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Tracing ^{15}N through landscapes: potential uses and precautions

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Abstract

Stable N isotopes are used to examine the source, flow and fate of N at scales ranging from greenhouse pots to landscapes. There are two main approaches: the ^{15}N-enriched method applies an artificially enriched source of ^{15}N and the ^{15}N natural abundance ($\delta^{15}N$) method uses natural ^{15}N differences between N sources and sinks.

The $\delta^{15}N$ method is good for semi-quantitative estimates of N flow in undisturbed ecosystems, for analyzing patterns, and for developing new hypotheses, particularly when spatial variability across a landscape or watershed can be explained. The spatial variability of $\delta^{15}N$ across a landscape is often non-random, following predictable spatial patterns. Topographic features control the rate of various hydrological and biological processes, resulting in significantly different $\delta^{15}N$ between lower and upper slope positions. However, if the difference between source-$\delta^{15}N$ and sink-$\delta^{15}N$ is small due to inherent background variability and/or if fractionating processes have a large effect on the isotopic signature of the N to be traced, $\delta^{15}N$ will not work as a tracer.

With the ^{15}N-enriched method, the isotopic signature of the enriched tracer can be pre-determined to ensure a significant difference in atom%^{15}N between source and background levels, even when fractionation occurs. In most situations, the ^{15}N-enriched method can be successfully used as a tracer to test hypotheses and to quantify N cycling through the landscape, regardless of background variability in $\delta^{15}N$. Limitations of the ^{15}N-enriched method include the cost associated with applying an enriched tracer, especially at the landscape scale, and the potentially confounding effects of applying N to a previously undisturbed landscape.

Keywords: Landscape; Ecosystem; Fractionation

1. Introduction

In many areas of the world, agricultural applications of organic and inorganic nitrogen increased rapidly during the second half of the 20th century (Mosier et al., 2001). FAO (1999) has estimated the total amount of N_2 fixed globally by industrial production of fertilizer at 85 million tones annually and increasing. Nitrogen is an essential macronutrient, second only to water availability in its importance for plant growth (Mengel and Kirkby, 1982). Unfortunately, increases in N use have had detrimental effects such as eutrophication of surface water and increases of NO_3^- in groundwater (Pierzynski et al., 1994).

Nitrogen is present in the soil as organic or inorganic N. Over 95% of soil N is in the organic pool and must be mineralized by microorganisms into inorganic N for plant uptake. Organic N compounds range in bioavailability from easily mineralized N to unavailable N (Kamprath, 2000). The two dominant forms of inorganic N are ammonium (NH_4^+) and

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nitrate (NO_3^-) Ammonium, applied as fertilizer-N or released in the soil solution by mineralization (Ladd and Jackson, 1982), undergoes microbiological nitrification into NO_3^- under aerobic conditions (Schmidt, 1982). Unlike NH_4^+, NO_3^- is highly soluble in water and is susceptible to leaching. Under anaerobic conditions, denitrification may occur, reducing NO_3^- into N_2O or N_2. Leaching and denitrification contribute to N losses from the agro-ecosystem, decreasing crop N use efficiency and increasing the potential for environmental problems.

Stable isotopes of N are used to trace the flow and fate of applied ^{15}N at several scales, from pot experiments to landscapes. The stable isotope technique can detect and quantify inputs or losses of new N (i.e. ^{15}N-enriched N) in a specific N pool, often without a change in total N. There are two commonly used stable N isotope methods: the ^{15}N-enriched method and the ^{15}N-natural abundance ($\delta\,^{15}N$) method. With the ^{15}N-enriched method, inorganic N (i.e. fertilizer) or organic N (i.e. manure or sludge) is enriched in ^{15}N to give it a unique isotopic signature. When this material is applied, it becomes part of the overall N-cycle and the flow of ^{15}N through the various soil and plant N pools can be followed. The ^{15}N-enrichment of a given N pool will depend on (1) the amount of N applied, (2) its background ^{15}N enrichment, (3) the turnover rates of the individual N pools, (4) the size of the N pools, and (5) the interconnectedness of the pools (i.e. the likelihood that ^{15}N from one pool will appear in another). The change in ^{15}N enrichment over time can be used to calculate the turnover rate of N in the various soil N pools. If all N in the vegetation accumulates following the application of the ^{15}N, the ^{15}N enrichment in the vegetation becomes an integrated measurement of the ^{15}N enrichment of available soil N during the growing season.

In contrast with the ^{15}N-enriched method, the $\delta\,^{15}N$ method does not add ^{15}N-enriched materials to the ecosystem. Instead, it uses the small difference between the $^{15}N/^{14}N$ ratio of the N source being examined and the $^{15}N/^{14}N$ ratio of N already in the system to follow the source N through the soil, water, and vegetation. The advantage of the $\delta\,^{15}N$ approach is that, in principle, it can be used in any ecosystem, but it has analytical and interpretative limitations.

1.1. Stable N isotopes: an overview

Nitrogen is composed of two stable isotopes with atomic masses of 14 and 15. The majority of N in the atmosphere is composed of ^{14}N (99.6337%) and the remainder is composed of ^{15}N (0.3663%) (Junk and Svec, 1958). All N-containing compounds on earth show a $^{14}N/^{15}N$ ratio close to 272, similar to the $^{14}N/^{15}N$ ratio observed in atmospheric N_2.

Natural ^{15}N abundance is expressed as delta $(\delta)^{15}N$ in per mill (‰) ^{15}N excess over a standard:

$$\delta^{15}N(‰) = \frac{\text{atom\%}^{15}N_{\text{sample}} - \text{atom\%}^{15}N_{\text{standard}}}{\text{atom\%}^{15}N_{\text{standard}}} 1000$$

A slightly different expression for $\delta\,^{15}N$ (‰) uses the R-values of the isotope ratios:

$$\delta^{15}N(‰) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} 1000$$

where $\delta\,^{15}N$ (‰) is the isotope ratio of the sample relative to the atmospheric air standard and R_{sample} and R_{standard} are the molar ratios of $^{15}N-^{14}N$. Both equations have been widely used at the $\delta\,^{15}N$ level. Atmospheric N_2 is the ultimate reference value for $\delta\,^{15}N$ measurements (Mariotti, 1983), but because it remains cumbersome to use atmospheric N_2 as a standard, researchers commonly use a more convenient, off-the-shelf working standard (usually back-calibrated to N_2).

1.2. Isotope fractionation

The $\delta\,^{15}N$ of the product and the substrate (assuming incomplete consumption) of a bio- or physio-chemical process differ when ^{15}N and ^{14}N react at different rates. This is called the isotope effect, expressed as the ratio of the rate constants (k_{14}/k_{15}) and equivalent to ($R_{\text{substrate}}/R_{\text{product}}$). The rate constant for ^{14}N (k_{14}) is larger than for ^{15}N (k_{15}), so the resulting value for the intrinsic kinetic isotope effect is typically >1 (Fry, 1970).

For biologically mediated processes, each step of a complete reaction sequence will have its own intrinsic kinetic isotope effect (Shearer and Kohl, 1986). For field studies, the overall observed isotope fractionation (β_{obs}) for the reaction is more relevant. In an

open system, with unlimited supply of the substrate:

$$\beta_{obs} = \frac{(^{15}N/^{14}N)_{substrate}}{(^{15}N/^{14}N)_{product}}$$

The value for β_{obs} depends on (1) the intrinsic isotope effects for each individual reaction in the sequence, (2) the relative rate of each reaction, and (3) the specific mechanism of the reaction (Shearer and Kohl, 1992a). Another commonly used fractionation factor is α:

$$\alpha = 1 + \frac{\delta^{15}N_{source} - \delta^{15}N_{product}}{1000}$$

which is susceptible to the same influences as β_{obs}.

Most, if not all, biotic processes show an isotope effect but the intensity of the isotope fractionation varies, particularly for biotic processes (Table 1). For example, the median isotope fractionation reported for denitrification is 1.0185 (Table 1), so denitrification depletes the N_2O or N_2 by 18.5‰ and enriches the unreacted NO_3^- with ^{15}N. In contrast, for N_2 fixation the depletion in ^{15}N of fixed N hovers around zero with β_{obs} between 0.9963 and 1.0090 (Table 1).

Isotopic fractionation of ^{15}N also occurs during abiotic processes such as the diffusion of solutes, volatilization of NH_3, and NH_4^+ exchange and fixation (Hübner, 1986). The volatilization of NH_3 shows a strong isotope fractionation with a β_{obs} of 1.0245. When the supply of NH_4^+ is unlimited, volatilization depletes NH_3 by 24.5‰ and enriches the remaining NH_4^+. In contrast, the diffusion of solutes and the exchange and fixation of NH_4^+ has a minimal isotope effect (Table 1).

Table 1
Fractionation values for biotic and abiotic N-cycle processes

	α			β			Reference
	Median	Minimum	Maximum	Median	Minimum	Maximum	
Biotic							
Ammonification				1.0025	1.0000	1.0050	1
Assimilation of NH_4^+	1.0158[a]	1.0091[a]	1.0200[a]	1.0050	1.0050	1.0050	2,3,4,5,6,7
Assimilation of NO_2^-	1.0210[a]	1.0000[a]	1.0360[a]				5,8,9
Assimilation of NO_3^-	1.0142[a]	1.0027[a]	1.0300[a]				3,4,5,9,10, 11, 12
Denitrification	1.0060[b]	1.0025[b]	1.0330[b]	1.0185	1.0000	1.0200	2,13,14,15,16,17,18,19,20,21, 22,23,24,26
N_2 fixation	1.0020[a]	0.9910[a]	1.0090[a]	1.0013	0.9963	1.0090	2,3,5,10,11,27,28,29,30,31,32, 33,34,35,36
N_2O/N_2 loss by denitrification	1.0305[a]	1.0280[a]	1.0330[a]				37
N_2O/N_2 loss by nitrification	1.0600[a]	1.0350[a]	1.0684				8,37
Nitrification	1.0285[a,c]	1.0150[a,c]	1.0350[a,c]	1.0250	1.0250	1.0250	2,8,38
Abiotic							
Exchange of NH_4^+				1.0014	1.0014	1.0014	39
Volatilization of NH_3	1.0400[c]	1.0400[c]	1.0400[c]	1.0245	1.0200	1.0268	38,40

1, Shi et al. (1992); 2, Delwiche and Steyn (1970); 3, Macko et al. (1987); 4, Yoneyama et al. (1991); 5, Wada and Hattori (1978); 6, Pennock et al. (1988); 7, Yoneyama et al. (1991); 8, Yoshida (1988); 9, Ledgard et al. (1985); 10, Mariotti et al. (1980); 11, Wada and Hattori (1978); 12, Medina and Schmidt (1982); 13, Aravena and Robertson (1998); 14, Böttcher et al. (1990); 15, Fryar et al. (2000); 16, Fustec et al. (1991); 17, Hinkle et al. (2001); 18, Koba et al. (1997); 19, Kreitler (1975); 20, Mariotti et al. (1981); 21, Mariotti et al. (1982); 22, Mariotti et al. (1988); 23, Smith et al. (1991); 24, Wellman et al. (1968); 25, Wilson et al. (1994); 26, Yoshida et al. (1989); 27, Minagawa and Wada (1986); 28, Yoneyama et al. (1987); 29, Yamazaki et al. (1987); 30, Hoering and Ford (1960); 31, Kohl and Shearer (1980); 32, Shearer and Kohl (1986); 33, Domenach and Corman (1984); 34, Steele et al. (1983); 35, Shearer et al. (1983); 36, Bergersen and Turner (1983); 37, Handley and Raven (1992); 38, Mariotti (1982); 39, Karamanos and Rennie (1978); 40, Hermes et al. (1985).

[a] Calculated as $\alpha = 1 + ((\delta^{15}N_{source} - \delta^{15}N_{product})/1000)$.

[b] Calculated from enrichment factor ε reported in papers, using $\alpha = 1 + (\varepsilon/1000)$.

[c] Calculated as $\alpha = 1 + (\delta^{15}N_{product}/\delta^{15}N_{source})$.

1.3. Isotopic signatures of N sources

The fractionation of ^{15}N through biotic and abiotic processes contributes to different ranges of ^{15}N:^{14}N ratios for different N sources. Common sources of NO_3^- include animal waste, soil N, and inorganic fertilizer (Heaton, 1986). Animal waste $\delta^{15}N$ is generally between 10 and 20‰, which is higher than the $\delta^{15}N$ of most of the vegetation animals consume (Fig. 1). Metabolic processes lead to ^{15}N enrichment in the animal and concurrent ^{15}N depletion of the excreted N (Steele and Daniel, 1978). Volatilization, a major pathway of N losses from manure, may increase the $\delta^{15}N$ of manure-N (Table 1), resulting in ^{15}N-enriched compounds (Wassenaar, 1995). Groundwater $NO_3^- - \delta^{15}N$ typically falls between 0 and 10‰ but values as low as −15‰ (Wilson et al., 1994) and as high as 80‰ (Böttcher et al., 1990) have been reported (Fig. 1). The typical range of $NO_3^- - \delta^{15}N$ is similar to the range to the $\delta^{15}N$ of soil organic N, which reflects the relative rate of the mineralization and nitrification processes. Non-fractionating mineralization occurs more slowly than nitrification, serving as the rate-limiting step (Wilson et al., 1994). Fertilizer-$\delta^{15}N$ ranges from 0.5 to 5‰ for oxidized N (NO_3^-), with lower values for the reduced form (i.e. NH_4^+ or urea; Fig. 1). Some of the variation presented in Fig. 1 may also be attributed to fractionations associated with sample preparation prior to analysis.

To follow an N source (e.g. manure or fertilizer-N), it must have a ^{15}N:^{14}N ratio distinct from the N already in the system. Based on the principles of the two pool mixing model, the percentage of N derived from the N source can be calculated once the source N mixes with the ^{15}N and ^{14}N present in the system:

$$\%N_{source} = \frac{\delta^{15}N_{sample}}{\delta^{15}N_{source}} 100$$

When using a ^{15}N-enriched source (e.g. enriched ^{15}N fertilizer or residue), the percentage N derived from the enriched N source ($\%N_{enriched\ source}$) in a particular N pool is calculated as:

$$\%N_{enriched\ source} = \frac{\text{atom}\%^{15}N\ \text{excess}_{sample}}{\text{atom}\%^{15}N\ \text{excess}_{enriched\ source}} 100$$

defining atom%^{15}N excess as atom%^{15}N minus background atom%^{15}N.

To trace and quantify the flow of applied N through the various N pools, the $\delta^{15}N$ or the atom%^{15}N of the source must be significantly different from the $\delta^{15}N$ or atom%^{15}N of the background N. This is an absolute requirement for ^{15}N-enriched and ^{15}N-natural abundance studies.

2. Using $\delta^{15}N$ to trace N in landscapes

The first widely reported study of the $\delta^{15}N$ approach to trace N at the landscape scale was carried out in the Mississippi Valley (Kohl et al., 1971). The total amount of fertilizer-derived N in the Mississippi River was estimated by measuring the $\delta^{15}N$ of NO_3^- upstream and downstream and the $\delta^{15}N$ of the fertilizer used. Virgin soil collected from fields in the study region was aerobically incubated, producing NO_3^- with a $\delta^{15}N$ of 13‰; N from fertilizers in the study region had a $\delta^{15}N$ of 3.7‰. Using those two values and measuring the $\delta^{15}N$ of NO_3^- in the surface water, they calculated that between 55 and 60% of the surface water N in the valley was derived from fertilizer. They also found a significant negative correlation between surface water NO_3^- concentration and $\delta^{15}N$–NO_3^-, reported as further evidence that a low $\delta^{15}N$ source (i.e. fertilizer-N) was present to increase NO_3^- concentrations and reduce $\delta^{15}N$ in surface water.

The authors' conclusions generated heavy criticism because the estimates of fertilizer-N input from $\delta^{15}N$ data were considered unreliable (Broadbent et al., 1980; Hauck et al., 1972). Because the $\delta^{15}N$ of inorganic soil N depends on several different N processes, each with its own fractionation (Table 1), the $\delta^{15}N$ of inorganic N in soil or water was thought to be too variable to provide a reliable indicator of an N source. The $\delta^{15}N$ of NO_3^- produced in a laboratory soil incubation study also may not reflect the average $\delta^{15}N$ of soil NO_3^- across a watershed. In follow-up studies, soil ^{15}N was shown to vary by as much as 20‰, further proving its limited use as a tracer (Black and Waring, 1977; Broadbent et al., 1980; Delwiche and Steyn, 1970). Following this early report on $\delta^{15}N$ and the ensuing controversy, the use of $\delta^{15}N$ to determine and

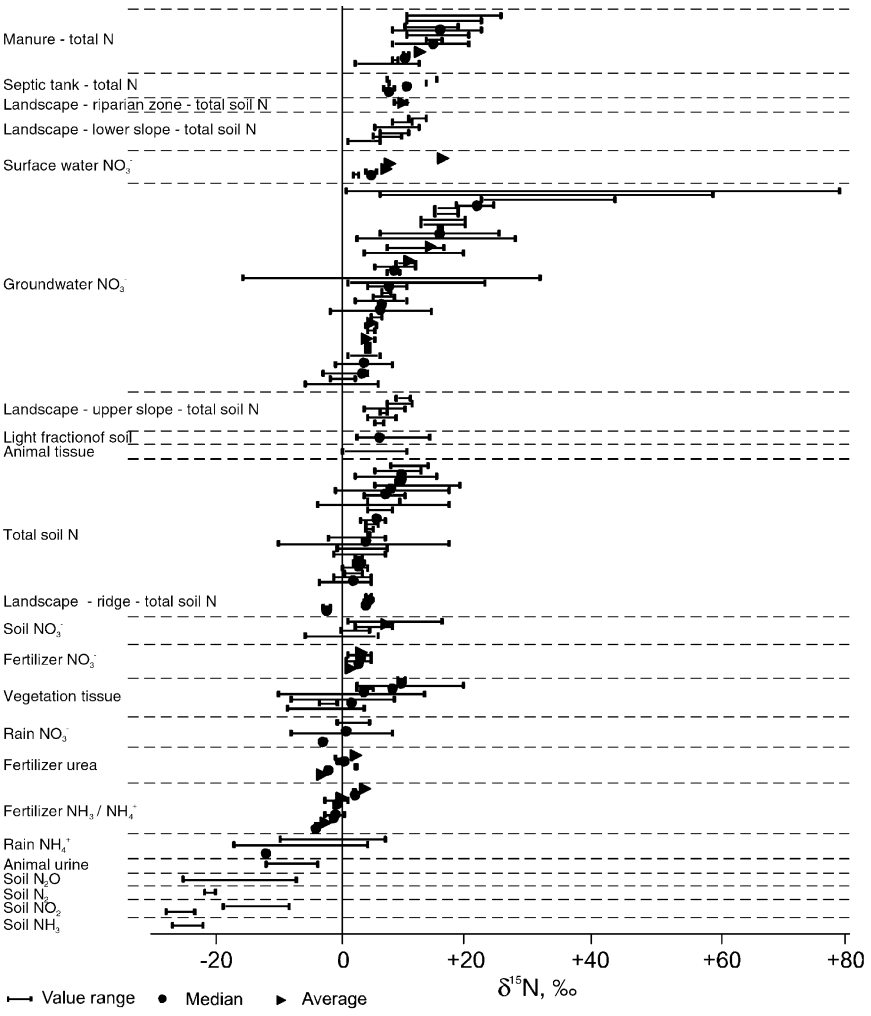

Fig. 1. Range of $\delta^{15}N$ for various organic and inorganic N sources and sinks. Animal—Shearer and Kohl (1992b); fertilizer (NH_3, NH_4)—Kohl et al. (1971), Black and Waring (1977), Farrell et al. (1996), Freyer and Aly (1974), Hübner (1986), Shearer and Kohl (1992b) and Wassenaar (1995); fertilizer (NO_3)—Freyer and Aly (1974), Hübner (1986), Kellman and Hillaire-Marcel (1998) and Shearer and Kohl (1992b); fertilizer (urea)—Black and Waring (1977), Farrell et al. (1996), Hübner (1986), Sutherland et al. (1993) and Wassenaar (1995); groundwater (NO_3)—Aravena et al. (1993), Aravena and Robertson (1998), Black and Waring (1977), Böttcher et al. (1990), Burg and Heaton (1998), Cey et al. (1999), Durka et al. (1994), Fogg et al. (1998), Fryar et al. (2000), Feast et al. (1998), Fustec et al. (1991), Herbel and Spalding (1993), Hinkle et al. (2001), Karr et al. (2001), Koba et al. (1997), Komor and Anderson (1993), Kreitler and Browning (1983), Lindau et al. (1997), Mengis et al. (1999), Panno et al. (2001), Wilson et al. (1994) and Wassenaar (1995); landscape—Fustec et al. (1991), Karamanos and Rennie (1980), Karamanos et al. (1981), Garten (1993) and Brandes et al. (1996); light fraction—van Groenigen and van Kessel (2002); manure—Fogg et al. (1998), Fryar et al. (2000), Wassenaar (1995), Heaton (1986), Karr et al. (2001), Kellman and Hillaire-Marcel (1998) and Kreitler and Browning (1983); N_2—Feast et al. (1998) and Handley and Scrimgeour (1997); N_2O—Hübner (1986) and Mariotti et al. (1981); N_2O—Kim and Craig (1993) and Macko and Ostrom (1994); NH_3—Freyer (1978); rain (NH_4)—Freyer (1978), Hübner (1986) and Shearer and Kohl (1992b); rain (NO_3)—Freyer (1978), Hübner (1986), Kellman and Hillaire-Marcel (1998) and Shearer and Kohl (1992b); septic—Fogg et al. (1998) and Fryar et al. (2000); soil (NO_3)—Black and Waring (1977), Farrell et al. (1996), Koba et al. (1997) and Kreitler and Browning (1983); soil (total N)—Black and Waring (1977), Broadbent et al. (1980), Cheng et al. (1964), Eshetu and Hogberg (2000), Fogg et al. (1998), Garten and van Miegroet (1994), Handley and Scrimgeour (1997), Handley et al. (1999a), Mariotti et al. (1980), Marriott et al. (1997), Shearer and Kohl (1992b), Shearer and Kohl (1986), Shearer et al. (1978) and Wassenaar (1995); surface water (NO_3)—Brandes et al. (1996), Feast et al. (1998), Harrington et al. (1998) and Mengis et al. (1999); vegetation—Brandes et al. (1996), Eshetu and Hogberg (2000), Garten and van Miegroet (1994), Handley et al. (1999a), Shearer and Kohl (1992b, 1986) and van Groenigen and van Kessel (2002).

quantify the source of N in groundwater or streams was largely abandoned for many years.

Recently, the $\delta^{15}N$ technique has been successfully applied to tracing N in landscapes, particularly for following potential sources of N to surface- and groundwater systems. The advancement of continuous flow stable isotope ratio mass spectrometers allows up to 150 samples per day to be processed at a precision of 0.2δ units for N and at a limited cost. Because landscape studies often require a large number of samples to reasonably capture and quantify the high degree of variability and because the differences in $\delta^{15}N$ between source and background may be very small, these advancements in sample preparation and increases in precision have been crucial in improving the feasibility of landscape scale ^{15}N studies.

Agricultural landscapes encompass multiple sources of NO_3^- and it is important to isolate the source posing the greatest risk to water supplies. When a single strong source dominates, the $\delta^{15}N$ method may determine the extent of N movement. For example, Karr et al. (2001) studied the effectiveness of riparian buffers in removing NO_3^- from land-applied swine waste. They found identical median $\delta^{15}N$ of 15‰ for samples taken from swine waste lagoons, groundwater wells, and local streams. The stream values were highest (15.4‰) adjacent to and downstream from the farm site, indicating that buffers were not protecting against NO_3^- leaching. The upstream and downstream differences were particularly pronounced when $\delta^{15}N$–NO_3^- measurements were taken the day of swine waste application: downstream $\delta^{15}N$ was 1.5–10 times greater than upstream $\delta^{15}N$.

The $\delta^{15}N$ technique has also been used to determine which of multiple potential N sources are contributing NO_3^- to surface and ground water. In some areas, a single source may dominate (Kreitler and Browning, 1983), but more frequently, several sources contribute (Iqbal et al., 1997; Kellman and Hillaire-Marcel, 1998; Wassenaar, 1995; Wilson et al., 1994). For example, Komor and Anderson (1993) found $\delta^{15}N$ greater than 10‰ in the groundwater beneath certain feedlots, cultivated-irrigated fields where manure was applied, and septic systems in residential areas, indicating that animal and human waste was a dominant source of NO_3^- in the sand-plain aquifers in central Minnesota. At all non-feedlot locations, $\delta^{15}N$ of less than 2‰ provided evidence that inorganic fertilizers from cultivated areas were also contributing NO_3^- to the aquifers.

Many studies do not report the results of tests of significance difference, but a wide range of differences have been construed as evidence of N sources (Table 2). Although the upstream and downstream

Table 2
Compilation of $\delta^{15}N$ differences between sources and sinks in landscape and watershed studies that report $\delta^{15}N$ as a successful tracer of N

Source	Sink	Sink mean $\delta^{15}N$ (‰)		Detected difference (‰)	Reference
		Without source	With source[a]		
Animal waste	Soil water	2.6	12.2	9.6[b]	Fogg et al. (1998)
Animal waste	Stream water	13.2	15.5 (15.4)	2.3[c]	Karr et al. (2001)
Animal waste	Groundwater	3.1	21.3	18.2	Komor and Anderson (1993)
Cultivated-irrigated	Groundwater	3.1	7.4	4.3	Komor and Anderson (1993)
Cultivated-non-irrigated	Groundwater	3.1	3.4	0.3	Komor and Anderson (1993)
Poultry manure	Soil	4.3	10.6 (8.3)	6.3	Wassenaar (1995)
Poultry manure	Soil water	10.8	15.2 (8.3)	4.4	Wassenaar (1995)
Poultry manure	Groundwater	<6	12 (8.3)	>6	Wassenaar (1995)
Residential with septic	Groundwater	3.1	6.0	2.9	Komor and Anderson (1993)
Septic	Groundwater	4.6	9.9	5.3[d]	Aravena et al. (1993)
Septic	Soil water	2.6	8.8	6.2[b]	Fogg et al. (1998)

[a] Source value, when reported, appears in parentheses.
[b] Significantly different with 95% confidence.
[c] Not significantly different with 95% confidence. Compares upstream values to stream values adjacent to fields with applied swine waste.
[d] Significantly different—confidence level not reported.

$\delta^{15}N$ were not significantly different, Karr et al. (2001) still suggested swine waste as the primary N source in a North Carolina Catchment. Several studies examining multiple N sources do not even report background values, but instead distinguish among possible N sources for an area by comparing the range of $\delta^{15}N$ present in the sinks to previously reported source values (Wilson et al., 1994). Many reportedly successful studies have relied on measures of central tendency to detect differences induced by N sources because individual measurements can be highly variable. Lindau et al. (1997) found that although the mean $\delta^{15}N$ for NH_4^+ and NO_3^- differed by 10‰, the individual values overlapped, Edwards (1973) observed that the innate variability of the $\delta^{15}N$ of fertilizer being applied was of the same order of magnitude as the mean difference between the $\delta^{15}N$ of the fertilizer and the $\delta^{15}N$ of the soil-derived NO_3^-, and Fogg et al. (1998) observed ranges of ≥ 10‰ within the animal sources, depending on the site. Variability within and among sites suggests that comparing $\delta^{15}N$ to previously reported source values may be misleading. To properly interpret $\delta^{15}N$ results, variability must be recognized and accounted for.

2.1. Sources of $\delta^{15}N$ variability and precautions

When NO_3^- moves from terrestrial to aquatic systems, the N-cycle may increase the $\delta^{15}N$ of N pools through nitrification, denitrification, ammonia volatilization and/or leaching of depleted NO_3^- (Handley and Raven, 1992). Denitrification and volatilization have particularly strong isotope effects, leading to large $\delta^{15}N$ differences. When the ^{15}N and ^{14}N isotopes undergo a series of fractionations, differences in $\delta^{15}N$ among the various source N pools may disappear, limiting the usefulness of $\delta^{15}N$ measurements (Böttcher et al., 1990; Lindau et al., 1997; Panno et al., 2001; Robinson, 2001; Wilson et al., 1994).

The type of fractionation occurring and the potential rate of the fractionating process under a given set of environmental conditions remain important. For example, Aravena et al. (1993) and Burg and Heaton (1998) suggested that limiting soil NH_4^+ or containing animal waste minimizes volatilization losses, resulting in lower $\delta^{15}N$ than that normally associated with animal waste subjected to extensive volatilization. When all factors influencing fractionation are not considered, it may be difficult to interpret the results correctly. Harrington et al. (1998) found $\delta^{15}N$ in streams that might suggest animal sources (7.1‰), but were unwilling to conclusively attribute these to the nearby dairy farm because they could not be certain of the extent of volatilization.

Temporal variability of N-cycle processes may also be a factor. Panno et al. (2001) observed an increase in groundwater $\delta^{15}N\text{-}NO_3^-$ from spring (4.69‰) through summer (6.21‰), fall (6.34‰), and winter (9.32‰) due to differing denitrification rates. Increasing the temporal frequency of sampling and incorporating seasonal land use data could minimize ambiguities due to temporal variability (Iqbal et al., 1997; Komor and Anderson, 1993).

The spatial variability of $\delta^{15}N$ for the various N sources should also be considered. Observed increases in $\delta^{15}N$ with soil profile and groundwater well depth have been attributed primarily to increased denitrification with depth. In soils, biodegradation, humification, and increased adsorption of NO_3^- by soil with depth may also alter the isotopic signature. Black and Waring (1977) observed soil $\delta^{15}N$ ranging from 5.5‰ near the surface to 10.6‰ at depths of 5 m. Similarly, in groundwater, Cey et al. (1999) observed a range for NO_3^- from 4.8‰ near the surface to 24.8‰ for the deepest sampling well and Böttcher et al. (1990) observed a range of nearly 80‰ between shallow and deep sampling wells. Although high $\delta^{15}N$ for NO_3^- should make it easy to trace, the quantities of NO_3^- are often too small due to denitrification losses (Fustec et al., 1991; Farrell et al., 1996).

Spatial variability of organic N mineralization may contribute to different $\delta^{15}N$ for surface mineral soil N across study sites (Garten and van Miegroet, 1994). Plant $\delta^{15}N$ reflects the tremendous variability of the available soil N (Sutherland et al., 1991, 1993; Garten, 1993), although other factors such as stress, genotype and mycorrhizal associations also influence plant $\delta^{15}N$ (Handley et al., 1999b; Handley and Scrimgeour, 1997; van Groenigen and van Kessel, 2002).

There are no clear or easy rules regarding the minimum difference required between the source and background $\delta^{15}N$ to trace N in a landscape. When there is only one source of N, and that source has stable and unique $\delta^{15}N$, the minimum difference in

$\delta^{15}N$ between the source and background can be small. For example, atmospheric N_2 is the only N source for N_2 fixation, and has known, stable $\delta^{15}N$ (typically assumed to be 0). Minimal isotopic fractionation occurs during N_2 fixation by legume symbioses (Table 1), so differences of only a few $\delta^{15}N$ units could be sufficient to determine the relative contribution of N_2 fixation, assuming the $\delta^{15}N$ of an adjacent non-N_2-fixing plant accurately reflects the $\delta^{15}N$ of the soil available N pool. If there are several N processes that can change the $\delta^{15}N$ of the N source or the N sink, a difference of only a few $\delta^{15}N$ units between the source and sink will not suffice. Although a survey of studies that used the $\delta^{15}N$ method across landscapes shows a mean difference of 5.9‰ (Table 2), variability in the type and number of fractionating processes dictates that no single number can be meaningfully applied in all cases without corresponding statistical analyses.

3. Landscape patterns of $\delta^{15}N$

Broadbent et al. (1980) found soil $\delta^{15}N$ spatial variability of the same magnitude as the difference between soil and atmospheric N, whereas Fogg et al. (1998) found negligible spatial variability in flat, uniform soils. Where large variations in $\delta^{15}N$ are present, they may be related to landscape-scale variations in topography within a given site (Karamanos et al., 1981). Soil $\delta^{15}N$ and plant $\delta^{15}N$ can have depression-centered patterns, suggesting that even very minor differences in drainage can dramatically influence not only soil profile characteristics, but also soil organic matter and N-cycle processes (Karamanos and Rennie, 1980).

By examining several studies, Shearer and Kohl (1986) observed that the $\delta^{15}N$ of soils on lower slopes and in riparian zones tends to be greater than on upper slopes and ridges (Fig. 1). In forest soils in Ohio, Morris and Boerner (1998) observed that topography was the best predictor of mineralization and nitrification differences, because it can control soil moisture, microclimate, nutrient levels and soil formation. Several studies have successfully illustrated the landscape pattern of N-mineralization using $\delta^{15}N$. Mineralization tends to be higher (30% higher as reported by Garten (1993)) in or adjacent to depressions where soil water converges than on the drier upper slopes (Pennock et al., 1992; Stevenson et al., 1995). This pattern contributes to the greater levels of soil inorganic N in depressions and valley bottoms (Stevenson et al., 1995).

Topography also indirectly influences denitrification (van Kessel et al., 1993; Corre et al., 1996; Velthof et al., 2000): it occurs where water table fluctuations result in anaerobic microsites within the soil (Fryar et al., 2000), within the hyporheic zone of streams (Hinkle et al., 2001), in the organic-rich soils of the riparian zone (Cey et al., 1999), or within the stream or groundwater (Aravena and Robertson, 1998; Iqbal et al., 1997; Kellman and Hillaire-Marcel, 1998; Komor and Anderson, 1993; Mengis et al., 1999; Wilson et al., 1994). Thus, denitrification displays a distinct landscape-scale pattern, with maximum values in the depressions (up to 0.48 kg N ha^{-1} d^{-1} as reported in Sutherland et al. (1993)) and minimum values on the knolls (Groffman and Tiedje, 1989; Pennock et al., 1992; van Kessel et al., 1993). Sutherland et al. (1993) observed this pattern in landscapes with gradients as gentle as 1°. Clay et al. (1997) attributed a net N loss of up to 95 kg ha^{-1} in the depressions to denitrification, suggesting that denitrification reduces the amount of NO_3^- available for leaching, consistent with lower median NO_3^- concentrations in depressions (Farrell et al., 1996). Denitrification enriches the unreacted NO_3^- in ^{15}N, which may also partially explain the higher foliar $\delta^{15}N$ in depressions. Failing to account for denitrification results in underestimation of gross nitrification (Kellman and Hillaire-Marcel, 1998).

In riparian landscapes, several N-cycle processes may occur over a very short distance, giving rise to a complex spatial pattern for $\delta^{15}N$. For example, Brandes et al. (1996) observed rapid changes in isotopic enrichment and N concentrations between the riparian zone and the stream channel. At the boundary between the uplands and the riparian zone, very low NO_3^- concentrations and high ^{15}N enrichment of the NH_4^+ suggested partial nitrification followed by nearly complete denitrification. Preferential removal of N by plants may also have been a factor. Gaseous NH_3 losses were considered unlikely due to the low pH (<6.0) of the groundwater. Within the riparian zone, the release of NH_4^+ by organic matter mineralization enhanced the rapid spatial transition from a NO_3^-

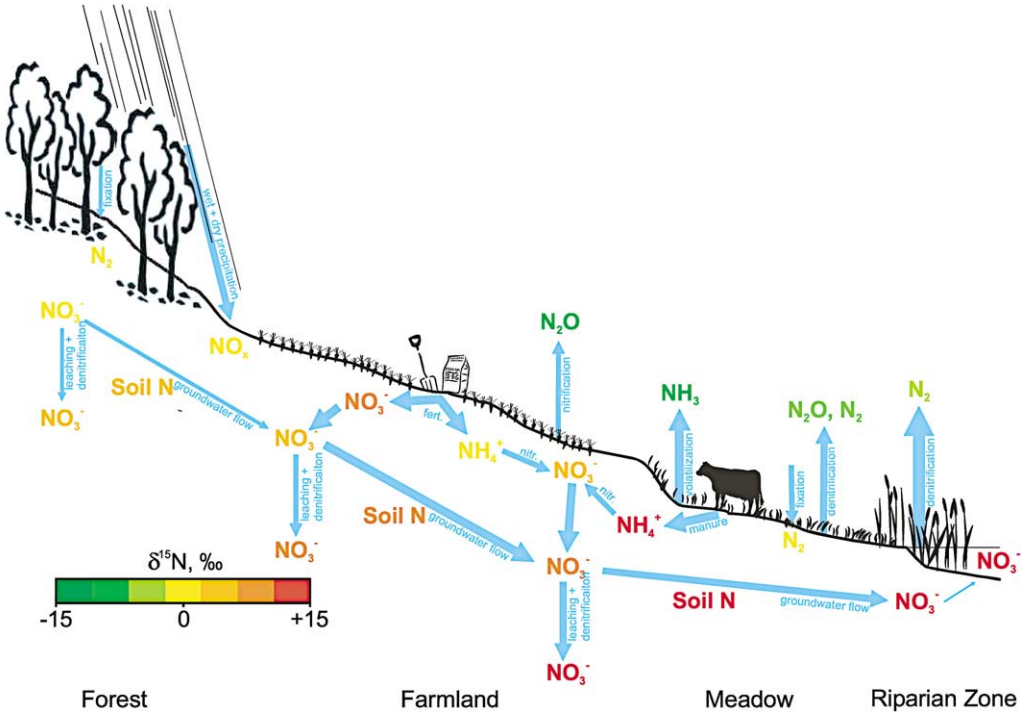

Fig. 2. A conceptual overview of landscape influence of $\delta^{15}N$ of organic and inorganic N.

($\delta^{15}N = 6.25 \pm 0.9‰$) dominated system upland to an NH_4^+ ($\delta^{15}N = 9.17 \pm 1.0‰$) dominated system in the riparian zone.

A conceptual model depicting changes in $\delta^{15}N$ of the various N sources and sinks along a landscape can be constructed (Fig. 2). The two main processes that can lead to major N losses along a landscape are denitrification and the volatilization of NH_3 (Table 1), which both show a high ^{15}N enrichment factor. The long-term effects of other processes, such as leaching and runoff, on the signature of the residual N are unknown. If NO_3^- and soluble C are present, higher soil moisture content in the lower landscape positions increases denitrification activity (Fig. 2), increasing the $\delta^{15}N$ of plant and soil organic matter-N (Sutherland et al., 1993; Fig. 2). Ammonia volatilization occurs mainly following the application of urea fertilizers and manure but remains largely independent of topographic influences with the exception of soil pH. In some landscapes, topographic controls on soil moisture affect depth to $CaCO_3$ contributing to higher pH in the upper landscape positions (Pennock et al., 1987, 1992). Because high soil pH increases volatilization (Tisdale et al., 1993), upper landscape positions may be more susceptible to N losses.

The N that enters the system by dry deposition has $\delta^{15}N$ of approximately 0‰, whereas wet deposition is depleted in ^{15}N (Fig. 1, rain), but in general the inputs would be too small to significantly alter soil-$\delta^{15}N$ across landscapes with high N fertilizer input. N_2 fixation by legume symbioses also lacks a clear landscape pattern because of its minimal isotopic effect and its $\delta^{15}N$ close to 0‰. The amount of fixed N, however, varies across the landscape, controlled by the amount of available soil N (Androsoff et al., 1995; Stevenson et al., 1995). Once fixed, the N can be denitrified or lost by volatilization thereby changing the isotopic composition of the available soil N pool.

4. ^{15}N enriched method

The material applied in the ^{15}N-enriched method has a ^{15}N concentration significantly different from unlabeled N in the system being examined. The ^{15}N enrichment or depletion of an N pool by adding

isotopically labeled material will reflect where and to what extent the enriched material has been incorporated (Hauck and Bremner, 1976). This allows determination of the rate of N cycling in the various N pools in the ecosystem, determination of the total applied N losses from the ecosystem, and refinement of ecosystem-scale models of N cycling (Nadelhoffer and Fry, 1994). This technique has been used extensively for a number of years, and has been accepted by the scientific community at large as the most reliable way to follow the flow and fate of N in systems. Researchers are increasingly using the ^{15}N-enriched technique at the large plot or small catchment scale (1–10 ha, Nadelhoffer and Fry, 1994). The cost of applying ^{15}N-enriched materials is a significant disadvantage, particularly at landscape scales. Depending on the compound, the current price (2002) of 99.9 atom%^{15}N is >$200US per g of ^{15}N.

4.1. Using ^{15}N enriched tracers in landscapes

The relatively narrow range of δ^{15}N for most natural materials (Fig. 1) allows application of slightly ^{15}N-enriched tracers with atom%^{15}N excess of 0.04–0.4 to be used as tracers in ecosystems (Nadelhoffer and Fry, 1994). Landscape-scale research using such slightly enriched tracers can improve the potential for isolating the various N-cycle processes and sinks within a given ecosystem, and allows for more precise determination of N turnover rates (Mulholland et al., 2000; Tank et al., 2000). Repeated watershed studies from ecosystems throughout North America used ^{15}N-enriched tracers ($^{15}NH_4^+$) in streams to examine N dynamics (Peterson et al., 2001). Overall, the stream bottom took up 70–80% of the $^{15}NH_4^+$, and 20–30% was removed by nitrification. While nitrification added NO_3^- to the stream, biological assimilation and denitrification removed NO_3^- from the stream, contributing to a dynamic balance among these processes. This study also demonstrated the importance of small streams for limiting NO_3^- input in large watersheds because their surface-to-volume ratio contributes to rapid N cycling (Peterson et al., 2001).

When potential sources of N are labeled with ^{15}N, their flow and fate in the landscape can be traced. For example, Di et al. (1999) examined relative leaching losses using ^{15}N-labeled dairy-shed effluent and NH_4^+ fertilizer. The autumn-applied N had higher potential for leaching from both sources than N applied in spring, but the dairy-shed effluent had lower leaching losses in the year following application. Between 4.5 and 8.1% of the ^{15}N-labeled mineral N from the effluent was lost, in contrast to 15.1–18.8% of the fertilizer N. The difference was accounted for by the stimulated microbial activities and increased immobilization associated with the application of dairy-shed effluent.

5. Relative advantages of the δ^{15}N and enriched ^{15}N methods

The δ^{15}N and ^{15}N-enriched methods both require a significant difference between the atom%^{15}N of the source being examined and the background ^{15}N (Table 3). If the N sources are not sufficiently distinct, the occurrence of isotopic fractionation could limit the inferences drawn (Shearer and Kohl, 1986). Hence, both methods require knowledge of the atom%^{15}N of the N pools in the ecosystem being studied (Robinson, 2001), including a characterization of spatial variability of the ^{15}N background (Chalk and Ladha, 1999). In particular, the δ^{15}N method requires an understanding of landscape-induced variability in δ^{15}N caused by isotopic fractionation during the N-cycle (Fig. 2).

The two methods differ in the amount of disturbance to the ecosystem and the type of information collected (Table 3). With the δ^{15}N method, the function of ecosystems can be examined without adding an external source of N (Garten and van Miegroet, 1994; Shearer and Kohl, 1992b). Adding N, whether labeled or at the natural abundance level (manure or fertilizer), will influence the N-cycle processes, limiting the validity of conclusions regarding the flow of N. This may be of little concern in agro-ecosystems with routine additions of fertilizer N, but could have a confounding effect in previously undisturbed ecosystems. When using the ^{15}N-enriched method in undisturbed ecosystems, it may be preferable to apply a minimal amount of a highly ^{15}N-enriched compound (i.e. easily detectable), but the cost of a highly enriched source may be restrictive (Table 3).

It has been argued that the use of δ^{15}N as a tracer requires great caution (Handley and Scrimgeour,

Table 3
Comparison of ^{15}N enriched and $\delta^{15}N$ natural abundance techniques for landscape-scale studies. Adapted and modified from Robinson (2001)

	^{15}N enriched	$\delta^{15}N$ natural abundance
Range of ^{15}N abundance	Greater than natural abundance range	Within natural abundance range
System perturbation	Minimal if typical N applications can be enriched	Minimal to zero if only examining existing N pools
	High if labeled N (i.e. manure, fertilizer) applied to a system that does not ordinarily have added N	High if unlabeled N (i.e. manure, fertilizer) applied to a system that does not ordinarily have added N
Cost of tracer	High, particularly at the landscape and watershed scales	Zero
Ease of detection	Excellent	Poor to good
Duration of study	<1 h to >1 year	>1 h to $\gg 1$ year
Scale of study	Pot to landscape	Pot to landscape
Conditions required	^{15}N range of enriched N tracer greater than natural range	Significant differences in $\delta^{15}N$ among all sources and sinks (mean in Table 2 = 5.9‰)
Isotopic information required	^{15}N of tracer before addition to the system	$\delta^{15}N$ of all potential N sources
	Amount of tracer added	^{15}N of the system before and after any N addition
	^{15}N of the system before and after tracer addition	N fluxes among pools if more than two sources are involved
		Landscape influences on fractionation and system values (must account for influences on $\delta^{15}N$ background range)
Other information required	Ecology, biology and history of system being studied	Ecology, biology and history of system being studied
Interpretive model	Mixing	Mixing
Information obtained	Amounts and rates of mixing of enriched N in potential pools	Amounts and rates of mixing of source N in potential pools
	Quantitative	Semi-quantitative to qualitative
Applications	Testing hypotheses	Pattern analysis
		Generating hypotheses

1997; Robinson, 2001). Nitrogen input by N_2 fixation across a landscape was used to emphasize this point because the source of fixed N is atmospheric N_2, which has a very stable isotopic signature (Mariotti, 1983). Assuming that only biological N_2 fixation changes the isotopic signature of an N_2-fixing legume compared to an adjacent non-N_2-fixing plant, estimating N input by N_2 fixation across a landscape should be well-suited to quantification by $\delta^{15}N$. Handley and Scrimgeour (1997) cited, inter alia, the absence of a significant correlation between concurrent estimates of N input by N_2 fixation across landscapes by both the ^{15}N enriched and the $\delta^{15}N$ approach (Androsoff et al., 1995; Stevenson et al., 1995) as evidence that the $\delta^{15}N$ approach is not suitable as a tracer of N.

Boddey et al. (2000) strongly disagreed with Handley and Scrimgeour's (1997) argument, countering that high variability of the N_2-fixing process across the landscape caused the lack of a significant correlation between the ^{15}N enriched and the $\delta^{15}N$ approach. Indeed, in a subsequent landscape study the spatial variation in N input by biological N_2 fixation was found to be too large to allow a comparison to be made between the ^{15}N enriched and the $\delta^{15}N$ approaches (Walley et al., 2001). However, the results by Walley et al. (2001) and Sutherland et al. (1991) also implied that the short-range variability in the $\delta^{15}N$ of plant available soil N across the landscape was high, the causes of which remain unknown. Such unpredictable short-range

variability in the $\delta^{15}N$ of available soil N lends support to Handley and Scrimgeour's (1997) argument of the limited suitability of the $\delta^{15}N$ approach for tracing N at the landscape scale.

When an artificially enriched source is used as a tracer, the occurrence of fractionating processes and the innate variability of $\delta^{15}N$ do not affect study outcome (Broadbent et al., 1980). In ^{15}N-enriched studies, the variability inherent to soil $\delta^{15}N$ is only a problem when the applied tracers are highly diluted (Cheng et al., 1964) and the difference between the atom% ^{15}N of the source and sinks becomes small. Typically, when using ^{15}N-enriched material as a tracer, the ^{15}N enrichment of the source or the ^{15}N depletion of the product will be well outside the experimental variability in $\delta^{15}N$ of the N in the background. Thus, ^{15}N enriched tracer methods are better suited to studies examining the fate and transformations of N and tracing the path of N from source to sink, whereas the $\delta^{15}N$ method may be limited to analyzing patterns and generating hypotheses (Table 3; Handley and Scrimgeour, 1997; Hauck and Bremner, 1976).

6. Conclusions

Several studies have reported success in using $\delta^{15}N$ at the landscape scale to determine the primary sources of N in a given system and to examine N cycling under different conditions. The average difference between source and background $\delta^{15}N$ was 5.9‰. Although it may be tempting to use this mean difference of approximately 6δ units as a measure of success, each study will have its own statistical measure of difference. As with ^{15}N-enriched studies, the amount of N applied, its $\delta^{15}N$, and the size and turnover rates of the various N pools will ultimately determine what constitutes a meaningful separation. Fractionation can alter the $\delta^{15}N$ of the source, thereby increasing variability and limiting the interpretability of the $\delta^{15}N$. The amount of fractionation can be affected by temporal or spatial variability of N-cycle processes across the landscape. Even when the spatial variability can be accounted for (i.e. by landscape patterns), it has been argued that the inherent short-range variability of $\delta^{15}N$ limits the usefulness of natural abundance techniques to qualitative or semi-quantitative interpretations. To overcome these limitations, some researchers have successfully applied artificially enriched tracers at the large plot or small catchment scale (Nadelhoffer and Fry, 1994). The ^{15}N levels of labeled tracers may be predetermined to ensure detection within the various N pools, allowing for more precise quantification of the rates and types of processes occurring.

Given the increasing levels of N in surface- and groundwater systems, identification of the primary sources of N and modification of management practices are important. The $\delta^{15}N$ and ^{15}N-enriched approaches may both contribute to this endeavor. The $\delta^{15}N$ technique can work well in undisturbed ecosystems where applying N tracers, enriched or non-enriched, may influence the normal functioning of the system. Furthermore, the lower cost of $\delta^{15}N$ studies can provide an excellent first approximation of N sources and cycling at a given site. In agroecosystems where the occurrence of NO_3^- movement has already been established, it may be most beneficial to use a combination of $\delta^{15}N$ and ^{15}N-enriched approaches to provide a complete, accurate picture of the flow and fate of N sources.

Overall, we concur with Robinson (2001) who stated in a recent review that the $\delta^{15}N$ approach should be used for semi-quantitative studies of $\delta^{15}N$ pattern analysis and for generating new hypotheses about N cycling, whereas the ^{15}N-enriched method can also be used to quantitatively test hypotheses. Both methods require an understanding of the variability of $\delta^{15}N$ across a landscape, whether innate or due to fractionating processes; when the majority of the variability in $\delta^{15}N$ can be explained, the $\delta^{15}N$ method provides a less invasive, more affordable way to identify sources of N. In all situations where variability in $\delta^{15}N$ cannot be thoroughly accounted for and where differences between the $\delta^{15}N$ of the source and sink are too small, the ^{15}N-enriched method is recommended.

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