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A theory for $^{15}N/^{14}N$ fractionation in nitrate-grown vascular plants

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Abstract. We present a theory describing how the $\delta^{15}N$ values of the nitrogen (N) pools in a vascular plant depend on that of its source N (nitrate), on $^{15}N/^{14}N$ fractionations during N assimilation, and on N transport within and N loss from the plant. The theory allows measured $\delta^{15}N$ values to be interpreted in terms of physiological processes. The $\delta^{15}N$ values of various N pools are calculated using three rules: (1) when a pool divides without transformation, there is no change in the $\delta^{15}N$ values of the N entering the resulting pools; (2) when nitrate is assimilated by nitrate reductase, the $\delta^{15}N$ values of the resulting pools (product and residual substrate) are described by a Rayleigh equation; (3) when two N pools mix, the $\delta^{15}N$ value of the mixture is a weighted average of the $\delta^{15}N$ values of the component pools. The theory is written as a spreadsheet and solved numerically. Potentially, it has multiple solutions. Some contravene physiological reality and are rejected. The remainder are distinguished, where possible, using additional physiological information. The theory simulated independent measurements of $\delta^{15}N$ in N pools of *Brassica campestris* L. var. *rapa* (komatsuna) and *Lycopersicon esculentum* Mill. cv. T-5 (tomato).

Key words: $^{15}N/^{14}N$ – Nitrate assimilation – Nitrogen isotope fractionation

Introduction

There is considerable genotypic and environmental variation among the $\delta^{15}N$ values of whole plants, plant parts and their constituent nitrogen (N) pools (Handley and Scrimgeour 1997). Variations in $\delta^{15}N$ values of leaves or whole plants have often been thought to reflect the chemical sources of N used by those plants, i.e. ^{15}N natural abundances have been assumed to be reliable tracers of N pools. However, it is now clear that ^{15}N is usually a poor natural tracer of N pools. One reason for this is that the $\delta^{15}N$ values of potential N sources probably overlap, varying temporally and spatially in unknown ways.

Evidence that variations in plant $\delta^{15}N$ values reflect something more than the $\delta^{15}N$ values of their N sources comes from experiments in which different plants are grown on the same N source. The $\delta^{15}N$ values of the roots and shoots of such plants may be very different. The $\delta^{15}N$ of their total N may differ from the $\delta^{15}N$ of the source. This was found by Mariotti et al. (1982), Yoneyama et al. (1991) and Evans et al. (1996) in *Pennisetum* spp. (pearl millet), *Oryza sativa* L. (rice) and *Lycopersicon esculentum* Mill. (tomato), respectively. Sometimes, there is no difference in $\delta^{15}N$ between a plant and its N source. This was found for *Brassica campestris* L. (komatsuna) by Yoneyama and Kaneko (1989). Environmental factors may influence the $\delta^{15}N$ values of plants even though the $\delta^{15}N$ value of the source N is constant. This has been observed in *Hordeum vulgare* L. (cultivated barley) and *H. spontaneum* C. Koch (wild barley) subjected to salinity (Handley et al. 1997). None of these observations can be explained by a simple N-source model.

A more realistic and inevitably more complex view is now emerging. It is that plant $\delta^{15}N$ values reflect interactions between N sources and metabolic $^{15}N/^{14}N$ fractionations. The $\delta^{15}N$ values of N sources, the effects of metabolic processes on $\delta^{15}N$ values, and the relative influences of source and metabolism on a plant's $\delta^{15}N$ value may all vary. The relation between a plant's $\delta^{15}N$ value and that of an external N source may, therefore, be complex and non-intuitive. A plant's $\delta^{15}N$ value is ultimately some function of all the source and metabolic effects to which its N pools have been subjected, i.e. it is a time- and mass-weighted average. Previously, this

Abbreviations: α = rate constant for ^{14}N-nitrate reduction/rate constant for ^{15}N-nitrate reduction; $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \cdot 10^3‰$, where R_{sample} is the $^{15}N/^{14}N$ ratio of a sample and $R_{standard}$ is that of atmospheric N_2 (0.0036765); NR = nitrate reductase

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function was largely implicit (see Handley and Scrimgeour 1997). Attempts to make it more explicit (e.g. Mariotti et al. 1982) considered only the influences of the $\delta^{15}N$ value of source N and $^{15}N/^{14}N$ fractionations occurring during the assimilation of that N. The transport of N within plants and losses of N from them were ignored. Here, we describe a theory describing how the $\delta^{15}N$ value of a plant and its N pools depend on all of these factors.

Description of the theory

Background. There are several reasons why $\delta^{15}N$ values of N pools in vascular plants vary. (1) Plants growing in soil have access to many N sources (nitrate, ammonium, organic N, N_2 in the case of species symbiotic with diazotrophic prokaryotes). (2) These sources vary in amount, and in $^{15}N/^{14}N$ composition, both of which may be influenced by the plants themselves. (3) The acquisition of N by plants is often mediated by microbes, e.g. mycorrhizal fungi. (4) Nitrogen acquisition may involve the partial removal of available N from soil close to root surfaces, so altering its effective concentration at the point of uptake. (5) Assimilated N effluxed from roots may change the $\delta^{15}N$ value of plant-available N in the soil. (6) Sites of $^{15}N/^{14}N$ fractionation during N assimilation may be in roots, shoots or both, depending on species, environmental conditions and N source. (7) Nitrogen may, to some extent, remain unassimilated and mix with N in pools that have been subjected to some $^{15}N/^{14}N$ fractionation during assimilation. (8) Assimilated N may be transported between roots and shoots, so that products of one assimilation event may become mixed with others of different $\delta^{15}N$ value.

Here, we concentrate on plant processes (i.e. 6–8 in the above list), which do not involve soil. Process 5 is included insofar as N efflux influences plant $\delta^{15}N$ values, but it is assumed not to alter those of soil N pools. We assume that the plant has access to only one external N source, nitrate.

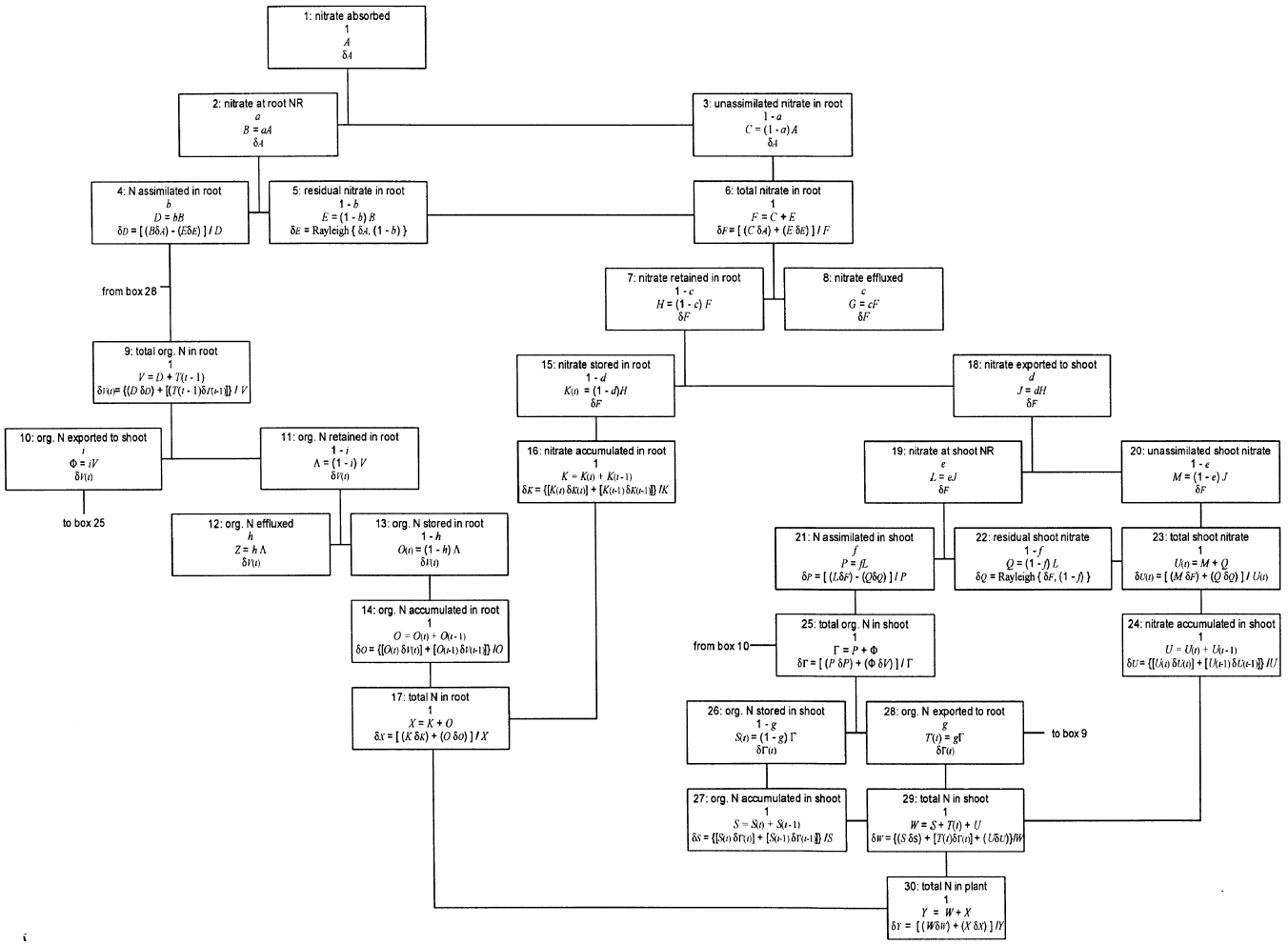

Fig. 1. Structure of the theory. Each box represents a pool of N. It contains: a description of the pool; the fraction of the preceding pool that has entered the current one; the amount of N in the current pool and how it is calculated; the $\delta^{15}N$ value of the pool and how it is calculated. Symbols are defined and the equations are listed in Table 1

Structure. The $\delta^{15}N$ values of N pools are assumed to vary according to three rules. (1) When a pool divides without transformation, there is no change in the $\delta^{15}N$ values of the N entering the resulting pools. This reflects there being negligible $^{15}N/^{14}N$ fractionation during

transport among pools (Handley and Raven 1992). (2) When nitrate is assimilated by nitrate reductase (NR), the $\delta^{15}N$ values of the resulting N pools (product and residual nitrate) are described by a Rayleigh equation (see below). (3) When two pools mix, the $\delta^{15}N$ value of the mixture is a weighted average, i.e. an isotope mass balance (Handley and Scrimgeour 1997), of the $\delta^{15}N$ values of the component pools.

Thirty sets of equations (shown diagramatically in Fig. 1, and listed in Table 1) describe the application of these rules as N is absorbed, assimilated and transported within a plant. These equations calculate the sizes and $\delta^{15}N$ values of the resulting N pools. From these, mean $\delta^{15}N$ values of roots, shoots and the whole plant are calculated as weighted averages.

Table 1. Definition of the symbols and a listing of the equations used in the 30 boxes of Fig. 1. Upper case Italic and Greek symbols (A, Γ, etc.) are the sizes (mol) of N pools. Lower case Italic symbols (a, b, etc.) are variable fractions of N pools transferred to other pools. Symbols including "δ" are the $\delta^{15}N$ values (‰) of the N pools

Box	Description	Fraction	N (mol)	$\delta^{15}N$ (‰)
1.	nitrate absorbed	1	A	δ_A
2.	nitrate at root NR	a	$B = aA$	δ_A
3.	unassimilated nitrate in root	$1 - a$	$C = (1 - a)A$	δ_A
4.	N assimilated in root	b	$D = bB$	$\delta_D = \frac{B\delta_A - E\delta_E}{D}$
5.	residual nitrate in root	$1 - b$	$E = (1 - b)B$	$\delta_E = \left[\left(\frac{\delta_A}{10^3} + 1\right)(1 - b)^{\left(\frac{1}{\alpha} - 1\right)} - 1\right] \cdot 10^3$
6.	total nitrate in root	1	$F = C + E$	$\delta_F = \frac{C\delta_A + E\delta_E}{F}$
7.	nitrate retained in root	$1 - c$	$H = (1 - c)\,F$	δ_F
8.	nitrate effluxed	c	$G = cF$	δ_F
9.	total organic N in root	1	$V = D + T_{(t-1)}$	$\delta_{V(t)} = \frac{D\delta_D + T_{(t-1)}\delta_{\Gamma(t-1)}}{V}$
10.	organic N exported to shoot	i	$\Phi = iV$	$\delta_{V(t)}$
11.	organic N retained in root	$1 - i$	$\Lambda = (1 - i)V$	$\delta_{V(t)}$
12.	organic N effluxed	h	$Z = h\Lambda$	$\delta_{V(t)}$
13.	organic N stored in root	$1 - h$	$O_{(t)} = (1 - h)\,\Lambda$	$\delta_{V(t)}$
14.	organic N accumulated in root	1	$O = O_{(t)} + O_{(t-1)}$	$\delta_O = \frac{O_{(t)}\delta_{V(t)} + O_{(t-1)}\delta_{V(t-1)}}{O}$
15.	nitrate stored in root	$1 - d$	$K_{(t)} = (1 - d)H$	δ_F
16.	nitrate accumulated in root	1	$K = K_{(t)} + K_{(t-1)}$	$\delta_K = \frac{K_{(t)}\delta_{K(t)} + K_{(t-1)}\delta_{K(t-1)}}{K}$
17.	total N in root	1	$X = K + O$	$\delta_X = \frac{K\delta_K + O\delta_O}{X}$
18.	nitrate exported to shoot	d	$J = dH$	δ_F
19.	nitrate at shoot NR	e	$L = eJ$	δ_F
20.	unassimilated nitrate in shoot	$1 - e$	$M = (1 - e)J$	δ_F
21.	N assimilated in shoot	f	$P = fL$	$\delta_P = \frac{L\delta_F - Q\delta_Q}{P}$
22.	residual nitrate in shoot	$1 - f$	$Q = (1 - f)L$	$\delta_Q = \left[\left(\frac{\delta_F}{10^3} + 1\right)(1 - f)^{\left(\frac{1}{\alpha} - 1\right)} - 1\right] \cdot 10^3$
23.	total nitrate in shoot	1	$U_{(t)} = M + Q$	$\delta_{U(t)} = \frac{M\delta_F + Q\delta_Q}{U_{(t)}}$
24.	nitrate accumulated in shoot	1	$U = U_{(t)} + U_{(t-1)}$	$\delta_U = \frac{U_{(t)}\delta_{U(t)} + U_{(t-1)}\delta_{U(t-1)}}{U}$
25.	total organic N in shoot	1	$\Gamma = P + \Phi$	$\delta_{\Gamma(t)} = \frac{P\delta_P + \Phi\delta_V}{\Gamma}$
26.	organic N stored in shoot	$1 - g$	$S_{(t)} = (1 - g)\Gamma$	$\delta_{\Gamma(t)}$
27.	organic N accumulated in shoot	1	$S = S_{(t)} + S_{(t-1)}$	$\delta_S = \frac{S_{(t)}\delta_{S(t)} + S_{(t-1)}\delta_{S(t-1)}}{S}$
28.	organic N exported to root	g	$T_{(t)} = g\Gamma$	$\delta_{\Gamma(t)}$
29.	total N in shoot	1	$W = S + T_{(t)} + U$	$\delta_W = \frac{S\delta_S + T_{(t)}\delta_{\Gamma(t)} + U\delta_U}{W}$
30.	total N in plant	1	$Y = W + X$	$\delta_Y = \frac{W\delta_W + X\delta_X}{Y}$

Each box in Fig. 1 represents a pool of N and contains four items: (1) a description of the pool; (2) the fraction of the preceding pool that has entered the current one; (3) the amount of N in the current pool and how it is calculated; (4) the $\delta^{15}N$ value of the pool and how it is calculated (see also Table 1). Flows from pools are assumed to be, at most, dichotomous, i.e. N in any pool has only one or two immediate destinations. Where two destinations are possible, defining how much goes to one pool, also defines the amount which goes to the other.

The plant's nitrate source has a fixed $\delta^{15}N$ value, δ_A (box 1, Fig. 1; Table 1). A moles of it are absorbed in each time step, t. Nitrate absorbed by roots has two immediate fates. It may reach sites of NR in roots (box 2) or it may not (box 3). Root nitrate is a mixture (box 6) of that in box 3 and any nitrate which reaches root NR, but which, during the time interval t, remains unreduced (box 5). Root nitrate is liable to be effluxed from the root (box 8), exported to the shoot via xylem transport (box 18) or stored in the root cells, mainly in vacuoles (box 15).

When a substrate is converted into a product during an enzyme-catalyzed reaction, the $\delta^{15}N$ values of both can be described by a Rayleigh equation (Mariotti et al. 1981, 1988; Hoefs 1987, p. 11). For example, when nitrate is reduced by NR in roots, the $\delta^{15}N$ value (δ_E) of the substrate remaining after a fraction b of the substrate pool has been assimilated is given by:

$$\delta_E = \left[\left(\frac{\delta_A}{10^3} + 1\right)(1-b)^{\left(\frac{1}{\alpha}-1\right)} - 1\right] \cdot 10^3$$

In box 5, the term "Rayleigh $\{\delta_A, (1-b)\}$" is shorthand for the above equation, δ_A and $(1-b)$ being the inputs appropriate for nitrate reduction by root NR. α is the ratio of the rate constant for ^{14}N-nitrate reduction to that for ^{15}N-nitrate reduction (see Handley and Raven 1992). The $\delta^{15}N$ value (δ_D) of the N assimilated via root NR is calculated by an isotope mass balance (box 4). (Alternatively, δ_D could be calculated directly by an equation equivalent to that shown above: Hoefs 1987, p. 11). Similar logic is applied to N assimilation in shoots (boxes 21 and 22, Table 1), with appropriate inputs.

Strictly, a Rayleigh equation describes isotopic changes in "closed" reactions where a finite supply of substrate is consumed and a product accumulates. Its use here assumes that, in the limit, nitrate reduction occurs as a closed reaction during a single, discrete time-step, t. No new substrate enters the pool being reduced, and reduced N and unreduced nitrate do not move into other pools. Integrated over many such time steps, however, the net process comes to approximate the true "open" system. The total fractionation in a series of related reactions is not the sum of their individual fractionations, but approximates that of the most rate-limiting step(s) (O'Leary 1988). A Rayleigh equation can give an approximate description of isotopic fractionations in a series of complex processes if there is a particularly rate-limiting step, even in open systems. So, for example, Mariotti et al. (1988) used a Rayleigh equation to describe and interpret $^{15}N/^{14}N$ fractionations during denitrification in an aquifer. We assume that nitrate reduction by NR is more rate-limiting than the reduction of nitrite and ammonium, the first and second products of nitrate assimilation, by nitrite reductase and glutamine synthetase, respectively.

The assumption that nitrate reduction is the only isotopic "branch point" at which significant $^{15}N/^{14}N$ fractionations can occur is probably true as far as the $\delta^{15}N$ values of total N are concerned. It is not necessarily true for individual N-containing molecules. When N is incorporated into amino acids and proteins, large $^{15}N/^{14}N$ fractionations may occur which are detectable at the molecular level (Yoneyama 1995). Such effects remain hidden at organ or whole-plant levels. Inter- and intramolecular variations in $\delta^{15}N$ are subsumed in $\delta^{15}N$ measurements of "total N" and so are not the concern of this theory. The importance of other branch points, relative to that at NR, is unclear. Suppose, for example, it were shown that organic N transported from shoots to roots had a $\delta^{15}N$ value significantly different from that of organic N remaining in the shoot. That would argue for the inclusion in our theory of $^{15}N/^{14}N$ fractionations prior to the phloem loading of amino acids. The small amount of available data indicates little difference in $\delta^{15}N$ between total N in leaves and in phloem (Yoneyama et al. 1997).

At any time, the organic N in the root is a mixture of that assimilated by root NR (box 4) and some exported to the root from the shoot via phloem transport. Nitrate is probably not phloem-mobile (Peuke et al. 1996). Initially (i.e. at $t = 1$), there is no contribution of shoot-derived organic N in roots (box 9). Later, some organic N is exported from shoot to the root (box 28). Equally, some of the organic N in roots may be exported to the shoot via xylem transport (box 10). This represents a cycling of organic N between root and shoot (Cooper and Clarkson 1989; Larsson 1992).

Organic N in roots that is not exported to the shoot (box 11) may enter storage pools (box 13) or be effluxed as, for example, amino-N (box 12). As with nitrate efflux (box 8), we assume no effect of effluxed organic N on the $\delta^{15}N$ of externally available nitrate.

The organic N which accumulates in roots over successive time intervals will be a mixture of root- and shoot-derived molecules, possibly differing in $\delta^{15}N$ value. To account for this, a time-averaged $\delta^{15}N$ is calculated for root organic N (box 14). The same is done for root nitrate (box 16), shoot nitrate (box 24) and shoot organic N (box 27). This also allows for any temporal changes in the $\delta^{15}N$ of externally available nitrate to be reflected in the integrated $\delta^{15}N$ of plant N pools.

The total pool of organic N in shoots (box 25) is a mixture of that assimilated by shoot NR (box 21) and that exported from the root (box 10). The mixture may be stored in the shoot (box 26) or exported to the root (box 28).

From the resulting amounts and time- and pool-weighted $\delta^{15}N$ values of total N (nitrate plus organic N) in roots (box 17) and shoots (box 29), the total amount of N in the whole plant and its $\delta^{15}N$ value are obtained (box 30). If no N is effluxed from the plant, whole-plant $\delta^{15}N$ must equal that of the absorbed nitrate, i.e. there is

Table 2. $\delta^{15}N$ values (‰) of various N pools measured by Yoneyama and Kaneko (1989) and Evans et al. (1996) in nitrate-grown *Brassica campestris* L. var. *rapa* (komatsuna) and *Lycopersicon esculentum* Mill. cv. T-5 (tomato), respectively. The data for komatsuna are averaged over several treatments in which the amounts and rates of nitrate supply varied; "organic N" is the average of $\delta^{15}N$ values given for amino acids plus "residue". The data for tomato are averaged over a 45-d growth period. "Shoot" data for both species are those reported originally for leaves

N pool		Komatsuna		Tomato	
		Mean	SE	Mean	SE
Nitrate source	δ_A	+10.3	0.0	+1.8	0.0
Total N, whole plant	δ_Y	+10.3	0.07	+2.5	0.04
Total N, shoot	δ_W	+10.5	0.16	+3.4	1.0
Total N, root	δ_X	+5.6	0.42	+0.1	0.7
Nitrate, shoot	δ_U	+26.1	0.60	+14.0	4.6
Organic N, shoot	δ_S	+6.6	0.43	+2.7	0.5
Nitrate, root	δ_K	+12.4	n.d.	+11.1	1.7
Organic N, root	δ_O	+3.7	0.63	−1.8	0.5

no net isotopic discrimination between the plant's N source and whole-plant N.

Solutions. Figure 1 exists as a Microsoft Excel™ 5.0 spreadsheet, available from d.robinson@scri.sari.ac.uk on request. Solutions are found numerically using Excel's Solver program. The inputs required are, first, the $\delta^{15}N$ of absorbed nitrate (δ_A) and the amount of nitrate absorbed (A). If only the $\delta^{15}N$ values of plant N pools, and not their absolute sizes, are of interest, any positive value of A will still generate correct $\delta^{15}N$ values. Then, relative sizes of N pools are calculated. Parameters δ_A and A are usually constants.

The second input required is α which has been measured to be, maximally, ≈1.017, i.e. +17‰ against ^{15}N-nitrate (see the values collated by Handley and Raven 1992); α is assumed to be constant.

Solutions to the equations in Fig. 1 are found by varying the values of the third group of inputs, the fractions a to i. Each of these has a value between 0 and 1. If information is available to constrain any to a narrower range, this reduces the number of possible solutions. Such information may come from experimental data or be imposed by biological reality. For example, neither c nor h (nitrate and organic N efflux, respectively) can equal 1 in a living plant. The variables a to i are, in principle, independent of one another, but this is not strictly true. For example, if no nitrate reaches root NR (i.e. $a = 0$), then the fraction of that nitrate which is assimilated in roots (b) cannot be anything other than 0. Further limits may be imposed, depending on the availability of suitable data. For example, if $\delta^{15}N$ values of roots and shoots are known, Solver can constrain the values of δ_X and δ_W accordingly; the same limitation can be applied to any variable.

As with all numerical methods, it is sensible to solve the equations repeatedly, using different initial input values to check if a solution is unique. This is done by assigning initial values of a to i at random each time the Solver program is executed. Solver adjusts the values of a to i repeatedly until a combination is found which, via the equations in Fig. 1, produces the match with observed $\delta^{15}N$ values to within a defined tolerance. The solutions obtained are for a steady-state, after temporal changes in $\delta^{15}N$ values caused by the build-up of N cycling between shoot and root have ceased. Less than ten iterations are usually required to reach this steady-state. Some initial combinations of a to i generate errors which abort the program. This is because the method by which Solver converges on a solution can, given certain initial inputs, diverge from any stable solution.

Testing the theory

The test of the theory is that it should reproduce, from biologically plausible inputs, the $\delta^{15}N$ values of the N pools for which data are available. If neither criterion is met (i.e. if no combination of calculated $\delta^{15}N$ values matches the observed $\delta^{15}N$ values, or if the variable inputs – the fractions a to i – do not reflect the physiology of the plants under investigation), then the theory is falsified. To illustrate the theory's applicability, we use data from experiments in which plants were grown hydroponically with only nitrate of constant $\delta^{15}N$ as an N source (Yoneyama and Kaneko 1989; Evans et al. 1996).

Komatsuna. Yoneyama and Kaneko (1989) supplied *Brassica campestris* var. *rapa* (komatsuna) with nitrate at various concentrations from 0.2 to 12 mM, with a $\delta^{15}N$ value of +10.3‰ (Table 2). The theory was able to simulate the $\delta^{15}N$ values of whole komatsuna plants, shoots and roots, and of N pools within these organs, as averaged across the treatments, to within ±1%. Specimen solutions (i.e. values of the fractions a to i which simulate the data) from 20 successful simulations are shown in Table 3 (a "successful" simulation is one which yielded no error values and converged on a solution within 100 s). So, the first criterion of the theory's validity is satisfied: it simulated experimental data. However, for these data, the theory did not produce a unique solution, i.e. more than one combination of a to i produced the same simulation.

The variation among solutions was large (usually ≥20%: Table 3). This does not reflect rounding errors nor those produced as different trajectories converged with a finite tolerance on the same solution. Rather, the variations suggest that solutions are discrete and that it is inappropriate merely to average these, the resulting combination of a to i then falling between genuine

Table 3. Specimen solutions from 20 simulations of nitrate assimilation by komatsuna. Each simulation involved a different, randomly generated combination of initial values for the variable fractions *a* to *i* (defined in Table 1). The values shown in this table are those for *a* to *i* which reproduce Yoneyama and Kaneko's (1989) data (see Table 2) to within 1%. Mean values of *a* to *i* are also shown, together with the coefficient of variation (c.v. = 100 × standard deviation/mean). The results shown in bold are those judged most likely to have applied to the plants in Yoneyama and Kaneko's experiment

Variable fraction	Solution																				Mean	c.v. (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
a	0.28	0.28	0.28	0.28	**0.58**	0.29	0.04	0.28	0.21	0.28	0.28	0.40	0.36	0.31	0.31	0.33	0.28	1.00	0.28	1.00	0.37	64
b	0.81	0.81	0.81	0.81	**0.20**	0.81	0.71	0.81	0.55	0.54	0.81	0.32	0.40	0.81	0.81	0.40	0.81	0.12	0.81	0.11	0.61	42
c	0.05	0.03	0.13	0.96	**0.12**	0.20	0.90	0.03	0.22	0.09	0.30	0.02	0.21	0.79	0.24	0.11	0.90	0.03	0.21	0.82	0.32	107
d	0.98	0.98	0.98	0.98	**0.98**	0.98	0.52	0.98	0.27	0.99	0.98	0.98	0.98	0.98	0.98	0.98	0.99	0.99	0.99	1.00	0.92	20
e	0.84	0.84	0.84	0.84	**0.86**	0.83	0.90	0.84	0.85	0.86	0.84	0.85	0.85	0.83	0.83	0.86	0.85	0.86	0.83	0.94	0.85	3
f	0.89	0.89	0.89	0.89	**0.92**	0.89	0.92	0.89	0.24	0.92	0.88	0.92	0.92	0.89	0.89	0.92	0.90	0.92	0.89	0.99	0.87	17
g	0.00	0.00	0.00	0.00	**0.36**	0.00	0.09	0.00	0.36	0.27	0.00	0.33	0.39	0.00	0.00	0.34	0.00	0.38	0.00	1.00	0.18	145
h	0.09	0.02	0.31	0.99	**0.36**	0.50	0.71	0.02	0.48	0.42	0.63	0.00	0.55	0.94	0.51	0.34	0.98	0.09	0.60	0.99	0.48	70
i	0.75	0.81	0.66	0.03	**0.84**	0.64	0.12	0.77	0.48	0.90	0.56	0.88	0.78	0.17	0.61	0.83	0.08	0.95	0.68	0.51	0.60	48

solutions. It is necessary to inspect all solutions and decide which combination of *a* to *i* is the most plausible.

We note that *e*, the fraction of nitrate exported from the root which reaches NR in the shoot, and *f*, the fraction of that nitrate assimilated by shoot NR, were relatively constant across the 20 solutions in Table 3; *e* and *f* varied by only 3 and 17% around means of 0.85 and 0.87, respectively. This suggests unique solutions for these variables. This conforms to expectations. Leaves are sites of vigorous nitrate reduction when young, well-illuminated plants are grown on nitrate (Smirnoff and Stewart 1985; Andrews 1986). That both *e* and $f \approx 1$ is a good indication that the theory is realistic. Had *e* and *f* been consistently ≈ 0, instead, that would have cast doubt on the extent to which the theory reflected known facts about N assimilation in nitrate-grown vascular plants. The small variability in *e* and *f* also means that they cannot be used to distinguish plausible solutions from the implausible. For that, we must look to variations in the other seven variable fractions in Table 3.

Several of the solutions in Table 3 can be rejected as being physiologically unlikely. For example, those which predicted zero export of organic N from shoot to root ($g = 0$) are unrealistic. It would be unusual for phloem elements to contain no nitrogenous solutes. We can eliminate solutions 1–4, 6, 8, 11, 14, 15, 17 and 19 from further consideration (they are, with small differences, essentially the same solution, but reached from different initial conditions). It would be equally unlikely for all the organic N in shoots to be transported to roots ($g = 1$): this eliminates solution 20.

To reduce the possibilities even further, extra information about the N metabolism of komatsuna is needed. This comes from Yoneyama et al. (1987). They reported nitrate efflux amounting to ≈10% of root nitrate (i.e. $c \approx 0.1$). In addition, the amount of nitrate reduced in komatsuna roots is ≤20% of that reduced in shoots (Yoneyama et al. 1987). So, *b* should be ≈0.2, as both *e* and *f* were close to 1 (see above). Solution number 5 in Table 3 meets all of these conditions. By applying the constraints that $b < 0.3$ and $c = 0.1$, the coefficients of variation for all fractions (apart from *h*, the fraction of organic N effluxed) were only < 5% (data not shown). This indicates that solution 5 in Table 3 has few plausible alternatives. When unconstrained, the model found this solution (number 5) among others; when constrained using the extra physiological information, it converged on only this solution.

If solution number 5 in Table 3 does reflect the physiology of komatsuna plants, it predicts several interesting things. The first is a substantial efflux of organic N from roots ($h = 0.36$). This process was not included in Yoneyama et al.'s (1987) otherwise comprehensive model of N partitioning among N pools in komatsuna. Our analysis indicates that the efflux of organic N from komatsuna roots could have been significant. Handley and Scrimgeour (1997) noted that few studies consider the influence of organic N efflux on plant $\delta^{15}N$ values. The second is a large export of organic N from root to shoot ($i = 0.84$). Measurements of organic N flux in the xylem and the $\delta^{15}N$ value of N in

Table 4. $\delta^{15}N$ values (‰) of N pools in komatsuna plants measured by Yoneyama and Kaneko (1989) and calculated by solution number 5 (Table 3) of the theory. Theoretical values in bold were matched to the experimental values (within ±1%). n.d. = not determined

		Experiment	Theory
Nitrate absorbed	δ_A	**+10.3**	**+10.3**
Nitrate at root NR	δ_A	n.d.	+10.3
Unassimilated nitrate in root	δ_A	n.d.	+10.3
Nitrate assimilated in root	δ_D	n.d.	−4.8
Residual nitrate remaining unassimilated in root	δ_E	n.d.	+14.1
Total nitrate in root	δ_F	**+12.4**	**+12.3**
Nitrate effluxed from root	δ_F	n.d.	+12.3
Nitrate retained in root	δ_F	n.d.	+12.3
Nitrate exported from root to shoot	δ_F	n.d.	+12.3
Nitrate stored and accumulated in root	δ_K	n.d.	+12.3
Nitrate at shoot NR	δ_F	n.d.	+12.3
Unassimilated nitrate in shoot	δ_F	n.d.	+12.3
Organic N stored and accumulated in root	δ_O	**+3.7**	**+3.6**
Nitrate assimilated in shoot	δ_P	n.d.	+8.5
Residual nitrate remaining unassimilated in shoot	δ_Q	n.d.	+56.2
Organic N stored and accumulated in shoot	δ_S	**+6.6**	**+6.7**
Organic N exported from shoot to root	δ_Γ	n.d.	+6.7
Total nitrate stored and accumulated in shoot	δ_U	**+26.1**	**+26.4**
Total organic N in root	δ_V	n.d.	+3.9
Total N in shoot	δ_W	**+10.5**	**+10.6**
Total N in root	δ_X	**+5.6**	**+5.5**
Total N in whole plant	δ_Y	**+10.3**	**+10.3**
Organic N effluxed from root	δ_V	n.d.	+3.9
Organic N exported from root to shoot	δ_V	n.d.	+10.6
Total organic N in shoot	δ_Γ	n.d.	+6.7
Organic N retained in root	δ_V	n.d.	+3.9

xylem sap would allow this export to be quantified. The third is a huge export of nitrate from root to shoot ($d = 0.98$), again quantifiable from analyses of xylem sap. Table 4 lists, for komatsuna, the $\delta^{15}N$ values calculated by solution number 5 (Table 3) for all the N pools shown in Fig. 1.

Tomato. Evans et al. (1996) grew *Lycopersicon esculentum* cv. T-5 (tomato) at a nitrate concentration of 0.05 mM. This concentration is unexceptional for solutions isolated from unfertilized soils, but far more dilute than those used for the commercial cultivation of tomato (e.g. >10 mM). The nitrate supplied to the plants had a $\delta^{15}N$ value of +1.8‰. Although Evans et al. claimed that their plants had the same $\delta^{15}N$ value as the source, they reported that the former was + 2.5 ± 0.04‰ (Table 2). The 0.7‰ enrichment in ^{15}N in whole plants relative to source is significant at $P < 0.001$, as a *t*-test indicates. It is unusual to find whole plants more enriched in ^{15}N than their source. When it has been reported, the most likely explanation is an unsuspected change in the $\delta^{15}N$ of the source N (Robinson and Conroy 1998). This probably does not explain Evans et al.'s data. Their hydroponic system added nitrate of constant $\delta^{15}N$ value to the nutrient solution in response to N absorption by the plants. Moreover, microbial contaminants were filtered from the solution, minimizing the likelihood of denitrification and the ^{15}N enrichment of residual nitrate that would result (Mariotti et al. 1988; Robinson and Conroy 1998). However, without measurements of the $\delta^{15}N$ value of nitrate in the solution in which the tomato plants were grown (as well as of the fresh solution), it is impossible to confirm that they had access only to nitrate whose $\delta^{15}N = +1.8‰$ throughout. Assuming that they did, we used our theory to simulate the tomato data (Table 2) to see if there could be a physiological explanation for the unexpected ^{15}N enrichment of tomato plants.

The simulations were carried out as for komatsuna, with appropriate inputs. Specimen solutions are shown in Table 5. As before, the simulations did not converge on a unique solution. Five of the nine variable fractions (*a, b, d, e* and *f*) had small variations around their means (c.v. <10%). These describe the assimilation of nitrate in roots and shoots, and the export of nitrate in the xylem. Cultivated tomato is a voracious nitrate assimilator and, apparently, rather inflexible in the extent to which it varies nitrate reduction between roots and shoots. In contrast to komatsuna, nitrate reduction in tomato roots was almost as powerful as in the shoot (a and $b \geq 0.77$; e and $f \geq 0.86$). This might be because of the weak concentration of nitrate with which the plants were supplied. As external concentration increases, proportionately less nitrate is reduced in roots and more exported to shoots (Smirnoff and Stewart 1985; Andrews 1986; Yoneyama and Kaneko 1989; Pueke et al. 1996).

Distinguishing among the possible solutions in Table 5 again depends on additional information. Evans et al. reported negligible nitrate efflux from tomato roots. Several solutions predict this ($c = 0$, solutions 3–6, 10, 13, 19 and 20). Solutions 4–6, 13 and 19 can be eliminated because they predict zero export of organic N from shoots ($g = 0$). This leaves only solutions 3, 10 and 20. Without further information, there is nothing to decide

Table 5. Specimen solutions from 20 simulations of nitrate assimilation by tomato. Each simulation involved a different, randomly generated combination of initial values for the variable fractions *a* to *i* (defined in Table 1). The values shown in this table are those for *a* to *i* which reproduce Evans et al.'s (1996) data (see Table 2) to within 1%. Mean values of *a* to *i* are also shown, together with the coefficient of variation (c.v. = 100 × standard deviation/mean). The results shown in bold are those judged most likely to have applied to the plants in Evans et al.'s experiment

Variable fraction	Solution																			Mean	c.v. (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
a	0.77	0.77	**0.72**	0.78	0.78	0.78	0.78	0.78	0.72	**0.75**	0.77	0.75	0.78	0.78	0.78	0.78	0.78	0.78	0.78	**0.72**	0.77	2.9
b	0.91	0.92	**0.75**	0.92	0.92	0.92	0.92	0.92	0.85	**0.89**	0.92	0.89	0.92	0.92	0.92	0.92	0.92	0.92	0.92	**0.79**	0.90	5.4
c	0.58	0.84	**0.00**	0.00	0.00	0.00	0.01	0.46	0.05	**0.00**	0.08	0.37	0.00	0.43	0.21	0.30	0.18	0.38	0.00	**0.00**	0.19	126
d	0.89	0.91	**0.93**	0.88	0.88	0.88	0.88	0.88	0.91	**0.90**	0.88	0.91	0.88	0.88	0.88	0.88	0.88	0.88	0.88	**0.92**	0.89	2.1
e	0.85	0.88	**0.90**	0.86	0.84	0.86	0.85	0.85	0.90	**0.87**	0.84	0.91	0.84	0.85	0.87	0.83	0.85	0.88	0.83	**0.90**	0.86	2.8
f	1.00	1.00	**1.00**	0.99	0.99	0.99	0.99	0.99	1.00	**0.99**	0.99	1.00	0.99	0.99	0.99	0.99	0.99	1.00	0.99	**1.00**	0.99	0.1
g	0.23	0.34	**0.53**	0.00	0.00	0.00	0.02	0.00	0.40	**0.24**	0.06	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.49**	0.13	143
h	0.87	0.96	**0.46**	0.45	0.45	0.45	0.46	0.79	0.51	**0.46**	0.53	0.79	0.45	0.78	0.64	0.71	0.61	0.76	0.45	**0.46**	0.60	28
i	0.23	0.09	**0.67**	0.50	0.50	0.50	0.50	0.27	0.58	**0.57**	0.47	0.37	0.50	0.28	0.39	0.36	0.41	0.32	0.50	**0.65**	0.43	34

among these. They are, in any case, very similar to one another. Interestingly, each predicts the same substantial efflux of organic N ($h = 0.46$). The efflux of organic N with a $\delta^{15}N$ value more negative than that of the source nitrate was not considered by Evans et al. as a possible cause of their plants' $\delta^{15}N$ values. Some loss from the plants of ^{15}N-depleted N must have occurred. This is the only way that they could have become more enriched in ^{15}N than the external N source, in the absence of nitrate efflux. The theory predicted that the $\delta^{15}N$ of the effluxed organic N (δ_V) would have been $\approx -1.5‰$ (Table 6); the $\delta^{15}N$ of organic N in whole root tissue (δ_O) was measured to be $-1.8‰$ (Table 2). Table 6 lists, for tomato, the $\delta^{15}N$ values calculated by solution number 3 (Table 5) for all the N pools shown in Fig. 1.

Discussion

The theory which we have described was not falsified by its comparison with independent experimental data. Therefore, we believe it to be a realistic description of $^{15}N/^{14}N$ fractionation in vascular plants grown with nitrate as a sole N source. Although the amount of data needed for rigorous, repeated tests of the theory is limited, its concordance with that which is available is encouraging. Experiments with the explicit aim of testing the theory's predictions are now in progress in our laboratory.

The main purpose of the theory is to interpret measured $\delta^{15}N$ values. However, it alone cannot provide definitive interpretations. Background knowledge of a particular plant's N metabolism and ecology must guide the theory's use. Given suitable data, the theory can suggest which explanations are most likely to be correct and worthy of closer examination. It seems that shoot and root $\delta^{15}N$ values alone will not be adequate to distinguish between alternative solutions to the theory. Raven's (1987) view that "the more that is known about other details of the process, the more useful are the δ values" certainly applies here. We suggest that the most useful item to measure, in addition to the $\delta^{15}N$ values of total N in shoot and root (and of nitrate isolated from them), is the $\delta^{15}N$ of total N in xylem sap (and of nitrate isolated from it). That information would allow δ_v and δ_F to be specified, reducing the number of possible solutions. Measuring rates of nitrate reduction in leaves is not likely to be useful in this context, because the relevant variable fractions were always similar, in both komatsuna and tomato. It is difficult to measure the export of N from shoot to root in many species. Obtaining good data for organic N efflux is also problematical. We anticipate that our theory may allow these processes to be estimated from measurements of $\delta^{15}N$ in N pools which are easier to measure, i.e. total N and nitrate in leaves, roots and xylem.

Alternatively, the theory can be used to explore the consequences for plant $\delta^{15}N$ values of changes in specific processes. For example, suppose we wished to know whether the magnitude of nitrate efflux was likely to influence shoot $\delta^{15}N$. This could be tested by constraining

Table 6. $\delta^{15}N$ values (‰) of N pools in tomato plants measured by Evans et al. (1996) and calculated by solution number 3 (Table 5) of the theory. Theoretical values in bold were matched to the experimental values (within ±1%). n.d. = not determined

		Experiment	Theory
Nitrate absorbed	δ_A	**+1.8**	**+1.8**
Nitrate at root NR	δ_A	n.d.	+1.8
Unassimilated nitrate in root	δ_A	n.d.	+1.8
Nitrate assimilated in root	δ_D	n.d.	−5.3
Residual nitrate remaining unassimilated in root	δ_E	n.d.	+28.0
Total nitrate in root	δ_F	**+11.1**	**+11.0**
Nitrate retained in root	δ_F	n.d.	+11.0
Nitrate exported from root to shoot	δ_F	n.d.	+11.0
Nitrate stored and accumulated in root	δ_K	n.d.	+11.0
Nitrate at shoot NR	δ_F	n.d.	+11.0
Unassimilated nitrate in shoot	δ_F	n.d.	+11.0
Organic N stored and accumulated in root	δ_O	**−1.8**	**−1.5**
Nitrate assimilated in shoot	δ_P	n.d.	+10.6
Residual nitrate remaining unassimilated in shoot	δ_Q	n.d.	+110
Organic N stored and accumulated in shoot	δ_S	**+2.7**	**+2.6**
Organic N exported from shoot to root	δ_Γ	n.d.	+2.6
Total nitrate stored and accumulated in shoot	δ_U	**+14.0**	**+14.2**
Total organic N in root	δ_V	n.d.	−1.5
Total N in shoot	δ_W	**+3.4**	**+3.4**
Total N in root	δ_X	**+0.1**	**+0.1**
Total N in whole plant	δ_Y	**+2.5**	**+2.5**
Organic N effluxed from root	δ_V	n.d.	−1.5
Organic N exported from root to shoot	δ_V	n.d.	−1.5
Total organic N in shoot	δ_Γ	n.d.	+2.6
Organic N retained in root	δ_V	n.d.	−1.5

the value of c to, for example, 0.1, 0.5 and 0.9 in successive runs, and observing how the value of δ_W varied.

The tolerance to which the theory simulated the komatsuna and tomato data was set, arbitrarily, at ± 1% of each measured $\delta^{15}N$ value. Another way of setting tolerances is to use the analytical precision with which the $\delta^{15}N$ values were determined. These are likely to differ among N pools. For example, the $\delta^{15}N$ of total N in plant tissues can be determined routinely to within < 0.2‰ (Scrimgeour and Robinson 1998). However, those of plant nitrate and of external N sources may be reliable to within only 1‰.

Several further developments to the theory are needed. The first is to find an objective way of minimising the number of solutions for a particular simulation and to assess whether any of the variables in Fig. 1 can be ignored, making the theory more compact. Cluster analysis seems to be a promising candidate for the first of these tasks. It is not yet possible to say if any of the variables included in the theory have so little effect on the convergence to a solution that they can be ignored. Those describing nitrate reduction in shoots might seem good candidates for exclusion because they hardly vary. However, what this really means is that the $\delta^{15}N$ values of N pools "downstream" from nitrate reduction in shoots are very sensitive to the values of d, e and f. Only relatively small changes in them are required for the model to converge on, or diverge from, a possible solution. While Fig. 1 appears unwieldy, it makes the physiological processes in the theory transparent. The convenience of reducing Fig. 1 into a single, approximate equation [cf. Farquhar et al.'s (1982) theory for $\delta^{13}C$ in C_3 species] would entail the loss of some of this transparency.

The second is to apply the theory to $\delta^{15}N$ data for a wider range of species and for plants grown in environments likely to affect nitrate acquisition and assimilation. Various abiotic stresses (e.g. salinity, drought, N starvation) produce genotype-specific changes in root and shoot $\delta^{15}N$ values (Handley et al. 1997, and unpublished data). These changes are now interpretable physiologically using our theory, as are several observations from non-isotopic studies. These observations include the following. (1) Salinity increases nitrate efflux from *Hordeum vulgare* cv. Kikaihadaka (Yamashita and Matsumoto 1996) and nitrate reduction in *Ricinus communis* L. (castor bean) roots (Peuke et al. 1996). (2) Osmotic stress diminishes nitrate reduction in *Triticum aestivum* L. cv. Drabant (wheat) roots, but not in shoots (Larsson 1992). (3) As the concentration of external nitrate decreases, so do those of nitrate in roots and shoots of komatsuna (Yoneyama and Kaneko 1989). Our theory should be able to indicate, from the $\delta^{15}N$ values measured in experiments similar to these, whether some plants are especially prone to respond to stress in these or in other ways.

The third development is to produce a parallel theory describing ammonium assimilation. The "ammonium only" version of the theory is largely embedded within that shown in Fig. 1. The assimilation of absorbed ammonium occurs almost exclusively in roots (Raven and Smith 1976). Ammonium is also assimilated in leaves as a step in the assimilation of nitrate and, in even larger amounts, during photorespiration (Wallsgrove et al. 1983). None of these processes is likely to cause large $^{15}N/^{14}N$ fractionations at the organ or whole-plant levels. The strong affinity of glutamine synthetase for ammonium results in near-complete in-vivo assimilation. This leaves little, if any, residual substrate to accumulate to influence whole-plant $\delta^{15}N$ values. We hypothesize that differences in $\delta^{15}N$ between roots and shoots of

ammonium-grown plants reflect mainly N effluxes and the export between roots and shoots of organic N rather than any $^{15}N/^{14}N$ fractionations associated with ammonium assimilation per se.

The fourth development is to extend the theory from its restriction to nitrate-grown, vegetative plants, to include N metabolism during reproductive development (e.g. grain filling in cereal crops) and of perennial species. The latter are particularly interesting as a significant fraction of their annual N budget comes not from the assimilation of soil-derived N, but from the remobilization of N from internal stores (Millard 1996). We hypothesize that if internal N remobilization precedes primary N assimilation, the $\delta^{15}N$ values of organic N stored and accumulated in roots and shoots (boxes 14 and 27, Fig. 1) in one season will initially dominate that of N in new roots and leaves produced in the following season.

The final development is to apply the theory to soil-grown plants. The main problems to be overcome are: (1) isolating nitrate, ammonium and available organic N from soil solutions without $^{15}N/^{14}N$ fractionation and in sufficient quantity for reliable $\delta^{15}N$ determinations (Scrimgeour and Robinson 1998); (2) dealing with $\delta^{15}N$ values of source N pools which change with plant size, are induced by the plants themselves and depend on environmental conditions (Robinson and Conroy 1998); (3) incorporating effects of soil microbes on plant $\delta^{15}N$ values (Handley and Scrimgeour 1997). It may then be possible to use $\delta^{15}N$ measurements to study ecological aspects of whole-plant N metabolism in ways which do not assume that ^{15}N natural abundances are tracers of N sources – $\delta^{15}N$ values are, potentially, much more informative than that.

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References

Andrews M (1986) The partitioning of nitrate assimilation between root and shoot of higher plants. Plant Cell Environ 9: 511–519

Cooper HD, Clarkson DT (1989) Cycling of amino-nitrogen and other nutrients between shoots and roots in cereals – a possible mechanism integrating shoot and root in the regulation of nutrient uptake. J Exp Bot 40: 753–762

Evans RD, Bloom AJ, Sukrapanna SS, Ehleringer JR (1996) Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill. cv. T-5) grown under ammonium or nitrate nutrition. Plant Cell Environ 19: 1317–1323

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9: 121–137

Handley LL, Raven JA (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell Environ 15: 65–985

Handley LL, Robinson D, Forster, BP, Ellis RP, Scrimgeour CM, Gordon DC, Nevo E, Raven JA (1997) Shoot $\delta^{15}N$ correlates with genotype and salt stress in barley. Planta 201: 100–102

Handley LL, Scrimgeour CM (1997) Terrestrial plant ecology and ^{15}N natural abundance: the present limits to interpretation for uncultivated systems with original data from a Scottish old field. Adv Ecol Res 27: 133–212

Hoefs J (1987) Stable isotope geochemistry, 3rd edn. Springer, Berlin

Larsson M (1992) Translocation of nitrogen in osmotically stressed wheat seedlings. Plant Cell Environ 15: 447–453

Mariotti A, Germon JC, Hubert P, Kaiser P, Letolle R, Tardieux A, Tardieux P (1981) Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. Plant Soil 62: 413–430

Mariotti A, Mariotti F, Champigny M-L, Amarger N, Moyse A (1982) Nitrogen isotope fractionation associated with nitrate reductase activity and uptake of nitrogen by pearl millet *Pennisetum* spp. Plant Physiol 69: 880–884

Mariotti A, Landreau A, Simon B (1988) ^{15}N isotope biogeochemistry and natural denitrification process in groundwater: application to the chalk aquifer of northern France. Geochim Cosmochim Acta 52: 1869–1878

Millard P (1996) Ecophysiology of the internal cycling of nitrogen for tree growth. Z Pflanzenernähr Bodenkd 159: 1–10

O'Leary MH (1988) Carbon isotopes in photosynthesis. BioScience 38: 328–336

Pueke AD, Glaab J, Kaiser WM, Jeschke WD (1996) The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L. IV. Flow and metabolism of inorganic nitrogen and malate depending on nitrogen nutrition and salt treatment. J Exp Bot 47: 377–385

Raven JA (1987) The application of mass spectrometry to biochemical and physiological studies. In: Davies DD (ed) The biochemistry of plants, vol 13, Methodology. Academic Press, London, pp 127–180

Raven JA, Smith FA (1976) Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytol 76: 415–431

Robinson D, Conroy JP (1998) A possible plant-induced feedback between elevated atmospheric CO_2 and denitrification identified using $\delta^{15}N$. Soil Biol Biochem, in press

Scrimgeour CM, Robinson D (1998) Stable isotope analysis and applications. In: Smith KA, Cresser MS (eds) Soil and environmental analysis, 3rd edn. Marcel Dekker, New York, in press

Smirnoff N, Stewart GR (1985) Nitrate assimilation and translocation by higher plants: comparative physiology and ecological consequences. Physiol Plant 64: 133–140

Wallsgrove RM, Keys AJ, Lea PJ, Miflin BJ (1983) Photosynthesis, photorespiration and nitrogen metabolism Plant Cell Environ 6: 301–309

Yamashita K, Matsumoto H (1996) Salt stress-induced enhancement of anion efflux and anion transport activity in plasma membrane of barley roots. Soil Sci Plant Nutr 42: 209–221

Yoneyama T (1995) Nitrogen metabolism and fractionation of nitrogen isotopes in plants. In: Wada E, Yoneyama T, Minagawa M, Ando T, Fry B (eds) Stable isotopes in the biosphere. Kyoto University Press, Kyoto. pp 92–102

Yoneyama T, 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

Yoneyama T, Yamada H, Yamagata M, Kouchi H (1987) Nitrate reduction and partitioning of nitrogen in komatsuna (*Brassica campestris* L. var. *rapa*) plants: compartmental analysis in combination with ^{15}N tracer experiments. Plant Cell Physiol 28: 679–696

Yoneyama T, Omata T, Nakata S, Yazaki J (1991) Fractionation of nitrogen isotopes during the uptake and assimilation of ammonia by plants. Plant Cell Physiol 32: 1211–1217

Yoneyama T, Handley LL, Scrimgeour CM, Fisher DB, Raven JA (1997) Variations of the natural abundances of nitrogen and carbon isotopes in *Triticum aestivum* L. with special reference to phloem and xylem exudates. New Phytol 137: 205–213