morphs of the intertidal snail *Littorina saxatilis*. *Evolution* 49, 1180–1190
31 Johannesson, K. *et al*. (2000) Hybrid fitness seems not to be an explanation for the partial reproductive isolation between ecotypes of Galician *Littorina saxatilis*. *J. Moll. Stud*. 66, 149–156
32 McCune, A.R. and Lovejoy, N.R (1998) The relative rate of sympatric and allopatric speciation in fishes: test using DNA sequence divergence between sister species and among clades. In *Endless Forms – Species and Speciation* (Howard, D.J. and Berlocher, S.H., eds), pp. 172–185, Oxford University Press
33 Bush, G.L. and Smith, J.J. (1998) The genetics and ecology of sympatric speciation: a case study. *Res. Popul. Ecol*. 40, 175–187
34 Galis, F. and Metz, A.J. (1998) Why are there so many cichlid species? *Trends Ecol. Evol*. 13, 1–2
35 Feder, J.L. (1998) The apple maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation? In *Endless Forms – Species and Speciation* (Howard, D.J. and Berlocher, S.H., eds), pp. 130–144, Oxford University Press
36 Simpson, G.G. (1944) *Tempo and Mode in Evolution*, Columbia University Press

δ^{15}N as an integrator of the nitrogen cycle

David Robinson

Natural abundances of the rare stable isotope of nitrogen, ^{15}N, are now being used widely in research on N cycling in organisms and ecosystems. ^{15}N natural abundances are used in fundamentally different ways from traditional ^{15}N tracers by integrating N cycle processes via N isotope fractionations and the mixing of various N-containing pools. This approach of using ^{15}N natural abundances still requires certain technical and conceptual advances before it can be used routinely in ecological research. Here, the basis of the natural abundance ^{15}N approach and opportunities for applying it in ecology are reviewed, and recent progress towards overcoming some of the key technical problems and in revealing large-scale patterns in N cycle processes is discussed.

Nitrogen (N) cycling is a fundamental ecological process, gaining in prominence because of concerns about the impact of excess N on ecosystems[1] and the contribution of gases, such as N_2O, to global warming[2,3]. Although traditionally probed with ^{15}N-enriched (ISOTOPIC) TRACERS (see Glossary; Ref. 4; Table 1), the N cycle is being studied increasingly by measuring NATURAL ABUNDANCES of the rare stable ISOTOPE ^{15}N relative to that of the more abundant ^{14}N (δ^{15}N: Box 1). The cost of ^{15}N enriched tracers (currently ~£200 g^{-1} N) and analysis (~£10 per sample) limits their use when heavy replication or large-scale measurements are required. Nevertheless, ^{15}N tracers are now being used to study N cycling in forests and catchments to great effect[5–7].

For many years, it was hoped that expensive ^{15}N-enriched tracers could be avoided by exploiting δ^{15}N as a 'natural' means to trace specific N SOURCES (Table 2) in biological systems. This idea has been applied extensively to detect and quantify N derived from the fixation (via the nitrogenase enzyme) of atmospheric N_2. By definition, the δ^{15}N of atmospheric N_2 is 0‰ (Box 1). Therefore, δ^{15}N of plants with access to N_2 should also be close to 0‰. If δ^{15}N of other N sources (e.g. soil NH_4^+ and NO_3^-) to which the plants also have access are different from 0‰, a MIXING MODEL (Box 2) can be used to estimate how much N the plants obtain from N_2. This technique can work reasonably well in some agricultural settings in which few potential N sources are available[8,9] (Fig. 1). It has a poor track record, however, in multi-species (especially woody) vegetation, where there is often uncertainty about the identity and δ^{15}N of the N sources potentially available to different species[10] (see Ref. 11, however, for an alternative view).

The Achilles heel of natural ^{15}N tracer approaches is that, if source N pools mix (Box 2), or ^{15}N and ^{14}N isotopes undergo fractionation (Box 3), differences in δ^{15}N among source N pools can be obliterated. This undermines the use of δ^{15}N as a tracer of any of those pools. The problem is compounded because it is impossible to predict when mixing or fractionation will occur, and it is difficult to measure these processes and the resulting δ^{15}N values of N sources *in situ*, leading to uncertainty about exactly what δ^{15}N measurements mean.

Only if there are differences in δ^{15}N among potential N sources can δ^{15}N be used as a tracer for any of those sources. This condition has been met in some ecological studies. For example, δ^{15}N has been used to detect the assimilation of atmospheric NH_3 by plants on a subantarctic island using the δ^{15}N of NH_3 derived from penguin guano (Fig. 2)[12]. Guano N was relatively ^{15}N enriched (δ^{15}N = +14.6‰), and the NH_3 derived from it was ^{15}N depleted (–10‰), giving an unusually wide δ^{15}N range between two potential N sources. The mean leaf δ^{15}N of plants growing on the penguin colony was +11‰, implying some plant assimilation of guano-derived soluble N (e.g. NH_4^+). In plants downwind of the colony, leaf δ^{15}N became progressively more ^{15}N depleted with distance from the colony, reaching minimum values of ~ –7‰ at upland sites, a pattern consistent with the vegetation at those sites intercepting and assimilating significant amounts of guano-derived, ^{15}N-depleted NH_3 from the atmosphere.

Another study[13] used δ^{15}N (with δ^{13}carbon and δ^{34}sulphur) to trace nutrients derived from municipal

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Table 1. ^{15}N tracer, $\delta^{15}N$ natural tracer and $\delta^{15}N$ integration approaches compared[a,b]

	^{15}N as a tracer	Refs	$\delta^{15}N$ as a natural tracer	Refs	$\delta^{15}N$ as an integrator	Refs
Range of ^{15}N abundance	Greater than natural abundance range		Within natural abundance range		Within natural abundance range	
System perturbation	Large		Small or zero		Zero	
Cost of tracer	Large		Zero		Zero	
Sensitivity of detection	Excellent		Poor to good		Poor to excellent	
Duration of study	<1 h to ~1 yr		⩾1 h to ~1 yr		Unlimited	
Scale of study	Pot, plot or small catchment		Pot to landscape		Molecular to global	
Conditions required	$\delta^{15}N$ of tracer > natural range, and steady-state labelling of sinks		Significant differences in $\delta^{15}N$ among all potential source pools		Reliable $\delta^{15}N$ measurements of main N sources	
Isotopic information required	$\delta^{15}N$ of tracer before addition to system, and of system N pools before and after tracer addition Amount of tracer added		$\delta^{15}N$ of all potential N sources, system containing no tracer, system pools containing tracer N fluxes among pools if more than two sources are involved		$\delta^{15}N$ and sizes of N pools measured on at least two occasions and/or in contrasting 'treatments' Isotope fractionation factors for main processes	
Other information required	The ecology, biology and history of the system being studied		The ecology, biology and history of the system being studied		The ecology, biology and history of the system being studied	
Interpretive model	Mixing		Mixing		Fractionation and mixing	
Information obtained	Amounts and rates of mixing of tracer N in nontracer pools		Amounts and rates of mixing of tracer N in nontracer pools		Quantitative identification of N cycle processes	
Examples of ecological applications	Interannual N cycling in grassland	41	Estimating N_2 fixation in ecologically simple systems	8,9	Detecting denitrification	23,24
	Plant uptake of organic N	42	Detecting NH_3 use by vegetation	12	Establishing global trends in plant and soil N cycling	15
	N cycling in catchments	5–7	Detecting pollutant transfer into food webs	13	Determining trophic relationships among soil invertebrates	38
					Identifying N inputs to, and detecting N losses, from ecosystems	22

[a]Ref. 43.

[b]Abbreviations: $\delta^{15}N$, the abundance of ^{15}N on the δ scale. Usually refers to the natural abundance of ^{15}N; N, nitrogen.

sewage into benthic fauna at a North Atlantic dumpsite. Measurable differences existed in $\delta^{15}N$ between sewage- and phytoplankton-derived organic matter, potential N sources for fauna such as the surface-deposit feeders *Echinus affinus* (a sea urchin) and *Benthodytes sanguinolenta* (a sea cucumber). These differences allowed the assimilation of sewage-derived material to be detected as a shift in faunal isotopic compositions, refuting earlier assessments that dumped sewage had minimal ecological impact.

$\delta^{15}N$ could be used as a tracer in these studies[12,13] only because large, verifiable differences in $\delta^{15}N$ existed among potential N sources. Yet, it is sobering to realize that it required the annual dumping of ~4000 tonnes of penguin excrement on a remote island[12] and of nine million tonnes of sewage into the Atlantic[13] to create N sources that were sufficiently distinct isotopically to make $\delta^{15}N$ tracing feasible in those systems. It is probably safest to assume as a default that $\delta^{15}N$ is not a good tracer for N sources in most circumstances. $\delta^{15}N$ can, however, be used in a different way, one that does not depend on it being a good tracer.

$\delta^{15}N$ as an integrator

The use of $\delta^{15}N$ as an 'integrator' of N cycle processes makes use of the fractionations of ^{15}N and ^{14}N, and N-pool mixing that can thwart attempts to exploit $\delta^{15}N$ as a tracer. It is based, essentially, on four rules (Box 4) that describe how $\delta^{15}N$ changes when N-containing pools are transformed, divided or mixed in any system.

Consider how the $\delta^{15}N$ of a whole plant (δ_{sink}) is related to that of its N source(s) (δ_{source}; Fig. 3). δ_{source} is the mean of the $\delta^{15}N$ values of all potential N sources, weighted by their availabilities (Eqn 3, Box 2). To a first approximation, δ_{sink} tracks δ_{source}: the more ^{15}N-enriched δ_{source}, the more ^{15}N-enriched δ_{sink}. But the relationship between δ_{source} and δ_{sink} is not constant; if it were, $\delta^{15}N$ would be a reliable tracer of N sources and that is not necessarily true.

Rule 1 describes what happens when a process that can fractionate N isotopes transforms an N pool. This rule allows the expression of N ISOTOPE FRACTIONATIONS to be flexible. Rather than assume that a specific process occurs with a certain amount of fractionation (ε), Rule 1 allows fractionation to vary between 0 and ε, depending on source supply and SINK demand, according to a RAYLEIGH MODEL (Box 3). Because of the

Box 1. Definitions and measurements of ^{15}N abundance

Of the N atoms on earth, 99.6337% are ^{14}N; the remaining 0.3663% are ^{15}N. These ATOM % ABUNDANCES (see Glossary: *A*) are defined as:

$$A = 100\left(\frac{n_{15}}{n_{15}+n_{14}}\right) \approx 100\left(\frac{R_{sample}}{R_{sample}+1}\right) \quad (1)$$

n_{15} and n_{14} are, respectively, the numbers of ^{15}N and ^{14}N atoms present in a sample. R_{sample} is the ^{15}N:^{14}N ISOTOPE RATIO (n_{15}:n_{14}). Equation 1 is used when *A* exceeds ~0.5 atom %, which applies to practically all studies in which ^{15}N is used as a tracer.

For convenience, isotope ratios in samples containing only slight ^{15}N enrichments, and in all natural abundance studies, are reported on the δ scale:

$$\delta^{15}N = 1000\left(\frac{R_{sample}-R_{standard}}{R_{standard}}\right) \quad (2)$$

The standard is atmospheric N_2 ($R = 0.0036765$, $\delta^{15}N = 0‰$). For most naturally occurring N containing materials, $-30‰ < \delta^{15}N < +30‰$ ($0.355 < A < 0.377$ atom%). The more ^{15}N-enriched a sample, the more positive (or less negative) its $\delta^{15}N$.

$\delta^{15}N$ of ecological materials (plants, animals, microbes, soil, waters, gases, etc.) is usually measured by a CONTINUOUS-FLOW ISOTOPE RATIO MASS SPECTROMETER (CF-IRMS)[a]. A sample (typically containing ~100 μg N) is combusted on-line in the analyzer of the CF-IRMS, reducing all forms of N in the sample to N_2, on which R_{sample} is determined. About 200 $\delta^{15}N$ results can be produced daily[a], sufficient for the statistical rigour required for ecological work. Analytical precisions better than ±0.2‰ can be achieved routinely[a] and, for that reason, $\delta^{15}N$ data should not be quoted to a precision better than ±0.1‰.

Reference

a Scrimgeour, C.M. and Robinson, D. Stable isotope analyses and applications. In *Soil and Environmental Analysis* (3rd edn) (Smith, K.A. and Cresser, M.S., eds), Marcel Dekker (in press)

Box Glossary

Atom % abundance: the percentage contribution of one isotope (e.g. ^{15}N) to the total number of atoms of that element in a sample. Usually refers to the abundance of a ^{15}N-enriched tracer, and symbolized as *A*.

Isotope ratio: the ratio of the number of heavy isotopes of an element to the number of light isotopes in a sample, for example, ^{15}N:^{14}N. Usually symbolized as *R*.

Continuous-flow isotope ratio mass spectrometer: an elemental analyzer coupled to a mass spectrometer designed to measure the isotope ratios of a few low molecular weight gases such as N_2 and CO_2. Often abbreviated to CF-IRMS.

differences between the closed physical systems for which Rayleigh models were developed and open biological systems, it is probably safer to apply Rule 1 over each of many infinitesimal time-intervals[14] than to expect it to apply over one long period during which many source–sink transitions would occur.

In our example (Fig. 3), suppose the plant has access to only one N source, NO_3^-, the assimilation of which incurs an N isotope fractionation (ε) of up to 19‰ (Box 3). During assimilation, organic N (the immediate sink for assimilated N) might, as a result of fractionation, become more ^{15}N depleted than the source NO_3^- (δ_{source}). If NO_3^- supply is ample relative to its assimilation rate ($f \sim 0$: Box 3), δ_{source} will remain almost constant and the $\delta^{15}N$ of residual NO_3^- in the plant will equal δ_{source}. If, however, most of the source NO_3^- is assimilated ($f \rightarrow 1$), residual NO_3^- becomes ^{15}N-enriched compared with δ_{source} (Box 3).

Table 2. N sources for various biological N sinks

Sinks	Sources
Microbes	NH_4^+, NO_3^-, and many forms of organic N including high molecular weight N compounds such as proteins; microbes such as cyanobacteria, rhizobia and actinomycetes that have the enzyme nitrogenase can fix N_2
Plants	NH_4^+, NO_3^-, and amino acids dissolved in soil solution or surrounding waters; NH_3 or N oxides intercepted by foliage from atmospheric deposition; the amounts of each form assimilated depends on its availability (external concentration and rate of supply, both of which vary with habitat) and on plant species; some land plants can access N from higher molecular weight sources (e.g. proteins) if associated with fungi able to form ecto- or ericoid mycorrhizas; other plants, such as members of the Fabaceae (the legumes), can access N derived from N_2 fixation by symbiotic microbes such as *Rhizobium*; carnivorous plants obtain some of their N from the tissues of trapped invertebrates
Herbivores	N in edible plant tissues, usually present at low concentrations per unit dry mass; some N compounds (e.g. alkaloids, glucosinolates and cyanogenic glycosides) can function as herbivore deterrents or toxins; termites (Insecta: Isoptera) and shipworms (Bivalvia: Teredinidae) contain symbiotic gut bacteria that can fix N_2 and to which the animals have access
Carnivores	N (mainly protein N) in the tissues of their prey
Detritivores	N in plant, animal and microbial remains; degradation of high molecular weight N compounds can occur via gut microbial activity

These changes are detectable only if the organic N and NO_3^- are isolated and their $\delta^{15}N$ values measured separately. They remain undetectable if whole-plant $\delta^{15}N$ is measured although, internally, fractionations can produce isotopically distinct N pools. Only if N enters or leaves a system can the $\delta^{15}N$ of the system change. Even then, only if the N entering or leaving is isotopically distinct from that in the system will a change in $\delta^{15}N$ occur; this is Rule 2.

Rule 3 says that, when an N pool divides without being transformed, there is no change in $\delta^{15}N$ of the resulting pools. This applies, for example, if a NO_3^- pool becomes divided if some NO_3^- crosses a membrane and the rest remains behind (a process that, according to current evidence, does not

Box 2. Isotope mixing and mass balances

Isotope mixing

Tracer studies are interpreted using mixing models. The simplest is a model for two N sources, a ^{15}N-enriched (or, less commonly, ^{15}N-depleted) tracer and the N in the environment (i.e. 'background' N). ^{15}N tracer is added to the system where it mixes with some of the background N. Samples are later collected. We need to know how much of the N in the samples was tracer-derived.

$\delta_{background}$, δ_{tracer} and δ_{sample} are, respectively, δ^{15}N of the system before tracer addition, of the tracer itself, and of the sample. [$\delta_{background}$ and δ_{tracer} are the END MEMBERS (see Glossary) of the mixing model]. The fraction of tracer N (x_{tracer}) in the sample is:

$$x_{tracer} = \frac{\delta_{sample} - \delta_{background}}{\delta_{tracer} - \delta_{background}} \quad (1)$$

Equation 1 requires that:

(1) $\delta_{background}$ and δ_{tracer} are known and are significantly different.

(2) Negligible N isotope fractionations (Box 3) occur as tracer N moves through the system.

To calculate the mass of tracer-derived N in the sample (m_{tracer}), the mass of total N in the sample (m_{sample}) must be known, so that:

$$m_{tracer} = x_{tracer} m_{sample} \quad (2)$$

Isotope mass balances

The δ^{15}N of a sample (δ_{sample}) is the mass-weighted mean of the δ^{15}N of each of its N-containing compounds. δ^{15}N of different compounds can differ because of N isotope fractionations during metabolism (Box 3). If a sample contains n N compounds,

$$\delta_{sample} = \frac{\delta_1 m_1 + \delta_2 m_2 + \ldots + \delta_n m_n}{m_1 + m_2 + \ldots + m_n} = \frac{\sum_1^n \delta_i m_i}{m_{sample}} \quad (3)$$

δ_1, δ_2, m_1, m_2 are δ^{15}N and mass of N in compound 1, 2, etc., up to $i = n$.

Equation 3 can also be applied to whole plants, animals and ecosystems. The δ^{15}N of a whole plant is the mean of the δ^{15}N values of its leaves, stems, roots, flowers, seeds, etc., weighted by the N content of each part. The mean δ^{15}N of the vegetation in a habitat is the mean of the δ^{15}N values of each plant weighted by its total N content. The mean δ^{15}N of soil is the mean of the δ^{15}N values of the various forms of N present in the soil, weighted by the amounts of N in each form. If the N pools in any of these systems are dynamic[a] or spatially heterogeneous[b,c], substantial sampling is required to account for these sources of variation.

An ISOTOPE MASS BALANCE is useful to check for N gains to or losses from a system, or for isotopic fractionations (Box 3) occurring in it. In general,

$$(\delta_{system}\, m_{system})_{t+1} = (\delta_{system}\, m_{system})_t + (\delta_{system}\, m_{gained}) - (\delta_{lost}\, m_{lost}) \quad (4)$$

The δ terms are, respectively, the δ^{15}N of the system, the N gained by and lost from it over the time interval t to $t+1$. The m terms are the corresponding masses of N. 'System' can mean a whole plant, an animal or an ecosystem, for example.

References

a Stark, J.M. and Hart, S.C. (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* 385, 61–64

b Stark, J.M. (1994) Causes of soil nutrient heterogeneity at different scales. In *Exploitation of Environmental Heterogeneity by Plants* (Caldwell, M.M. and Pearcy, R.W., eds), pp. 255–284, Academic Press

c Marriott, C.A. *et al.* (1997) Spatial variability of soil total C and N and their stable isotopes in an upland Scottish grassland. *Plant Soil* 196, 151–162

Box Glossary

End members: The isotopic abundances of the sources used in a mixing model.

Isotope mass balance: A means of calculating if the amount of an element in a system is conserved over time.

fractionate N isotopes[15]). It also applies when foliage N is 'divided' by some of it being removed from the plant by a herbivore. It does not apply, however, if leaf N becomes 'divided' by NH_3 volatilizing into the atmosphere (NH_3 volatilization can strongly fractionate N isotopes: Box 3), which would count as a transformation ($NH_4^+ \rightarrow NH_3$) subject to Rule 2.

Rule 4 restates Eqn 3 in Box 2: when N pools mix, the resulting δ^{15}N is a mass-weighted average of the δ^{15}N of each component. So, whole-plant δ^{15}N reflects the δ^{15}N of the organic N, of residual NO_3^-, and of previously accumulated N pools within its tissues. This does not imply physical mixing of these pools inside the plant, rather that when the δ^{15}N of the plant is measured, the components contribute to the measurement as if they were mixed. (They actually do mix during sample combustion during δ^{15}N analysis: Box 1).

To summarize, the δ^{15}N of a system (e.g. a plant, an animal or an ecosystem) not only reflects the δ^{15}N of the N source, but also any N isotope fractionations, N gains and losses and N pool mixing.

These rules have formed the basis of one model[14] for plant δ^{15}N. Their generality makes them applicable to any system at any scale, although the processes they describe will differ. N is lost from a plant by efflux from roots, volatilization from leaves and herbivory, and transformed in plants during N assimilation, amino acid synthesis and protein

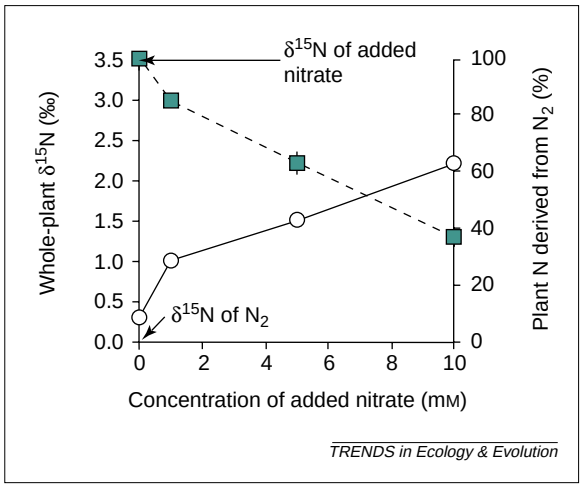


Fig. 1. An example of the use of natural ^{15}N abundances ($\delta^{15}N$) to estimate the proportion of plant N derived from the atmosphere by symbiotic N_2 fixation[9]. The tree legume *Chamaecytisus proliferus* was grown in sand containing different amounts of added NO_3^- with a $\delta^{15}N$ value of +3.5‰. $\delta^{15}N$ of N_2 is, by definition (Box 1), 0‰. Whole-plant $\delta^{15}N$ values (circles) varied within these limits, depending on how much N was derived from N_2 and NO_3^-, becoming more ^{15}N enriched as the availability of source NO_3^- increased. From these data, the proportion of plant N derived from N_2 (squares) was estimated using a mixing model (Box 2).

turnover. N is lost from ecosystems by migration, DENITRIFICATION and burning and N transfers through ecosystem pools occur by ingestion, excretion and decomposition. The rules are simple and flexible enough to apply to all of these systems, provided that information is available about the key isotope fractionations (Box 3) and mixing processes.

Interpreted on this basis, the $\delta^{15}N$ of a system integrates the operation of recent and, to some extent, historical processes, all of which are difficult to measure directly. The conceptual challenge is to decipher $\delta^{15}N$ in terms of those processes, providing information that helps us to understand how ecosystems work; Table 1 emphasizes the distinctions between ^{15}N tracer and $\delta^{15}N$ integrative approaches.

Measuring the $\delta^{15}N$ of N sources

An equally important technical challenge is the measurement of $\delta^{15}N$ of putative N sources in water, soil, sediments and cells. Standard methods exist to measure the concentrations of specific N compounds and their ^{15}N abundances if isotopically enriched tracers are used (Box 1, Table 1), but not their natural ^{15}N abundances ($\delta^{15}N$). The N compounds must be isolated from their matrices for $\delta^{15}N$ analysis, avoiding two potential sources of error that have trivial effects on ^{15}N abundances in enriched tracer studies, but which can cause significant alterations in $\delta^{15}N$ values.

N isotope fractionation during isolation

Suppose that we have a sample comprising a 1 mM solution of NH_4NO_3 in water, and we wish to determine the $\delta^{15}N$ of the NH_4^+ (the 'target' N). We must isolate the NH_4^+ from the NO_3^-. One way to do this is to raise the pH of the sample in a gas-tight container, causing NH_3 to volatilize. The NH_3 is trapped as NH_4^+ on an acidified N-free filter. The $\delta^{15}N$ of the dried filter plus NH_4^+ is determined[16]. NH_3 volatilization, however, can fractionate N isotopes according to Rayleigh kinetics (Box 3). Only if most of the NH_4^+ is isolated will $\delta^{15}N$ of the trapped NH_4^+ approximate that of the target N. How much is 'most'?

If an accuracy of ±1‰ is acceptable (i.e. measured $\delta^{15}N$ is within 1‰ of the true value), at least 92% of the NH_4^+ must be trapped if the fractionation (ε) for the isolation process is 5‰ (Fig. 4a). If ε exceeds 20‰, >98% of the NH_4^+ must be trapped. For maximum accuracy (i.e. to within 0.2‰ of the true $\delta^{15}N$ value), at least 99% recovery of the target N is required. That is, achieving only 95% isolation of the NH_4^+ would generate a 3‰ error in the $\delta^{15}N$ result if ε for the isolation process was 20‰. This might be unacceptable if $\delta^{15}N$ varies by <20‰ among samples, as is often the case in ecological studies.

Similar arguments apply to all distillation, ion exchange or diffusion techniques that potentially fractionate N isotopes, and to all forms of soluble N, not just NH_4^+. Most published measurements of $\delta^{15}N$ of soil NH_4^+ and NO_3^-, for example, were obtained using such techniques.

Contamination

To continue with the NH_4^+ example, suppose that instead of the 100 µg target N expected (from an NH_4^+-specific assay such as a colorimetric determination), 120 µg were recovered in the acid trap. If the isolation method was not NH_4^+-specific, some organic N in the sample might be converted to volatile organic N and trapped with the target N, contributing rogue N and $\delta^{15}N$ to the isolate. This is a particular problem when isolating inorganic N from some soils, but how serious is it?

Suppose that the difference in $\delta^{15}N$ between the target N and that of any contaminants was 1‰. Then, according to an isotope mass balance (Box 2), the $\delta^{15}N$ of the target could be determined to within 0.2‰ if the target–contaminant mixture was ~90% target N (Fig. 4b). To achieve similar accuracy with a 20‰ difference between target N and contaminant N would demand that the mixture be at least 99% pure target N. Unless contaminant $\delta^{15}N$ is very different from that of the target, contamination generates relatively small $\delta^{15}N$ errors if sample purification is stringent. For example, if the $\delta^{15}N$ of target and contaminant N differed by 50‰ and the mixture was 99% target N, the $\delta^{15}N$ error would be only 0.5‰. This is acceptable for most ecological applications but, then, $\delta^{15}N$ results differing by ≤0.5‰ should be interpreted carefully.

If traditional methods for isolating N compounds from their matrices cannot be avoided, it is worth subjecting the $\delta^{15}N$ data obtained using them to quality controls:

(1) Compare the amounts of target N in the original sample (measured by a target-specific method, e.g. colorimetry) with those measured by mass spectrometry.

(2) Use Fig. 4a to estimate the minimum recoveries required to achieve a given precision (error).

(3) If N recoveries are significantly less than the minimum required, the data could be unsound.

(4) If recoveries are less than, but still close to, the minimum required, assess the potential error and analyse the $\delta^{15}N$ data accordingly.

Box 3. Fractionation and discrimination

Variations in $\delta^{15}N$ among samples reflect N isotope fractionations. These occur because more energy is needed to break or form chemical bonds involving ^{15}N than ^{14}N. On average, ^{14}N-containing molecules react faster (e.g. with an enzyme) than those containing ^{15}N. By definition, if a substrate and a product maintain the same $^{15}N/^{14}N$ ratio (R: Box 1), no fractionation occurs, that is α, the $R_{product}$:$R_{substrate}$ ratio, or FRACTIONATION FACTOR (see Glossary), is unity. Deviations in α from unity indicate isotopic fractionation. To allow fractionations to be expressed on the δ scale (Box 1), α values are transformed as follows:

$$\varepsilon = 1000(\alpha - 1) \quad (1)$$

ε is the difference in $\delta^{15}N$ between a substrate and its immediate product. For a given reaction, ε is not constant[a,b], but depends on external conditions, for example, temperature. Representative ε values that have been measured for major N cycle processes are shown in Table I.

During a reaction, the $\delta^{15}N$ of residual substrate (δ_S) changes according to[c]:

$$\delta_s = \delta_0 + \varepsilon \ln(1-f) \quad (2)$$

δ_0 is the initial $\delta^{15}N$ of the substrate. f is the fraction of the substrate that has been consumed in the reaction [Fig. I: Theoretical changes in $\delta^{15}N$ when a substrate is converted to a product with an N isotope fractionation (ε) of 10‰ and an initial $\delta^{15}N$ of substrate (δ_0, broken line) of 0‰ as a function of the fraction of substrate converted (f). The $\delta^{15}N$ of residual substrate (upper curve) is described by Eqn 2; that of accumulated product (δ_P, lower curve) by Eqn 3. When conversion of substrate is complete, $f = 1$ and $\delta_P = \delta_0$]. Products become simultaneously ^{15}N depleted relative to the substrate. The $\delta^{15}N$ of the accumulated product is:

$$\delta_p = \frac{\varepsilon(1-f)[\ln(1-f)]}{f} \quad (3)$$

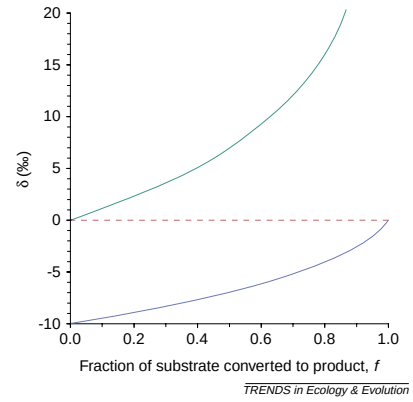
If all the substrate is consumed, $f = 1$ and $\delta_P = \delta_0$, and the final product has the same $\delta^{15}N$ as the initial substrate (Fig. I).

This Rayleigh model[d] applies to unidirectional reactions in closed systems. Although natural systems are neither closed nor unidirectional, ecosystem $\delta^{15}N$ variations can be interpreted using Rayleigh equations, but with important differences:

(1) Because ecologists are often interested in N fluxes between whole organisms, it is meaningless at that scale to refer to 'substrates' and 'products'. These are usually replaced by the more relevant 'sources' and 'sinks'.

(2) In an ecological context, f in Eqns 2 and 3 is the extent to which the N source has been consumed. If $f = 0$, the N supply is effectively infinite relative to the demand for N by potential sinks. If $f = 1$, the N source has been totally consumed by the various sinks that are present.

Fig. I



(3) In natural systems, unlike closed vessels, a consumed N source can be replenished (e.g. by decomposition of organic matter), and f varies between 0 and 1 depending on how source supply and consumption vary over time.

(4) If the supply of an N source is effectively infinite relative to demand, $f \rightarrow 0$ and the $\delta^{15}N$ of source N remains unaffected by fractionations, that is $\delta_{source} \approx \delta_0$. If such conditions apply, and recalling that:

$$\delta^{15}N = 1000\left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \quad (4)$$

Eqns 3 and 4 can be combined to give:

$$\varepsilon = 1000(\alpha - 1) = 1000\left(\frac{\delta_{source} - \delta_{sink}}{1 + \frac{\delta_{sink}}{1000}}\right) \quad (5)$$

$$\approx \delta_{source} - \delta_{sink} = \Delta$$

Δ is the isotopic discrimination between N source(s) and sink(s), and is, to a good approximation, the difference in $\delta^{15}N$ between them.

Not every N cycle process always fractionates N isotopes: it depends on the process, on external conditions and the extent to which the N source has been consumed.

A process that fractionates N isotopes can be distinguished from one involving the mixing of isotopically distinct pools[e]. A fractionating process produces a linear relation between $\delta^{15}N$ of the source pool and ln$C$, where C is the concentration of the source pool. A mixing process produces a linear relation between $\delta^{15}N$ and $1/C$.

Table I. Representative N isotope fractionations (ϵ) that have been measured for major N cycle processes[a]

Process	ϵ (‰)
N_2 fixation via nitrogenase	0–6
NH_3 volatilization	40–60
N_2O and NO production during NH_4^+ oxidation (nitrification)	35–60
N_2O and N_2 production during NO_3^- reduction (denitrification)	28–33
NO_3^- assimilation into organic N by plants	0–19
NH_4^+ assimilation into organic N by plants	9–18
NO_3^- or organic N assimilation by microbes	13
NH_4^+ assimilation by microbes	14–20
NH_4^+ production from organic matter decomposition (ammonification)	0–5
NO_3^- production during nitrification	15–35
Organic N assimilation by animals (deamination and transamination)	1–6

[a]Data collated from various sources[a,b]

References

a Handley, L.L. *et al.* (1999) The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects measures of water availability. *Aust. J. Plant Physiol.* 26, 185–199

b Adams, T.S. and Sterner, R.W. (2000) The effect of dietary nitrogen content on trophic level ^{15}N enrichment. *Limnol. Oceanogr.* 45, 601–607

c 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

d Rayleigh, J.W.S. (1896) Theoretical considerations respecting the separation of gases by diffusion and similar processes. *Philos. Mag.* 42, 493

e Mariotti, A. *et al.* (1988) ^{15}N isotope biogeochemistry an natural denitrification process in groundwater: application to the chalk aquifer of northern France. *Geochem. Cosmochim. Acta* 52, 1869–1878

Box Glossary

Fractionation factor: The ratio of the isotope ratio of a product to that of the substrate in a given reaction. (Sometimes defined as the inverse of this). If the ratio is unity, there is no fractionation. Usually symbolized as α.

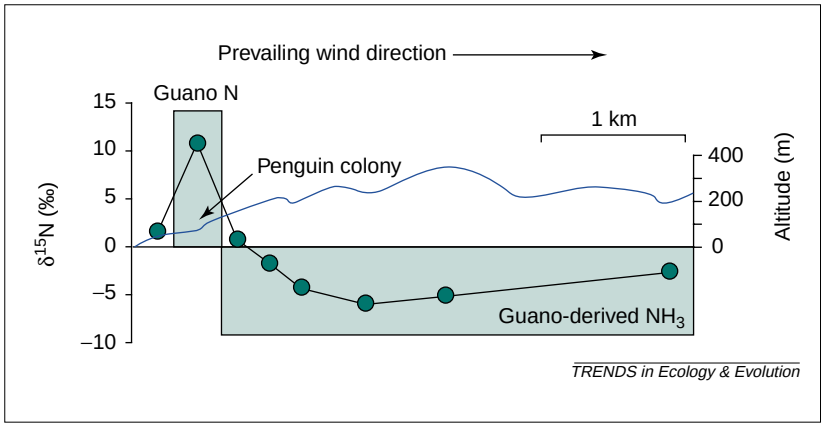


Fig. 2. An example of the use of natural ^{15}N abundances ($\delta^{15}N$) to trace an N source in vegetation on a subantarctic island[12]. The assimilation of atmospheric NH_3 derived from penguin guano was detected using the $\delta^{15}N$ values of vegetation (circles) at eight sites along an altitudinal transect (curved line), using the wide difference in $\delta^{15}N$ between guano N ($\delta^{15}N = +14.6‰$) and the NH_3 ($\delta^{15}N = -10‰$) derived from it and volatilized into the atmosphere (green rectangles). The ^{15}N enrichment of plants growing on the penguin colony suggests some assimilation of guano-derived soluble N (e.g. NH_4^+). The relatively ^{15}N-depleted plants downwind of, and at higher altitudes than, the colony suggest the interception of guano-derived, ^{15}N-depleted NH_3 from the atmosphere.

Box 4. $\delta^{15}N$ pool rules

(1) When a pool is (bio)chemically transformed, the $\delta^{15}N$ of the product and residual pools change according to Rayleigh kinetics over an infinitesimal time interval (Box 3, Fig. I).
(2) The $\delta^{15}N$ of a system can change only if N with a $\delta^{15}N$ different from that of the system enters or leaves it.
(3) When a pool divides, but is not transformed, there is no change in $\delta^{15}N$ of either pool.
(4) When pools mix, the resulting $\delta^{15}N$ is a mass-weighted mean of the $\delta^{15}N$ of each pool (Box 2).

(5) If recoveries significantly exceed expectations, contamination is likely. Samples should be cleaned up more stringently before repeating the isolation protocol.

(6) If recoveries are acceptable, but there are large inter-replicate variations in $\delta^{15}N$, contamination by trace contaminants with 'exotic' $\delta^{15}N$ values is possible, but probably not serious.

Rather than depend on such methods, it is preferable to use others that have been developed explicitly to overcome the problems of fractionation and contamination. Only three have been developed, all for NO_3^-: one[17] uses NO_3^--specific dye-coupling; the others[18,19], ion exchange. These methods and their eventual counterparts for other N compounds should become standard items in the N ecologist's toolkit.

Inferring processes from $\delta^{15}N$ patterns

Despite the necessity and importance of these methods, it is still possible to do ecologically meaningful $\delta^{15}N$ work without them if experiments, data collection and pattern analysis are done using appropriate models to interpret the $\delta^{15}N$ data.

To measure the extent of N ISOTOPE DISCRIMINATION by plants, it is, according to Eqn 5 (Box 3), necessary to measure the $\delta^{15}N$ of their N source(s), δ_{source}, which is a nontrivial task. However, it is possible to detect discrimination even if δ_{source} is unknown. If a range of plants has access to the same N source, and if there is significant variation in whole-plant $\delta^{15}N$, that variation would signify N isotope discrimination by some of the plants.

For example, wild barley, *Hordeum spontaneum*, genotypes grown hydroponically on the same NO_3^- source varied significantly in whole-plant $\delta^{15}N$ ($= \delta_{sink}$, Box 3), by 1.3‰ (–1.0 to +0.3‰)[20]. Genotypic variation in whole-plant $\delta^{15}N$ implies that at least some of the plants discriminated against ^{15}N. Had no discrimination occurred in any genotype, the δ_{sink} values would have tracked that of the common N source. (In the unlikely event of all plants discriminating equally, there would have been no variation in δ_{sink} and it would have been impossible to infer discrimination.) In this study[20], δ_{source} was known and constant (~+1‰), suggesting that discrimination had indeed occurred, at least in those genotypes where $\delta^{15}N$ was at the negative end of the range. The mechanism of discrimination was unknown, but the loss of certain products of N metabolism (and that were isotopically distinct from δ_{sink}: Rule 2, Box 4) via root efflux seems the most plausible explanation[14].

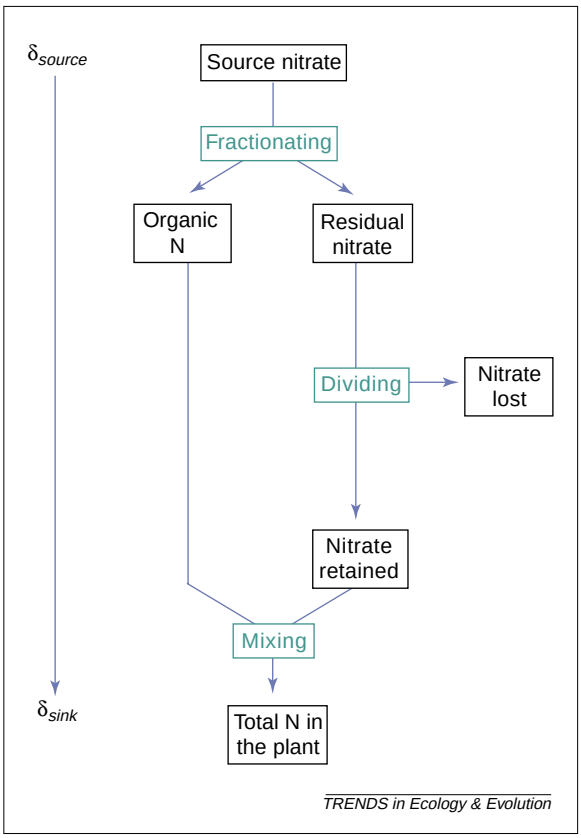


Fig. 3. The main types of process that can affect the relationship between the $\delta^{15}N$ of a plant (δ_{sink}) and that of its N source (δ_{source}). δ_{sink} reflects not only δ_{source}, but also N isotope fractionation during NO_3^- assimilation, N losses from the plant, and the mixing of different N pools within the plant[14]. N isotopes can be fractionated according to a Rayleigh model (Box 3) when NO_3^- is assimilated, that is, the $\delta^{15}N$ values of organic assimilation products can be different from that of any NO_3^- remaining unassimilated. The pool of unassimilated NO_3^- in the plant divides if some NO_3^- is lost from the plant by, for example, efflux from roots. The total N left in the plant is a mixture of assimilated N and unassimilated NO_3^-, so that δ_{source} is a mass-weighted mean of the $\delta^{15}N$ values of these pools (Box 2), and does not necessarily have a simple relationship with δ_{source}.

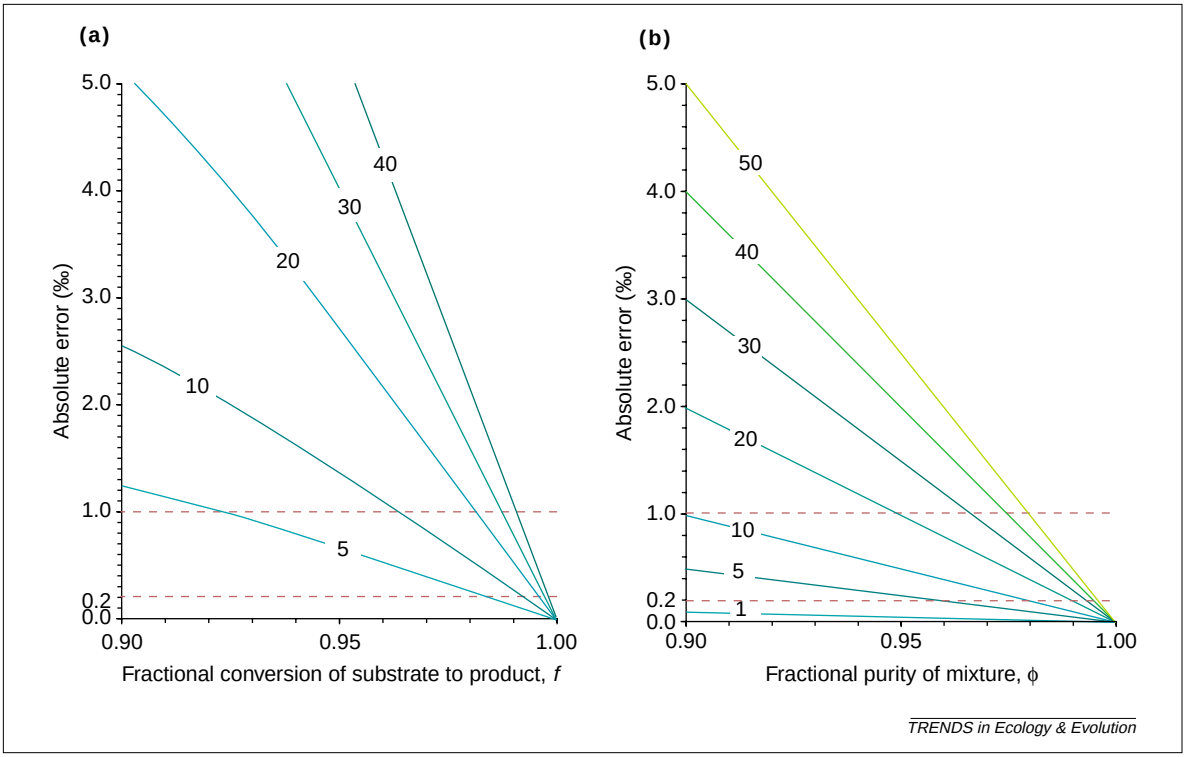


Fig. 4. Examples of absolute errors in $\delta^{15}N$ measurements. (a) Absolute errors (‰) in $\delta^{15}N$ measurement as a function of the fraction of substrate converted to product (f), for different N isotope fractionations (ε, ‰, numbers on curves). Error is calculated as the difference in $\delta^{15}N$ between the initial substrate and the product, according to a Rayleigh model (Box 3). For the error to fall below 1‰ (upper broken line), at least 92% of the substrate must be converted if the absolute magnitude of ε exceeds 5‰. The corresponding conversion for the error to fall below 0.2‰, the maximum routine accuracy of $\delta^{15}N$ analysis (lower broken line), is at least 98.5%. (b) Absolute errors (‰) in $\delta^{15}N$ measurement of a certain N compound (the 'target N') in a mixture of N compounds. Error is calculated as the difference in $\delta^{15}N$ between that of the target (δ_T) and that of the mixture (δ_{mix}). Each curve shows how the error depends on the fractional purity (ϕ) of the mixture with respect to the target, for a given difference (numbers on curves) between δ_T and the net $\delta^{15}N$ of the contaminants (δ_C). These curves were derived from an isotope mass balance (Box 2) and are described by $\delta_T - \delta_{mix} = (\delta_T - \delta_C)(1-\phi)$. If the $\delta^{15}N$ of the target differs by >10‰ from that of the contaminants, the mixture must be at least 90% pure target N for the error to be <1‰ (upper broken line). The corresponding purity for the error to be <0.2‰ (lower broken line) is at least 98%.

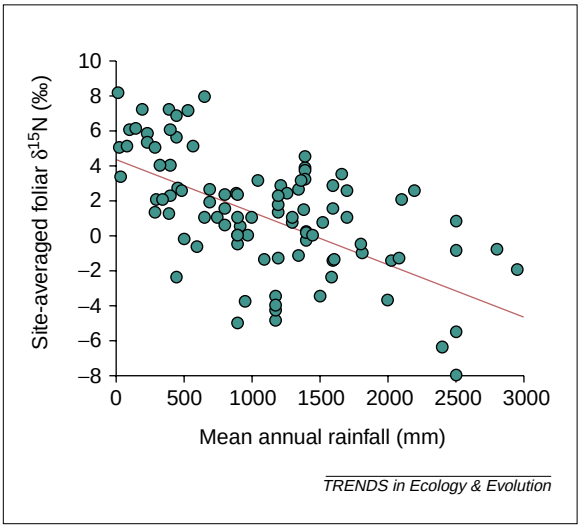


Fig. 5. Correlation between foliar $\delta^{15}N$ (averaged across the plant taxa at each site) and the mean annual rainfall at those sites; data collated from numerous sources[15]. The regression foliar $\delta^{15}N = 4.49 - 0.003$. Rainfall is significant at $P<0.000$, $r=-0.59$, $n=97$.

When δ_{source} is unknown, N isotopic discrimination by plants can be inferred if the following precautions are taken which, although of little use in natural settings, can be applied in controlled environments:

(1) Grow plants on a common N source that has a known $\delta^{15}N$ (i.e. use hydroponics[20] or sand[21]; avoid soil containing many potential sources of unknown $\delta^{15}N$).

(2) Compare many plants to reveal interplant $\delta^{15}N$ variations when growing on that common N source.

(3) Compare genetically similar plants (i.e. plants unlikely to have evolved different N source preferences and microbial, especially mycorrhizal, symbionts).

(4) Avoid comparisons among relatively unrelated taxa and disparate life forms.

(5) Measure whole-plant $\delta^{15}N$, not just foliar $\delta^{15}N$ (i.e. roots must be harvested).

(6) Avoid treatments (e.g. N starvation[20]) that deprive plants of the opportunity to discriminate (because $f \rightarrow 1$ and, therefore, $\Delta \rightarrow 0$: Box 3).

This study[20] illustrates that the information provided by $\delta^{15}N$ is often circumstantial rather than definitive. $\delta^{15}N$ can provide clues about, or reveal the 'footprint' of, a process, but not necessarily deliver conclusive evidence for its cause. Another example where this applies is the use of Rayleigh models (Box 3) to detect N transformation processes in the field, and to distinguish such processes from others that involve the mixing of isotopically distinct N sources. In an arid juniper woodland in Utah, there was an inverse relationship between the total N content of the soil and soil $\delta^{15}N$ (Ref. 22). The data fitted a version of Eqn 2 (Box 3), implying that N had originally entered the ecosystem from one source,

Glossary

^{14}N: the more abundant of the two stable isotopes of nitrogen, atomic mass = 14.
^{15}N: the less abundant of the two stable isotopes of nitrogen, atomic mass = 15.
Cryptobiotic: referring to organisms that are concealed in crevices or by stones.
Denitrification: the microbial reduction of NO_3^- to N_2O, and of N_2O to N_2.
Isotope: Atoms of an element whose nuclei contain the same number of protons but different numbers of neutrons. ^{14}N nuclei contain seven neutrons; ^{15}N nuclei, eight neutrons, both contain seven protons. Unstable isotopes are radioactive and potentially hazardous to work with; stable isotopes are not radioactive.
Isotope abundance: the amount of an isotope, whether expressed on the atom % or δ scale.
Isotope fractionation: the extent to which the isotopes of an element are separated between a substrate and a product during a reaction. Usually symbolized as ε.
Isotope discrimination: the difference in δ value between a source and a sink. Usually symbolized as Δ.
Isotopic tracer: an isotopically distinct, but chemically indistinguishable substance, added to a system to follow the substance as it is transformed and transported.
Mixing model: a way of calculating the extent to which the isotopic abundance of a sample is caused by the mixing of isotopically distinct sources.
N source: a form of N that is captured from the environment and which can be used in metabolism by living organisms.
N sink: an organism or group of organisms that consume certain N sources in response to a metabolic demand, or a non-biological process (e.g. leaching or ion exchange) that removes N from a source pool.
Natural abundance: the abundance of an isotope in samples to which no tracer has been added.
Rayleigh model: a mathematical description of how the isotopic compositions of a substrate and a product change as a reaction proceeds. Developed originally by Lord Rayleigh.
δ scale: differences in isotope ratio between sample and a standard, normalized to the isotope ratio of the standard. Usually expressed in units of parts per thousand (‰).
$\delta^{15}N$: the abundance of ^{15}N on the δ scale. Usually refers to the natural abundance of ^{15}N.

probably via the N_2-fixing cyanobacteria and lichens in a CRYPTOBIOTIC crust covering the soil surface.

The data also suggested that N had been lost from the soil by, at least in part, a process that caused N isotopic fractionation. Had significant mixing of N sources caused the observed changes in soil $\delta^{15}N$, the data would have fitted a mixing model (Box 2) rather than a Rayleigh model (Box 3). This study[22] provided evidence for the importance of cryptobiotic crusts to the N cycle in arid ecosystems. The partial loss of the cryptobiotic crusts, and their N inputs, by soil disturbance has caused significant heterogeneity in soil N content, and could jeopardize local N dynamics over the long term.

Similar approaches have been used to infer the occurrence of denitrification in groundwater[23] and soil[24]. In each of these studies[22–24], $\delta^{15}N$ data allowed the likely importance of alternative N cycle process to be evaluated. To attempt this by direct measurement of those processes would have required far greater effort and, possibly, the risk of measuring the wrong processes.

Although most field-based use of $\delta^{15}N$ has been at plot or community scales (Table 1), $\delta^{15}N$ might have greater utility at biome or global scales where some of the subtleties of N metabolism and N cycling can be ignored. For example, in a global survey of terrestrial vegetation[15], site-averaged foliar $\delta^{15}N$ decreased significantly as mean annual rainfall increased (Fig. 5). Similar trends occur at subcontinental[25] and insular[26] scales, but these might be difficult to detect at landscape scales[27]. At large spatial scales, foliar $\delta^{15}N$ seems to reflect mainly ecosystem water availability, which must influence local N cycle processes, especially those involving soil microbes[28]. Some repeatable $\delta^{15}N$ patterns do occur locally: for example, increases in $\delta^{15}N$ with soil depth[10], or correlations between foliar $\delta^{15}N$ and type of mycorrhizal symbiont[29].

Mechanistic explanations for local or global $\delta^{15}N$ patterns will require models that include rules such as those in Box 4 applied to specific processes. It will then be possible to use $\delta^{15}N$ approaches to say, for example, whether N cycling in a certain ecosystem is 'open' or 'closed'[26], that is, the extent to which N is retained within an ecosystem over time. This is important when considering the mechanisms for the turnover and transport of N within ecosystem N pools, including that from atmospheric pollution.

Another repeatable $\delta^{15}N$ pattern is the increase in the $\delta^{15}N$ of animals with their trophic position. The more ^{15}N enriched the tissues, the higher the trophic level of the animal[30]. This pattern has been best characterized in rivers, lakes and seas. The mean ^{15}N enrichment of 3.4‰ with trophic level in those systems[31] has become the 'rule-of-thumb' for subsequent studies[32]. Trophic level ^{15}N enrichment occurs because excess dietary N is excreted as waste products that are ^{15}N depleted compared with residual N in the tissues (as a result mainly of deamination and transamination reactions[33]).

Isotopic differences among animals have been exploited in studies of feeding behaviour. For example, $\delta^{15}N$ (with $\delta^{13}C$) analyses of tissue composition were used to track temporal shifts in the diet of a generalist predator (the American marten, *Martes americana*) as the availabilities of alternative prey fluctuated[34]. When preferred prey (small rodents) were scarce, salmon carcasses formed a larger fraction of the diet of the martens, as deduced from a mixing model (Box 2).

The trophic level ^{15}N enrichment is not constant, however[35]. It can change with the dietary state of an animal (e.g. if it has been metabolizing N reserves rather than assimilating recently caught food[36,37]). It can also depend on the ecosystem. The 3.4‰ 'rule' does not hold for soil invertebrates[38], for reasons that remain unknown, but could reflect the chemical heterogeneity of soil organic matter. Nevertheless, the utility of $\delta^{15}N$ (with $\delta^{13}C$ and, sometimes, $\delta^{34}S$: Refs 13,30) to indicate trophic relations will be greatest where direct observations of feeding behaviour are difficult, as in soil[36,37] and within host–parasite associations[39], or impossible, as in the distant past[40].

Conclusions

The emergence of robust $\delta^{15}N$ patterns within and among ecosystems, at small and large scales, and across diverse taxa and life forms, illustrates the opportunities for using $\delta^{15}N$ as an integrator of N cycle processes. The challenge lies in finding ways to interpret $\delta^{15}N$ data in terms of those processes, using the power of isotope fractionation and mixing rules (Box 3 and 4) to constrain the range of ecological interpretations that can be made.

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References

1 Moffat, A.S. (1998) Global nitrogen overload problem grows critical. *Science* 279, 988–989
2 Yoshida, N. and Toyoda, S. (2000) Constraining the atmospheric N_2O budget from intramolecular site preference in N_2O isotopomers. *Nature* 405, 330–334
3 Pérez, T. *et al.* (2000) Isotopic variability of N_2O emissions from tropical soils. *Global Biogeochem. Cycles* 14, 525–535
4 Mary, B. *et al.* (1998) A model for calculating nitrogen fluxes in soil using ^{15}N tracing. *Soil Biol. Biochem.* 30, 1863–1979
5 Nadelhoffer, K.J. *et al.* (1999) Sinks for ^{15}N enriched additions to an oak forest and a red pine plantation. *Ecol. Appl.* 9, 72–86
6 Hughes, J.E. *et al.* (2000) Nitrogen flow through the food web in the oligohaline zone of a New England estuary. *Ecology* 81, 433–452
7 Mulholland, P.J. *et al.* (2000) Nitrogen cycling in a forest stream determined by a N 15 tracer addition. *Ecol. Monogr.* 70, 471–493
8 Pate, J.S. and Unkovich, M.J. (1999) Measuring symbiotic nitrogen fixation: case studies of natural and agricultural ecosystems in a Western Australian setting. In *Physiological Plant Ecology* (Press, M.C. *et al.*, eds), pp. 153–173, Blackwell Science
9 Unkovich, M.J. *et al.* (2000) Nitrogen isotope fractionation in the fodder tree legume tagasaste (*Chamaecytisus proliferus*) and assessment of N_2 fixation inputs in deep sandy soils of Western Australia. *Aust. J. Plant Physiol.* 27, 921–929
10 Handley, L.L. and Scrimgeour, C.M. (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
11 Boddey, R.M. *et al.* (2000) Use of the ^{15}N natural abundance technique to quantify biological nitrogen fixation by woody perennials. *Nutrient Cycling Agroecosyst.* 57, 235–270
12 Erskine, P.D. *et al.* (1998) Subantarctic Macquarie Island – a model ecosystem for studying animal-derived nitrogen sources using ^{15}N natural abundance. *Oecologia* 117, 187–193
13 Van Dover, C.L. *et al.* (1993) Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web. *Nature* 360, 153–156
14 Robinson, D. *et al.* (1998) A theory for $^{15}N/^{14}N$ fractionation in nitrate-grown vascular plants. *Planta* 205, 397–406
15 Handley, L.L. *et al.* (1999) The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects measures of water availability. *Aust. J. Plant Physiol.* 26, 185–199
16 Sørensen, P. and Jensen, E.S. (1991) Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for ^{15}N determination. *Anal. Chim. Acta* 252, 201–203
17 Johnston, A.M. *et al.* (1999) Isolation of NO_3^-N as 1-phenylazo-2-naphthol (Sudan-1) for measurement of $\delta^{15}N$. *Rapid Commun. Mass Spectrom.* 13, 1531–1534
18 Silva, S.R. *et al.* (2000) A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. *J. Hydrol.* 228, 22–36
19 Stickrod, R.D. and Marshall, J.D. (2000) On-line nitrate-$\delta^{15}N$ extracted from groundwater determined by continuous-flow elemental analyzer/isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* 14, 1266–1268
20 Robinson, D. *et al.* (1999) 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
21 Azcon-G-Aguilar, R. *et al.* (1998) The $\delta^{15}N$ of lettuce and barley are affected by AM status and external concentration of N. *New Phytol.* 138, 19–26
22 Evans, R.D. and Ehleringer, J.R. (1993) A break in the nitrogen cycle in aridlands? Evidence from $\delta^{15}N$ of soils. *Oecologia* 94, 314–317
23 Mariotti, A. *et al.* (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
24 Robinson, D. and Conroy, J.P. (1999) A possible plant-induced feedback between elevated atmospheric CO_2, denitrification and the enhanced greenhouse effect. *Soil Biol. Biochem.* 31, 43–53
25 Schulze, E-D. *et al.* (1998) Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. *Aust. Plant Physiol.* 25, 413–425
26 Austin, A.T. and Vitousek, P.M. (1998) Nutrient dynamics on a rainfall gradient in Hawai'i. *Oecologia* 113, 519–529
27 Sutherland, R.A. *et al.* (1993) Landscape-scale variations in plant and soil nitrogen-15 natural abundance. *Soil Sci. Soc. Am. J.* 57, 169–178
28 Garten, C.T. (1993) Variation in foliar ^{15}N abundance and the availability of soil nitrogen on Walker Branch watershed. *Ecology* 74, 2098–2113
29 Michelsen, A. *et al.* (1998) Vascular plant ^{15}N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. *Oecologia* 115, 406–418
30 Ehleringer, J.R. *et al.* (1986) Stable isotopes in physiological ecology and food web research. *Trends Ecol. Evol.* 1, 42–45
31 Minagawa, M. and Wada, E. (1984) Stepwise enrichment of ^{15}N along food-chains – further evidence and the relation between $\delta^{15}N$ and animal age. *Geochim. Cosmochim. Acta* 48, 1135–1140
32 Eggers, T. and Jones, T.H. (2000) You are what you eat…or are you? *Trends Ecol. Evol.* 15, 265–266
33 Adams, T.S. and Sterner, R.W. (2000) The effect of dietary nitrogen content on trophic level ^{15}N enrichment. *Limnol. Oceanogr.* 45, 601–607
34 Ben-David, M. *et al.* (1997) Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. *Oecologia* 111, 280–291
35 Ponsard, S. and Arditti, R. (2001) Detecting omnivory with $\delta^{15}N$. *Trends Ecol. Evol.* 16, 20–21
36 Scrimgeour, C.M. *et al.* (1995) Trophic levels and anomalous $\delta^{15}N$ of insects on raspberry (*Rubus idaeus* L.). *Isot. Environ. Health Stud.* 31, 107–115
37 Gannes, L.Z. *et al.* (1998) Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comp. Biochem. Physiol.* 119A, 725–737
38 Neilson, R. *et al.* (2000) $\delta^{13}C$ and $\delta^{15}N$ analyses suggest that putative functional classifications of earthworms are site-specific and may also indicate habitat diversity. *Soil Biol. Biochem.* 32, 1053–1061
39 Neilson, R. and Brown, D.J.F. (1999) Feeding on different host plants alters the natural abundances of $\delta^{13}C$ and $\delta^{15}N$ in Longidoridae (Nemata). *J. Nematol.* 31, 20–26
40 Dickson, J.H. *et al.* (2000) The omnivorous Tyrolean Iceman: colon contents (meat, cereals, pollen, moss and whipworm) and stable isotope analyses. *Philos. Trans. R. Soc. London Ser. B* 355, 1843–1849
41 Clark, F.E. (1977) Internal cycling of ^{15}nitrogen in a shortgrass prairie. *Ecology* 58, 1322–1333
42 Näsholm, T. *et al.* (1998) Boreal forest plants take up organic nitrogen. *Nature* 392, 914–916
43 Scrimgeour, C.M. and Robinson, D. Stable isotope analyses and applications. In *Soil and Environmental Analysis* (3rd edn) (Smith, K.A. and Cresser, M.S., eds), Marcel Dekker (in press)

Other *Trends* and *Current Opinion*s

Articles of ecological or evolutionary interest in recent issues of other *Trends* and *Current Opinion* journals

Evolution of genome size: new approaches to an old problem, *Dmitiri Petrov* ***Trends in Genetics*** 17, 23–28 (January 2001)

Effects of low atmospheric CO_2 on plants: more than a thing of the past, *Rowan F. Sage and John R. Coleman* ***Trends in Plant Science*** 6, 18–24 (January 2001)

The evolution of plant architecture, *Ian M. Sussex and Nancy M. Kerk* ***Current Opinion in Plant Biology*** 4, 33–37 (February 2001)

Duplicate and diverge: the evolution of plant genome microstructure, *Ian Bancroft* ***Trends in Genetics*** 17, 88–93 (February 2001)