

Research review

Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability

Author for correspondence:

Joseph Craine

Tel: +1 785 532 3062

Email: jcraine@ksu.edu

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Joseph M. Craine¹, Andrew J. Elmore², Marcos P. M. Aida³, Mercedes Bustamante⁴, Todd E. Dawson^{5,6}, Erik A. Hobbie⁷, Ansgar Kahmen⁶, Michelle C. Mack⁸, Kendra K. McLaughlan⁹, Anders Michelsen¹⁰, Gabriela B. Nardoto¹¹, Linda H. Pardo¹², Josep Peñuelas¹³, Peter B. Reich¹⁴, Edward A. G. Schuur⁸, William D. Stock¹⁵, Pamela H. Templer¹⁶, Ross A. Virginia¹⁷, Jeffrey M. Welker¹⁸ and Ian J. Wright¹⁹

¹Division of Biology, Kansas State University, Manhattan, KS 66506, USA; ²University of Maryland Center for Environmental Science, Appalachian Laboratory, 301 Braddock Road, Frostburg, MD 21532, USA; ³Department of Plant Physiology and Biochemistry, Institute of Botany, PB 4005 CEP 01061-970 São Paulo, Brazil; ⁴Departamento de Ecologia, Universidade de Brasília, Brasília, DF, Brazil; ⁵Division of Ecosystem Sciences, Mulford Hall, University of California, Berkeley, CA 94720, USA; ⁶Center for Stable Isotope Biogeochemistry, Department of Integrative Biology, University of California, Valley Life Sciences Building, Berkeley, CA 94720, USA; ⁷Institute for the Study of Earth, Oceans, and Space, Morse Hall, University of New Hampshire, 39 College Road, Durham, NH 03824, USA; ⁸Department of Botany, University of Florida, PO Box 118526, Gainesville, FL 32611, USA; ⁹Department of Geography, Kansas State University, Manhattan, KS 66506; ¹⁰Department of Terrestrial Ecology, Institute of Biology, Oester Farimagsgade 2D, DK-1353 Copenhagen K, Denmark; ¹¹Lab. Ecologia Isotópica – CENA/USP, Universidade de São Paulo, Av. Centenário, 303, Piracicaba SP 13416-000, Brazil; ¹²USDA Forest Service, PO Box 968, Burlington, VT 05402, USA; ¹³Unitat d'Ecofisiologia CSIC-CREAF-CEAB, Centre de Recerca Ecològica i Aplicacions Forestals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain; ¹⁴Department of Forest Resources, University of Minnesota, 115 Green Hall, 1530 Cleveland Avenue North, St Paul, MN 55108, USA; ¹⁵Centre for Ecosystem Management, School of Natural Sciences, Edith Cowan University, Joondalup 6027, Western Australia, Australia; ¹⁶Department of Biology, Boston University, 5 Cummington Street, Boston, MA 02215, USA; ¹⁷Environmental Studies, Dartmouth College, Hanover, NH 03755, USA; ¹⁸Environment and Natural Resources Institute, University of Alaska, 707 A Street, Anchorage, AK 99501, USA; ¹⁹Department of Biological Sciences, Macquarie University 2109, Sydney, Australia

Summary

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Ratios of nitrogen (N) isotopes in leaves could elucidate underlying patterns of N cycling across ecological gradients. To better understand global-scale patterns of N cycling, we compiled data on foliar N isotope ratios ($\delta^{15}\text{N}$), foliar N concentrations, mycorrhizal type and climate for over 11 000 plants worldwide. Arbuscular mycorrhizal, ectomycorrhizal, and ericoid mycorrhizal plants were depleted in foliar $\delta^{15}\text{N}$ by 2‰, 3.2‰, 5.9‰, respectively, relative to nonmycorrhizal plants. Foliar $\delta^{15}\text{N}$ increased with decreasing mean annual precipitation and with increasing mean annual temperature (MAT) across sites with $\text{MAT} \geq -0.5^\circ\text{C}$, but was invariant with MAT across sites with $\text{MAT} < -0.5^\circ\text{C}$. In independent landscape-level to regional-level studies, foliar $\delta^{15}\text{N}$ increased with increasing N availability; at the global scale, foliar $\delta^{15}\text{N}$ increased with increasing foliar N concentrations and decreasing foliar phosphorus (P) concentrations. Together, these results suggest that warm, dry ecosystems have the highest N availability, while plants with high N concentrations, on average, occupy sites with higher N availability than plants with low N concentrations. Global-scale comparisons of other components of the N cycle are still required for better mechanistic understanding of the determinants of variation in foliar $\delta^{15}\text{N}$ and ultimately global patterns in N cycling.

Introduction

The natural abundance nitrogen (N) stable isotope ratio ($\delta^{15}\text{N}$) of leaves can serve as an integrator of terrestrial N cycling (Robinson, 2001). As such, they have the potential to reveal spatial and temporal patterns of N cycling as well as how disturbances alter the N cycle. Foliar $\delta^{15}\text{N}$ varies by over 32‰ among plants found in natural ecosystems (Yoneyama *et al.*, 1993; Aranibar *et al.*, 2004; Codron *et al.*, 2005; Hobbie *et al.*, 2005) and is well known to vary somewhat systematically along gradients of climate and nutrient cycling (Schulze *et al.*, 1994; Handley *et al.*, 1999a; Martinelli *et al.*, 1999; Amundson *et al.*, 2003; Pardo *et al.*, 2006), among species (Michelsen *et al.*, 1996; Hobbie *et al.*, 2005; Templer *et al.*, 2007; Kahmen *et al.*, 2008) and in response to experimental manipulations of resource availability (Högberg, 1990). In addition, there is good mechanistic knowledge about the degrees of fractionation of N for different processes (Högberg, 1997). For example, denitrification and nitrification discriminate much more against ^{15}N than N_2 fixation. Overall, foliar $\delta^{15}\text{N}$ could become an important metric for generalizing the global patterns of N cycling (Amundson *et al.*, 2003) as well as an important indicator to monitor changes in N cycling that might accompany anthropogenic influences on ecosystems such as increasing atmospheric CO_2 or disturbance (BassiriRad *et al.*, 2003; McLauchlan *et al.*, 2007).

Despite its potential, interpreting global-scale patterns of foliar $\delta^{15}\text{N}$ remains a challenge. Plants can acquire N from a number of sources, each of which could have a unique signature and vary over time. For example, the $\delta^{15}\text{N}$ of soil organic matter (SOM) often increases with depth, ammonium is

generally more enriched than nitrate, while depositional N is variable both spatially and temporally. The N cycle also contains a large number of fractionating steps, which generates multiple competing interpretations for any pattern of observed foliar signatures. In general, there are three sets of unresolved relationships that currently limit the use of foliar $\delta^{15}\text{N}$ in inferring patterns of N cycling at the global scale. First, foliar $\delta^{15}\text{N}$ values appear to consistently differ among plants that are associated with different types of mycorrhizal fungi. For example, at a given site, plant species associated with ericoid and ectomycorrhizal fungi can have foliar $\delta^{15}\text{N}$ signatures that are 3–8‰ lower than nonmycorrhizal plants (Michelsen *et al.*, 1996, 1998). It is uncertain whether arbuscular mycorrhizal fungi contribute to variation in foliar $\delta^{15}\text{N}$ (Handley *et al.*, 1999b), largely because they are thought to be involved mainly in plant P nutrition. In addition, although the results of individual studies comparing mycorrhizal types are robust, general patterns among mycorrhizal types have never been synthesized at global scales, and there are differences among studies in the general ranking of signatures among mycorrhizal types (Michelsen *et al.*, 1998; Pardo *et al.*, 2006).

Second, relationships between foliar $\delta^{15}\text{N}$ and climate need to be clarified. With respect to rainfall, Austin & Sala (1999) observed that foliar $\delta^{15}\text{N}$ increased with decreasing mean annual precipitation (MAP) in data collected by Schulze *et al.* (1998) in northern Australia. The increase in foliar $\delta^{15}\text{N}$ with decreasing MAP was later shown to exist at global scales (Handley *et al.*, 1999a). With respect to temperature, Martinelli *et al.* (1999) showed that foliar $\delta^{15}\text{N}$ was greater for tropical forests than temperate forests, suggesting a global relationship between temperature and foliar $\delta^{15}\text{N}$. Amundson *et al.* (2003) later

quantified positive linear relationships between mean annual temperature (MAT) and soil $\delta^{15}\text{N}$, foliar $\delta^{15}\text{N}$ and the difference between leaf and soil $\delta^{15}\text{N}$.

Although the climate patterns appear robust at multiple spatial scales, these relationships need to be improved in three ways. First, little data from cold ecosystems were included in these regional and global reviews. Coverage of foliar $\delta^{15}\text{N}$ in ecosystems with low MAT has recently expanded (Welker *et al.*, 2003), allowing for better examination of foliar $\delta^{15}\text{N}$ patterns in cold ecosystems, i.e. $\text{MAT} < 0^\circ\text{C}$. Second, although the relationship between foliar $\delta^{15}\text{N}$ and temperature or precipitation seems well-established, no global synthesis of foliar $\delta^{15}\text{N}$ has accounted for potential covarying changes in the types of mycorrhizal fungi along climate gradients. For example, increases in foliar $\delta^{15}\text{N}$ with increasing MAT might be caused by a shift from ectomycorrhizal or ericoid mycorrhizal species to arbuscular mycorrhizal species and it is important to separate the influence of mycorrhizal fungi out of general climate relationships. Lastly, patterns of foliar $\delta^{15}\text{N}$ for plants that potentially fix N_2 have not, to our knowledge, been examined across climatic gradients. Because of the ability of these plants to obtain N from symbiotic N_2 -fixing bacteria, a process that does not discriminate against ^{15}N , their acquisition of N can be somewhat independent from soil N dynamics (Högberg, 1997). As the ^{15}N signatures of N_2 -fixing plants might be independent of climate and not reflect soil processes, these species are often excluded from syntheses, although it is unknown how the inclusion or exclusion of these species would bias the relationships between foliar $\delta^{15}\text{N}$ and climate.

For the third major area of improvement, foliar $\delta^{15}\text{N}$ and foliar N concentrations appear to be correlated, but it is uncertain how robust this pattern is at the global scale and whether it is independent of climate. Multiple studies at various spatial scales have shown a consistent positive relationship between foliar N concentrations and $\delta^{15}\text{N}$: across 15 temperate and tropical forest sites (Martinelli *et al.*, 1999); within and among plant species at Glacier Bay, Alaska (Hobbie *et al.*, 2000), across 67 grasslands in four regions of the world (Craine *et al.*, 2005), and within and among species and temperate forest stands (Pardo *et al.*, 2006). The relationship has the potential to serve as another strong constraint on the pattern of N cycling across stands if it could be demonstrated to hold at the global scale.

Once the global patterns of foliar $\delta^{15}\text{N}$ are established, they will be useful only inasmuch as they can be used to infer underlying characteristics of the N cycle. One of the most promising potential interpretations of foliar $\delta^{15}\text{N}$ is that it might be correlated with soil N availability to plants – the supply of N to terrestrial plants relative to their N demands (McLauchlan *et al.*, 2007). Although multiple studies have shown positive relationships between an index of N availability and foliar $\delta^{15}\text{N}$ (Garten & Van Miegroet, 1994), it is currently unknown whether regional or global patterns of foliar $\delta^{15}\text{N}$ consistently index N availability to plants. There are two main reasons why foliar $\delta^{15}\text{N}$ might index soil N availability to

plants. First, when N availability is high, N lost from the ecosystem is more likely to be depleted in ^{15}N , which increases the $\delta^{15}\text{N}$ of leaves. When N availability is low, N is cycled and lost primarily as organic N (Hedin *et al.*, 1995; Neff *et al.*, 2003). As N availability increases, the production of ammonium and then nitrate increases. Gaseous N loss during nitrification (Firestone & Davidson, 1989) and the leaching of ^{15}N -depleted nitrate (Högberg, 1997; Koba *et al.*, 2003) can cause the remaining N pool (and subsequently plants) to be enriched in ^{15}N . With further increases in N availability, denitrification begins to consume a greater fraction of nitrate (Hall & Matson, 2003), further enriching inorganic N pools, provided that denitrification does not completely consume the nitrate pool (Houlton *et al.*, 2006). Ammonia volatilization also discriminates strongly against ^{15}N (Högberg, 1997). In ecosystems where ammonia volatilization is prevalent, an analogous pattern of increasing ammonia volatilization with increasing N availability would lead to a similar positive relationship between N availability and foliar $\delta^{15}\text{N}$, provided that the ammonia is not taken up by leaves (Frank & Evans, 1997).

The second main reason that foliar $\delta^{15}\text{N}$ might index soil N availability is that plants experiencing low N availability might be more likely to be dependent on mycorrhizal fungi for N acquisition than at high N availability, and the N that mycorrhizal fungi transfer to plants is depleted in ^{15}N (Taylor *et al.*, 2000; Hobbie & Colpaert, 2003). Current evidence suggests that N-containing transfer compounds such as glutamine are depleted in ^{15}N relative to bulk fungal N, presumably owing to the large discrimination against ^{15}N associated with processes such as transaminase reactions (Macko *et al.*, 1986; Stoker *et al.*, 1996). In addition, mycorrhizal fungi at low N availability should transfer a lower proportion of the N that they take up than at high N availability (Hobbie *et al.*, 2000; Hobbie & Colpaert, 2003). As N availability decreases, the benefit to plants to use mycorrhizal fungi for N acquisition increases. Hence, the dependence of plants on mycorrhizal fungi for N could increase consistently with decreasing N availability. Plant $\delta^{15}\text{N}$ would decrease with decreased N availability if the proportion of nitrogen acquired by plants that is supplied by fungi increases or if the proportion of nitrogen taken up by fungi that is transferred to the host plants decreases (Hobbie *et al.*, 2000).

To better understand the global patterns of foliar $\delta^{15}\text{N}$ and N cycling, we compiled a data set of over 11 000 geo-referenced observations of foliar $\delta^{15}\text{N}$ and foliar N concentrations from wild-grown, unfertilized plants. Data were matched with climate parameters and mycorrhizal types. We then examined the global relationships between foliar $\delta^{15}\text{N}$ and the type of mycorrhizal fungi association, mean climate parameters, and plant N and P concentrations. The relationship between foliar $\delta^{15}\text{N}$ and climate was determined separately for plants that are not associated with N_2 -fixing bacteria and from those species that are known to be or might be (e.g. plants of the Fabaceae family). We then examined the relationships between

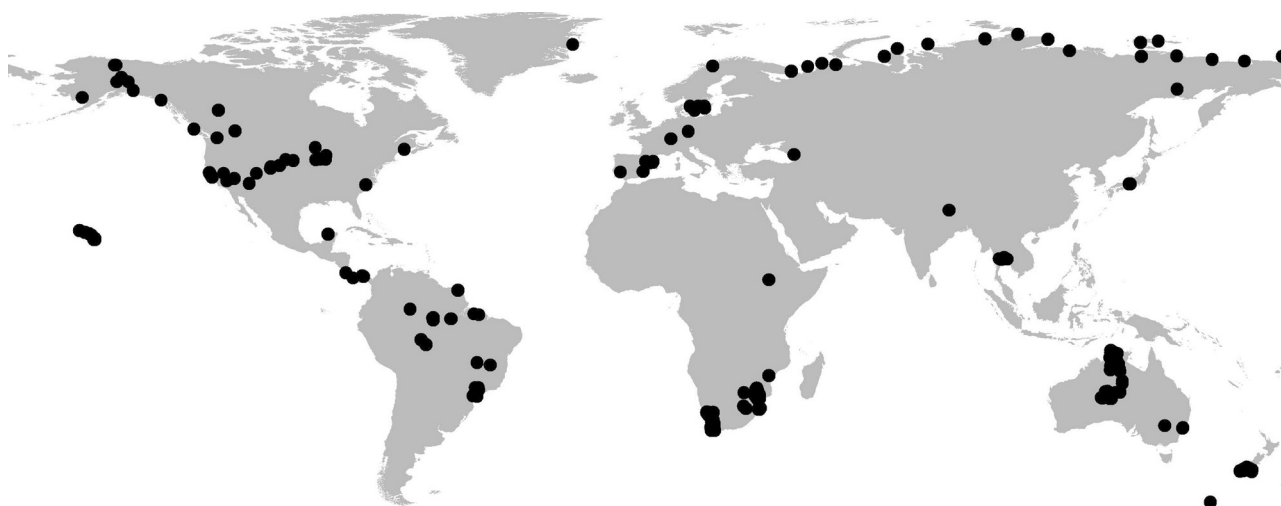


Fig. 1 Geographic distribution of sites in this study.

foliar $\delta^{15}\text{N}$ and different measures of N availability for 15 studies to determine if there are consistent positive correlations between N availability and foliar $\delta^{15}\text{N}$. In general, we expect that sites with greater N availability, for example, warm, dry sites with plants with high foliar N concentrations, should have leaves that are enriched in ^{15}N . That said, we are uncertain of the specific relationships between climate or foliar N concentration and foliar $\delta^{15}\text{N}$.

Materials and Methods

Data on foliar $\delta^{15}\text{N}$ and foliar N concentrations of non N_2 -fixing plants were identified from: previous published reviews; searching ISI Science Citation Index (www.isiknowledge.com) for papers that included terms such as 'nitrogen isotopes' or ' ^{15}N '; and contacting individuals that were thought to have been likely to have collected data on foliar ^{15}N . Data on non- N_2 -fixing plants were acquired from 91 studies distributed from around the world (Fig. 1) encompassing 900 sites, 1103 taxa and 9757 plants. Data for potential N_2 -fixing plants (those known to fix atmospheric N_2 and all members of the Fabaceae family, unless verified not to fix atmospheric nitrogen) were found for 35 studies, 120 sites, and 283 taxa. The combined data set encompassed 95 studies, 916 sites, and 1386 taxa (see the Supporting Information, Notes S1, for a list of published studies used here). Data for each study were generally at the level of the individual plant, although for a few records, data from multiple individuals had been originally summarized at the site level. All data come from unfertilized wild-grown plants whose signatures are likely to reflect soil N cycling. Based on this criterion, nonvascular, wetland, agricultural, carnivorous, mycoheterotrophic and epiphytic plants were excluded. In addition, we also did not attempt to review nonepiphytic orchids because of their unique mycorrhizal symbiosis combined with a lack of data on the global scale. The $^{15}\text{N} : ^{14}\text{N}$ ratios are expressed

using δ notation where foliar $^{15}\text{N} : ^{14}\text{N}$ ratios are expressed relative to the ratio of $^{15}\text{N} : ^{14}\text{N}$ in atmospheric N_2 : $\delta^{15}\text{N}$ (‰) = $(R_{\text{sample}}/R_{\text{atm}} - 1) \times 1000$, where $R_{\text{atm}} = 0.0036765$. The $\delta^{15}\text{N}$ of atmospheric N_2 by definition is 0.0‰.

In addition to foliar $\delta^{15}\text{N}$, we compiled data on foliar N concentration ($[\text{N}_L]$), the type of mycorrhizal association, MAT and MAP from published and unpublished studies. A total of 6627 of the plants were categorized as arbuscular, 1761 as ectomycorrhizal, 650 as ericoid and 699 as nonmycorrhizal. We note that in our dataset all mycorrhizal associations were found in ecosystems with MAT from -10 to 23°C and MAP from 200 mm yr^{-1} to 1400 mm yr^{-1} (Fig. 2).

Data on mean annual temperature and mean annual precipitation were derived from the original publications, if available. If no climate data were published, 30-yr means (1961–90) for annual temperature and precipitation were obtained from site location data and a global climate database at 10-min resolution (New *et al.*, 2002). Mycorrhizal associations were occasionally assessed by the authors in some cases and derived from various published sources (Brundrett, 2004, 2008; Smith & Read, 2008). Arbutoid mycorrhizas were classified as ectomycorrhizal.

Foliar $\delta^{15}\text{N}$ was first analysed with a general linear model that included MAT, MAP, mycorrhizal association and $[\text{N}_L]$. Log-transformed MAP and $[\text{N}_L]$ were found to explain more variation in foliar ^{15}N than the untransformed data and were used in all subsequent models. Observing relationships between MAT and foliar $\delta^{15}\text{N}$ revealed a potential breakpoint around which there would be two different relationships between MAT and foliar $\delta^{15}\text{N}$. To determine the temperature around which to divide ecosystems, we first calculated the residuals of foliar $\delta^{15}\text{N}$ with respect to $[\text{N}_L]$, mycorrhizal association, and log-transformed MAP. We then fitted a nonlinear model with the residuals and MAT that fits two linear segments end to end (i.e. a piecewise linear regression) (Toms & Lesperance,

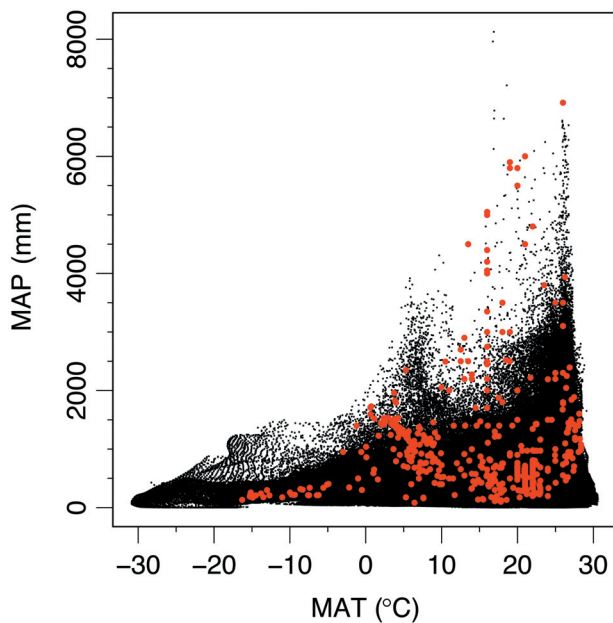


Fig. 2 Relationship between mean annual temperature (MAT) and mean annual precipitation (MAP) for samples included in this study relative to a global climate envelope. Open circles are from data in this study. Black dots represent the terrestrial climate space with each point representing the combination of MAT and MAP at 18.5×18.5 km resolution with data derived from (New *et al.*, 2002). Certain combinations of MAT and MAP are less well represented in the foliar ^{15}N dataset. These climates include cool, high rainfall ecosystems (MAT approx. 4°C , MAP > 2000 mm), such as the temperate rainforests of coastal Alaska, and hot, high rainfall ecosystems (MAT $> 28^\circ\text{C}$, MAP > 4000 mm) that occur in equatorial areas on the western edges of continents. Hot, xeric ecosystems (MAT $> 25^\circ\text{C}$, MAP < 100 mm), such as the west-central Sahara, and cold, xeric systems of Greenland (MAT $< -15^\circ\text{C}$, MAP < 750 mm) have low primary productivity with the latter largely covered by ice.

2003). The nonlinear model solves for four parameters: a y -intercept, the slope of the first linear relationship, a breakpoint and the slope of the second linear relationship. Including a constant to alter the intercept of the second line did not markedly change its elevation. Because the model did not independently converge on a single answer, we ran multiple models that each had a different breakpoint temperature set a priori and selected the temperature that produced the lowest error sums of squares. Data from Falkengren-Grerup *et al.* (2004) were removed from the dataset as the data altered the breakpoint for MAT from -0.5 to 8.5°C and they appeared to be extreme outliers in other relationships (see below).

After determining the MAT breakpoint, the main regression model for foliar $\delta^{15}\text{N}$ included log-transformed $[\text{N}_\text{L}]$, mycorrhizal association, log-transformed MAP, MAT and a categorical factor that divided ecosystems into those with MAT $< -0.5^\circ\text{C}$ and those with MAT $\geq -0.5^\circ\text{C}$. A more complicated model that included all pairwise interactions among the main factors only explained an additional 3% of the total variation

in foliar $\delta^{15}\text{N}$ and complicated the interpretations with little real additional insight into relationships. For MAP, MAT and $[\text{N}_\text{L}]$, we also calculated foliar $\delta^{15}\text{N}$ residuals of multifactor regression models to more specifically examine the relationships between the variables and foliar $\delta^{15}\text{N}$.

After attempting to explain variation in foliar $\delta^{15}\text{N}$ with the four main variables, we tested the ability of the identity of the continent, seasonality of precipitation, and soil pH to explain residual variation in foliar $\delta^{15}\text{N}$. Soil pH data was taken from the IGBP Global Soils Data Task (Belward *et al.*, 1999). Relationships between foliar $\delta^{15}\text{N}$ and foliar N and P concentrations were assessed for 1014 plant samples for which both N and P concentrations were provided. With fewer data points, the explanatory model only included $[\text{N}_\text{L}]$, foliar P concentration ($[\text{P}_\text{L}]$), and the interaction between the two. Results were similar whether elemental concentrations were log-transformed or not ($r^2 = 0.34$ for both) and additional covariates such as MAT, MAP or mycorrhizal type did not qualitatively alter the relationships between foliar $\delta^{15}\text{N}$ and nutrient concentrations.

To determine whether patterns among potentially N_2 -fixing plants were similar to those that are not N_2 -fixing, the last set of analyses examined the patterns of foliar $\delta^{15}\text{N}$ for potentially N_2 -fixing plants. We examined the 1604 records of foliar $\delta^{15}\text{N}$ of this data set in a general linear model that was similar to the one used for non- N_2 -fixing species. Mean annual temperature, MAP and $[\text{N}_\text{L}]$ were included in the model, but mycorrhizal associations were not because all the plants are associated with arbuscular fungi. No categorical differences in the relationship between MAT and $\delta^{15}\text{N}$ at different temperatures were found and therefore no categorical variable for MAT was included.

To better understand relationships between foliar $\delta^{15}\text{N}$ and soil N availability, we examined two additional datasets. First, for studies that provided data on bulk soil $\delta^{15}\text{N}$ as well as foliar $\delta^{15}\text{N}$, we examined the relationship between foliar $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$, and the difference between foliar and soil $\delta^{15}\text{N}$. Since foliar $\delta^{15}\text{N}$ is initially dependent on the signature of soil organic nitrogen from which the N made available to plants is derived, the offset between soil and plant signatures is considered an index of the short-term enrichment or depletion of ^{15}N available to plants and a potentially better index of short-term processes than foliar $\delta^{15}\text{N}$ alone (Emmett *et al.*, 1998; Amundson *et al.*, 2003). For each data point, soil $\delta^{15}\text{N}$ was calculated from 0 to 20 cm depth where possible. Second, 14 studies measured both an index of N supply and foliar or aboveground biomass $\delta^{15}\text{N}$ over landscape to regional scales. In these studies, N supply data were measured as: *in situ* net N mineralization; laboratory incubations of soils under standard conditions; *in situ* net N mineralization with resin bags; or inorganic N leaching with lysimeters (Hogbom *et al.*, 2002). For each study, we determined the relationships (generally linear) between N supply and $\delta^{15}\text{N}$ of leaves or aboveground biomass. A few studies had measured N supply with multiple techniques (*in situ* and laboratory incubations) or for different

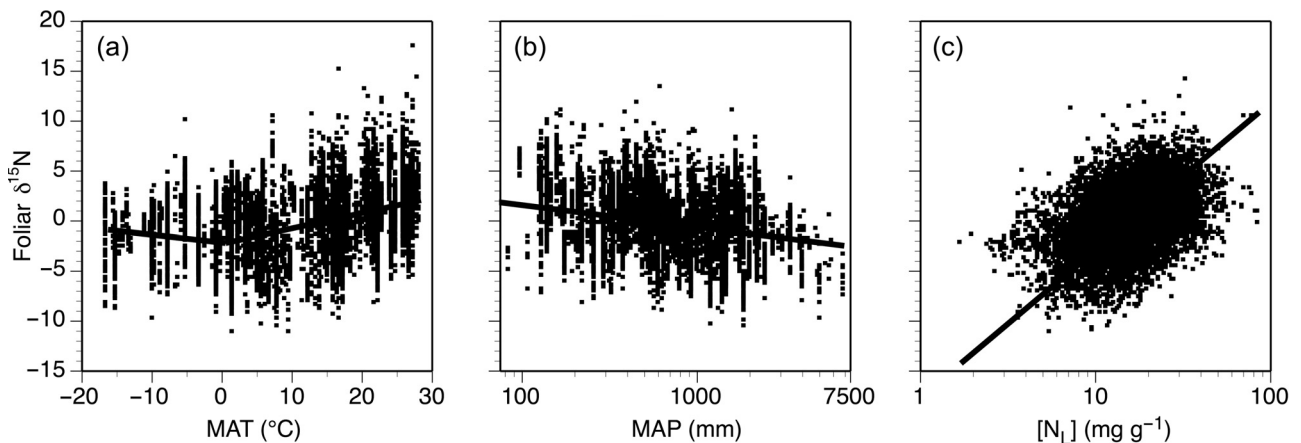


Fig. 3 Relationships between foliar $\delta^{15}\text{N}$ of non- N_2 -fixing plants and temperature, precipitation and foliar N concentrations. (a) Residual foliar $\delta^{15}\text{N}$ vs mean annual temperature (MAT), (b) mean annual precipitation (MAP), and (c) foliar N concentration ($[\text{N}_L]$). Residuals were taken from a model that included all the variables listed in Table 1, except for the target variable or derivative variables (i.e. the categorical separation of sites *c.* -0.5°C when analysing the residuals for MAT). Relationship with $[\text{N}_L]$ is from a model II regression.

portions of the soil (e.g. O vs A horizon). In these cases, the relationship with the highest coefficient of determination was selected for display after ensuring that this selectivity did not qualitatively alter the overall synthesis. Likely associated with the variability in how N supplies were assessed, there was little relationship between N supply and foliar $\delta^{15}\text{N}$ when the data for all studies were joined into a single dataset (data not shown). All statistical analyses were conducted in JMP 5.1 (SAS Institute, Cary, NC, USA).

Results

Global patterns

In the global data set of non- N_2 -fixing plants, the variables MAT, MAP, mycorrhizal association, and $[\text{N}_L]$ explained 56% of the total variation in foliar $\delta^{15}\text{N}$. In contrast to previous analyses, with this larger data set, foliar $\delta^{15}\text{N}$ did not increase monotonically with MAT (Fig. 3a). Foliar $\delta^{15}\text{N}$ showed no trend with MAT for ecosystems with $\text{MAT} < -0.5^\circ\text{C}$, implying similar reliance on mycorrhizal fungi or similar relative importance of different loss pathways across the range of sites with $\text{MAT} < -0.5^\circ\text{C}$. For ecosystems with $\text{MAT} \geq -0.5^\circ\text{C}$, foliar $\delta^{15}\text{N}$ increased at a rate of $0.23\text{‰ }^\circ\text{C}^{-1}$ (Fig. 3a). Foliar $\delta^{15}\text{N}$ declined with increasing MAP with no evidence of a breakpoint in the relationship analogous to the one observed for the relationship between MAT and foliar $\delta^{15}\text{N}$ (Fig. 3b). On average, foliar $\delta^{15}\text{N}$ decreased by 2.6‰ for every order of magnitude increase in MAP.

The main groups of mycorrhizal fungi differed in foliar $\delta^{15}\text{N}$ by almost 6‰ (Fig. 4). The type of mycorrhizal association explained the second-largest fraction of the explained variation in foliar $\delta^{15}\text{N}$ (29%, Table 1), even though 68% of the species were associated with arbuscular mycorrhizal fungi. Standardized to a common MAT (13.2°C , arithmetic mean),

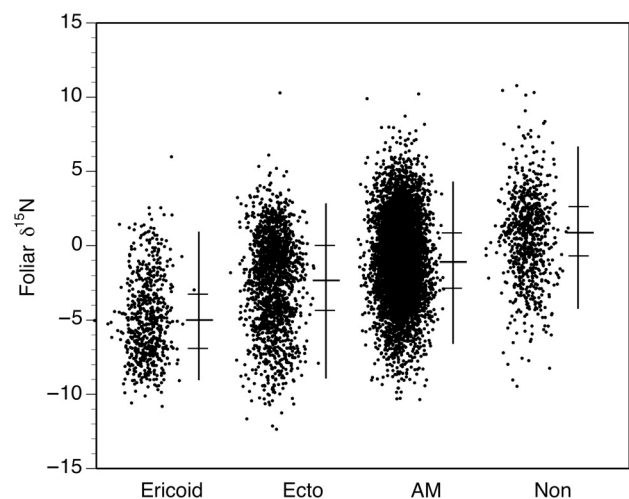


Fig. 4 Distribution of foliar $\delta^{15}\text{N}$ (‰) values among different mycorrhizal types. Data were first standardized to a common mean annual temperature (13.2°C), mean annual precipitation (751 mm yr^{-1}) and foliar N concentration (15.8 mg g^{-1}) using relationships in Table 1. Adjacent bars represent range of 95% of the data with horizontal bars representing the 25% quantile, mean, and 75% quantile, from top to bottom. All means are significantly different at $P < 0.001$. AM, arbuscular mycorrhizal; Non, nonmycorrhizal.

MAP (751 mm yr^{-1} , geometric mean) and $[\text{N}_L]$ (15.8 mg g^{-1} , geometric mean), nonmycorrhizal plants had the highest average foliar $\delta^{15}\text{N}$ ($0.9 \pm 0.2\text{‰}$). Among mycorrhizal plants, arbuscular mycorrhizal plants had the highest mean foliar $\delta^{15}\text{N}$ ($-1.1 \pm 0.1\text{‰}$), ericoid mycorrhizal plants the lowest ($-5.0 \pm 0.2\text{‰}$) and ectomycorrhizal plants were intermediate in mean foliar $\delta^{15}\text{N}$ ($-2.3 \pm 0.2\text{‰}$).

After controlling for mycorrhizal type and climate, foliar $\delta^{15}\text{N}$ increased linearly with the logarithm of $[\text{N}_L]$ (Fig. 3c) with $[\text{N}_L]$ accounting for 44% of the explainable variation in

	NonN ₂ -fixing			Potentially N ₂ -fixing		
	SS	%Model SS	P	SS	%Model SS	P
MAT	1638	3.7	< 0.001	2434	67	< 0.001
MAT Break	1748	3.9	< 0.001			
Log MAP	5980	13.5	< 0.001	0.09	0	0.92
Mycorrhizal	12 657	28.6	< 0.001			
Log [N _L]	19 549	44.1	< 0.001	1218	33	< 0.001
MAT×MAT Break	2708	6.1%	< 0.001			

MAT, mean annual temperature; MAT Break, a categorical separation of sites based on a MAT of -0.5°C . MAP, mean annual precipitation; [N_L], leaf N concentration.

For nonN₂-fixing plants, $r^2 = 0.56$, $n = 9757$. For potentially N₂-fixing plants, $r^2 = 0.23$; $n = 1604$; SS = sums of squares.

Table 2 Relationships between foliar $\delta^{15}\text{N}$ and concentrations of nitrogen ([N_L]) and phosphorus ([P_L]) in leaves

	Coefficient (‰ per mg g ⁻¹)	P
Intercept	-3.05 ± 0.35	< 0.001
[N _L]	0.45 ± 0.02	< 0.001
[P _L]	-4.72 ± 0.32	< 0.001
[N _L] × [P _L]	-0.038 ± 0.026	0.13

Model $r^2 = 0.34$, $n = 1014$.

foliar $\delta^{15}\text{N}$ (Table 1). Doubling [N_L] increased foliar $\delta^{15}\text{N}$ by 2.1‰. Among the 1014 foliar samples from nonN₂-fixing species where both N and P had been measured, after controlling for variation in P concentrations, foliar $\delta^{15}\text{N}$ increased at a rate of 4.5‰ for every 10 mg g⁻¹ increase in N concentrations (Table 2), similar to the increase for the greater dataset that constrained for other factors. By contrast, after controlling for variation in N concentrations, foliar $\delta^{15}\text{N}$ decreased at a rate of 4.7‰ for every 1 mg g⁻¹ increase in P concentration. Relationships between N concentrations and foliar $\delta^{15}\text{N}$ were independent of P concentrations and vice versa.

Almost half of the unexplained variation in foliar $\delta^{15}\text{N}$ was found within sites (16% of the total variance, data not shown). Among sites, little additional variation could be explained by incorporating other factors, although there often were significant differences. For example, foliar $\delta^{15}\text{N}$ differed significantly among continents, with leaves from Australia and Europe depleted in ¹⁵N relative to the global mean (1.1 and 2.0‰, respectively) and North America enriched by 0.6‰ after accounting for the other four variables. Sites with primarily winter rainfall had 0.7‰ lower site-mean $\delta^{15}\text{N}$ than other sites. Soil pH explained only 0.2% of the residual variation in foliar $\delta^{15}\text{N}$, but was not a significant predictor of foliar $\delta^{15}\text{N}$ after taking into account the seasonality of precipitation.

Excluding N₂-fixing plants did not strongly bias relationships between climate and foliar $\delta^{15}\text{N}$. Among potentially

Table 1 Regression results for foliar $\delta^{15}\text{N}$ of nonN₂-fixing plants and potentially N₂-fixing plants

N₂-fixing plants, patterns of foliar $\delta^{15}\text{N}$ fell within the general envelopes delineated by the relationships between foliar $\delta^{15}\text{N}$ of non-fixing plants and MAT, MAP and [N_L]. Potentially N₂-fixing species were more prevalent at high MAT than low MAT but spanned most of the precipitation gradients. By contrast, potentially N₂-fixing species did not span the full range of foliar N concentrations, having relatively few observations at low [N_L]. Mean annual temperature, MAP and [N_L] explained 23% of the variation in foliar $\delta^{15}\text{N}$ for potentially N₂-fixing plants, with about half of the total variation explained by those three factors for the larger dataset. For these plants, foliar $\delta^{15}\text{N}$ increased with increasing MAT at approximately the same rate as non-N₂-fixing plants from ecosystems with $\text{MAT} \geq -0.5^{\circ}\text{C}$ (0.24 vs $0.23^{\circ}\text{C}^{-1}$; Fig. 5a), while MAP and foliar $\delta^{15}\text{N}$ were unrelated ($P > 0.92$; Fig. 5b). Increasing [N_L] an order of magnitude for potentially N₂-fixing species increased foliar $\delta^{15}\text{N}$ by 5.8‰ (Fig. 5c).

Nitrogen availability

Foliar $\delta^{15}\text{N}$ and the difference in $\delta^{15}\text{N}$ between leaf and soil were positively correlated (Fig. 6), reinforcing the idea that the general patterns of N availability can be assessed with foliar $\delta^{15}\text{N}$ alone. When averaged for each site, soil $\delta^{15}\text{N}$ correlates with foliar $\delta^{15}\text{N}$, with foliar $\delta^{15}\text{N}$ increasing at a faster rate than soil $\delta^{15}\text{N}$ as soil $\delta^{15}\text{N}$ increases (Fig. 6a). As such, foliar $\delta^{15}\text{N}$ was positively correlated with the difference between the $\delta^{15}\text{N}$ of leaves and soil (Fig. 6b).

Foliar $\delta^{15}\text{N}$ also increased with increasing N supply in 13 of 15 multi-site studies of unmanaged ecosystems (Fig. 7, Table 3), averaging a 4.9‰ increase in foliar $\delta^{15}\text{N}$ across the range of N availability in each study. For each of 13 studies, foliar $\delta^{15}\text{N}$ increased monotonically with N availability regardless of whether it was measured as *in situ* mineralization, *ex situ* potential net mineralization or *in situ* mineralization from resin bags. In the two studies that did not fit the trend, one examined herbaceous understory plants in Swedish forests (Falkengren-Grerup *et al.*, 2004). In this study, foliar $\delta^{15}\text{N}$

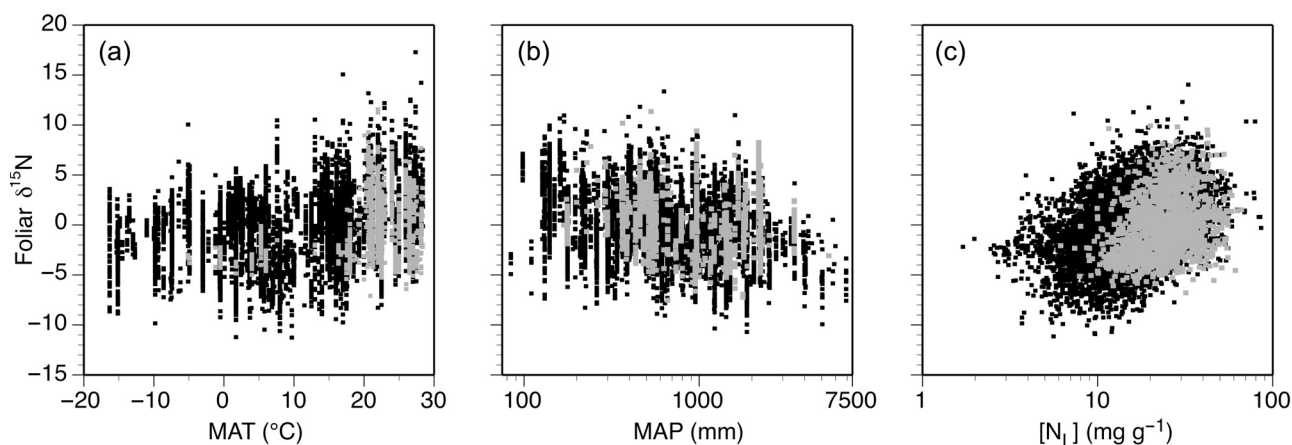


Fig. 5 Relationships between foliar $\delta^{15}\text{N}$ of potentially N_2 -fixing plants (gray dots) and temperature, precipitation, and foliar N concentrations. (a) Residual foliar $\delta^{15}\text{N}$ vs mean annual temperature (MAT), (b) mean annual precipitation (MAP), (c) foliar N concentration ($[\text{N}_\text{L}]$). Residuals were taken from a model that included MAT, MAP and $[\text{N}_\text{L}]$, except for the target variable. Included are also the data for both non- N_2 -fixing plants (black dots) for reference.

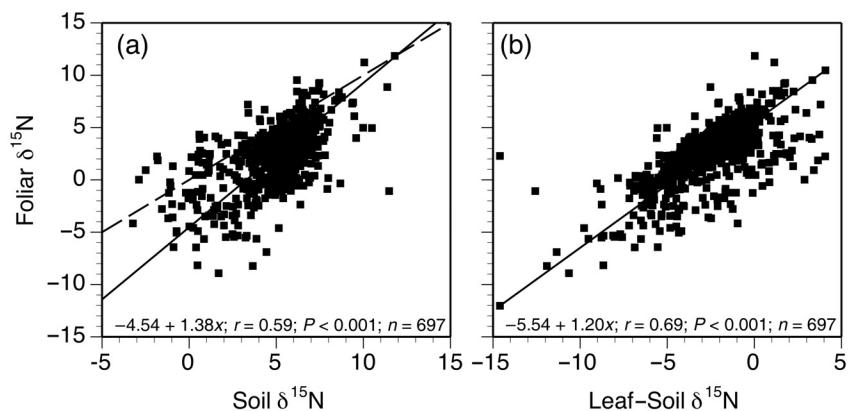


Fig. 6 Comparisons between site-averaged leaf and soil $\delta^{15}\text{N}$. (a) Relationship between leaf and soil $\delta^{15}\text{N}$, and (b) the relationship between leaf $\delta^{15}\text{N}$ and the difference between leaf and soil $\delta^{15}\text{N}$. For (a) solid line is a model II regression with a 95% CI for slope = 1.25–1.53. Dashed line is 1 : 1 line. For (b) 95% CI for slope is 1.14–1.32.

Table 3 Details on the studies of N availability and foliar $\delta^{15}\text{N}$ presented in Fig. 3

Study	Location	Soil depth	Temperature	Incubation duration
Hobbie <i>et al.</i> (2000) ^a	Alaska	0–5 cm	Ambient	2 × 30 d
Kitayama & Iwamoto (2001) ^b	Borneo	0–15 cm	Ambient	10 d
Makarov <i>et al.</i> (2003) ^c	Caucasus Mountains	0–10 cm	Ambient	1 yr
Kahmen <i>et al.</i> (2008) ^d	Germany	0–10 cm	Ambient	2 × 30 d
Tan <i>et al.</i> (2006) ^e	British Columbia	0–10 cm	Ambient	5 × 30–60 d
Schuur & Matson (2001) ^f	Hawaii	0–30 cm	Ambient	9 × 7 d
Falkengren-Grerup <i>et al.</i> (2004) ^g	Sweden	0–5 cm	20 $^{\circ}\text{C}$	77 d
Vitousek <i>et al.</i> (2003) ^h	Hawaii	0–10 cm	22 $^{\circ}\text{C}$	30 d
Garten & Van Miegroet (1994) ⁱ	Smoky Mountains, TN, USA	0–10 cm	30 $^{\circ}\text{C}$	42 d
Pardo <i>et al.</i> (2006) ^j	North American and European temperate forests	Variable	Variable	Variable
Craine <i>et al.</i> (2009) ^k	South Africa	0–20 cm	25 $^{\circ}\text{C}$	30 d
Craine & Lee (2003) ^l	New Zealand	10 cm	Ambient	1 yr
McLauchlan <i>et al.</i> (2006) ^m	Minnesota, USA	10 cm	Ambient	160 d
Schmidt & Stewart (2003) ⁿ	Northern Australia	5 cm	Ambient	4–7 d
Hogbom <i>et al.</i> (2002) ^o	Sweden	50 cm	Ambient	c. 120 d

^{a–o}Superscripts refer to panels in Fig. 7.

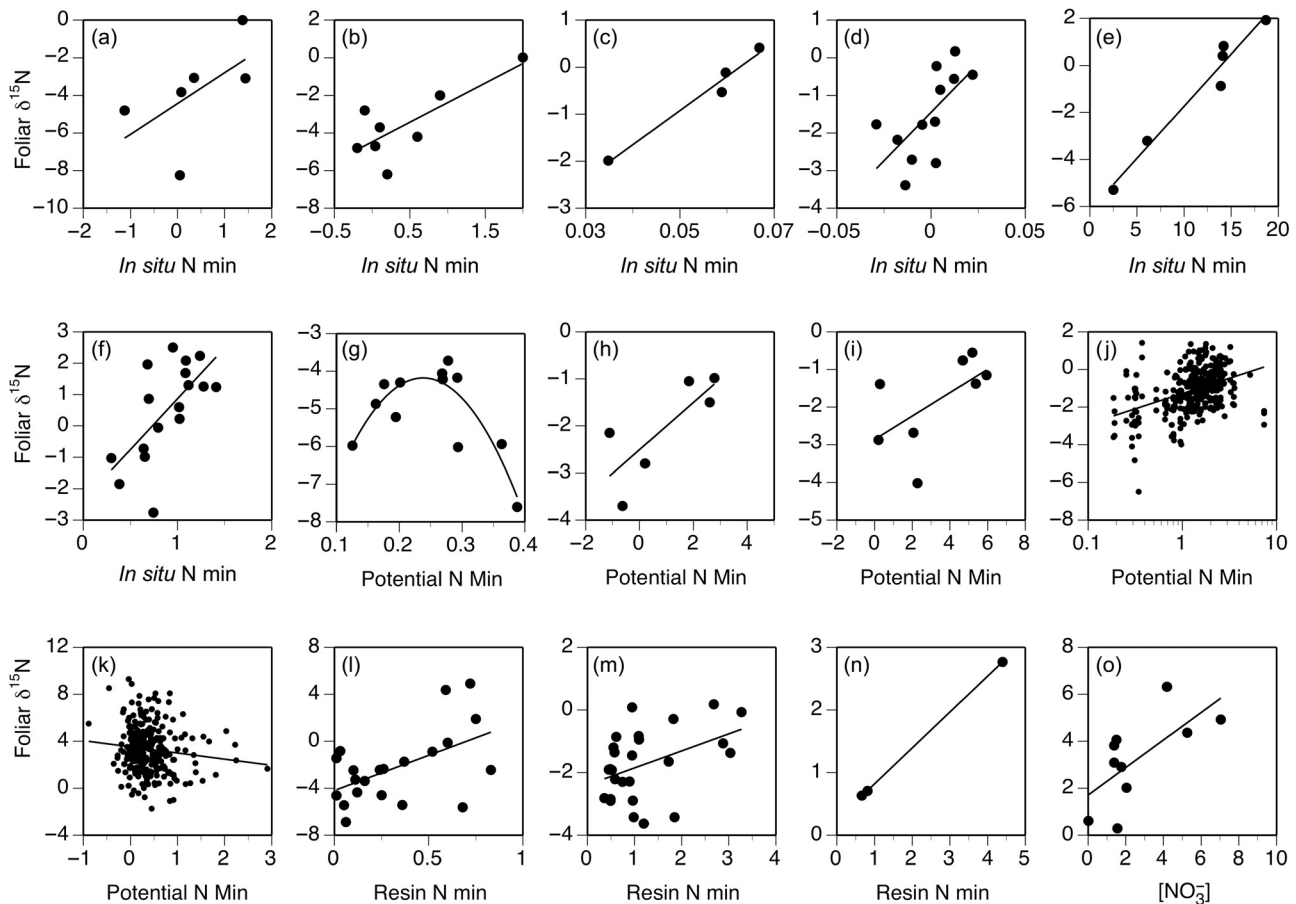


Fig. 7 Relationships between measures of N supply and foliar $\delta^{15}\text{N}$ among 14 studies. Panel identifier (a–o) refers to identity of study, as listed in Table 3. *In situ* N mineralization and potential N mineralization are both expressed as $\mu\text{g N g}^{-1} \text{ soil d}^{-1}$, *in situ* resin mineralization as $\mu\text{g N g}^{-1} \text{ resin d}^{-1}$ and nitrate as mg N l^{-1} .

increased with increasing potential N mineralization for the nine sites with the lowest mineralization rates, but declined for the three sites with the highest mineralization rates. In one grassland study where N availability was associated more with differences in grazing than supply (Craine *et al.*, 2009), potential N mineralization (PotNmin) explained little variation in foliar $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_L = 3.53 - 0.52 * \text{PotNmin}$, $P = 0.03$, $r^2 = 0.01$).

Discussion

The analyses presented here provide new insights into global patterns of foliar $\delta^{15}\text{N}$ and the N cycle. First, this study is the first review to incorporate mycorrhizal fungi into global-scale analyses of foliar $\delta^{15}\text{N}$, thereby explicitly addressing a potential covariate influencing relationships between foliar $\delta^{15}\text{N}$ and climate. Second, this review showed different relationships between temperature and foliar $\delta^{15}\text{N}$ for cold and warm ecosystems. Third, the positive relationships reported here between $[\text{N}_L]$ and foliar $\delta^{15}\text{N}$ at the global scale across a number of ecosystem types extends the generality of previous relationships quantified on smaller scales. Fourth, although

relationships between foliar $\delta^{15}\text{N}$ and climate are not biased by excluding potentially N_2 -fixing plants, MAP and foliar $\delta^{15}\text{N}$ were uncorrelated for potentially N_2 -fixing plants. Lastly, foliar $\delta^{15}\text{N}$ consistently increases along natural N supply gradients from landscape to regional scales.

In past reports of global patterns of foliar $\delta^{15}\text{N}$, the ability of mycorrhizal fungi to deliver ^{15}N -depleted N to plants has been acknowledged (Amundson *et al.*, 2003) but had not been explicitly incorporated in analyses that determined relationships between climate and foliar $\delta^{15}\text{N}$. The effect of mycorrhizal type on foliar $\delta^{15}\text{N}$ observed in this study extends the patterns of foliar $\delta^{15}\text{N}$ dependence upon mycorrhizal associations previously observed in tundra, boreal, alpine and heath ecosystems (Michelsen *et al.*, 1998; Schmidt & Stewart, 2003) to the global scale. The differences among plants with different mycorrhizal fungi could be influenced by differences in sources of N among types (Smith & Read, 2008). Yet, these differences in foliar $\delta^{15}\text{N}$ among plants with different mycorrhizal fungi associations are broadly congruent with the degree of plant reliance on the fungi for N – ericoid plants are more reliant on mycorrhizal fungi for N than ectomycorrhizal plants than

arbuscular plants (Hobbie *et al.*, 2005; Jin *et al.*, 2005). The lower average foliar $\delta^{15}\text{N}$ in arbuscular mycorrhizal plants compared with nonmycorrhizal plants suggests that N transfer from arbuscular mycorrhizal fungi to host plants favors ^{15}N -depleted N in a similar fashion to the well-established transfer pathways in ectomycorrhizal symbioses. In addition, the absence of proteolytic capabilities in arbuscular mycorrhizal fungi (Chalot & Brun, 1998) suggests that the potential N sources for nonmycorrhizal and arbuscular mycorrhizal plants should be similar. If true, then ^{15}N depletion in arbuscular mycorrhizal plants relative to co-occurring nonmycorrhizal plants may reflect preferential transfer by arbuscular mycorrhizal fungi of ^{15}N -depleted compounds. Recent experiments support arbuscular mycorrhizal fungi transferring N to plants (Leigh *et al.*, 2009), but culture studies investigating the effects of arbuscular mycorrhizal colonization on plant $\delta^{15}\text{N}$ have been inconclusive (Hobbie & Hobbie, 2008).

The relationships between MAT and foliar $\delta^{15}\text{N}$ for ecosystems with $\text{MAT} \geq -0.5^\circ\text{C}$ were similar to previous results (Martinelli *et al.*, 1999; Amundson *et al.*, 2003), but with a greater slope than was found for temperate forests alone (Pardo *et al.*, 2006). In contrast to sites with $\text{MAT} \geq -0.5^\circ\text{C}$, foliar $\delta^{15}\text{N}$ did not change with increasing MAT across sites with $\text{MAT} < -0.5^\circ\text{C}$. Two facts are important in interpreting the patterns of foliar $\delta^{15}\text{N}$ for cold sites. First, soil $\delta^{15}\text{N}$ values in cold regions are typically close to those of atmospheric N_2 (0‰) (Amundson *et al.*, 2003). Second, dissolved organic N (DON) likely dominates the cycling of N in these soils (Neff *et al.*, 2003) and losses of N in ecosystems with MAT of $< -0.5^\circ\text{C}$ are likely to be dominated by DON. With little fractionation during solubilization, DON loss should not lead to enrichment of the available N pool. As such, the lack of increase in $\delta^{15}\text{N}$ with MAT below -0.5°C implies that the signature of N being lost from the ecosystem or the dependence of plants on mycorrhizal fungi for N does not change with temperature over this range.

As observed before (Handley *et al.*, 1999a; Schuur & Matson, 2001; Amundson *et al.*, 2003), foliar $\delta^{15}\text{N}$ declined with increasing MAP for non N_2 -fixing plants. In addition to finding a better fit between log-transformed MAP and foliar $\delta^{15}\text{N}$ than untransformed MAP, and extending relationships to higher precipitation sites than before, there is little qualitative difference between our results here regarding relationships with MAP and earlier studies. That said, more research is necessary to understand whether the high foliar $\delta^{15}\text{N}$ can be directly linked to high gaseous N loss, lower dependence on mycorrhizal fungi and/or changes in cycling of N within the plant. For example, for non N_2 -fixing plants, high foliar $\delta^{15}\text{N}$ at low precipitation suggests that these sites might experience relatively high rates of gaseous N loss through ammonium volatilization, gaseous N loss during nitrification or denitrification. Although no study has yet compared gaseous N loss rates across a precipitation gradient, denitrification rates can be high in xeric ecosystems (Peterjohn & Schlesinger, 1991) and are higher for tropical

forests than temperate forests (Stehfest & Bouwman, 2006). Although requiring anaerobic microsites, denitrification is often limited by nitrate availability (Groffman *et al.*, 1993), which could explain why dry sites that might have soils that are less anaerobic can have greater denitrification. The lack of elevated foliar $\delta^{15}\text{N}$ at high precipitation might reflect strong N limitation and low N availability (Schuur & Matson, 2001), but could also reflect complete denitrification of soil nitrate pools, which does not leave behind ^{15}N -enriched substrate (Houlton *et al.*, 2006), or consistently high reliance by plants on mycorrhizal acquisition of N. Future research on the dual controls of nitrification and denitrification across precipitation gradients on the $\delta^{15}\text{N}$ of available N could provide new insights into patterns of foliar $\delta^{15}\text{N}$ with increasing MAP.

At the local to regional scale, foliar $\delta^{15}\text{N}$ consistently increased with increasing N supply. The one study that did not show a monotonically positive relationship between foliar $\delta^{15}\text{N}$ and N availability (Falkengren-Grerup *et al.*, 2004) only measured understory species, and the patterns observed may not have represented stand-level N dynamics. As such, understory plants in sites with high rates of mineralization might have relied more on nitrate than sites with lower mineralization, while other singular explanations such as signatures of N deposition could also explain this anomaly. With multiple ways to quantify aspects of the supply of N to plants, how best to represent N supply, much less N availability to plants, is still an open question. For example, in some cases Pardo *et al.* (2006) found better relationships for temperate forests between foliar $\delta^{15}\text{N}$ and the fraction of mineralized N that was nitrified than laboratory or field measures of mineralization rate. Also, soil N supply likely has to be coupled with plant N demand to best represent N availability. In Craine *et al.* (2009), variation in N availability among 330 South African grassland sites was driven more by variation in plant demand than supply. Sites with high foliar $\delta^{15}\text{N}$ did not necessarily have higher N supplies, but instead were associated with higher grazing intensity. Sodic sites and private protected areas known to have high herbivore densities had grasses with high foliar $\delta^{15}\text{N}$. These sites were associated with lower grass biomass and species that tend to increase with grazing pressure.

Separating proximal and distal drivers of variation in $\delta^{15}\text{N}$ is a long-standing question that ultimately cannot be answered without additional data and/or modeling. That said, the patterns that we are attempting to explain span approx. 5‰ (MAT), 4‰ (MAP), 20‰ ($[\text{N}_\text{L}]$) and 6‰ (types of mycorrhizal fungi). Although still a valid hypothesis, there is currently no evidence that within-plant fractionations change markedly along these gradients. For example, for grasses collected from a wide range of grasslands (Craine *et al.*, 2005), the difference in $\delta^{15}\text{N}$ between leaves and roots changed by only 1‰ across an order of magnitude of foliar N concentrations (J. Craine, unpublished). With regard to other aspects of within-plant fractionation, often no discrimination is observed during resorption of N from leaves (Kolb & Evans, 2002), while differences

between leaves and stems are often *c.* 1‰ (Gebauer & Schulze, 1991; Hobbie *et al.*, 2008). Differences in signatures of depositional N might add variation to our dataset, but do not appear to vary consistently with climate at regional scales (Bragazza *et al.*, 2005; Elliott *et al.*, 2007). Differences in plant preference for forms of N can explain variations within a site, but not stand-level differences in signatures. Other distal factors might be controlled by climate, but it is unknown whether climate directly affects the N cycle or does so indirectly by altering patterns of disturbance. For example, there are unresolved debates about the relative importance in xeric grasslands of low precipitation *per se* or greater grazing in enriching plants in ^{15}N (Schulze *et al.*, 1998; Austin & Sala, 1999; Cook, 2001).

If regional-scale variation in N supply is generally tied to foliar $\delta^{15}\text{N}$, one cannot necessarily assume that global-scale patterns in foliar $\delta^{15}\text{N}$ or differences between any small number of sites within a region can be interpreted as representing N availability. Yet, if the general regional relationships between N availability and foliar $\delta^{15}\text{N}$ extend to the global scale, then the global foliar $\delta^{15}\text{N}$ patterns suggest that: warm and dry sites have high N availability relative to cold and wet sites; plants with high N concentrations occupy sites with high N supply; and N availability might be relatively high in sites with low P availability. Whether this greater N availability also translates to less N limitation to plant production is uncertain. Foliar $\delta^{15}\text{N}$ increased with increasing foliar N : P ratio, regardless of whether a result of increasing N concentrations or decreasing P concentrations. Although foliar N : P ratio has been considered to index the relative limitation of N and P to plants in terrestrial ecosystems (Güsewell, 2004), at least for grasslands this is not necessarily true (Craine *et al.*, 2008).

With important questions still remaining about the patterns of foliar $\delta^{15}\text{N}$, future research should turn to understanding the potential underlying determinants of plant $\delta^{15}\text{N}$ that can be used to interpret the global foliar $\delta^{15}\text{N}$ patterns. Because multiple levels of causation may exist for a given pattern, future research should begin to identify what underlying processes are correlated with signatures. More comparative data at the global scale are needed to evaluate the potential drivers of foliar $\delta^{15}\text{N}$ with climate or increasing $[\text{N}_\text{L}]$, such as data on the signatures of available N (Houlton *et al.*, 2006; Kahmen *et al.*, 2008). Measurements of the biomass or colonization rates of mycorrhizal fungi and their isotopic signatures would assist in testing the importance of changes in the reliance of plants on mycorrhizal fungi, while measurements of root $\delta^{15}\text{N}$ would evaluate whether within-plant fractionation is responsible for any of the changes in foliar $\delta^{15}\text{N}$ seen across gradients. The patterns laid out here will help constrain the potential mechanisms that underlie changes in N cycling along ecological gradients, such as the directional change in the reliance of plants on mycorrhizal fungi or the signature of N lost from ecosystems. Knowing the patterns of foliar $\delta^{15}\text{N}$ at the global scale might help constrain models of gaseous N loss, while there is

now real potential to routinely use plant $\delta^{15}\text{N}$ to reconstruct past N availability as well as to monitor changing soil N availability.

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