37 McKim, K.S. and Hayashi-Hagihara, A. (1998) *mei-W68* in *Drosophila melanogaster* encodes a Spo11 homologue: evidence that the mechanism for initiating meiotic recombination is conserved. *Genes Dev.* 12, 2932–2942
38 Hartung, F. and Puchta, H. (2000) Molecular characterisation of two paralogous *SPO11* homologues in *Arabidopsis thaliana*. *Nucleic Acids Res.* 28, 1548–1554
39 Garcia, V. *et al.* (2000) An *ATM* homologue from *Arabidopsis thaliana*: complete genomic organisation and expression analysis. *Nucleic Acids Res.* 28, 1692–1699
40 Hartung, F. and Puchta, H. (1999) Isolation of the complete cDNA of the *MRE11* homologue of *Arabidopsis* indicates conservation of DNA recombination mechanisms between plants and other eukaryotes. *Plant Physiol.* 121, 312
41 Kolodner, R. and Marsischky, G.T. (1999) Eukaryotic DNA mismatch repair. *Curr. Opin. Genet. Dev.* 9, 89–96
42 Ade, J. *et al.* (1999) Four mismatch repair paralogues coexist in *Arabidopsis thaliana*: *AtMSH2*, *AtMSH3*, *AtMSH6-1* and *AtMSH6-2*. *Mol. Gen. Genet.* 262, 239–249
43 Culligan, K. and Hays, J.B. (2000) *Arabidopsis* MutS homologues – AtMSH2, AtMSH3, AtMSH6, and a novel AtMSH7 – form three distinct protein heterodimers with different specificities for mismatched DNA. *Plant Cell* 12, 991–1002
44 Jean, M. *et al.* (1999) Isolation and characterization of *AtMLH1*, a *MutL* homologue from *Arabidopsis thaliana*. *Mol. Gen. Genet.* 262, 633–642
45 Starr, D.A. *et al.* (1997) Conservation of the centromere/kinetochore protein ZW10. *J. Cell Biol.* 138, 1289–1301
46 Dawe, R.K. *et al.* (1999) A maize homologue of the mammalian CENPC is a constitutive component of the inner kinetochore. *Plant Cell* 11, 1227–1238
47 Yu, H-G. *et al.* (1999) The maize homologue of the cell cycle checkpoint protein MAD2 reveals kinetochore substructure and contrasting mitotic and meiotic localization patterns. *J. Cell Biol.* 145, 425–435
48 Kaszas, E. and Birchler, J. (1998) Meiotic transmission rates correlate with physical features of rearranged centromeres in maize. *Genetics* 150, 1683–1692
49 Round, E.K. *et al.* (1997) *Arabidopsis thaliana* centromere regions: genetic map positions and repetitive DNA structure. *Genome Res.* 7, 1045–1053
50 Copenhaver, G.P. *et al.* (1999) Genetic definition and sequence analysis of *Arabidopsis* centromeres. *Science* 286, 2468–2474
51 Fransz, P.F. *et al.* (2000) Integrated cytogenetic map of chromosome arm 4S of *A. thaliana*: structural organization of heterochromatic knob and centromere region. *Cell* 100, 367–376
52 Rieder, C.L. and Salmon, E.D. (1998) The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* 8, 310–318
53 Chan, A. and Cande, W.Z. (1998) Maize meiotic spindles assemble around chromatin and do not require paired chromosomes. *J. Cell Sci.* 111, 3507–3515
54 West, C.E. *et al.* (2000) *Arabidopsis* DNA ligase IV is induced by γ irradiation and interacts with an *Arabidopsis* homologue of the double strand break repair protein XRCC4. *Plant J.* 24, 67–78

Physiological mechanisms influencing plant nitrogen isotope composition

R. Dave Evans

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.

R. Dave Evans
University of Arkansas Stable Isotope Laboratory, Biological Sciences, University of Arkansas, Fayetteville, AR 72701, USA.
e-mail: devans@uark.edu

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[1]. Anthropogenic activity has altered the amount and relative abundance of the forms of nitrogen (NH_4^+, NO_3^- and amino acids) that are available for plant absorption[1,2]. The forms of nitrogen absorbed by plants can have different isotope compositions[3], and many studies now routinely measure foliar $\delta^{15}N$ (Box 1) in an attempt to understand differences in patterns of nitrogen use among co-occurring species. Many studies assume that ^{15}N 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 $\delta^{15}N$ of leaf tissues reflects that of the nitrogen source in the soil). This assumption is important because although the reported variation in plant $\delta^{15}N$ 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 ^{15}N. This review addresses how physiological transformations of nitrogen can influence whole-plant and leaf $\delta^{15}N$. Ecological aspects of $\delta^{15}N$ measurements and $\delta^{15}N$ of plant nitrogen sources are addressed in Refs 3,5.

Mechanisms of NH_4^+ and NO_3^- uptake and assimilation

The three steps of NH_4^+ and NO_3^- acquisition are:

- Uptake of nitrogen from the soil solution.
- Translocation to the site of assimilation.
- Assimilation of inorganic nitrogen into organic nitrogen[6].

 1360-1385/01/$ – see front matter PII: S1360-1385(01)01889-1

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):

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

where $\delta^{15}N$ is the isotope ratio relative to the atmospheric air standard, and R_{sample} and $R_{standard}$ are the molar ratios of the heavier to the lighter isotope. The value for $R_{standard}$ is 0.0036765. Differences in $\delta^{15}N$ between a substrate and product will occur when ^{15}N and ^{14}N react at different rates. The ratio of the rate constants (k^{14}/k^{15}) is the isotope effect (α), and is equivalent to ($R_{substrate}/R_{product}$) (Ref. a). Discrimination (Δ) is the deviation of α from unity ($\Delta = \alpha - 1$)(Ref. a). Discrimination can be stated in relation to the $\delta^{15}N$ of the substrate ($\delta^{15}N_s$) and product ($\delta^{15}N_p$) (Eqn II):

$$\Delta = 1000\left(\frac{\delta^{15}N_s - \delta^{15}N_p}{1 + \frac{\delta^{15}N_p}{1000}}\right) \quad [II]$$

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

$$\Delta = \delta^{15}N_s - \delta^{15}N_p \quad [III]$$

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

Reference

a Farquhar, G.D. *et al.* (1989) Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Mol. Biol.* 40, 503–537

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 $\delta^{15}N$ of the product to be less than that of the substrate, but this will also cause the $\delta^{15}N$ of the remaining substrate to increase over time (Fig. I). This causes a corresponding increase in the $\delta^{15}N$ of the next increment of product, therefore the $\delta^{15}N$ of both substrate and product will increase over time. It is important to note that because of mass balance, the $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ will not change over time. The isotope effect will always be expressed, and the $\delta^{15}N$ 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[a], or by growing plants in containers with large volumes so that plant uptake does not alter solution concentration.

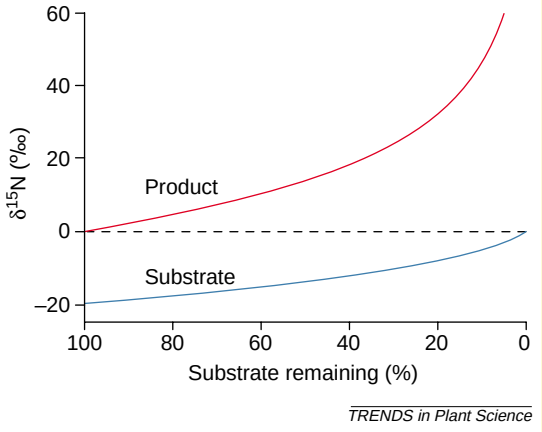

Fig. I. The $\delta^{15}N$ of accumulated product and remaining substrate in a closed nutrient system. The $\delta^{15}N$ of the initial substrate is 0‰, and fractionation during the reaction is 20‰. Isotope ratios were calculated using the equations given in Ref. b.

References

a Smart, D.R. and Bloom, A.J. (1993) Relationships between kinetics of NH_4^+ and NO_3^- absorption and growth in the cultivated tomato (*Lycopersicon esculentum* Mill. cv T-5). *Plant Cell Environ.* 16, 259–267

b Mariotti, A. *et al.* (1981) Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. *Plant Soil* 62, 413–430

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

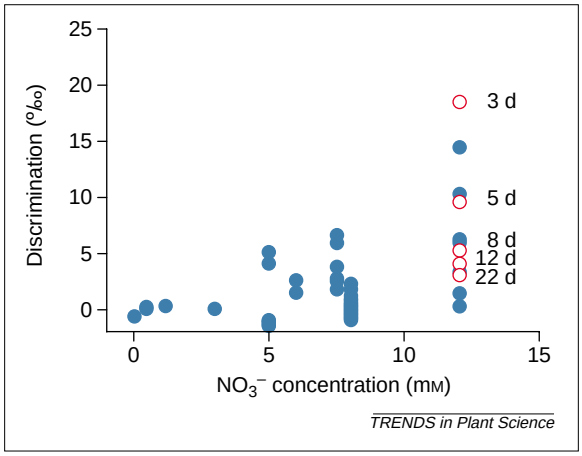

Fig. 1. Discrimination observed with NO_3^- uptake in studies[9,12,15,24,36,37] using open nutrient systems (blue filled circles). Unfilled red circles represent plants of different ages grown at 12 mM NO_3^- (Ref. 12). Abbreviation: d, days.

result, NH_4^+ is seldom present in the xylem[6]. Nitrate assimilation can occur in either the roots or leaves by the nitrate reductase–nitrite reductase pathway. The product of this assimilation event is NH_4^+, which subsequently enters the GS–GOGAT pathway.

Fractionation with NH_4^+ and NO_3^- uptake and assimilation

Significant discrimination has been observed for plants grown on NO_3^- using open nutrient systems (Box 2). A general pattern is that the discrimination increases with external NO_3^- concentration and decreases with plant age (Fig. 1). Similar patterns have been observed for plants grown with NH_4^+ as their sole nitrogen source. For example, discrimination was not significantly different from 0‰ for *Lycopersicon esculentum* grown at 50 μM NH_4^+ (Ref. 9). Discrimination varied from 0.9 to 5.8‰ for *Pinus sylvestris* grown with 4.6 mM NH_4^+ (Ref. 10), and the observed discrimination for *Oryza sativa* grown at 1.4 mM NH_4^+ and 7.3 mM NH_4^+ was 4.1‰ and 12.6‰, respectively[11].

Two trends emerge from the data in Fig. 1 and comparable studies of plants grown on NH_4^+. 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 observed 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]:

Uptake Assimilation

$$(N)_{out} \underset{F_2}{\overset{F_1}{\rightleftarrows}} (N)_{in} \xrightarrow{F_3} (N)_{assimilated} \qquad [1]$$

where $(N)_{out}$ and $(N)_{in}$ are the inorganic nitrogen forms (NO_3^-, NH_4^+) outside and inside of the cell, respectively, and $N_{assimilated}$ is assimilated nitrogen. F_1, F_2, and F_3 are the influx, efflux and assimilation rates of nitrogen, respectively. $F_1 = F_2 + F_3$ under steady-state conditions.

The underlying assumption of this model is that nitrogen enters into a pool (N_{in}) that is being assimilated into organic nitrogen. Nitrate reductase and glutamine synthetase both fractionate against ^{15}N; observed discrimination by nitrate reductase and glutamine synthetase is 15‰ and 17‰, respectively[4,13]. This fractionation event causes the N_{in} to become enriched in ^{15}N compared with N_{out}, whereas the organic nitrogen product is depleted in ^{15}N. Discrimination is observed when nitrogen enriched in ^{15}N is lost from N_{in} through efflux (F_2 in Eqn 1).

Influx and efflux associated with an enzyme-substrate system can be described by an equation modified from Tom Sharkey and Joe Berry[14] (Eqn 2):

$$\Delta = b - \left(b\frac{F_3}{F_1}\right) \qquad [2]$$

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 $\delta^{15}N$ of the plant and nitrogen in solution will occur if the rate of uptake exceeds assimilation, so that efflux of enriched nitrogen occurs from (N_{in}). This model assumes steady-state conditions and ignores compartmentation of inorganic N within the root or efflux of compounds other than NH_4^+ and NO_3^-, yet it demonstrates the relationship between nitrogen availability and demand in controlling discrimination.

Experiments with *Brassica campestris* and *L. esculentum* support this general model[9,15]. In both cases, plant $\delta^{15}N$ values were not significantly different than the $\delta^{15}N$ of the source NO_3^-, even though the $\delta^{15}N$ of internal NO_3^- 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 NO_3^- (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 Robinson[16] for plants grown on NO_3^- predicts that plant $\delta^{15}N$ might deviate from the nitrogen source if there is a substantial efflux of

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*

Species	Source	Plant	Root	Root NO_3^-	Leaf	Leaf NO_3^-	Refs
Brassica	10.3	10.1	4.9	12.4	10.6	25.0	15
Lycopersicon	1.8 ± 0.1	2.5 ± 0.4	–0.1 ± 0.4	11.1 ± 1.7	3.3 ± 0.6	14.0 ± 4.6	9

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 with their host plants has 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 to aspartic 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 fungi values were enriched 4–6‰, compared with soil NH_4^+ on sites with low nitrogen availability. It was concluded[23] 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.

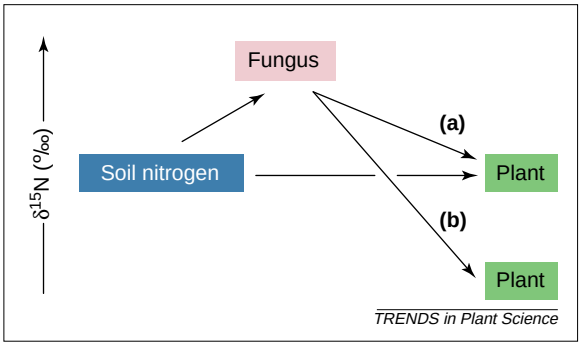

Fig. 2. Potential influences of mycorrhizae on plant $\delta^{15}N$. Field and modeling studies[21–23] suggest that when soil inorganic nitrogen is abundant, plants will acquire little nitrogen from mycorrhizae and should reflect the $\delta^{15}N$ of the inorganic nitrogen source [arrow (a)]. Plants 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)].

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‰ 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

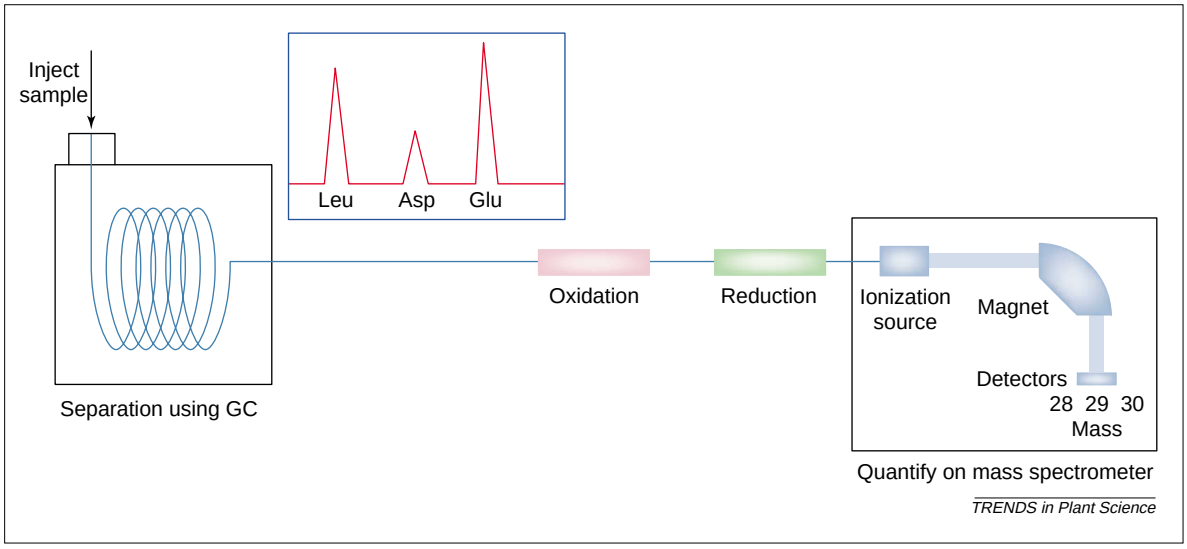

Fig. 3. Individual compounds for $\delta^{15}N$ analysis can be separated and analyzed by coupling a gas chromatograph (GC) to a mass spectrometer. They are then carried in a helium stream through oxidation and reduction reactors that convert nitrogen in each compound to N_2. The N_2 is then analyzed for isotope composition on the mass spectrometer.

actively growing plants[25]. Attempts have been made to measure the $\delta^{15}N$ of volatilized nitrogen, but levels of NH_3 were below limits of detection[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[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[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[25,26]. A recent model[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[26–28] are compelling. The $\delta^{15}N$ of nitrogen in phloem is lower than that of xylem[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[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*[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[8]; genotypes can have nitrate reductase present only in roots, shoots or both. Discrimination should not be observed if nitrate reductase is absent

Acknowledgements
This work was supported by grants from the National Science Foundation (Awards IBN 97-23913, DEB 98-14510 and DEB 98-14358) and the NRI Competitive Grants Program/USDA (Award 94-01759). Sharon Billings, Jim Ehleringer, Debbie LeRoux-Swarthout, David Robinson and Cindy L. Sagers provided helpful comments on the manuscript.

from the root unless there is an efflux of organic nitrogen, and multiple assimilation events should result in greater intra-plant variation in $\delta^{15}N$.

Future prospects

Progress has been made in understanding the mechanisms that control plant and foliar $\delta^{15}N$. It is clear that plant $\delta^{15}N$ is not a tracer of nitrogen source; instead, it provides a synthesis of the $\delta^{15}N$ 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[16] provide valuable tools to identify processes that could control plant $\delta^{15}N$, but more thorough, theoretical models such as those developed for plant carbon isotope composition[31] are necessary. Future research must also be directed at quantifying the $\delta^{15}N$ 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 ^{15}N (Ref. 3). A more thorough understanding of fractionations that occur within plants is also necessary. Controlled studies with NH_4^+ and NO_3^- 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[32–35]. 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 $\delta^{15}N$, they also have the potential to provide valuable insight into plant nitrogen dynamics.

References

1 Vitousek, P.M. (1994) Beyond global warming: ecology and global change. *Ecology* 75, 1861–1876
2 Evans, R.D. and Belnap, J. (1999) Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. *Ecology* 80, 150–160
3 Robinson, D. (2001) $\delta^{15}N$ as an integrator of the nitrogen cycle. *Trends Ecol. Evol* 16, 153–162
4 Handley, L.L. and Raven, J.A. (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant Cell Environ.* 15, 965–985
5 Högberg, P. (1997) Tansley review no. 95: ^{15}N natural abundance in soil–plant systems. *New Phytol.* 137, 179–203
6 Bloom, A.J. (1988) Ammonium and nitrate as nitrogen sources for plant growth. *ISI Atlas of Science* 1, 55–59
7 Ullrich, W.R. (1992) Transport of nitrate and ammonium through plant membranes. In *Nitrogen Metabolism of Plants* (Mengel, K. and Pilbeam, D.J., eds), pp. 121–137, Clarendon Press
8 Warner, R.L. and Huffaker, R.C. (1989) Nitrate transport is independent of NADH and NAD(P)H nitrate reductases in barley seedlings. *Plant Physiol.* 91, 947–953
9 Evans, R.D. *et al.* (1996) Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill. cv. T-5) grown under ammonium or nitrate nutrition. *Plant Cell Environ.* 19, 1317–1323
10 Högberg, P. *et al.* (1999) Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal *Pinus sylvestris*. *New Phytol.* 142, 569–576
11 Yoneyama, T. *et al.* (1991) Fractionation of nitrogen isotopes during the uptake and assimilation of ammonia by plants. *Plant Cell Physiol.* 32, 1211–1217
12 Mariotti, A. *et al.* (1982) Nitrogen isotope fractionation associated with nitrate reductase activity and uptake of NO_3^- by pearl millet. *Plant Physiol.* 69, 880–884
13 Yoneyama, T. *et al.* (1993) Fractionation of nitrogen isotopes by glutamine synthetase isolated from spinach leaves. *Plant Cell Physiol.* 34, 489–491
14 Sharkey, T.D. and Berry, J.A. (1985) Carbon isotope fractionation of algae as influenced by an inducible CO_2 concentrating mechanism. In *Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms* (Lucas, W.J. and Berry, J.A., eds), pp. 389–401, American Society of Plant Physiologists
15 Yoneyama, T. and Kaneko, A. (1989) Variations in the natural abundance of ^{15}N in nitrogenous fractions of komatsuna plants supplied with nitrate. *Plant Cell Physiol.* 30, 957–962
16 Robinson, D. *et al.* (1998) A theory for $^{15}N/^{14}N$ fractionation in nitrate-grown vascular plants. *Planta* 205, 397–406
17 Taylor, A.F.S. *et al.* (1997) Natural ^{15}N abundance in fruit bodies of ectomycorrhizal fungi from boreal forests. *New Phytol.* 136, 713–720
18 Gebauer, G. and Dietrich, P. (1993) Nitrogen isotope ratios in different compartments of a mixed stand of spruce, larch and beech trees and of understory vegetation including fungi. *Isotopenpraxis* 29, 35–44
19 Högberg, P. *et al.* (1996) ^{15}N abundance of surface soils, roots and mycorrhizas in profiles of European forest soils. *Oecologia* 108, 207–214
20 Macko, S.A. *et al.* (1986) Kinetic fractionation of stable nitrogen isotopes during amino acid transamination. *Geochimica et Cosmochimica Acta* 50, 2143–2146
21 Hobbie, E.A. *et al.* (1999) Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. *Oecologia* 118, 353–360
22 Hobbie, E.A. *et al.* (1999) Interpretation of nitrogen isotope signatures using the NIFTE model. *Oecologia* 120, 405–415
23 Hobbie, E.A. *et al.* (2000) Correlations between foliar $\delta^{15}N$ and nitrogen concentrations may indicate plant-mycorrhizal interactions. *Oecologia* 122, 273–283
24 Bergersen, F.J. *et al.* (1988) Isotopic discriminations during the accumulation of nitrogen by soybeans. *Aus. J. Plant Physiol.* 15, 407–420
25 Shearer, G. and Kohl, D.H. (1986) N_2-fixation in field settings: estimations based on natural ^{15}N abundance. *Aus. J. Plant Physiol.* 13, 699–756
26 Yoneyama, T. *et al.* (1998) Natural abundance of ^{15}N in amino acids and polyamines from leguminous nodules: unique ^{15}N enrichment in homospermidine. *J. Exp. Bot.* 49, 521–526
27 Yoneyama, T. *et al.* (1997) Variations of the natural abundances of nitrogen and carbon isotopes in *Triticum aestivum*, with special reference to phloem and xylem exudates. *New Phytol.* 137, 205–213
28 Yoneyama, T. *et al.* (2000) Weather and nodule mediated variations in $\delta^{13}C$ and $\delta^{15}N$ values in field-grown soybean (*Glycine max* L.) with special interest in the analyses of xylem fluids. *J. Exp. Bot.* 51, 559–566
29 Handley, L.L. *et al.* (1997) Shoot $\delta^{15}N$ correlates with genotype and salt stress in barley. *Planta* 201, 100–102
30 Robinson, D. *et al.* (2000) Using stable isotope natural abundances ($\delta^{15}N$ and $\delta^{13}C$) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes. *J. Exp. Bot.* 51, 41–50
31 Farquhar, G.D. *et al.* (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aus. J. Plant Physiol.* 9, 121–137
32 Chapin, F.S. *et al.* (1993) Preferential use of organic nitrogen for growth by a non-mycorrhizal sedge. *Nature* 361, 150–153
33 Raab, T.K. *et al.* (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides*: implications for the alpine nitrogen cycle. *Oecologia* 108, 488–494
34 Näsholm, T. *et al.* (1998) Boreal forest plants take up organic nitrogen. *Nature* 392, 914–916
35 Kielland, K. (1994) Amino acid absorption by Arctic plants: implications for plant nutrition and nitrogen cycling. *Ecology* 75, 2373–2383
36 Kohl, D.H. and Shearer, G. (1980) Isotopic fractionation associated with symbiotic N_2 fixation and uptake of NO_3^- by plants. *Plant Physiol.* 66, 51–56
37 Mariotti, A. *et al.* (1980) Fractionnements isotopiques de l'azote lors des processus d'absorption des nitrates et de fixation de l'azote atmospherique par les plantes. *Physiol. Veget.* 18, 163–181