). The mycorrhizal-assoicated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytol.*, 199, 41-51.
70.
Phillips R.P. & Fahey T.J. (2006). Tree species and mycorrhizal associations influence the magnitude of rhizosphere effects. *Ecology*, 87, 1302-1313.

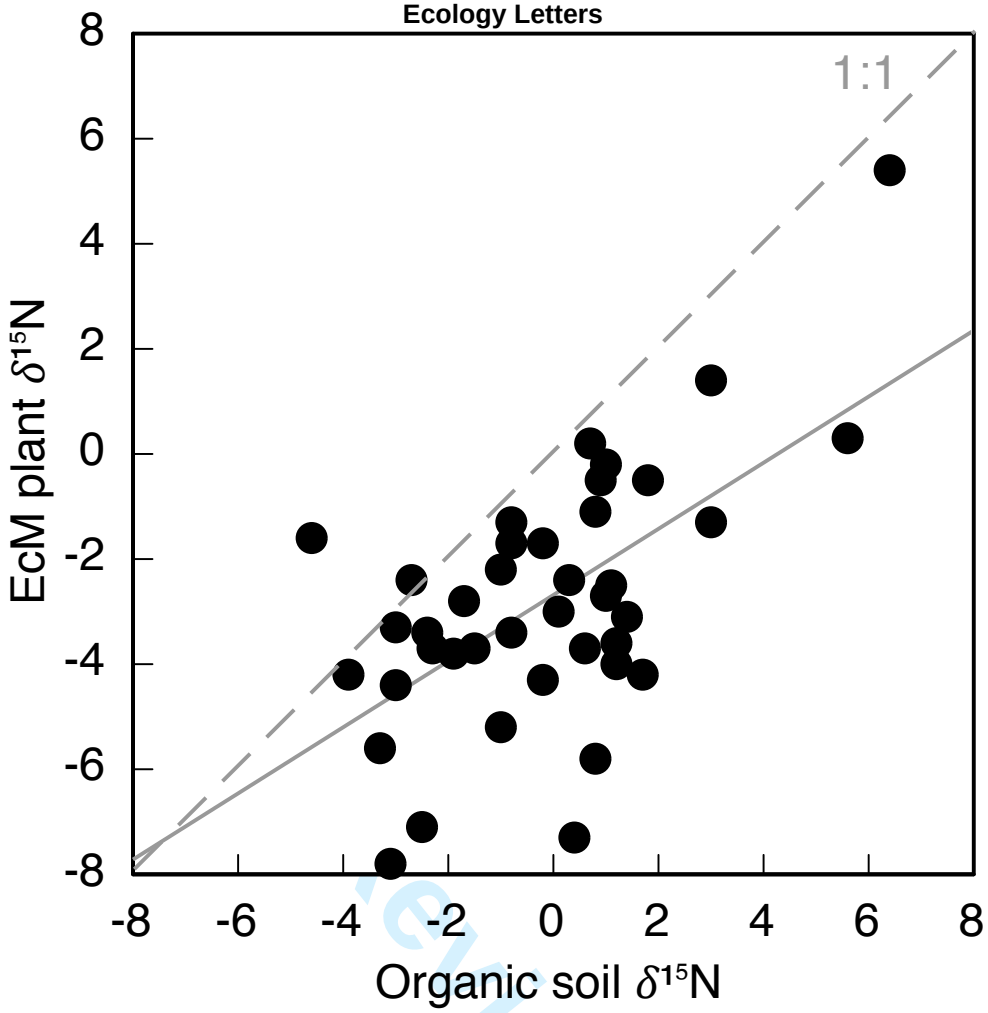
71.
Pinheiro J., Bates D., DebRoy S., Sarkar D. & EISPACK authors. (2011). nlme: Linear and Nonlinear Mixed Effects Models. *R package version 3.1-104.*
72.
Pritsch K. & Garbaye J. (2011). Enzyme secretion by ECM fungi and exploitation of mineral nutrients from soil organic matter. *Annal. For. Sci.*, 68, 25-33.
73.
Read D.J. & Perez-Moreno J. (2003). Mycorrhizas and nutrient cycling in ecosystems - a journey towards relevance? *New Phytol.*, 157, 475-492.
74.
Schelsinger W.H. & Bernhardt E.S. (2013). *Biogeochemistry: An analysis of global change*. 3rd Edition. Academic Press, New York, USA, pp. 203-210.
75.
Schulze E.D., Chapin F.S. & Gebauer G. (1994). Nitrogen nutrition and isotope differences among life forms at the northern treeline of Alaska. *Oecologia*, 100, 406-412.
76.
Shipley B. (2000). *Cause and Correlation in Ecology – A User's Guide to Path Analysis, Structural Equations and Causal Inference*. Cambridge University Press, Cambridge, UK, pp. 1-332.
77.
Smith S.E. & Read D.J. (2008). *Mycorrhizal Symbiosis*, 3rd edn. Academic Press, New York, USA, pp. 1-769.
78.
Smith S.E. & Smith F.A. (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosytem scales. *Annu. Rev. Plant Biol.*, 62, 227-250.
79.
Talbot J.M., Allison S.D. & Treseder K.K. (2008). Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Funct. Ecol.*, 22, 955-963.
80.
Taylor A.F.S. & Alexander I.J. (2005). The ectomycorrhizal symbiosis: life in the real world. *New Phytol.*, 159, 102-112.
81.
Team R.D.C. (2012). R: A language and environment for statistical computing. *R package version 2.15.0; R Foundation for Statistical Computing, Vienna, Austria*, ISBN 3-900051-07-0, http:/ci/http://www.R-project.org.
82.
Tedersoo L., Bahram M., Toots M., Diédhiou A.G., Henkel T.W., Kjøller R., *et al.* (2012a). Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Mol. Ecol.*, 21, 4160-4170.
83.
Tedersoo L., Naadel T., Bahram M., Pritsch K., Buegger F., Leal M., *et al.* (2012b). Enzymatic activities and stable isotope patterns of ectomycorrhizal fungi in relation to phylogeny and exploration types in an afrotropical rain forest. *New Phytol.*, 195, 832-843.
84.
Tedersoo L. & Smith M.E. (2013). Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biol. Rev.*, http://dx.doi.org/10.1016/j.fbr.2013.09.001.
85.
Torti S.D., Coley P.D. & Kursar T.A. (2001). Causes and consequences of monodominance in tropical lowland forests. *Am. Nat.*, 157, 141-153.

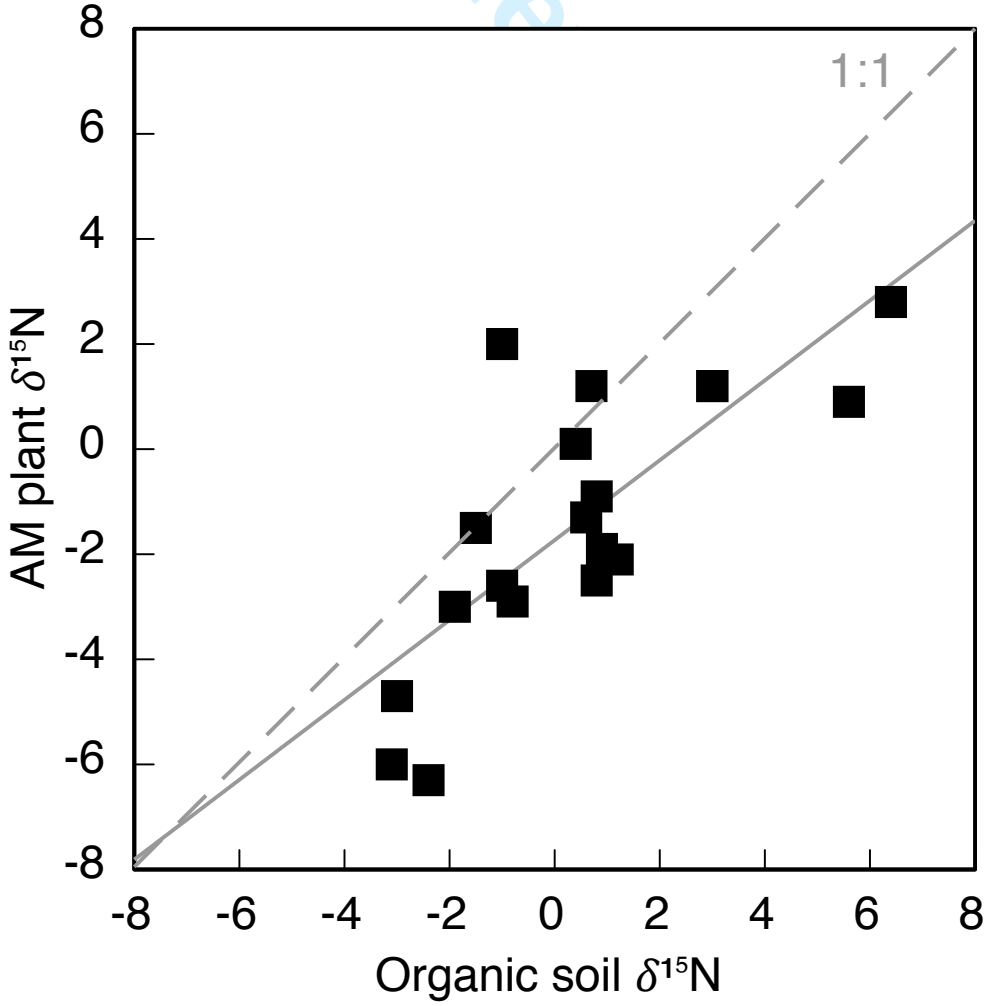
86.
Vitousek P.M., Porder S., Houlton B.Z. & Chadwick O.A. (2010). Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecol. Appl.*, 20, 5-15.
87.
Wurzburger N. & Hendrick R.L. (2009). Plant litter chemistry and mycorrhizal roots promote a nitrogen feedback in a temperate forest. *J. Ecol.*, 97, 528-536.

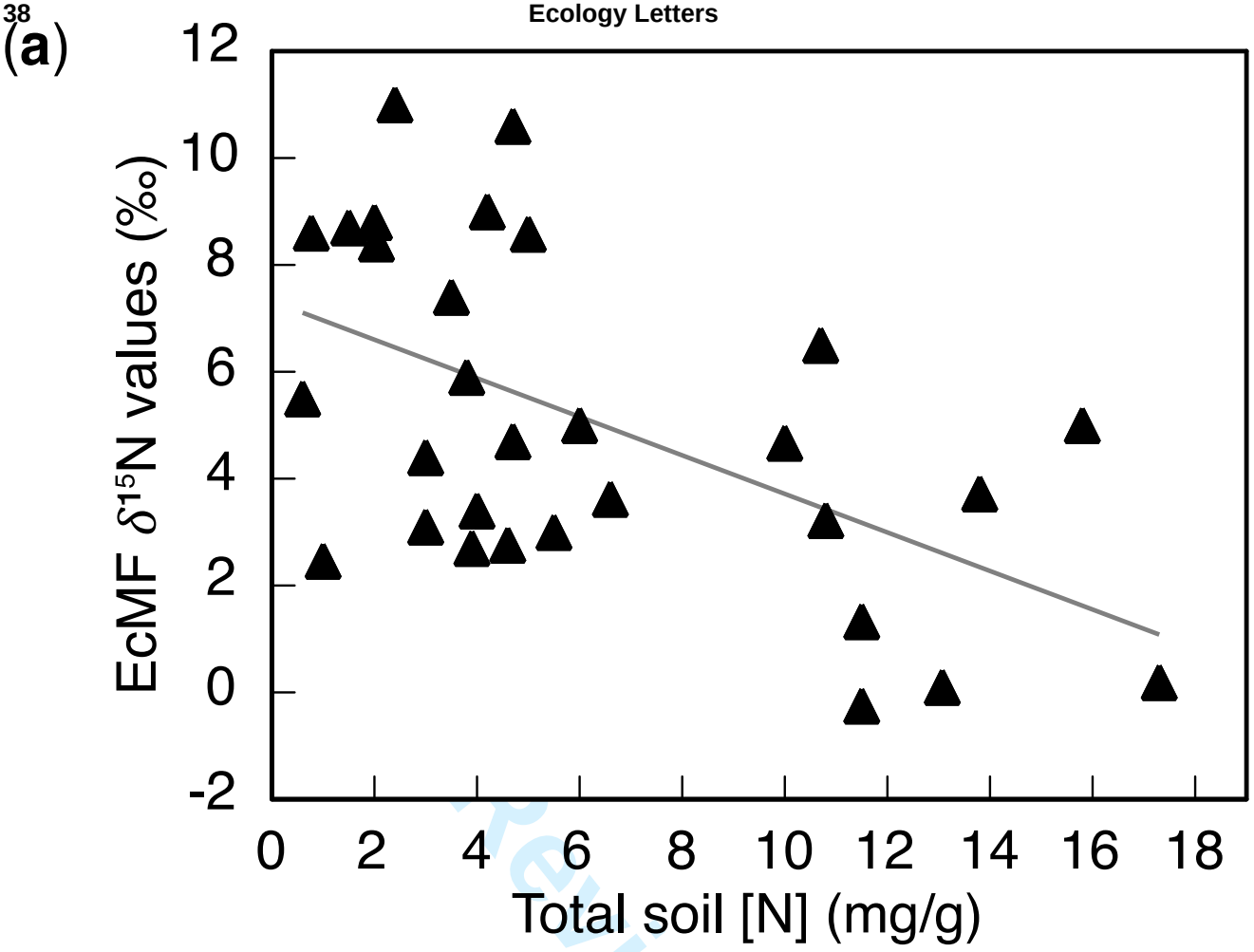

(a)
Soil δ15N (‰)
Org Soil
Min Soil
(b)
Plant δ15N (‰)
EcM Plant
AM Plant
(c)
Sporocarp δ15N (‰)
EcM Fungi
SAP Fungi
Latitude
Low-Lat.
High-Lat.

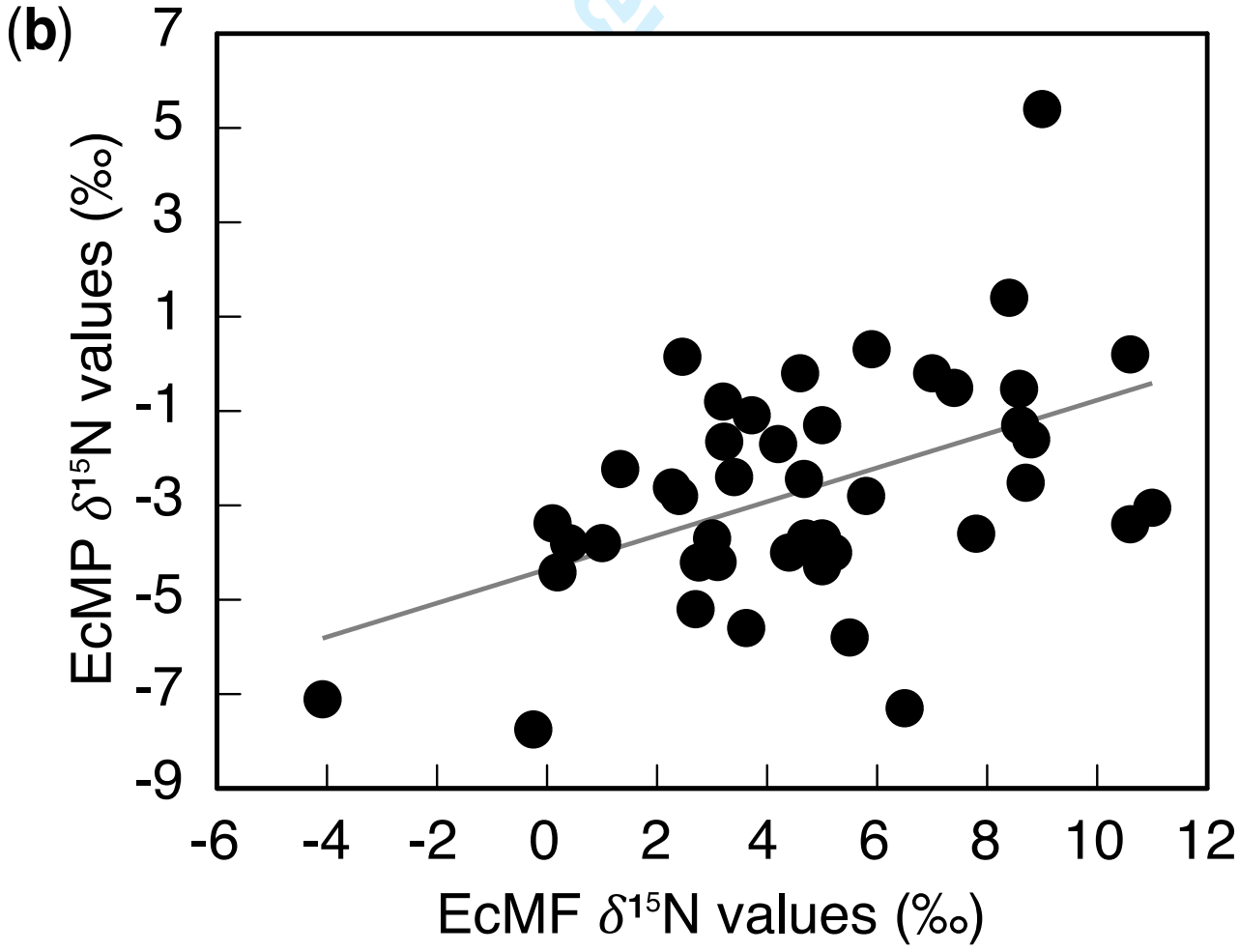

(a)
EcM – AM plant δ15N values (‰)
4
2
0
-2
-4
-6
-8
0
10
20
30
40
50
60
70
80
Latitude

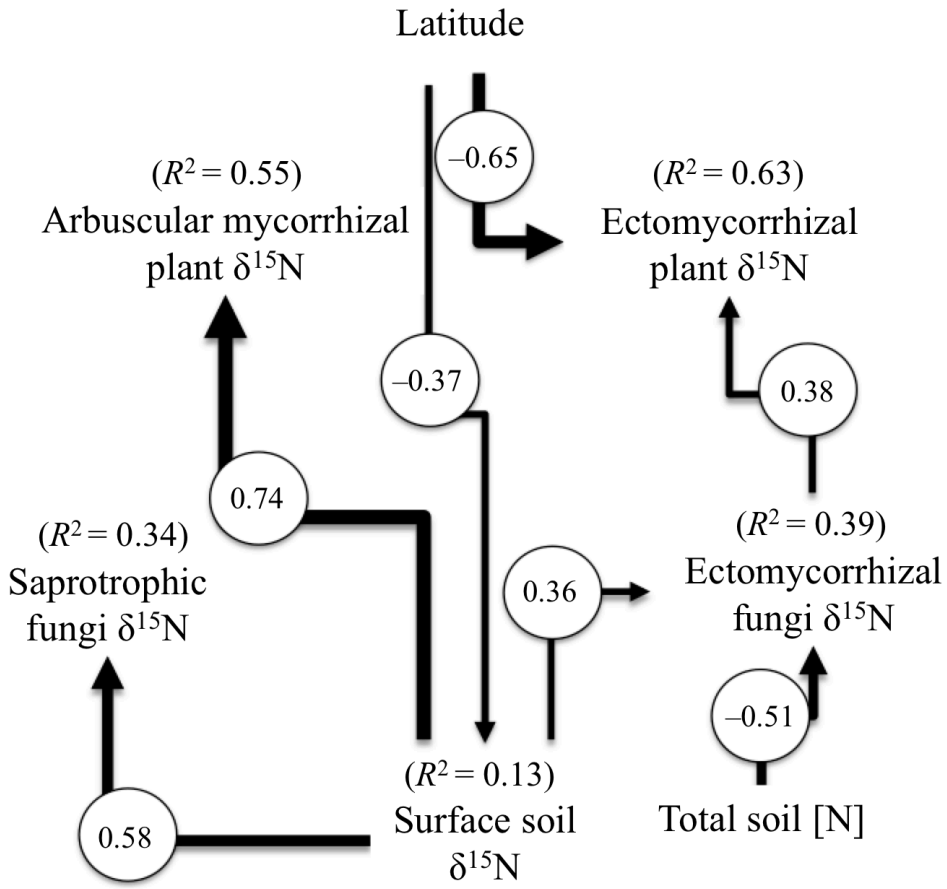

(b)
EcMF – EcMP δ15N values (‰)
16
14
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
Latitude


1:1
EcM plant δ^{15}N
Organic soil δ^{15}N
8
6
4
2
0
-2
-4
-6
-8


1:1
AM plant δ^{15}N
Organic soil δ^{15}N
8
6
4
2
0
-2
-4
-6
-8


(a)
EcMF δ^{15}N values (‰)
Total soil [N] (mg/g)
12
10
8
6
4
2
0
-2
0
2
4
6
8
10
12
14
16
18


(b)
EcMP δ^{15}N values (‰)
EcMF δ^{15}N values (‰)
7
5
3
1
-1
-3
-5
-7
-9
-6
-4
-2
0
2
4
6
8
10
12


Latitude
–0.65
(R^2 = 0.55)
Arbuscular mycorrhizal plant δ^{15}N
(R^2 = 0.63)
Ectomycorrhizal plant δ^{15}N
–0.37
0.38
0.74
(R^2 = 0.34)
Saprotrophic fungi δ^{15}N
0.36
(R^2 = 0.39)
Ectomycorrhizal fungi δ^{15}N
–0.51
0.58
(R^2 = 0.13)
Surface soil δ^{15}N
Total soil [N]