

Plant $\delta^{15}\text{N}$ Correlates with the Transpiration Efficiency of Nitrogen Acquisition in Tropical Trees^{1[OA]}

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Based upon considerations of a theoretical model of $^{15}\text{N}/^{14}\text{N}$ fractionation during steady-state nitrate uptake from soil, we hypothesized that, for plants grown in a common soil environment, whole-plant $\delta^{15}\text{N}$ (δ_{p}) should vary as a function of the transpiration efficiency of nitrogen acquisition (F_{N}/v) and the difference between δ_{p} and root $\delta^{15}\text{N}$ ($\delta_{\text{p}} - \delta_{\text{R}}$). We tested these hypotheses with measurements of several tropical tree and liana species. Consistent with theoretical expectations, both F_{N}/v and $\delta_{\text{p}} - \delta_{\text{R}}$ were significant sources of variation in δ_{p} , and the relationship between δ_{p} and F_{N}/v differed between non- N_2 -fixing and N_2 -fixing species. We interpret the correlation between δ_{p} and F_{N}/v as resulting from variation in mineral nitrogen efflux-to-influx ratios across plasma membranes of root cells. These results provide a simple explanation of variation in $\delta^{15}\text{N}$ of terrestrial plants and have implications for understanding nitrogen cycling in ecosystems.

Variation in the natural abundance of stable nitrogen isotopes ($\delta^{15}\text{N}$) in terrestrial plants can provide valuable information about plant nitrogen acquisition and ecosystem nitrogen cycling (Handley and Raven, 1992; Höglberg, 1997; Evans, 2001; Robinson, 2001). However, interpretation of foliar or whole-plant $\delta^{15}\text{N}$ data is complicated by the existence of multiple sources of nitrogen in the soil and the possibility for variable discrimination against ^{15}N during the assimilation of each source (Handley and Raven, 1992; Evans, 2001). Analytical equations describing discrimination against ^{15}N during uptake of nitrogenous solutes from the soil could greatly enhance the application of plant $\delta^{15}\text{N}$ data in physiological and ecological investigations.

Comstock (2001) presented a theoretical model of $^{15}\text{N}/^{14}\text{N}$ fractionation during steady-state uptake of nitrate, a primary source of nitrogen for terrestrial plants. Here, we extend this model to formulate the hypothesis that plant $\delta^{15}\text{N}$ should vary as a function of the transpiration efficiency of nitrogen acquisition for plants growing under similar soil conditions, and we test this with measurements on several tropical tree and liana species. Figure 1 shows a simplified model of nitrate uptake in plants based on that presented pre-

viously (Comstock, 2001). In the steady state, the following relationship can be defined:

$$F_{\text{In}} = F_{\text{En}} + F_{\text{Rn}} + F_{\text{Xn}} \quad (1)$$

where F_{In} is the influx of nitrate from the soil into root cells, F_{En} is the efflux of nitrate from root cells back to the soil, F_{Rn} is the assimilation of nitrate into organic molecules in root cells, the first step of which is reduction of nitrate to nitrite by nitrate reductase, and F_{Xn} is the flux of nitrate from root cells into the xylem sap, which results in the transport of nitrate from the root to the shoot in the transpiration stream. Because nitrate is essentially absent from phloem sap exported from leaves in higher plants (Peoples and Gifford, 1997), the flux of nitrate into xylem sap in the roots is equal to the assimilation rate of nitrate in the shoot in the steady state. Assuming nitrate as the sole nitrogen source and that there is no nitrogen isotope fractionation associated with F_{In} , F_{En} , or F_{Xn} , discrimination against ^{15}N during nitrogen uptake (Δ_{P}) can be expressed as (Comstock, 2001):

$$\Delta_{\text{P}} = \frac{F_{\text{Rn}}}{F_{\text{Nn}}} \left(1 - \frac{F_{\text{Nn}}}{F_{\text{In}}} \right) b \quad (2)$$

where F_{Nn} is the net uptake of nitrate from the soil by the plant, defined as $F_{\text{In}} - F_{\text{En}}$, or equivalently as $F_{\text{Rn}} + F_{\text{Xn}}$, and b is the discrimination constant for reduction of nitrate to nitrite by nitrate reductase, estimated to be about 15‰ (Ledgard et al., 1985; Handley and Raven, 1992; Tcherkez and Farquhar, 2006). The assumption of no isotopic discrimination associated with F_{In} , F_{En} , or F_{Xn} means that transport of nitrate across the plasma-membranes of root cells and transport of nitrate and organic nitrogen into the xylem sap are assumed to be nondiscriminating processes. A full derivation of Equation 2 and justification for the assumptions involved is given by Comstock (2001). The Δ_{P} is defined as:

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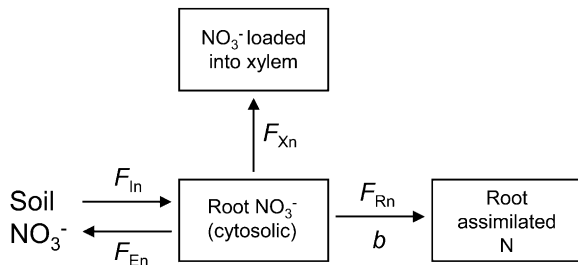


Figure 1. A simplified model of steady-state nitrate uptake, based on the conceptual model of Comstock (2001). F_{in} is the influx of nitrate from the soil solution into root cells; F_{En} is the efflux of nitrate from root cells back to the soil solution; F_{Rn} is the assimilation flux of nitrate into organic molecules within root cells; and F_{Xn} is the flux of nitrate from root cells into the xylem. Nitrate loaded into the xylem is carried to the leaf in the transpiration stream, where it can also be assimilated into organic molecules. Because nitrate is not exported from leaves in the phloem, the flux of nitrate into the xylem in the root is equal to the assimilation flux in the shoot in the steady state. The discrimination against ^{15}N during nitrate reduction in both the root and the shoot is defined as b , which is a constant. F_{in} , F_{En} , and F_{Xn} are assumed to proceed without discrimination against ^{15}N .

$$\Delta_p = \frac{R_{Sn}}{R_p} - 1 = \frac{\delta_{Sn} - \delta_p}{1 + \delta_{Sn}} \approx \delta_{Sn} - \delta_p \quad (3)$$

where R_{Sn} is $^{15}\text{N}/^{14}\text{N}$ of soil nitrate, R_p is $^{15}\text{N}/^{14}\text{N}$ of plant nitrogen, δ_{Sn} is $\delta^{15}\text{N}$ of soil nitrate, and δ_p is $\delta^{15}\text{N}$ of plant nitrogen. Combining Equations 2 and 3 gives:

$$\delta_p = \delta_{Sn} - \frac{F_{Rn}}{F_{Nn}} \left(1 - \frac{F_{Nn}}{F_{in}} \right) b \quad (4)$$

Equation 2 results from the combination of individual discrimination expressions for root-assimilated nitrate and leaf-assimilated nitrate (Comstock, 2001). The expression for discrimination against ^{15}N during assimilation of nitrate in the root (Δ_{Rn}) is given by $\Delta_{Rn} = (1 - F_{Rn}/F_{in})b$. That for discrimination against ^{15}N during assimilation in the leaf (Δ_{Ln}) is given by $\Delta_{Ln} = -(F_{Rn}/F_{in})b$. Subtracting Δ_{Ln} from Δ_{Rn} yields the expression $\Delta_{Rn} = \Delta_{Ln} + b$. Finally, applying the approximations that $\Delta_{Rn} \approx \delta_{Sn} - \delta_{Rn}$ and $\Delta_{Ln} \approx \delta_{Sn} - \delta_{Ln}$ leads to the following simple expression: $\delta_{Rn} = \delta_{Ln} - b$, where δ_{Rn} is $\delta^{15}\text{N}$ of root-assimilated, nitrate-derived nitrogen and δ_{Ln} is $\delta^{15}\text{N}$ of leaf-assimilated, nitrate-derived nitrogen.

The relationship between δ_{Rn} and δ_{Ln} can be intuitively understood by considering two contrasting examples. As a first example, consider the situation when F_{Rn} is small compared with F_{in} . In this case, the cytosolic nitrate pool in the root cells will be little enriched compared with nitrate in the soil solution, while at the same time the discrimination by nitrate reductase in the roots will be nearly fully expressed, because the supply of nitrate is large compared with consumption by the discriminating enzyme. In this case, root-assimilated nitrogen will show a large discrimination relative to soil nitrate, whereas nitrate loaded into the xylem, destined for assimilation in the shoot, will show a small enrichment relative to soil

nitrate. In the steady state, assimilation of nitrate in the shoot proceeds without discrimination, because there is no branch point in the reaction sequence. That is to say, there is only one possible fate for nitrate that has been loaded into the xylem under steady-state conditions.

As a second example, consider the situation when F_{Rn} approaches the value of F_{in} . Here, the cytosolic nitrate pool will show a large enrichment relative to soil nitrate that approaches b , while at the same time discrimination by nitrate reductase in the root will be very small, because nearly all of the available substrate is being consumed. In this case, root-assimilated nitrogen will have a $\delta^{15}\text{N}$ only slightly depleted compared with soil nitrate, whereas nitrate loaded into the xylem will show a large enrichment that approaches b relative to soil nitrate. In both examples, the difference between δ_{Rn} and δ_{Ln} will be approximated by the value of b .

We further suggest that for plants grown on nitrate, the nitrogen pool in the plant (N_p) can be partitioned into a root-assimilated pool (N_{Rn}) and a shoot-assimilated pool (N_{Ln}).

$$N_p = N_{Ln} + N_{Rn} \quad (5)$$

Equation 5 assumes that the pool of unassimilated nitrate in the plant, stored in vacuoles, for example, is negligible. Storage of nitrate typically accounts for less than 1% of total plant nitrogen in uncultivated plants (Smirnov and Stewart, 1985; Pate et al., 1993; Schmidt and Stewart, 1997; Aidar et al., 2003); thus, this assumption should generally be valid. However, exceptions can occur, for example, in crop plants grown in hydroponics (Evans et al., 1996; Seginer, 2003). Equation 5 can be written for ^{15}N as:

$$R_p N_p = R_{Ln} N_{Ln} + R_{Rn} N_{Rn} \quad (6)$$

where R_p is $^{15}\text{N}/^{14}\text{N}$ of the plant nitrogen pool, R_{Ln} is $^{15}\text{N}/^{14}\text{N}$ of the shoot-assimilated pool, and R_{Rn} is $^{15}\text{N}/^{14}\text{N}$ of the root-assimilated pool. Using the relationship $\delta_x = (R_x/R_{St}) - 1$, where δ_x is $\delta^{15}\text{N}$ of component X , R_x is $^{15}\text{N}/^{14}\text{N}$ of component X , and R_{St} is $^{15}\text{N}/^{14}\text{N}$ of a standard (N_2 in air for nitrogen), Equation 6 can be written as:

$$(1 + \delta_p)N_p = (1 + \delta_{Ln})N_{Ln} + (1 + \delta_{Rn})N_{Rn} \quad (7)$$

Subtracting Equation 5 from Equation 7 gives:

$$\delta_p N_p = \delta_{Ln} N_{Ln} + \delta_{Rn} N_{Rn} \quad (8)$$

Substituting from Equation 5, and from the relationship $\delta_{Rn} = \delta_{Ln} - b$ derived above, Equation 8 can be expressed as:

$$\delta_p N_p = (\delta_{Rn} + b)(N_p - N_{Rn}) + \delta_{Rn} N_{Rn} \quad (9)$$

Expanding Equation 9, canceling terms, and rearranging leads to:

$$\frac{N_{Rn}}{N_p} = 1 - \left(\frac{\delta_p - \delta_{Rn}}{b} \right) \quad (10)$$

Having assumed that the pool of unreacted nitrate in the plant is negligible compared with the organic pool and that nitrate uptake proceeds at steady state, the ratio of pools $N_{\text{Rn}}/N_{\text{P}}$ can be set equal to the ratio of fluxes $F_{\text{Rn}}/F_{\text{Nn}}$. If we make the further assumption that the nitrogen pool in roots contains only root-assimilated nitrogen, that is to say, that any shoot-assimilated nitrogen transported to the roots in phloem sap does not remain in the roots but is transported back to the shoot, then δ_{Rn} can be set equal to δ_{R} , where δ_{R} is the $\delta^{15}\text{N}$ of root organic material. Applying these assumptions, Equation 10 can be written as:

$$\frac{F_{\text{Rn}}}{F_{\text{Nn}}} = 1 - \left(\frac{\delta_{\text{P}} - \delta_{\text{R}}}{b} \right) \quad (11)$$

Finally, combining Equations 4 and 11 yields the following expression for δ_{P} :

$$\delta_{\text{P}} = \delta_{\text{Sn}} - \left(1 - \frac{F_{\text{Nn}}}{F_{\text{In}}} \right) [b - (\delta_{\text{P}} - \delta_{\text{R}})] \quad (12)$$

Equation 12 suggests that the $\delta^{15}\text{N}$ of plant nitrogen absorbed from the soil as nitrate should vary as a function of three terms, assuming that b is a constant: δ_{Sn} , $F_{\text{Nn}}/F_{\text{In}}$, and $\delta_{\text{P}} - \delta_{\text{R}}$. For situations where δ_{Sn} can reasonably be assumed to be similar among plants (e.g. for plants growing at a common site), δ_{P} for nitrate-derived plant nitrogen should thus vary as a function of $F_{\text{Nn}}/F_{\text{In}}$ and $\delta_{\text{P}} - \delta_{\text{R}}$. The $F_{\text{Nn}}/F_{\text{In}}$ is the ratio of the net uptake of nitrate by the plant to the influx of nitrate from the soil into the roots. The F_{In} represents a one-way, or gross, flux. The importance of recognizing one-way fluxes in isotopic modeling has been recently highlighted (Cernusak et al., 2004; Farquhar and Cernusak, 2005). It is necessary to decompose net fluxes into component one-way fluxes, because the one-way fluxes can carry different isotopic signatures.

Dalton et al. (1975) and Fiscus (1975) suggested the following expression for a net solute flux (F_{Ns}) across a membrane in the steady state ($\text{mol m}^{-2} \text{s}^{-1}$):

$$F_{\text{Ns}} = \omega RT(C_{\text{Os}} - C_{\text{Is}}) + (1 - \sigma)C_{\text{Os}}\nu + F_{\text{s}}^* \quad (13)$$

where ω is the permeability coefficient of the membrane ($\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$), R is the gas constant ($\text{J mol}^{-1} \text{K}^{-1}$), T is temperature (K), C_{Os} is the solute concentration outside the membrane (mol m^{-3}), C_{Is} is the solute concentration inside the membrane (mol m^{-3}), σ is the membrane reflection coefficient (dimensionless), ν is the flux of water across the membrane ($\text{m}^3 \text{m}^{-2} \text{s}^{-1}$), and F_{s}^* is the active uptake of solute across the membrane ($\text{mol m}^{-2} \text{s}^{-1}$). For a charged solute, the active uptake term would include both the metabolically supported transport across the membrane and the flow of ions down the resultant electrochemical gradient (Fiscus and Kramer, 1975). Following this approach, we suggest the following conceptual expression for the one-way flux of nitrate from the soil into root cells (F_{In}):

$$F_{\text{In}} = \omega RTC_{\text{Rn}} + (1 - \sigma)C_{\text{Rn}}\nu + F_{\text{n}}^* \quad (14)$$

where C_{Rn} is the nitrate concentration at the root surface (mol m^{-3}), F_{n}^* is the active uptake of nitrate ($\text{mol m}^{-2} \text{s}^{-1}$) as defined above for an ionic solute, and the other terms are as described for Equation 13. Equation 14 expresses the influx of nitrate into root cells as the sum of three component fluxes: a diffusive flux (ωRTC_{Rn}), a convective flux $[(1 - \sigma)C_{\text{Rn}}\nu]$, and an active uptake flux (F_{n}^*). Note that this formulation assumes that the three component fluxes operate independently, such that the efflux is not affected by the convective component (Dalton et al., 1975; Fiscus, 1977, 1986). Assuming that efflux occurs as a result of passive leakage of nitrate out of root cells (Peoples and Gifford, 1997; Crawford and Glass, 1998), F_{En} could then be defined as:

$$F_{\text{En}} = \omega RTC_{\text{Cn}} \quad (15)$$

where C_{Cn} is the cytosolic nitrate concentration. The efflux would thus result from diffusion of nitrate across the plasmalemma. Equations 14 and 15 represent the component one-way fluxes that combine to give the net solute flux described by Equation 13. Thus, one obtains Equation 13 by subtracting Equation 15 from Equation 14.

We suggest that the influx of nitrate into the roots partly depends upon the flux of water into the roots (ν), such that $F_{\text{Nn}}/F_{\text{In}}$ should correlate with F_{N}/ν for plants growing in soil of similar nitrate concentration. The F_{N} is net nitrogen uptake from all nitrogen sources but is assumed to result from only nitrate in this example. Equation 14 indicates that at a given value of C_{Rn} , F_{In} would be expected to increase with increasing ν , due to an increase in the convective flux, also referred to as the solvent drag flux (Fiscus, 1975), so long as σ is less than unity. The ν is expressed as $\text{m}^3 \text{water m}^{-2} \text{root surface s}^{-1}$ in Equations 13 and 14. It can also be expressed as $\text{mol water m}^{-2} \text{root surface s}^{-1}$. In this paper, we employ the latter, such that F_{N}/ν has units of $\mu\text{mol nitrogen mol}^{-1} \text{water}$.

In addition to the influence of ν on F_{In} due to the convective component of the influx, the soil solution nitrate concentration at the root surface, C_{Rn} , also partly depends upon ν . Nitrate can be transported to the root surface both by diffusion and by mass flow. For the simplified case of an isolated root in one dimension in the steady state, C_{Rn} can simply be described as $C_{\text{Rn}} = C_{\text{Sn}}\nu/\alpha$, where C_{Sn} is the nitrate concentration of the soil solution outside the disturbance zone associated with the root and α is the root absorbing power, defined as $F_{\text{Nn}}/C_{\text{Rn}}$ (Nye and Tinker, 1977). This relationship can be understood by considering the situation where a depletion zone has spread from the root surface outward for some distance into the soil. In the steady state, the flux of nitrate entering the depletion zone from the soil must be the same as the net flux of nitrate leaving the depletion zone at the root surface. Under such conditions, C_{Rn} will be a function of the interplay between nitrate supply, represented by $C_{\text{Sn}}\nu$, and root demand for nitrate, represented by α . If the α

does not change with changing C_{Rn} , C_{Rn} will vary as a function of v , assuming constant C_{Sn} . As shown in Equation 14, an increase in C_{Rn} would be expected to cause an increase in F_{In} .

To summarize, we suggest that there are two components to the dependence of F_{In} on v . First, at a given C_{Rn} , the convective component of F_{In} would be expected to increase with increasing v , as shown in Equation 14. Second, C_{Rn} partly depends upon v , such that if C_{Rn} increases as a function of increasing v , this should also cause an increase in F_{In} .

The above theoretical considerations lead us to suggest that for plants deriving their nitrogen from soil nitrate and growing in soil with similar C_{Sn} and δ_{Sn} , δ_p should vary as a function of $\delta_p - \delta_R$ and F_N/v , which we term the transpiration efficiency of nitrogen acquisition (Cernusak et al., 2007a). Furthermore, these correlations may hold for plants with multiple nitrogen sources if a significant fraction of plant nitrogen is derived from soil nitrate. In addition, if soil ammonium is the plant's nitrogen source, the same argument can be constructed suggesting that F_{Na}/F_{Ia} should correlate with F_N/v , where F_{Na} is net uptake of ammonium from the soil and F_{Ia} is the influx of ammonium from the soil into root cells.

A full description of the effects of multiple nitrogen sources on plant $\delta^{15}N$ can be developed by expanding the above treatment. For plants with multiple nitrogen sources, the following mass balance equation can be written for net uptake of nitrogen:

$$F_N = F_{Nn} + F_{Na} + F_{Nd} + F_{No} \quad (16)$$

where F_N is net uptake of nitrogen from all nitrogen sources, F_{Nn} is net uptake of soil nitrate, F_{Na} is net uptake of soil ammonium, F_{Nd} is dinitrogen (N_2) fixation from the atmosphere, and F_{No} is net uptake of organic nitrogen from the soil. Expressing Equation 16 for ^{15}N and applying similar approximations to those applied above leads to the following expression for the $\delta^{15}N$ of nitrogen uptake from all four nitrogen sources:

$$\delta_p = \frac{F_{Nn} \left[\delta_{Sn} - \frac{F_{Rn}}{F_{Nn}} \left(1 - \frac{F_{Nn}}{F_{In}} \right) b \right] + F_{Na} \left[\delta_{Sa} - \left(1 - \frac{F_{Na}}{F_{Ia}} \right) c \right] - F_{Nd} \Delta_{Nd} + F_{No} (\delta_{So} - \Delta_{No})}{F_{Nn} + F_{Na} + F_{Nd} + F_{No}} \quad (17)$$

where δ_{Sa} is the $\delta^{15}N$ of soil ammonium, c is the discrimination constant for assimilation of ammonium by Gln synthetase, estimated to be about 17‰ (Yoneyama et al., 1993), Δ_{Nd} is discrimination against ^{15}N during N_2 fixation, which ranges from approximately 0‰ to 2‰ (Yoneyama et al., 1986), δ_{So} is $\delta^{15}N$ of the soil organic nitrogen pool available to the plant, and Δ_{No} is discrimination during net uptake of soil organic nitrogen. Little is known about Δ_{No} , but it appears to vary depending on the type of organic molecule taken up (Schmidt et al., 2006). Equation 17 assumes that nitrate and ammonium assimilation pro-

ceed independently and that atmospheric N_2 has a $\delta^{15}N$ of 0‰ (Mariotti, 1983). Free ammonium is generally not detected in xylem sap, except in trace amounts (Peoples and Gifford, 1997), so all ammonium assimilation is assumed to take place in roots.

For plants incapable of dinitrogen fixation and taking up little or no nitrogen as organic molecules from the soil, Equation 12 may provide a reasonable approximation to Equation 17, in that positive relationships between δ_p and F_N/v and between δ_p and $\delta_p - \delta_R$ could be maintained, despite variable uptake of soil nitrogen as nitrate versus ammonium. We tested the generality of these predictions using a diverse suite of tropical tree and liana species. To provide further insight into the effects of variable uptake of nitrate versus ammonium on the predicted relationship between δ_p and F_N/v , we conducted a sensitivity analysis using Equation 17 and assuming a range of values for F_{Nn} and F_{Na} .

RESULTS

Figure 2 shows correlations between δ_p and F_N/v and between δ_p and $\delta_p - \delta_R$ for 15 species of tropical trees and lianas, the former including both conifers and angiosperms. These plants were grown individually in 38-L pots under well-watered conditions at a Smithsonian Tropical Research Institute field site in the Republic of Panama. Soil was homogenized at the beginning of the experiment, and the bulk soil had a $\delta^{15}N$ of 5.1‰. We observed positive correlations between both δ_p and F_N/v (Fig. 2A) and between δ_p and $\delta_p - \delta_R$ (Fig. 2B). The dashed lines in Figure 2 represent least-squares linear regressions. The linear regression in Figure 2A does not include the species *Platymiscium pinnatum*, a leguminous tree species that formed nitrogen-fixing nodules on its roots. Fixation of atmospheric nitrogen would be expected to alter the correlation between δ_p and F_N/v , both by increasing F_N/v and by shifting δ_p toward 0‰. Such a trend can

be seen for *P. pinnatum* in relation to the correlation for the other 14 species shown in Figure 2A.

Table I summarizes a multiple regression analysis of the data set presented in Figure 2, with δ_p as the dependent variable and F_N/v and $\delta_p - \delta_R$ as independent variables. The regression model explained 55% of variation in δ_p , and both F_N/v and $\delta_p - \delta_R$ were significant terms in the model (Table I). The standardized coefficients indicate that F_N/v was a slightly stronger term than $\delta_p - \delta_R$. *P. pinnatum* was excluded from the analysis presented in Table I. With *P. pinnatum* included in the analysis, the regression model

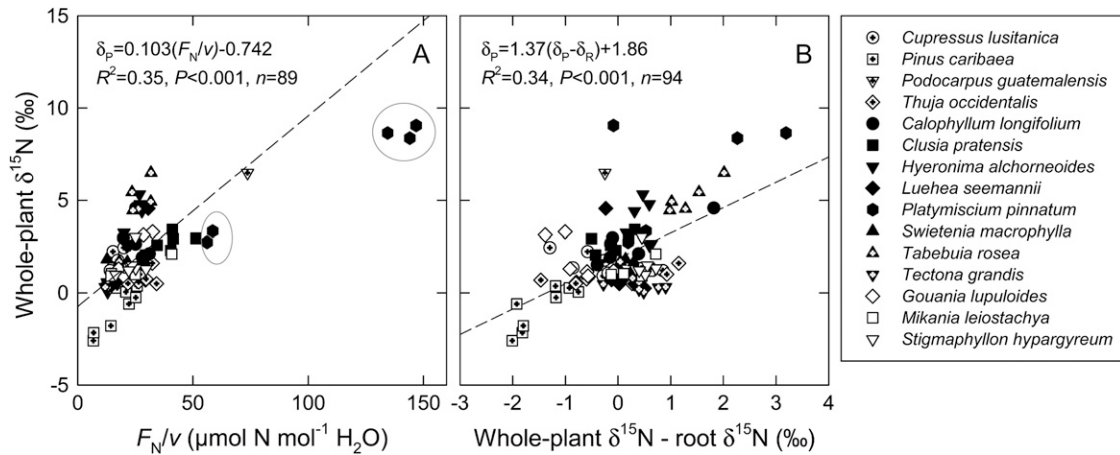


Figure 2. δ_p plotted against F_N/v (A) and $\delta_p - \delta_R$ (B). Dashed lines represent least-squares linear regressions. Regression equations and statistics are given in each panel. The regression analysis in A does not include the leguminous tree species *P. pinnatum*, represented by black hexagons, which are enclosed in gray circles to make them more discernible. This species forms N_2 -fixing nodules on its roots, which is expected to alter δ_p through an additional process not present in the other species. White symbols with internal cross-hairs refer to conifer tree species; completely white symbols refer to angiosperm liana species; black symbols and black symbols with internal cross-hairs refer to angiosperm tree species.

explained 66% of variation in δ_p , with most of the increase in explanatory power attributed to F_N/v .

Figure 3 shows an example of the interplay between $\delta_p - \delta_R$ and F_N/v in determining δ_p for two tropical tree species, *Tectona grandis* and *Swietenia macrophylla*. Five seedlings of each species were grown individually in 19-L pots under well-watered conditions in homogenously mixed, unfertilized forest topsoil. Both *T. grandis* and *S. macrophylla* showed positive correlations between δ_p and $\delta_p - \delta_R$ (Fig. 3A). The offset between species in these correlations was explained by variation in F_N/v (Fig. 3B). Results of a multiple regression analysis for the data presented in Figure 3 are shown in Table II. For this data set, the multiple regression model explained 91% of variation in δ_p , with both $\delta_p - \delta_R$ and F_N/v being significant terms. As in the analysis of data presented in Figure 1, F_N/v was a slightly stronger term in the model than $\delta_p - \delta_R$, as can be seen from the standardized coefficients for the two independent variables (Table II).

DISCUSSION

Application of a theoretical model of nitrogen isotope fractionation during nitrate uptake (Comstock, 2001) led us to predict that δ_p would correlate with F_N/v and $\delta_p - \delta_R$ for plants grown in a common soil environment. We tested these hypotheses with measurements of δ_p in tropical tree and liana seedlings grown in homogenized soil under well-watered conditions. In agreement with theoretical predictions, results suggested that both F_N/v and $\delta_p - \delta_R$ were significant sources of variation in δ_p .

In addition to influences on δ_p associated with uptake of nitrate, Equation 17 clearly indicates the

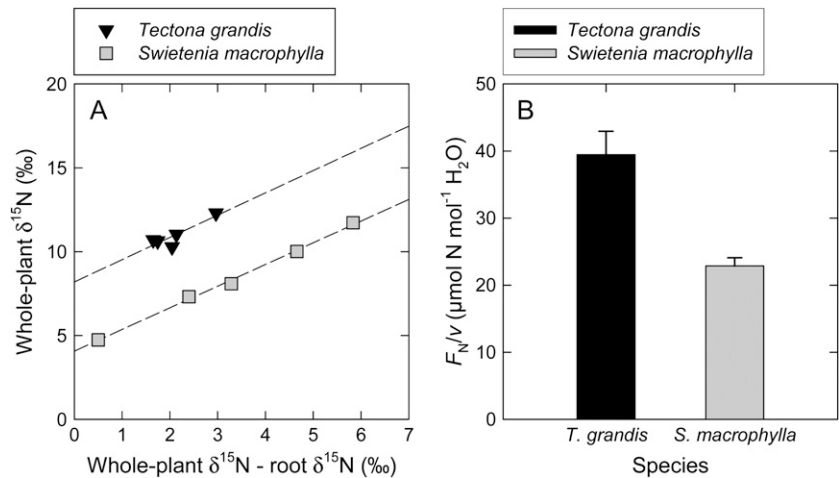
potential for influences associated with uptake of soil ammonium, uptake of soil organic nitrogen, and fixation of atmospheric nitrogen. The influence of the latter can be seen in Figure 2A in relation to *P. pinnatum*, as noted above. In addition, it is possible that some of the unexplained variation in the multiple regression analysis presented in Table II could have resulted from variation among species in preference for ammonium versus nitrate, or possibly from uptake of organic nitrogen from the soil. The importance of organic nitrogen uptake in woody tropical plants is largely unknown (Näsholm et al., 2009), although a capacity for uptake of Gly has been demonstrated in a small number of such species (Schmidt and Stewart, 1999; Wanek et al., 2002). On the other hand, woody tropical plants show a range of preferences for uptake of nitrate relative to ammonium (Stewart et al., 1988; Schmidt and Stewart, 1999; Arndt et al., 2002; Wanek et al., 2002; Aidar et al., 2003; Schimann et al., 2008).

Table I. Multiple regression analysis with δ_p as the dependent variable and F_N/v and $\delta_p - \delta_R$ as independent variables

Data included in the analysis are plotted in Figure 2, which also gives the species identities. The analysis shown here did not include the leguminous tree species *P. pinnatum*, which formed N_2 -fixing nodules on its roots. For the analysis, $r^2 = 0.55$, $F = 52.0$, $P < 0.001$, and $n = 89$.

| Independent Variable | Coefficient | SE | Standardized Coefficient | P |
|--|-------------|-------|--------------------------|--------|
| Constant | -0.521 | 0.325 | | 0.11 |
| F_N/v ($\mu\text{mol nitrogen mol}^{-1}$ water) | 0.094 | 0.013 | 0.537 | <0.001 |
| $\delta_p - \delta_R$ (‰) | 0.963 | 0.156 | 0.452 | <0.001 |

Figure 3. A, δ_p plotted against $\delta_p - \delta_R$ for five individuals each of two angiosperm tree species, *T. grandis* and *S. macrophylla*. B, Variation between the two species in F_N/v of the same plants. Dashed lines in A are least-squares linear regressions. Error bars in B represent 1 sd.



Assuming soil nitrate and soil ammonium as available nitrogen sources for a non- N_2 -fixing plant, Equation 17 indicates that variation in δ_p could result from differences in the proportion of F_N accounted for by F_{Nn} versus F_{Na} , from differences between δ_{Sn} and δ_{Sa} , and from differences in discriminatory processes associated with the two net fluxes. Variation in the $\delta^{15}N$ of leaves caused by variation in F_{Nn} relative to F_{Na} is consistent with some recent analyses (Garten, 1993; Miller and Bowman, 2002; Falkengren-Grerup et al., 2004; Houlton et al., 2007; Kahmen et al., 2008), and the potential for discrimination associated with nitrate and ammonium uptake is well recognized (Mariotti et al., 1982; Handley and Raven, 1992; Robinson et al., 2000; Evans, 2001; Yoneyama et al., 2001; Kolb and Evans, 2003; Pritchard and Guy, 2005).

A sensitivity analysis of the impact of preference for uptake of nitrate versus ammonium on the relationship between δ_p and F_N/v is shown in Figure 4A. The key assumptions underlying the relationships shown in Figure 4 are that F_{Nn}/F_{In} and F_{Na}/F_{Ia} are linearly correlated with F_{Nn}/v and F_{Na}/v , respectively. If these assumptions hold true, Figure 4 indicates that the positive relationship between δ_p and F_N/v is relatively robust in the face of a variable preference for nitrate versus ammonium uptake. Altering the proportion of F_N accounted for by F_{Nn} versus F_{Na} (Fig. 4A), the partitioning of nitrate assimilation between root and shoot (Fig. 4B), or the difference between δ_{Sn} and δ_{Sa} (Fig. 4C) can lead to moderate variation in the slope and/or intercept of the predicted relationship between δ_p and F_N/v ; however, the positive correlation between the two parameters remains.

Discrimination against ^{15}N during uptake of either nitrate or ammonium can occur when there is a significant efflux of that ion from root cells. Significant variation has been observed among species in efflux-to-influx ratios for both nitrate ($1 - F_{Nn}/F_{In}$) and ammonium ($1 - F_{Na}/F_{Ia}$) (Min et al., 1999; Scheurwater et al., 1999; Britto et al., 2001; Kronzucker et al., 2003). In general, $1 - F_{Nn}/F_{In}$ and $1 - F_{Na}/F_{Ia}$ appear to

increase with increasing concentration of nitrate or ammonium, respectively, in the rooting solution (Teyker et al., 1988; Siddiqi et al., 1991; Wang et al., 1993; Glass, 2003; Britto and Kronzucker, 2006). This supports the suggestion that an increase in nitrate or ammonium concentration at the root surface caused by an increase in v should also lead to an increase in $1 - F_{Nn}/F_{In}$ or $1 - F_{Na}/F_{Ia}$. Because nitrogen availability is generally assumed to be higher in tropical than in temperate forests (Vitousek and Howarth, 1991; Houlton et al., 2008), discrimination associated with efflux of nitrate and ammonium from root cells may be more pronounced in tropical than in temperate trees.

Equation 17 indicates that in the case of nitrate the possibility for discrimination associated with efflux from root cells can be completely canceled if all nitrate reduction takes place in the shoot rather than in roots. This is because nitrate loading into the xylem is assumed to be a nondiscriminating process. In this situation, even if there was significant efflux of nitrate from root cells, it would not be recorded in δ_p . Plants show a variable partitioning of nitrate assimilation between roots and shoots (Andrews, 1986; Stewart et al., 1993), and this is likely to cause variation in δ_p (Fig. 4B). Results presented in Figure 3A can be interpreted to suggest that *S. macrophylla* displayed a greater variability among individuals in F_{Rn}/F_{Nn}

Table II. Multiple regression analysis with δ_p as the dependent variable and F_N/v and $\delta_p - \delta_R$ as independent variables

The data set comprised five individuals each of *T. grandis* and *S. macrophylla*. Data included in the analysis are plotted in Figure 3. For the analysis, $r^2 = 0.91$, $F = 37.3$, $P < 0.001$, and $n = 10$.

| Independent Variable | Coefficient | SE | Standardized Coefficient | P |
|--|-------------|-------|--------------------------|--------|
| Constant | -1.047 | 1.280 | | 0.44 |
| F_N/v ($\mu\text{mol nitrogen mol}^{-1}$ water) | 0.232 | 0.031 | 0.915 | <0.001 |
| $\delta_p - \delta_R$ (‰) | 1.282 | 0.182 | 0.861 | <0.001 |

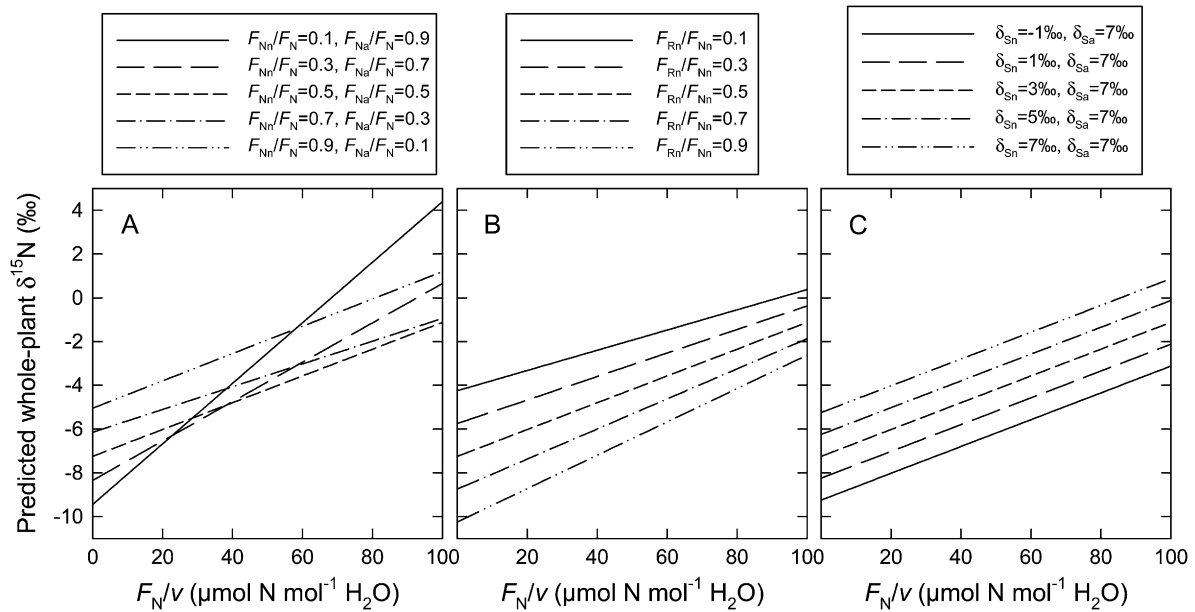


Figure 4. A sensitivity analysis of the predicted relationship between whole-plant $\delta^{15}\text{N}$ and F_N/v as a function of the proportion of nitrogen uptake from the soil as nitrate versus ammonium (A), the proportion of nitrate assimilation in roots versus leaves (B), and the $\delta^{15}\text{N}$ difference between soil nitrate and soil ammonium (C). Equation 17 was used to predict whole-plant $\delta^{15}\text{N}$, assuming no fixation of atmospheric N_2 and no uptake of organic nitrogen from the soil (i.e. $F_{\text{Nd}} = 0$, $F_{\text{No}} = 0$). Ratios of net flux to influx for soil nitrate and ammonium were assumed to correlate linearly with the transpiration efficiency of nitrogen acquisition for each nitrogen source, according to the following relationships: $(F_{\text{Nr}}/F_{\text{Nl}}) = (F_{\text{Nr}}/v)/100$ and $(F_{\text{Na}}/F_{\text{Na}}) = (F_{\text{Na}}/v)/100$. The range of parameter values considered for each analysis is given above each panel. If a parameter value is not given above the panel, the following were assumed: $(F_{\text{Nr}}/F_{\text{Nl}}) = 0.5$, $(F_{\text{Na}}/F_{\text{Na}}) = 0.5$, $b = 15\text{‰}$, $c = 17\text{‰}$, $(F_{\text{Rr}}/F_{\text{Rl}}) = 0.5$, $\delta_{\text{Sn}} = 3\text{‰}$ and $\delta_{\text{Sa}} = 7\text{‰}$.

than *T. grandis*, because the former species showed a larger range of $\delta_p - \delta_R$ than the latter.

Intriguingly, some of the highest δ_p values in the data set shown in Figure 2 were for the potentially N_2 -fixing species *P. pinnatum*. Symbiotic N_2 fixation in legumes may show a slight discrimination against ^{15}N , in the range of 0‰ to 2‰ (Yoneyama et al., 1986), such that plant nitrogen derived from this source should have a $\delta^{15}\text{N}$ in the range of approximately 0‰ to -2‰. Roots of the *P. pinnatum* plants shown in Figure 2A were nodulated, so some degree of N_2 fixation seems likely. As noted previously, N_2 fixation should shift the relationship between δ_p and F_N/v by pulling δ_p toward 0‰ and increasing F_N/v compared with values for non- N_2 -fixing plants. Consistent with this suggestion, the relationship between δ_p and F_N/v appears to differ for *P. pinnatum* than for the rest of the species in Figure 2A, although the difference is admittedly rather subtle. A more dramatic illustration of the difference in relationships between δ_p and F_N/v for N_2 -fixing, leguminous tree species compared with non- N_2 -fixing tree species is shown in Figure 5, based on a previously published data set (Cernusak et al., 2007a). A multiple regression analysis for the non- N_2 -fixing species in Figure 5 is shown in Table III. Results are similar to those shown in Table I. Figure 5 clearly shows a negative slope between δ_p and F_N/v for the leguminous tree species *P. pinnatum* and *Dalbergia retusa*, whereas the slope for the non- N_2 -fixing species is positive.

The intersection of the regression lines in Figure 5 for the leguminous tree species and the non- N_2 -fixing tree species suggests that in the absence of N_2 fixation, the leguminous tree species would have δ_p values near the highest observed for the non- N_2 -fixing species. In combination with Figure 2, these data suggest that the leguminous tree species examined may have low rates of nitrate and ammonium efflux from root cells, associated with a high plant demand for nitrogen. This would be consistent with the nitrogen-demanding lifestyle that characterizes legumes more generally (Mckey, 1994).

The theoretical argument put forward in the introduction to explain the correlation between δ_p and F_N/v is conceptually consistent with existing paradigms concerning ^{15}N discrimination during nitrogen uptake. It is generally assumed that whole-plant $\delta^{15}\text{N}$ is unlikely to show significant discrimination with respect to soil nitrogen sources when plant demand for nitrogen strongly exceeds nitrogen supply, and opportunities for discrimination increase as soil nitrogen supply increases relative to plant nitrogen demand (Evans, 2001). For plants growing in a common soil environment, F_N/v is an expression of nitrogen demand relative to nitrogen supply. At steady state, for a given F_N , an increase in v will increase the concentration of nitrogenous solutes at the root surface and their transport into the roots (assuming $\sigma < 1$), thus increasing nitrogen supply. Similarly, for a fixed F_N ,

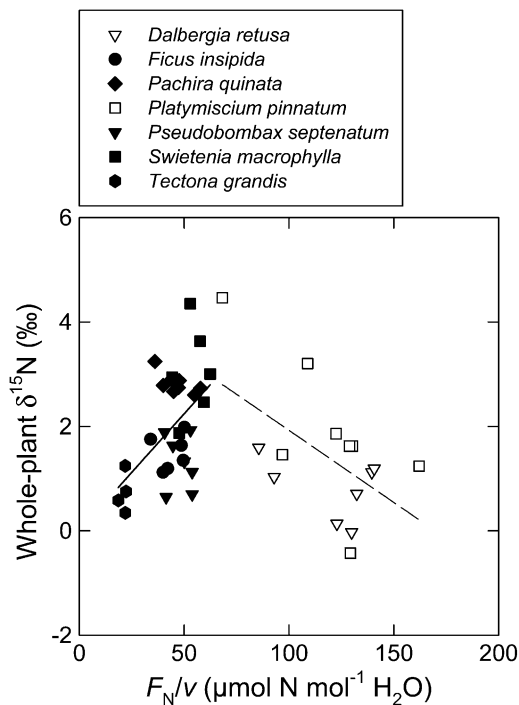


Figure 5. δ_p plotted against F_N/v for seven tropical tree species. White symbols refer to leguminous tree species capable of forming N_2 -fixing nodules on their roots, and black symbols refer to tree species incapable of N_2 fixation. The solid line and the dashed line represent least-squares linear regressions for non- N_2 -fixing species and N_2 -fixing species, respectively. Data presented in this figure were originally published by Cernusak et al. (2007a).

a decrease in v would decrease the concentration of nitrogenous solutes at the root surface and the convective flux of such solutes into the roots (assuming $\sigma < 1$), thus decreasing supply relative to demand. It is worth pointing out that a weaker relationship would be expected between δ_p and F_N/v for plants grown in hydroponics compared with soil, because solute concentrations at the root surface in a well-stirred hydroponic solution are largely uncoupled from variation in F_N/v .

It should be emphasized that a positive correlation between δ_p and F_N/v among a population of non- N_2 -fixing plants would only be expected when the plants are exposed to inorganic soil nitrogen pools of similar concentration and isotopic composition. If the plants are growing in differing soils in which the nitrate and ammonium concentrations in the soil solution are very different, nitrate and ammonium concentrations at the root surface will vary independently of variation in F_N/v . As a consequence, nitrate and ammonium efflux-to-influx ratios will also be uncoupled from F_N/v , as will discrimination during nitrogen uptake. This situation can be demonstrated by an analysis of the data set presented by Cernusak et al. (2007b). In that study, seedlings of a tropical pioneer tree (*Ficus insipida*) were grown in soil containing variable proportions of rice husks, an organic substrate with a high

carbon-nitrogen ratio and therefore expected to encourage microbial immobilization of soil nitrogen. Nitrogen availability differed strongly among the treatments, and as a result, no correlation was observed between δ_p and F_N/v ($P = 0.12$, $n = 24$).

It has been suggested that root symbioses with mycorrhizas can influence δ_p (Handley et al., 1993; Högberg, 1997; Hobbie et al., 2000; Schmidt et al., 2006). Most of the tropical tree and liana species examined in this study were likely associated with arbuscular mycorrhizal fungi (Wang and Qiu, 2006), with the exception of *Pinus caribaea*, which could have supported ectomycorrhizas (Tedersoo et al., 2007). Nonetheless, we are unable to draw any conclusions about the influence, or lack thereof, of mycorrhizal status on δ_p in our experiment, because we do not have information on mycorrhizal infection levels. However, it will likely prove useful in future work to incorporate a mycorrhizal compartment into the modeling framework for δ_p . This could be accomplished using the generalized approach for isotope reactions in branched pathways presented by Comstock (2001), assuming that appropriate information about fractionations associated with nitrogenous fluxes between mycorrhizas and roots becomes available.

Interpretation of plant $\delta^{15}N$ in ecological investigations is often a complex exercise (Robinson, 2001). It can be simplified if the assumption can be made that mineral nitrogen sources are assimilated without discrimination during uptake, as might be expected when plant demand for nitrogen strongly exceeds nitrogen supply to roots (Evans, 2001). We interpret the positive correlations between δ_p and F_N/v shown in Figures 2, 3, and 5 for non- N_2 -fixing tropical tree and liana species as resulting from variation in efflux-to-influx ratios for nitrate and ammonium from root cells. We provide a theoretical justification in the introduction for this interpretation. The implication is that, for tropical forest trees and lianas, it cannot simply be assumed that nitrate and ammonium will be taken up from the soil without discrimination against ^{15}N . Moreover, we observed a range of δ_p values among species (Fig. 2A), which suggests significant variation in mineral nitrogen efflux rates from roots. Such variation could have important implications for ecosystem nitrogen cycling and interspecific competition (Glass, 2003; Kronzucker et al., 2003), because it suggests that tropical tree and

Table III. Multiple regression analysis with δ_p as the dependent variable and F_N/v and $\delta_p - \delta_R$ as independent variables

The analysis includes data for the non- N_2 -fixing species shown in Figure 5 ($r^2 = 0.49$, $F = 13.2$, $P < 0.001$, and $n = 31$).

| Independent Variable | Coefficient | SE | Standardized Coefficient | P |
|--|-------------|-------|--------------------------|-------|
| Constant | 0.422 | 0.560 | | 0.46 |
| F_N/v ($\mu\text{mol nitrogen mol}^{-1}$ water) | 0.041 | 0.012 | 0.468 | <0.01 |
| $\delta_p - \delta_R$ (‰) | 0.547 | 0.157 | 0.473 | <0.01 |

liana species vary in their abilities to rapidly absorb available nitrogen from the soil.

CONCLUSION

We have presented experimental evidence from several tropical tree and liana species in support of the hypothesis that variation in δ_p should correlate with variation in F_N/v and $\delta_p - \delta_R$ for plants grown in a similar soil environment. Furthermore, we have demonstrated contrasting behavior in the relationship between δ_p and F_N/v in non- N_2 -fixing compared with N_2 -fixing species, also consistent with theoretical expectations. These results make a novel contribution to the theoretical framework that can be brought to bear upon the interpretation of $\delta^{15}\text{N}$ variations in terrestrial plants.

MATERIALS AND METHODS

Experiments were carried out at the Santa Cruz Experimental Field Facility, Smithsonian Tropical Research Institute, Republic of Panama (9°07' N, 79°42' W), at an altitude of approximately 28 m above sea level. For data presented in Figure 2, a full account of plant growth conditions and experimental procedures was given previously (Cernusak et al., 2008). Briefly, plants were grown for several months individually in 38-L pots that did not drain. Cumulative plant water use over the experimental period was determined by weighing the pots at weekly or subweekly intervals and replacing the water consumed in the preceding interval. Soil evaporation was estimated by weighing control pots that contained no plants. At harvest, plants were separated into roots, stems, and leaves and dried to constant mass at 70°C. Plant material was ground to a fine powder for elemental and isotopic analyses. The $\delta^{15}\text{N}$ and nitrogen concentrations of dry matter were determined on subsamples of approximately 3 mg that were combusted in an elemental analyzer (ECS 4010; Costech Analytical Technologies) coupled to a continuous flow isotope ratio mass spectrometer (Delta XP; Finnigan MAT) at the Stable Isotope Core Laboratory, Washington State University. The F_N/v was calculated as $[(M_F - M_I)N_F]/E_T$, where M_F and M_I are final and initial dry mass of the plants, respectively, N_F is the whole-plant nitrogen mass fraction at final harvest, and E_T is cumulative transpiration. Thus, the initial nitrogen mass fraction was assumed to be equal to that at harvest. The mean M_I was estimated as 1.8 g, and the mean M_F was 50.1 g, such that any variation in initial nitrogen concentration would have had a negligible influence on estimates of F_N/v .

The experimental procedure for measurements shown in Figure 3 was similar to that described above, except that plants were grown in 19-L pots. Full experimental details, including descriptions of growth and physiology, are provided elsewhere (Cernusak et al., 2009). The $\delta^{15}\text{N}$ data for data sets presented in Figures 2 and 3 are presented for the first time in this paper. Statistical analyses were performed in SYSTAT 11.0 (SYSTAT Software).

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