Physiological mechanisms influencing plant nitrogen isotope composition

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Whole-plant and leaf nitrogen isotope composition are determined by the isotope ratio of the external nitrogen source and physiological mechanisms within the plant. Whole-plant isotope composition can reflect that of the nitrogen source when plant demand exceeds nitrogen supply. Uptake by mycorrhizae can cause the isotope ratio of the plant to deviate from the source. Intra-plant variation in isotope composition can be caused by multiple assimilation events, organ-specific loss of nitrogen, and resorption and reallocation of nitrogen. Future work must address acquisition of organic nitrogen from the soil solution, the role of mycorrhizae, and internal transformations within the plant.

Understanding the mechanisms controlling whole-plant and foliar nitrogen isotope composition will advance our knowledge of plant nitrogen acquisition and allocation. Nitrogen is the element that most often limits plant growth in many terrestrial ecosystems. Anthropogenic activity has altered the amount and relative abundance of the forms of nitrogen (NH₄⁺, NO₃⁻ and amino acids) that are available for plant absorption into the plant. The forms of nitrogen absorbed by plants can have different isotope compositions, and many studies now routinely measure foliar δ¹⁵N (Box 1) in an attempt to understand differences in patterns of nitrogen use among co-occurring species. Many studies assume that δ¹⁵N at natural abundance levels acts as a tracer (i.e. the isotope ratio of source nitrogen is preserved during nitrogen absorption, assimilation and translocation, and that the δ¹⁵N of leaf tissues reflects that of the nitrogen source in the soil). This assumption is important because although the reported variation in plant δ¹⁵N can be between −10‰ and +10‰, the difference among co-occurring species is often less (0–10‰), and biologically significant differences can be ~1‰ (Ref. 4). However, it is clear that this assumption could be invalid because physiological factors, such as different nitrogen uptake mechanisms, different pathways of assimilation, and recycling of nitrogen in the plant, can discriminate against δ¹⁵N. This review addresses how physiological transformations of nitrogen can influence whole-plant and leaf δ¹⁵N. Ecological aspects of δ¹⁵N measurements and δ¹⁵N of plant nitrogen sources are addressed in Refs 3, 5.

Mechanisms of NH₄⁺ and NO₃⁻ uptake and assimilation

The three steps of NH₄⁺ and NO₃⁻ acquisition are:

- Uptake of nitrogen from the soil solution.
- Translocation to the site of assimilation.
- Assimilation of inorganic nitrogen into organic nitrogen.

42 Ade, J. et al. (1999) Four mismatch repair paralogues coexist in Arabidopsis thaliana: AtMSH2, AtMSH3, AtMSH6-1 and AtMSH6-2.
Uptake of both NH$_4^+$ and NO$_3^-$ occurs by two different mechanisms that are active at different concentrations. Ammonium uptake at low external concentrations is usually by a constitutive carrier system with high-substrate affinity$^7$. A second, non-saturable transport mechanism with low-substrate affinity is dominant at high concentrations. Uptake of NO$_3^-$ at relatively low external NO$_3^-$ concentrations (0–500 μM) is also by a high-affinity, inducible carrier system. It saturates at <500 μM and the $K_m$ is <100 μM (Ref. 8). A second, constitutive system operates at relatively high concentrations (>500 μM). Uptake by this system is a linear function of external NO$_3^-$ concentration, and kinetic studies indicate that it is a passive, channel-mediated transport system.

Assimilation of NH$_4^+$ occurs by the glutamine synthetase–glutamate synthase (GS–GOGAT) pathway. Assimilation occurs in the root near to the site of uptake to avoid toxic accumulation. As a

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**Box 1. Terminology**

Nitrogen exists as two naturally occurring stable isotopes, $^{15}$N and $^{14}$N. Variation in the absolute abundance of $^{15}$N is small, therefore nitrogen isotope composition is expressed using δ notation in parts per thousand (Eqn I):

$$\delta^{15}\text{N} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) \times 1000 \text{‰}$$  \[\text{I}\]

where δ$^{15}$N is the isotope ratio relative to the atmospheric air standard, and $R_{\text{sample}}$ and $R_{\text{standard}}$ are the molar ratios of the heavier to the lighter isotope. The value for $R_{\text{standard}}$ is 0.0036765.

Differences in δ$^{15}$N between a substrate and product will occur when $^{15}$N and $^{14}$N react at different rates. The ratio of the rate constants ($k^{15}_s/k^{14}_s$) is the isotope effect (α, and is equivalent to ($R_{\text{substrate}}/R_{\text{product}}$)) (Ref. a). Discrimination (Δ) is the deviation of α from unity ($\Delta = \alpha - 1$) (Ref. a).

Discrimination can be stated in relation to the δ$^{15}$N of the substrate (δ$^{15}$N$_s$) and product (δ$^{15}$N$_p$) (Eqn II):

$$\Delta = 1000 \left( \frac{\delta^{15}\text{N}_s - \delta^{15}\text{N}_p}{1 + \delta^{15}\text{N}_p/1000} \right)$$  \[\text{II}\]

The term (1 + δ$^{15}$N$_p/1000$) does not differ significantly from 1, therefore an approximation is (Eqn III):

$$\Delta = \delta^{15}\text{N}_s - \delta^{15}\text{N}_p$$  \[\text{III}\]

Discrimination is positive in most biological systems, therefore the product should have a lower δ$^{15}$N value than the substrate.

**Reference**


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**Box 2. Open versus closed systems**

A closed system has a limited quantity of substrate, therefore the absolute amount of substrate declines as it is converted to product. Fractionation during the reaction will cause the δ$^{15}$N of the product to be less than that of the substrate, but this will also cause the δ$^{15}$N of the remaining substrate to increase over time (Fig. I). This causes a corresponding increase in the δ$^{15}$N of the next increment of product, therefore the δ$^{15}$N of both substrate and product will increase over time. It is important to note that because of mass balance, the δ$^{15}$N of the product will be identical to the original substrate once the reaction is complete, even though fractionation occurs during the reaction.

Many greenhouse studies are carried out using closed systems; nutrients are added in pulses and the plants are allowed to assimilate all the nitrogen. Plant δ$^{15}$N will be equal to that of the original substrate when all the nitrogen is assimilated, even though discrimination might have been observed while there was still residual substrate. By contrast, an open system has an unlimited amount of substrate, therefore substrate δ$^{15}$N will not change over time. The isotope effect will always be expressed, and the δ$^{15}$N of the product will always be less than the substrate if fractionation does occur. This can be accomplished in experimental studies using specially designed hydroponic systems that regulate solution concentrations, or by growing plants in containers with large volumes so that plant uptake does not alter solution concentration.

**Fig. I.** The δ$^{15}$N of accumulated product and remaining substrate in a closed nutrient system. The δ$^{15}$N of the initial substrate is 0‰ and fractionation during the reaction is 20‰. Isotope ratios were calculated using the equations given in Ref. b.

**References**


result, NH₄⁺ is seldom present in the xylem⁶. Nitrate assimilation can occur in either the roots or leaves by the nitrate reductase–nitrite reductase pathway. The product of this assimilation event is NH₄⁺, which subsequently enters the GS–GOGAT pathway.

**Fractionation with NH₄⁺ and NO₃⁻ uptake and assimilation**

Significant discrimination has been observed for plants grown on NO₃⁻ using open nutrient systems (Box 2). A general pattern is that the discrimination increases with external NO₃⁻ concentration and decreases with plant age (Fig. 1). Similar patterns have been observed for plants grown with NH₄⁺ as their sole nitrogen source. For example, discrimination was not significantly different from 0‰ for *Lycopersicon esculentum* grown at 50 μM NH₄⁺ (Ref. 9). Discrimination varied from 0.9 to 5.8‰ for *Pinus sylvestris* grown with 4.6 mM NH₄⁺ (Ref. 10), and the observed discrimination for *Oryza sativa* grown at 1.4 mM NH₄⁺ and 7.3 mM NH₄⁺ was 4.1‰ and 12.6‰, respectively¹¹.

Two trends emerge from the data in Fig. 1 and comparable studies of plants grown on NH₄⁺. First, discrimination values near 0‰ were observed at all concentrations, indicating that there is not an inherent fractionation associated with uptake by either the high- or low-affinity transport mechanisms. Second, the range of discrimination values at each concentration suggests that external nitrogen concentration is not the only factor that determines discrimination.

The discrimination observed with plant acquisition of nitrogen was originally described as (Eqn 1):¹²:

\[
\text{Uptake} \quad \text{Assimilation} \\
(N)_{\text{out}} \xrightarrow{F_2} (N)_{\text{in}} \xrightarrow{F_3} (N)_{\text{assimilated}} \\
\text{where (N)}_{\text{out}} \text{ and (N)}_{\text{in}} \text{ are the inorganic nitrogen forms (NO}_3^-, \text{NH}_4^+) \text{ outside and inside of the cell, respectively, and (N)}_{\text{assimilated}} \text{ is assimilated nitrogen.} \quad F_2, F_3, \text{ and } F_4 \text{ are the influx, efflux and assimilation rates of nitrogen, respectively. } F_1 = F_2 + F_3 \text{ under steady-state conditions.}
\]

The underlying assumption of this model is that nitrogen enters into a pool (Nₑ) that is being assimilated into organic nitrogen. Nitrate reductase and glutamine synthetase both fractionate against ¹⁵N; observed discrimination by nitrate reductase and glutamine synthetase is 15‰ and 17‰, respectively⁴,¹³. This fractionation event causes the Nₑ to become enriched in ¹⁵N compared with Nₑ, whereas the organic nitrogen product is depleted in ¹⁵N. Discrimination is observed when nitrogen enriched in ¹⁵N is lost from Nₑ through efflux (F₂ in Eqn 1).

Influx and efflux associated with an enzyme-substrate system can be described by an equation modified from Tom Sharkey and Joe Berry¹⁴ (Eqn 2):

\[
\Delta = b - \left(\frac{b F_2}{F_1}\right) \\
\text{where b is fractionation associated with the assimilatory enzyme. This model yields two predictions. First, that discrimination will be 0‰ if assimilation does not occur in the root (b = 0) so that enriched pools are not available for efflux. Second, significant differences between the δ¹⁵N of the plant and nitrogen in solution will occur if the rate of uptake exceeds assimilation, so that efflux of enriched nitrogen occurs from (Nₑ). This model assumes steady-state conditions and ignores compartmentation of inorganic N within the root or efflux of compounds other than NH₄⁺ and NO₃⁻; yet it demonstrates the relationship between nitrogen availability and demand in controlling discrimination.}
\]

Experiments with *Brassica campestris* and *L. esculentum* support this general model⁹,¹⁵. In both cases, plant δ¹⁵N values were not significantly different than the δ¹⁵N of the source NO₃⁻, even though the δ¹⁵N of internal NO₃⁻ pools was significantly enriched compared with both source and whole-plant values (Table 1). The relationship between nitrogen uptake and demand has also been illustrated in experiments with *P. sylvestris* grown on 4.6 mM NO₃⁻ (Ref. 10); discrimination was only observed when plant nitrogen demand was relatively low, compared with the nitrogen available in solution. According to this model, discrimination during nitrogen uptake will probably not be observed in most natural ecosystems, where external concentrations are low and plant demand will exceed nitrogen supply. A more comprehensive whole-plant modeling effort by David Robinson¹⁶ for plants grown on NO₃⁻ predicts that plant δ¹⁵N might deviate from the nitrogen source if there is a substantial efflux of...
organic nitrogen from the root, a mechanism that has not been considered in previous studies.

**Role of mycorrhizae**

Previous work with uptake and assimilation of NH$_4^+$ and NO$_3^-$ has been conducted under controlled conditions in greenhouses or growth chambers, but in natural ecosystems, many plants also acquire nitrogen through fungal symbionts. Field studies have shown that host plants and mycorrhizal associates differ in their $\delta^{15}$N values by as much as 8‰ (Ref. 5). Three possible explanations for this pattern have been provided $^{10}$:

- Uptake of nitrogen sources with different $\delta^{15}$N values.
- Differences in fractionation during uptake.
- Host plants and mycorrhizal symbionts having different physiologies.

The observation that sporocarps$^{17,18}$ and sheaths$^{19}$ of ectomycorrhizal fungi are enriched in $^{15}$N compared to leaves and roots of their host plants, led researchers to hypothesize that much of the variation in plant $\delta^{15}$N among species is because of the internal metabolism of the fungi$^{10}$. Amino acid biosynthesis often results in amino acids that have a lower $\delta^{15}$N than their precursors; for example, transamination of glutamic acid results in a product with a $\delta^{15}$N value 9‰ lower than the source$^{20}$. Translocation of amino acids with a relatively lower $\delta^{15}$N to the host plant causes the fungus to become enriched, and the plant depleted, in $^{15}$N compared with the original soil nitrogen source (Fig. 2). This hypothesis was supported in an elegant series of field and modeling studies along a nitrogen availability gradient at Glacier Bay (AK, USA)$^{21–23}$. The $\delta^{15}$N of soil NH$_4^+$ remained relatively constant along the gradient, and was similar to foliar $\delta^{15}$N values on sites with the highest nitrogen availability. By contrast, foliar values were depleted by 2–6‰, and mycorrhizal fungal values were enriched 4–6‰, compared with soil NH$_4^+$ on sites with low nitrogen availability. It was concluded$^{21}$ that plants acquire NH$_4^+$ when availability is high, but have increased reliance on mycorrhizal fungi when NH$_4^+$ availability is low.

Possible mechanisms for the enrichment of mycorrhizae compared with their host plants were tested in a concurrent modeling study$^{22}$. Fractionation during mycorrhizal transfer of nitrogen provided the best explanation for patterns observed in the field.

A direct test of this hypothesis was conducted using ectomycorrhizal and non-mycorrhizal seedlings of P. sylvestris$^{10}$. No differences in fractionation were observed between mycorrhizal and non-mycorrhizal plants, yet fungal rhizomorphs were enriched by as much as 5‰ when compared with host plants (Fig. 2). Mass balance calculations show that even though the fungus is enriched relative to the plant, this enrichment causes little (~0.1‰) change in plant $\delta^{15}$N because a high level of efficiency of transfer ensures that almost all nitrogen acquired by the fungus is translocated into the plant. Clearly, more experiments are needed to reconcile field and laboratory studies.

**Intra-plant variation in $\delta^{15}$N**

Intra-plant variation in $\delta^{15}$N can confound interpretation of leaf $\delta^{15}$N values. Observed differences in leaf $\delta^{15}$N among co-occurring species can result from different patterns of nitrogen acquisition or simply differences in $\delta^{15}$N between plant organs. Intra-plant variation in $\delta^{15}$N has been observed in both laboratory and field studies. The $\delta^{15}$N of leaves can be 3–7‰ greater than roots in controlled experiments using hydroponic or pot systems$^{9,15,24}$. Observed variation in field studies is ecosystem specific; for example, differences in $\delta^{15}$N between leaves and roots is often less than 3‰ in deciduous forest and tallgrass prairie ecosystems, but can be as great as 7‰ in warm and cold desert ecosystems.

Organ-specific loss of nitrogen, different patterns of nitrogen assimilation, and reallocation of nitrogen can cause intra-plant variation in $\delta^{15}$N. Loss of NH$_3$ could enrich leaves in $^{15}$N but this is not likely in

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**Table 1.** The $\delta^{15}$N of source NO$_3^-$, whole-plants, roots, leaves and root and leaf NO$_3^-$ for Brassica campestris and Lycopersicon esculentum

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
<th>Plant $\delta^{15}$N</th>
<th>Root $\delta^{15}$N</th>
<th>Root NO$_3^-$ $\delta^{15}$N</th>
<th>Leaf $\delta^{15}$N</th>
<th>Leaf NO$_3^-$ $\delta^{15}$N</th>
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<td>4.9</td>
<td>12.4</td>
<td>10.6</td>
<td>25.0</td>
<td>15</td>
</tr>
<tr>
<td>Lycopersicon</td>
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<td>2.5 ± 0.4</td>
<td>-0.1 ± 0.4</td>
<td>11.1 ± 1.7</td>
<td>3.3 ± 0.6</td>
<td>14.0 ± 4.6</td>
<td>9</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Potential influences of mycorrhizal on plant $\delta^{15}$N. Field and modeling studies$^{21–23}$ suggest that when soil inorganic nitrogen is abundant, plants will acquire little nitrogen from mycorrhizae and should reflect the $\delta^{15}$N of the inorganic nitrogen source (arrow (a)). Plants will shift to mycorrhizae when nitrogen is limiting, and fractionation during nitrogen transfer will cause the plant to have a lower $\delta^{15}$N, and the fungus a greater $\delta^{15}$N, than the nitrogen source (arrow (b)). Greenhouse studies$^{10}$ indicate that the mass of the fungus is small compared with the plant therefore the effect of the mycorrhizae will be negligible. Plant $\delta^{15}$N should reflect the nitrogen source in all cases (arrow (a)).
actively growing plants\textsuperscript{25}. Attempts have been made to measure the $\delta^{15}N$ of volatilized nitrogen, but levels of NH$_3$ were below limits of detection\textsuperscript{24}. Efflux of organic nitrogen from roots could also alter their $\delta^{15}N$ (Ref. 16).

Nitrogen source and patterns of assimilation can strongly influence intra-plant patterns in $\delta^{15}N$ (Refs 9,11,15). Significant intra-plant variation can be observed when NO$_3^-$ is the primary nitrogen source, but little variation is observed when NH$_4^+$ is the source. The contrasting patterns of intra-plant variation are probably caused by different patterns of assimilation. NH$_4^+$ is assimilated immediately in the root\textsuperscript{6}, therefore organic nitrogen in shoots and roots is the product of a single assimilation event. NO$_3^-$ assimilation can occur in both roots and shoots. Fractionation during assimilation by nitrate reductase causes the $\delta^{15}N$ of unassimilated NO$_3^-$ to become enriched relative to organic nitrogen (Table 1). The $\delta^{15}N$ of leaves can be greater than roots because the NO$_3^-$ available for assimilation is enriched relative to root NO$_3^-$ because it originates from a pool that has already been exposed to assimilation\textsuperscript{9,15}.

Reallocation of nitrogen during growth can cause differences among organs because most reactions discriminate against $^{15}N$; reactions such as NO$_3^-$ reduction, GS–GOGAT, transaminations, and other enzymatic reactions should all result in products with lower $\delta^{15}N$ values than the original source\textsuperscript{25,26}. A recent model\textsuperscript{16} provides a theoretical framework to understand transformations within the plant and their effects on plant $\delta^{15}N$, but direct analysis of xylem and phloem fluids is necessary to elucidate source–sink relationships. These are difficult measurements to obtain, but recent results\textsuperscript{26–28} are compelling. The $\delta^{15}N$ of nitrogen in phloem is lower than that of xylem\textsuperscript{26,27}, and $\delta^{15}N$ of xylem fluids is greater than the $\delta^{15}N$ of water-extractable soil N. This has been attributed to an increased proportion of organic N compounds in the xylem that have higher $\delta^{15}N$ than soil NO$_3^-$ (Ref. 28). Detailed analysis of individual compounds in xylem and phloem using compound-specific isotope analysis (CSIA) is necessary to further address these important questions (Fig. 3).

**Genotypic differences in $\delta^{15}N$**

Few studies have addressed genetic variation in physiological traits that could cause differences in whole-plant and foliar $\delta^{15}N$. Twenty-eight genotypes of Hordeum spontaneum exhibited a range of 2.2–2.4‰ in shoot $\delta^{15}N$ for control and salt-stressed treatments respectively\textsuperscript{29}, but as source and root $\delta^{15}N$ were not measured it was not clear if these differences were because of fractionation during uptake and assimilation or because of intra-plant variation. Recent attempts have been made to correlate whole-plant and root $\delta^{15}N$ to water and nitrogen stress for 30 genotypes of H. spontaneum\textsuperscript{30}. Whole-plant $\delta^{15}N$ varied by almost 1.5‰ among genotypes within a treatment. Genotypes that were most tolerant of nitrogen stress had the lowest whole-plant $\delta^{15}N$ values and the greatest difference between shoot and root $\delta^{15}N$. It was argued that more stress-tolerant plants retain greater amounts of nitrogen, and the lower $\delta^{15}N$ values were caused by efflux of nitrogen compounds with positive $\delta^{15}N$ (Ref. 30).

Differences among genotypes in the distribution of NO$_3^-$ reductase within a plant could also result in variation in discrimination during uptake and differences between shoot and root $\delta^{15}N$ for plants grown with NO$_3^-$. Experiments with Hordeum vulgare show genotypic differences in the relative distribution of nitrate reductase\textsuperscript{2}; genotypes can have nitrate reductase present only in roots, shoots or both. Discrimination should not be observed if nitrate reductase is absent.
from the root unless there is an efflux of organic nitrogen, and multiple assimilation events should result in greater intra-plant variation in δ15N.

**Future prospects**

Progress has been made in understanding the mechanisms that control plant and foliar δ15N. It is clear that plant δ15N is not a tracer of nitrogen source; instead, it provides a synthesis of the δ15N of the nitrogen source, fractionation events that occur during nitrogen absorption and by different mycorrhizal associations and during assimilation, allocation and loss of nitrogen from the plant. Recent modeling efforts provide valuable tools to identify processes that could control plant δ15N, but more thorough, theoretical models such as those developed for plant carbon isotope composition are necessary. Future research must also be directed at quantifying the δ15N of plant-available nitrogen under field conditions. Conventional methods that proved reliable for enriched studies are unsatisfactory at natural abundance levels because each might discriminate against δ15N (Ref. 3). A more thorough understanding of fractionations that occur within plants is also necessary. Controlled studies with NH4+ and NO3− have greatly expanded our knowledge, but they could have limited relevance to some ecosystems, where it is becoming increasingly clear that plants can assimilate organic nitrogen or rely on mycorrhizae. Expanding our knowledge into the areas of organic nitrogen uptake, the role of mycorrhizae, and fractionation events associated with nitrogen allocation will require further development of CSIA of soil and plant solutions (Fig. 3). Although the further development of theoretical models, coupled with recent advances in CSIA, are prerequisites for our complete understanding of the mechanisms controlling plant and foliar δ15N, they also have the potential to provide valuable insight into plant nitrogen dynamics.

**References**