

Tracing anthropogenic inputs of nitrogen to ecosystems

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Introduction

Nitrate (NO_3^-) concentrations in public water supplies have risen above acceptable levels in many areas of the world, largely as a result of overuse of fertilizers and contamination by human and animal waste. The World Health Organization and the U.S. Environmental Protection Agency have set a limit of 10 mg L^{-1} nitrate (as N) for drinking water because nitrate poses a health risk, especially for children, who can contract methemoglobinemia (blue-baby syndrome). Nitrate in lower concentrations is non-toxic, but the risks from long-term exposure are unknown, although nitrate is a suspected carcinogen. High concentrations of nitrate in rivers, lakes, and coastal areas can cause eutrophication, often followed by fish-kills, due to oxygen depletion. Increased atmospheric loads of anthropogenic nitric and sulfuric acids have caused many sensitive, low-alkalinity streams in North America and Europe to become acidified. Still more streams that are not yet chronically acidic could undergo acidic episodes in response to large rain storms and/or spring snowmelt, seriously damaging sensitive local ecosystems. Future climate changes may exacerbate the situation by affecting biogeochemical controls on the transport of water, nutrients, and other materials from land to freshwater ecosystems.

The development of effective management practices to preserve water quality, and remediation plans for sites that are already polluted, requires the identification of actual N sources and an understanding of the processes affecting local nitrate concentrations. In particular, a better understanding of hydrologic flowpaths and solute sources is required to determine the potential impact of contaminants on water supplies. Determination of the relation between nitrate concentrations in groundwater and surface water and the quantity of nitrate introduced from a particular source is complicated by:

- 1 the occurrence of multiple possible sources of nitrate in many areas;
- 2 the presence of overlapping point and non-point sources;
- 3 the co-existence of several biogeochemical processes that alter nitrate and other chemical concentrations.

In many circumstances, isotopes offer a direct means of source identification because different sources of nitrate often have distinct isotopic compositions. In addition, biological cycling of nitrogen often changes isotopic ratios in predictable and recognizable directions that can be reconstructed from the isotopic compositions. Nitrogen isotopes ($\delta^{15}\text{N}$) have been used to identify N sources and processes in hundreds of studies over the past several decades (Heaton 1986). Since the early 1990s, nitrate isotope studies have often included analysis of the oxygen isotopes of nitrate ($\delta^{18}\text{O}$), especially in studies of the role of atmospheric deposition in watersheds (Kendall 1998). Analysis of nitrate $\delta^{17}\text{O}$ is a promising new tool for determining nitrate sources and reactions, and complements conventional uses of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. But the most promising forensic isotopic approaches combine nitrate isotopes with multi-isotope and multi-tracer approaches that track trace elements and organics specific to different sources of nitrate.

The primary goal of this chapter is to examine recent progress in the use of natural abundance isotopes of nitrate and other N-bearing species for identifying and quantifying the relative contributions of N from different anthropogenic sources (including fertilizer, sewage and animal waste, and atmospheric deposition) to various ecosystems, with an emphasis on applications to watersheds. This chapter contains sections on (i) the isotopic compositions of major N reservoirs, (ii) major processes affecting the isotopic composition of these reservoirs, (iii) how to distinguish the effects of mixing of sources from the effects of processes, (iv) applications to major ecosystem settings, and (v) a summary of the status of various "isotope tools".

Isotope techniques are a subset of tools available for hydrologists and biogeochemists studying nitrogen cycling in ecosystems; they are not a panacea. Most of the uses of nitrate isotopes are for source identification and qualitative estimations of source contributions, not quantitative determinations. The greatest problems for isotope studies are:

- 1 that different sources can have partially overlapping isotopic compositions;
- 2 sources can have considerable spatial and temporal variation in isotopic composition;
- 3 isotope fractionations can blur initially distinctive isotopic compositions.

These problems can often be minimized or eliminated by a multi-isotope, multi-tracer approach which also takes advantage of hydrologic and chemical data.

Why are stable isotope techniques underused in surface water studies in large agricultural basins?

While stable isotopes have become common tools for tracing sources of waters and solutes in small watersheds (e.g., Kendall & McDonnell 1998) and groundwater systems (e.g., Cook & Herczeg 2000), they are currently

underutilized in larger basins, especially in agricultural rivers. This is probably because one of the first attempts to use natural abundance $\delta^{15}\text{N}$ to understand the causes of the increases in nitrate concentrations in surface waters in many agricultural areas (Kohl et al. 1971) elicited a very critical response by 10 prominent soil scientists and agronomists (Hauck et al. 1972), which concluded that use of natural abundance $\delta^{15}\text{N}$ was a "questionable approach."

The abundant publications over the next two decades that used natural abundance ^{15}N to determine the source of nitrate in groundwater evoked little such controversy. However, it appears that the critical response to the Kohl et al. study effectively inhibited similar investigations in rivers in agricultural areas in the USA until the late 1990s when some technological advances resulted in renewed interest in attempting to use stable isotopes to quantify nitrate sources in agricultural basins.

Since the study by Kohl et al. (1971) appears to have had such a dramatic and continuing impact, it is useful to briefly examine the nature of the original controversy. Kohl et al. (1971) investigated sources of nitrate in drainage waters of the Sangamon River (Illinois, USA). As part of their study, several dozen nitrate samples were collected from drain tile effluent, plus samples from a nearby lake, the Sangamon River, and a drainage ditch. The $\delta^{15}\text{N}$ of the two potential end-members, soils and fertilizer, was determined. A linear regression through the data on a plot of $\delta^{15}\text{N}$ vs. concentration intersected the values measured for fertilizer and incubated soils. The trends were attributed to mixing of NO_3^- from nitrification of soil N and fertilizer N, and they concluded that about half the nitrate was derived from soil sources and half from unfractionated fertilizer nitrate.

This conclusion was strongly criticized by Hauck et al. (1972) and others because they contended that fractionation effects and natural variability in soil systems would make it impossible to apply simple mixing models to the $\delta^{15}\text{N}$ values. Specifically, the response by Hauck et al. (1972) made five main points:

- 1** analytical precision of natural abundance measurements is insufficient for quantifying sources over the small range of differences in ^{15}N between fertilizer and soil end-members (0.004 atom % or 10‰);
- 2** fertilizer NH_4^+ mixes with soil N before it is oxidized to NO_3^- , thus losing its isotopic signature;
- 3** insufficient soil samples were analyzed to assess the true variability in the $\delta^{15}\text{N}$ of soils within the >900 square mile basin;
- 4** it is difficult to correct for the biological fractionation effects that cause great variability in $\delta^{15}\text{N}$ in soils;
- 5** the $\delta^{15}\text{N}$ of NO_3^- produced by nitrification of soil organic N is best determined by short-term incubations of soil, not the long-term incubations performed by Kohl et al.

The response by Kohl et al. (1972) to Hauck et al. (1972) carefully considered but ultimately dismissed most of the criticisms, responding that despite all

the possible confounding complications, the surface water sample data themselves strongly supported their interpretation of mixing of soil and fertilizer NO_3^- , and that their evaluation method probably underestimated the true proportion of fertilizer-derived NO_3^- . The Sangamon River data, along with data from a somewhat similar study on the small Yerres River (France) described in Mariotti & Létolle (1977) and Létolle (1980), were reanalyzed by Hübner (1986). He noted that a logarithmic relation could be fitted to both data sets, with apparent enrichment factors of ca. 5‰, suggesting that denitrification or assimilatory fractionation effects could also be a factor. Because of the complications noted in both of these studies, Shearer & Kohl (1993) suggested that perhaps the best use of natural abundance ^{15}N research is not to try to estimate contributions from different sources, but to study N transformations. A recent review of agricultural $\delta^{15}\text{N}$ studies concluded that the most appropriate applications were for semi-quantitative to qualitative estimates of source proportions, pattern analysis, and generating hypotheses (Bedard-Haughn et al. 2003). However, this may be an overly conservative conclusion.

In the past few decades, numerous studies have shown that stable isotopic techniques are a powerful tool for determining sources and sinks of nutrients and organic matter in relatively small watersheds. Recently, these and newer isotope techniques have been successfully applied to tracing sources and sinks in large river basins, including the Mississippi River (Battaglin et al. 2001a,b; Kendall et al. 2001; Chang et al. 2002; Panno et al. 2006), large rivers in the northeastern USA (Mayer et al. 2002), the San Joaquin River in California (Kratzer et al. 2004), the Oldman River in Alberta (Canada; Rock & Mayer 2004), and the Seine River (France; Sebiló et al. 2006).

In reality, the isotopic compositions of nitrate are often the result of both mixing and cycling, and thus, a multi-tracer approach is usually the best approach. In the late 1980s and 1990s, a number of new approaches for studying the impact of agricultural sources of N on groundwater and surface water were developed. Most are based on using a multi-isotope and/or multi-tracer approach to resolve N source vs. cycling questions. The result has been scores of studies tracing sources of N and investigating N transformations in agricultural, urban, and forested watersheds, ranging from small to very large basins.

Perhaps the five most successful new isotopic approaches are:

- 1** Analysis of the N_2 gas produced by denitrification as a means for “correcting” for the fractionating effects of denitrification so that the initial $\delta^{15}\text{N}$ of the NO_3^- (and hence its source) can be determined. Examples: Vogel et al. (1981), Wilson et al. (1990), Böhlke & Denver (1995), McMahon & Böhlke (1996).
- 2** Development of methods for age-dating groundwater recharged in the past ca. 50 years with precisions of 1–3 years using chlorofluorocarbons (CFCs), T^3He , etc., and applying this to understanding the history of agricultural N

contamination. Examples: Böhlke & Denver (1995), Böhlke (2002), McMahon & Böhlke (2006).

3 The analysis of nitrate for $\delta^{18}\text{O}$ as well as $\delta^{15}\text{N}$. Examples: Böttcher et al. (1990), Aravena & Robertson (1998), Campbell et al. (2002); Mayer et al. (2002), Wankel et al. (2006), Wassenaar et al. (2006).

4 Using the $\delta^{15}\text{N}$ of algae and fish as “proxies” for (or integrators of) the $\delta^{15}\text{N}$ of NO_3 contributed by different land uses. Examples: Harrington et al. (1998), Koerner et al. (1999); Hebert & Wassenaar (2001), Anderson & Cabana (2005, 2006).

5 The analysis of nitrate for $\Delta^{17}\text{O}$. While this approach is in its infancy, with most of the publications dealing with atmospheric processes, $\Delta^{17}\text{O}$ promises to be valuable in ecosystem studies because it is an unambiguous tracer of atmospheric NO_3^- . Examples: Michalski et al. (2003, 2004, 2005).

Perhaps one reason that isotope techniques have not yet become a mainstream tool in agricultural basins is that the extent of temporal and spatial variability in the biogeochemistry and isotopic composition of various soil components makes it seem improbable that isotopic compositions could meaningfully integrate the myriad of environmental variability inherent to natural systems. To the contrary, many researchers have found that isotopes indeed have a unique ability to integrate environmental variability such that major natural patterns emerge and can be meaningfully interpreted. In the following section we consider how methodological advances have allowed us to answer increasingly complex questions regarding N isotopes in environmental systems.

Methodological advances in analyzing nitrogen isotopes

Until recently, almost all NO_3^- for both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ were analyzed using modifications of the silver nitrate method (Silva et al. 2000), where samples are concentrated on anion exchange resins, eluted, purified to produce silver nitrate, and then analyzed. The $\delta^{15}\text{N}$ of the silver nitrate can be measured using EA-IRMS or by pyrolysis. The original method used sealed-tube combustion to generate CO_2 for $\delta^{18}\text{O}$ measurement. However, automated pyrolysis systems that generate CO are now more commonly used. A number of modifications aimed at improved removal of dissolved organics have been described, including Chang et al. (1999), Hwang et al. (1999), and Heaton et al. (2004).

However, many laboratories are now analyzing nitrate, using the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002), where samples are inoculated with a pure culture of denitrifying bacteria lacking the enzyme to reduce nitrate beyond N_2O . The resulting N_2O is stripped from the samples using an automated headspace analyzer, purified, and then analyzed for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. This method is a significant improvement over the previous silver nitrate method because samples are about three

orders of magnitude smaller and high-salinity seawater samples are easily analyzed.

With the ongoing refinement of our understanding of $\delta^{18}\text{O}_{\text{NO}_3}$ and limitations of previous methods, it is evident that earlier $\delta^{18}\text{O}_{\text{NO}_3}$ data generated using sealed-tube combustions were potentially biased because of exchange of O with the glass and/or contamination by O-bearing contaminants in the silver nitrate (Revesz & Böhlke 2002), especially for samples that produced less than the recommended minimum of 100–200 $\mu\text{mol CO}_2$ (unpublished U.S. Geological Survey data). There is some speculation that even data produced using pyrolysis may be affected by O contamination from organic material. If so, the earlier $\delta^{18}\text{O}_{\text{NO}_3}$ data may have been subject to a “permil-scale contraction”. This topic will be discussed in more detail later in the section on **denitrification**.

Other recent methodological advances include methods for analyzing nitrite (NO_2^-) for $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ (McIlvin & Altabet 2003; Casciotti et al. 2007), nitrate for $\Delta^{17}\text{O}$ (Michalski et al. 2002; Kaiser et al. 2007), and marine dissolved organic N (DON) for $\delta^{15}\text{N}$ (Knapp et al. 2005).

Isotopic compositions of major N sources to ecosystems

Different sources of N to ecosystems have a wide range of $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ values (Figure 12.1). There is a vastly greater amount of $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NH}_4}$ data available than $\delta^{18}\text{O}_{\text{NO}_3}$ data. Recent compilations of $\delta^{15}\text{N}$ data include Kendall (1998, which includes a compilation of $\delta^{18}\text{O}_{\text{NO}_3}$ data), Fogg et al. (1998), and Bedard-Haughn et al. (2003). The sections below provide brief discussions of the major sources.

Atmospheric N

Since the tightening regulation of SO_2 emissions in the USA and in Europe, nitrate has become an increasingly important component of acidic deposition. For example, sulfate concentrations in precipitation have decreased throughout most of the USA (Butler et al. 2001; Lehmann et al. 2005). As a result, NO_3^- has become a more significant contributor to soil acidification, stream acidification, and forest degradation, particularly in eastern USA. Moreover, NO_3^- concentrations have increased in many western states, in some cases by up to 20–50% (Nilles & Conley 2001; Lehmann et al. 2005).

Advances in analytical methods have had a tremendous influence on our understanding of atmospheric nitrate isotopes. Up until the early 1990s, only $\delta^{15}\text{N}$ data were available for precipitation. It had been generally assumed that the $\delta^{18}\text{O}$ of atmospheric nitrate would be similar to the isotopic composition of atmospheric O_2 (ca. +23‰) because the $\delta^{15}\text{N}$ of atmospheric nitrate was similar to the composition of N_2 (ca. 0‰). For this reason, it was not thought

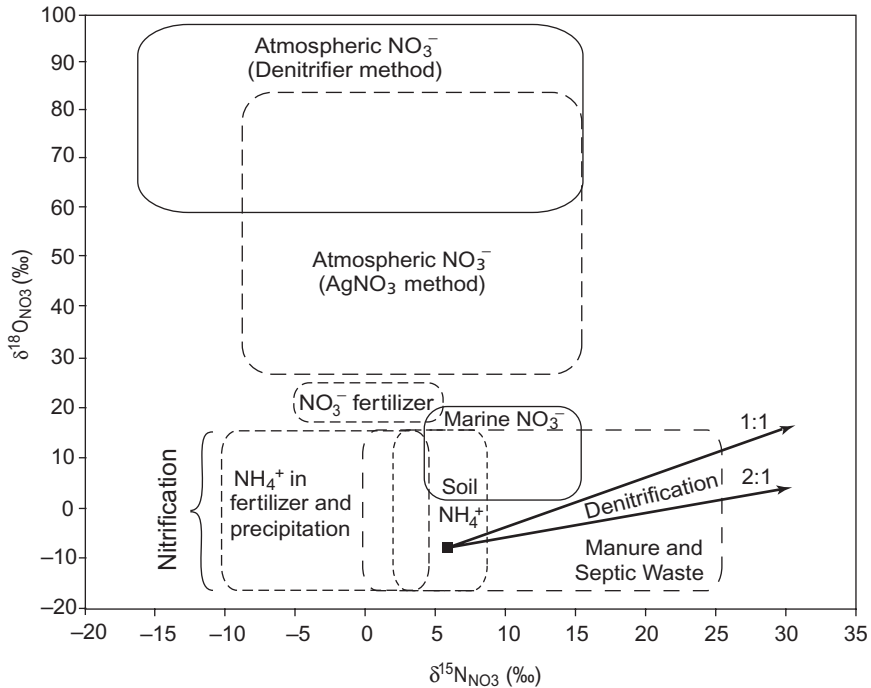


Figure 12.1 Typical values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate derived or nitrified from various N sources. Atmospheric $\delta^{18}\text{O}_{\text{NO}_3}$ data are divided into the ranges observed for samples analyzed using the denitrifier and AgNO_3 (non-denitrifier) methods. The two arrows indicate typical expected slopes for data resulting from denitrification of nitrate with initial $\delta^{15}\text{N} = +6\text{‰}$ and $\delta^{18}\text{O} = -9\text{‰}$. The typical ranges of $\delta^{18}\text{O}_{\text{NO}_3}$ values produced by nitrification of ammonium and organic matter are denoted by “nitrification”.

that analysis of $\delta^{18}\text{O}$ would provide much additional information. Since then, our understanding of the isotopic composition of atmospheric nitrate has experienced several major revisions, first in the mid-1990s with the development of silver nitrate methods for the $\delta^{18}\text{O}$ (first used by Kendall et al. (1995a,b) and Wassenaar (1995), but not published until Silva et al. (2000) and Chang et al. (1999)); then in the mid-2000s with the development of a denitrifier method for $\delta^{18}\text{O}$ (Sigman et al. 2001; Casciotti et al. 2002), and again in the early 2000s with the development of the methods for analyzing nitrate for $\delta^{17}\text{O}$ (Michalski et al. 2002; Kaiser et al. 2007).

The section below contains a brief discussion of whether different anthropogenic sources of atmospheric nitrate (e.g., power plant, vehicle, and agricultural emissions) may have distinguishable isotopic signatures and what is known about the $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\delta^{17}\text{O}$ of wet and dry precipitation. Later in the chapter, we discuss our current understanding of the atmospheric

processes and **causes of variability** in the isotopic compositions of atmospheric nitrate.

Isotopic composition of sources contributing to atmospheric nitrate

NO_x is released to the atmosphere from human activities (e.g., fossil fuel combustion) and natural processes (e.g., biogenic soil emissions, lightning, biomass burning). Fossil fuel combustion from mobile (e.g., vehicles) and stationary sources (e.g., electricity generation, industrial processes) constitute the largest global NO_x input. The major sink for NO_x in the atmosphere is the oxidation to nitric acid (HNO_3), which readily dissociates to nitrate (NO_3^-) where it can be deposited as wet deposition. Dry deposition can contribute significant loads of atmospherically derived N to ecosystems as dry gases (HNO_3 vapor, NH_3 , NO_2 , HONO, NO, peroxyacetyl nitrate (PAN)), dry aerosols (particulate NO_3^- , particulate NH_4^+), or in fogwater.

Isotopic composition of NO_x sources

For the past several decades, $\delta^{15}\text{N}$ of nitrogen oxides (NO_x) has been identified as a potential means for distinguishing air pollution sources. Anthropogenic NO_x sources generally have positive $\delta^{15}\text{N}$ values. In the 1970s, Moore (1977) characterized the isotopic composition of vehicle exhaust (average = +3.7‰, $n = 3$). A subsequent study (Heaton 1990) reported negative $\delta^{15}\text{N}$ in vehicle NO_x emissions (-13‰ to -2‰, $n = 8$). More recently, roadside denuders (average = +5.7‰, $n = 9$; Ammann et al. 1999) and roadside vegetation (average = +3.8‰, $n = 10$) (Pearson et al. 2000) have been used to illustrate characteristic $\delta^{15}\text{N}$ values associated with vehicle NO_x emissions. The isotopic composition of NO_x from stationary source fossil fuel combustion has also been characterized in several studies. Heaton (1990) reported that NO_x emissions from coal-fired power plants have $\delta^{15}\text{N}$ values ranging from +6‰ to +13‰ ($n = 5$; Heaton 1990). In a more recent study, Kiga et al. (2000) reported $\delta^{15}\text{N}$ values in NO_x produced from coal combustion ranging from +4.8‰ to +9.6‰ ($n = 6$). In both vehicle and stationary source fossil fuel combustion, the isotopic value of the resulting NO_x is suggested to be a function of the N present in the original fuel (negligible in the case of gasoline), the N_2 pumped through the engine, and the fractionations associated with thermal NO_x production (oxidation of atmospheric N_2 at high temperatures). Because thermally produced NO_x is assumed to have lower $\delta^{15}\text{N}$ values than fuel-derived NO_x , it is generally assumed that vehicle NO_x emissions have lower $\delta^{15}\text{N}$ values compared with stationary source NO_x emissions.

Natural sources of NO_x to the atmosphere, including lightning, biogenic soil emissions, and biomass burning are not as well characterized as anthropogenic sources. However, it has been documented that relatively pristine sites generally have lower $\delta^{15}\text{N}_{\text{NO}_2}$ values than highly polluted or heavily

traveled sites (Moore 1977; Ammann et al. 1999). Although $\delta^{15}\text{N}$ values of NO_x produced from biogenic soil emissions and biomass burning have not been directly characterized, as a volatile by-product of nitrification (and/or denitrification) and combustion respectively, $\delta^{15}\text{N}$ values are expected to be $<0\%$ due to the preferential volatilization of ^{14}N . The high temperature associated with lightning generates NO_x from the thermal oxidation of atmospheric N_2 and constitutes the other major natural NO_x source. The $\delta^{15}\text{N}_{\text{NO}_x}$ generated from laboratory discharges of lightning ranges from -0.5 to $+1.4\%$ (Hoering 1957). Although more extensive isotopic analyses are needed to more thoroughly characterize $\delta^{15}\text{N}_{\text{NO}_x}$ from various N sources, existing studies generally suggest that natural NO_x sources, including lightning and soil NO_x emissions, have lower $\delta^{15}\text{N}$ values than anthropogenically derived NO_x from fossil fuel combustion.

Variations in $\delta^{15}\text{N}$

Wet deposition

Wet deposition refers to all processes that transfer atmospheric N to the Earth's surface in aqueous form including rain, snow, and fog (Seinfeld & Pandis 1998). Complex chemical reactions in the atmosphere result in a large range of $\delta^{15}\text{N}$ values of N-bearing compounds depending on the reactants involved, the season, meteorological conditions, ratio of NH_4^+ to NO_3^- in the precipitation, types of anthropogenic inputs, proximity to pollution sources, distance from ocean, etc. (Hübner 1986; Heaton et al. 1997, 2004). Natural atmospheric sources of N-bearing gases (e.g., N_2O , HNO_3 , NH_3 , NO , NO_2 , etc.) include volatilization of ammonia from soils and animal waste (with fractionations as large as -40%), nitrification and denitrification in soils and surface waters, biomass burning, and lightning.

The $\delta^{15}\text{N}$ values of atmospheric NO_3^- and NH_4^+ are usually in the range of -15 to $+15\%$ (Figure 12.1), relative to atmospheric N_2 (0%), however lower nitrate $\delta^{15}\text{N}$ values in polar regions have been observed in snow (Heaton et al. 2004) and minerals (Michalski et al. 2005). In general, NO_3^- in rain appears to have a higher $\delta^{15}\text{N}$ value than the co-existing NH_4^+ , with the lower values for NH_4^+ attributed to washout of atmospheric NH_3 (Freyer 1978, 1991; Garten 1992). There is considerable literature on the $\delta^{15}\text{N}$ of N-bearing compounds in the atmosphere (see a review by Heaton et al. 1997). However, there have been few comprehensive studies of $\delta^{15}\text{N}$ of precipitation until recently, in part because of the difficulty of analyzing such dilute waters, prior to the development of the denitrifier method (Sigman et al. 2001). Below is a brief summary of the major findings of earlier studies.

Studies in Germany (Freyer 1978, 1991; Freyer et al. 1993), the USA (Russell et al. 1998), and South Africa (Heaton 1986, 1987) document that $\delta^{15}\text{N}_{\text{NO}_3}$ values show a seasonal cycle of low $\delta^{15}\text{N}$ values in spring and summer rain, and higher values in the winter. Russell et al. (1998) also showed

seasonal shifts in $\delta^{15}\text{N}_{\text{NH}_4}$ that were attributed to increased springtime agricultural emissions. The $\delta^{15}\text{N}_{\text{NO}_3}$ value of throughfall (rain that intercepts the tree canopy before falling “through”) was found to be higher than in open-air rain, whereas the $\delta^{15}\text{N}_{\text{NH}_4}$ in throughfall had a variable composition relative to rain in studies in Tennessee (Garten 1992) and Yorkshire (UK; Heaton et al. 1997). Although precipitation often contains unequal quantities of ammonium and nitrate, because ammonium is preferentially retained by the canopy relative to atmospheric nitrate (Garten & Hanson 1990), most of the atmospheric nitrogen that reaches the soil surface is in the form of nitrate.

More recent studies of nitrate isotopes in precipitation and snowpack using the denitrifier method also report seasonal $\delta^{15}\text{N}_{\text{NO}_3}$ variability, however, direction of the seasonal shifts vary. Hastings et al. (2004) report that $\delta^{15}\text{N}$ values are higher in spring and summer snowpack than in snowpack from fall and winter months in Greenland. Elliott et al. (2004; in preparation) observed strong seasonal and inter-event variability in $\delta^{15}\text{N}$ in a study of over 100 precipitation events at Connecticut Hill, New York (USA). Average $\delta^{15}\text{N}$ values in precipitation from this site were approximately 5‰ higher in the winter than in the summer. Back trajectory models, coupled with cluster analyses, indicate that inter-event variability in $\delta^{15}\text{N}$ at the site can be partially attributed to source areas of individual air masses. In comparison, in a series of 65 precipitation samples from Bermuda, Hastings et al. (2003) report opposite seasonal trends in $\delta^{15}\text{N}_{\text{NO}_3}$, with lower $\delta^{15}\text{N}$ values during the cool season (−5.9‰) than the warm season (−2.1‰). This pattern was explained by seasonal shifts in NO_x source and source region, which is dominated by lightning inputs during the warm season.

At a larger spatial scale, a recent investigation of temporal and spatial variations in the $\delta^{15}\text{N}$ of nitrate in wet deposition at ca. 150 precipitation monitoring sites across the USA showed values ranging from −11‰ to +3.5‰, with a mean value of −3.1‰ ($n = 883$; Elliott et al. 2006; in preparation). This range in $\delta^{15}\text{N}$ values is similar to those recently reported for rain in Bermuda (Hastings et al. 2003) and snow in Greenland (Hastings et al. 2004). Even at this large scale, seasonality was pronounced, with mean $\delta^{15}\text{N}$ values 3‰ higher during January-February than during May-June. For sites where bimonthly samples are available throughout the year, average annual $\delta^{15}\text{N}$ is calculated and the spatial distribution of these values is shown in Figure 12.2. The most prominent features of the data are several “hotspot” areas where $\delta^{15}\text{N}$ is consistently higher than the surrounding region, including areas in the Midwest, south of the Great Lakes, the central Front Range, and near Seattle. The lowest $\delta^{15}\text{N}$ values are generally located west of the Mississippi River and include sites in the Dakotas, Minnesota, Nebraska, and Texas.

At a subset of these sites spanning the nitrate and sulfate deposition gradient spanning the midwestern to northeastern USA, Elliott et al. (in press)

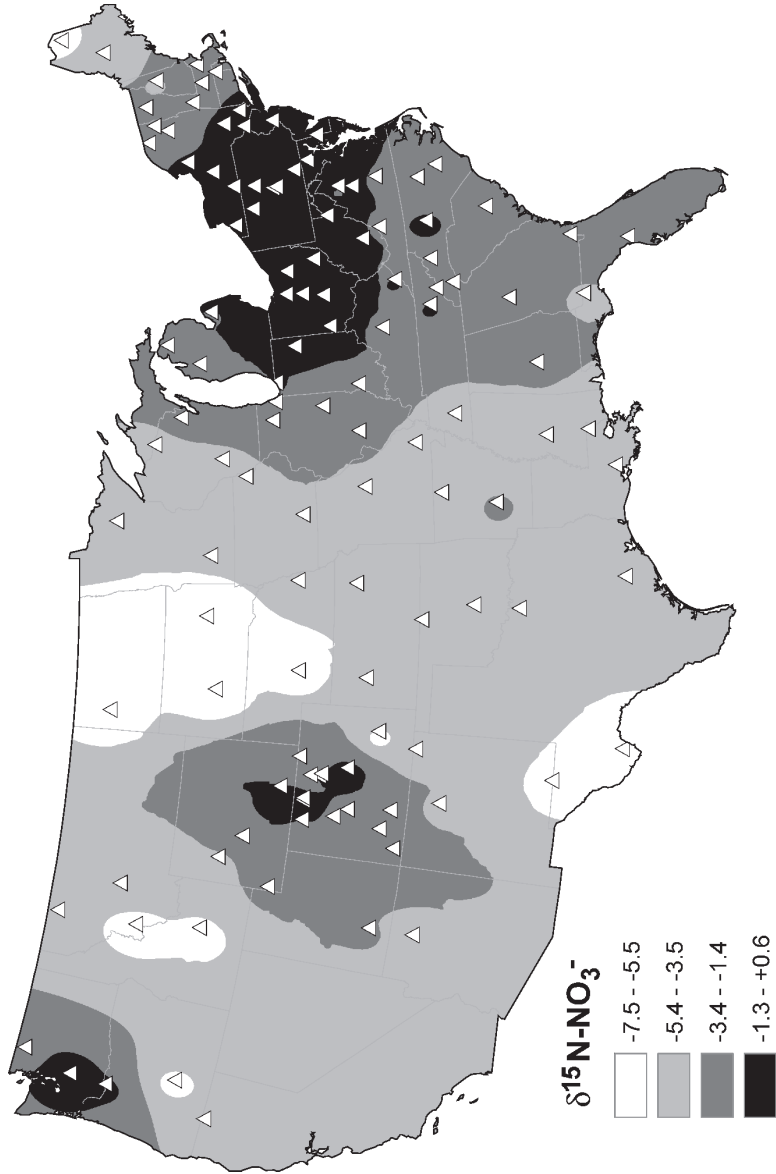


Figure 12.2 Spatial variability in the average $\delta^{15}\text{N}_{\text{NO}_3^-}$ of precipitation collected at National Atmospheric Deposition Program (NADP) sites in the USA in 2000. (Elliott & Kendall, unpublished U.S. Geological Survey data; in preparation.)

demonstrated that spatial variations in $\delta^{15}\text{N}$ are strongly correlated with amounts of NO_x emissions from surrounding electric generating units (Figure 12.3). Although vehicles comprise the single largest NO_x emission source in the eastern USA, $\delta^{15}\text{N}$ was not correlated with county-level vehicle NO_x emissions, suggesting that vehicle NO_x may not be as regionally distributed as stationary source NO_x . The results from this regional study suggest that nitrate isotopes in precipitation may be a “sharper tool” than concentration measurements and atmospheric transport models for assessing relative magnitude of various NO_x sources to landscapes, at any spatial or temporal scale.

Dry deposition and aerosols

Dry deposition is defined as the direct transfer of gaseous and particulate species to the Earth’s surface without the aid of precipitation (Seinfeld & Pandis 1998) and can include dry gases (HNO_3 vapor, NH_3 , NO_2 , HONO, NO, peroxyacetyl nitrate (PAN)) or dry aerosols (particulate NO_3^- , particulate NH_4^+). In general, dry deposition is poorly understood relative to wet deposition, partially because of the complexity of measuring dry deposition, the array of dry deposited N compounds, and the limited distribution of dry deposition monitoring networks. However, dry deposition is the dominant form of N deposition in arid climates, such as the western USA (Fenn et al. 2003) and can contribute 20–50% of N deposition in the eastern USA (Butler et al. 2005).

The $\delta^{15}\text{N}$ values of NO_3^- and NH_4^+ in dry deposition are usually higher than in wet deposition (Heaton et al. 1997). Equilibrium exchange reaction of

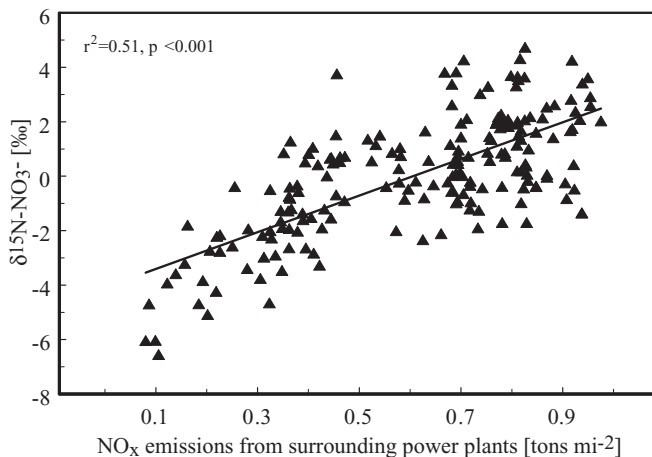


Figure 12.3 Correlation between precipitation $\delta^{15}\text{N}_{\text{NO}_3}$ from NADP sites in the northeastern and midwestern USA and NO_x emissions from power plants within 400 miles of individual NADP sites. (Modified from Elliott et al. in press.)

gaseous NO or NO₂ with dissolved NO₃ would likely result in ¹⁵N enrichment of the NO₃. However, other studies have illustrated various complicated relations (Moore 1977; Heaton 1987), and considerable interstorm and seasonal variability in δ¹⁵N. In a recent study in France, Widory (2007) analyzed δ¹⁵N of bulk N (NO₃⁻ + NH₄⁺) of particulate matter less than 10 μm in diameter (PM10) and determined that δ¹⁵N was higher for particulates generated from unleaded and diesel fuels (+4.6‰, *n* = 4), coal (+5.3‰, *n* = 1), natural gas (+7.7‰, *n* = 5), and waste incineration (+6.7, *n* = 3), than in fuel oil (-7.8‰, *n* = 8).

Freyer (1991) examined δ¹⁵N in particulate NO₃⁻ and HNO₃ vapor collected using both low volume and high volume samplers. Similar seasonal patterns (higher δ¹⁵N in winter) were observed between fine and coarse particulate NO₃⁻ and accompanying wet nitrate. Seasonality in δ¹⁵N of wet and particulate NO₃⁻ was strongly correlated with both temperature and solar radiation. δ¹⁵N of particulate NO₃⁻ was always higher than wet deposition, and δ¹⁵N of the coarse particulate fraction was generally lower than that of the fine particulate fraction. No seasonality was observed in HNO₃. In comparison, consistent seasonal variations in δ¹⁵N of particulate NO₃⁻ and HNO₃ were observed at dry deposition sampling sites in New York, Ohio, and Pennsylvania (Elliott & Kendall, unpublished U.S. Geological Survey data).

Yeatman et al. (2001) measured δ¹⁵N of aerosol NO₃⁻ and NH₄⁺ using high volume samplers at two coastal sites in the UK and Ireland, and also near livestock sources, roadways, and in remote areas. Similar δ¹⁵N_{NH₄⁺ and δ¹⁵N_{NO₃⁻ values were observed near chicken, cow, and pig livestock (δ¹⁵N_{NH₄⁺ = +13.5, *n* = 7 and δ¹⁵N_{NO₃⁻ = +10.6‰, *n* = 7), whereas samplers deployed near three roadways resulted in lower δ¹⁵N values (δ¹⁵N_{NH₄⁺ = +3.6‰, *n* = 3; and δ¹⁵N_{NO₃⁻ = +11‰, *n* = 2). Interestingly, samplers deployed in rural "remote" areas, not adjacent to immediate sources, have relatively high δ¹⁵N for both NH₄⁺ (+4.5‰) and NO₃⁻ (+11‰). Both δ¹⁵N_{NH₄⁺ and δ¹⁵N_{NO₃⁻ were higher at the coastal UK site (+6‰, *n* = 37 and +7‰, *n* = 25, respectively) than at the coastal site in Ireland (-9‰, *n* = 36 and -1‰, *n* = 21, respectively). The proximity of the UK site to anthropogenic sources, coupled with differences in the relative influence of marine-derived N, are suggested as potential causes for the observed spatial differences.}}}}}}}}

Variations in δ¹⁸O

Wet deposition

There is much less known about the δ¹⁸O of atmospheric NO₃⁻, mainly because there were no methods for analyzing it until the late 1980s (Amberger & Schmidt 1987; Silva et al. 2000). There have been multiple investigations of the δ¹⁵N and δ¹⁸O of precipitation NO₃⁻ in localized areas (Durka et al. 1994; Russell et al. 1998; Burns & Kendall 2002; Campbell et al. 2002; Xiao & Liu 2002; Pardo et al. 2004), with many focusing on snowpack samples

during winter months. Several of these studies (e.g., Williard 1999; Hastings et al. 2003; Pardo et al. 2004) observed seasonal differences in the $\delta^{18}\text{O}_{\text{NO}_3}$ in precipitation, with higher values in the winter than in the summer.

A survey of existing nitrate $\delta^{18}\text{O}$ values of precipitation in the late 1990s observed values ranging from +14 to +75‰, with a highly non-normal distribution of values (Kendall 1998). More recently, in a spatially extensive survey of nitrate isotopes in precipitation across the USA, Elliott et al. (2006; in preparation) document $\delta^{18}\text{O}_{\text{NO}_3}$ values ranging from +63‰ to +94‰, with a mean value of +76.3‰ ($n = 883$) across ca. 150 precipitation monitoring sites. Similar to the case with $\delta^{15}\text{N}$, Elliott et al. determined that $\delta^{18}\text{O}$ is seasonally variable, with mean $\delta^{18}\text{O}$ values 9.5‰ higher during January–February than during May–June. This range in $\delta^{18}\text{O}$ values reported by Elliott et al. is similar to those recently reported for rain in Bermuda (Hastings et al. 2003), snow in Greenland (Hastings et al. 2004), and snow in the Arctic (Heaton et al. 2004).

To date, it appears that all $\delta^{18}\text{O}$ values for atmospheric nitrate samples produced thus far using the denitrifier method are >60‰ (Figure 12.1). Hence, the $\delta^{18}\text{O}_{\text{NO}_3}$ values observed using the denitrifier method are higher than those analyzed using either the closed-tube or pyrolysis methods for converting silver nitrate to gases. It is possible that some of the lower precipitation $\delta^{18}\text{O}$ values observed using earlier methods are a result of reaction with glass during combustion, exchange with O in the glass, or contamination by other O-bearing materials in the silver oxide (e.g., organic compounds, sulfate, carbonate), all of which would probably lower the observed range in $\delta^{18}\text{O}$ values (Revesz & Böhlke 2002). Samples that produced the recommended minimum of 100–200 $\mu\text{mol CO}_2$ during sealed-tube combustions usually show minimal offset (unpublished U.S. Geological Survey data).

Variations in $\delta^{17}\text{O}$ (or $\Delta^{17}\text{O}$) in wet and dry deposition

In all oxygen bearing terrestrial materials, there is a consistent relationship between $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ values because kinetic and equilibrium isotope fractionations depend on the relative differences in atomic mass. However, ozone (O_3) formation exhibits a unique kinetic isotope effect, producing $\delta^{17}\text{O}$ values higher than statistically expected (Mauersberger et al. 2003). This “mass independent fractionation” (MIF) results in ozone having anomalous or excess ^{17}O (beyond that expected from the abundance of ^{18}O). Further, because ozone is a photochemically reactive species, this isotopic anomaly is transferred to several other oxygen-bearing atmospheric compounds (Thiemens 1999, 2006; Lyons 2001).

Figure 12.4, a triple oxygen isotope plot, illustrates the concept of this mass independent anomaly. On this plot, mass **dependent** fractionations (MDF) for nitrate result in values approximated by the relation: $\delta^{17}\text{O} = 0.52$

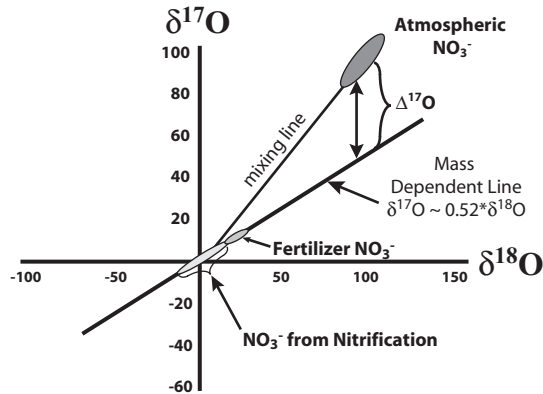


Figure 12.4 Schematic of relationship between $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ values. (Modified from Michalski et al. 2002.)

$\times \delta^{18}\text{O}$ (Michalski et al. 2002). This line is essentially fixed by the interactions of all conventional mass dependent kinetic and equilibrium isotopic fractionations involving oxygen. In contrast, MIFs cause values that deviate from this relation, and thus lie above the mass dependent line. Hence, MDF results in $\Delta^{17}\text{O} = 0$, whereas MIF results in $\Delta^{17}\text{O} \neq 0$ and $\Delta^{17}\text{O}$ values $>0\text{‰}$ are a useful tracer of O derived from atmospheric processes.

Current theory is that nitrate obtains its high $\delta^{17}\text{O}$ (and $\delta^{18}\text{O}$) due to chemical reactions with tropospheric ozone which has a $\Delta^{17}\text{O}$ of ca. $+35\text{‰}$ (Johnston & Thiemens 1997). Atmospheric nitrate $\Delta^{17}\text{O}$ values as high as ca. $+30\text{‰}$ have been observed (Michalski et al. 2003; Wankel 2006). Seasonal variation in the $\Delta^{17}\text{O}$ of aerosol nitrate (from $+20$ to $+30\text{‰}$) observed in Southern California (USA) was explained by a shift from nitric acid production by the $\text{OH}^* + \text{NO}_2$ reaction, which is predominant in the spring and summer, to N_2O_5 hydrolysis reactions, which are more important in the winter (Michalski et al. 2003). Figure 12.5 illustrates seasonal patterns in $\Delta^{17}\text{O}$ of bimonthly volume-weighted precipitation samples across New England (USA) and the strong correlation of $\Delta^{17}\text{O}$ with $\delta^{18}\text{O}$ (Wankel 2006), presumably due to a seasonality in the relative proportions of NO_x oxidation by OH^* or O_3 (Hastings et al. 2003; Michalski et al. 2003, 2004).

As discussed previously, many watershed studies have interpreted the seasonally high $\delta^{18}\text{O}$ of stream NO_3^- as an indicator of significant contributions of atmospheric nitrate. However, because of the strong isotopic discrimination involved in many processes that consume NO_3^- , there can also be increases in $\delta^{18}\text{O}_{\text{NO}_3^-}$ values which cannot be attributed to inputs by atmospheric nitrate (e.g., denitrification). Besides providing an unequivocal quantification of atmospheric inputs, the non-zero $\Delta^{17}\text{O}$ of atmospheric NO_3^- offers a unique tracer of N cycling as well. The $\Delta^{17}\text{O}$ value of nitrate derived

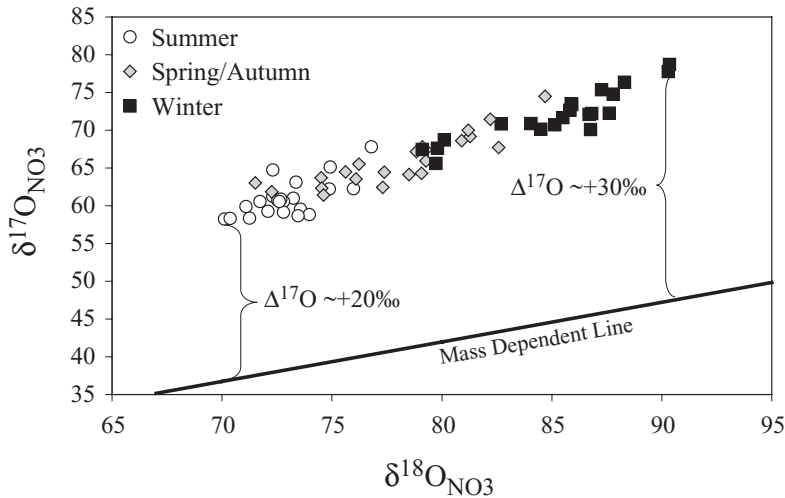


Figure 12.5 Seasonal variation of $\Delta^{17}\text{O}$ values in rain from NADP sites in northeastern USA. (Modified from Wankel & Kendall, unpublished U.S. Geological Survey data; in preparation.)

from atmospheric sources will remain unchanged regardless of the effects of the fractionating process (e.g., denitrification and assimilation), because all of these processes are strictly mass dependent and follow the slope of the mass dependent line (Figure 12.4). Thus, the triple oxygen isotopic composition of NO_3^- can be used to both calculate the proportion of atmospheric NO_3^- present and to estimate the relative amount of NO_3^- consumed (by back calculating the original $\delta^{18}\text{O}$ value). Production of new nitrate (nitrification) can dilute the $\Delta^{17}\text{O}$ signal of the original atmospheric NO_3^- to the point of being undetectable, and the recycling of atmospheric NO_3^- to organic matter and then back to NO_3^- will overwrite the original atmospheric NO_3^- with the terrestrial $\Delta^{17}\text{O}$ signal (i.e., 0‰).

Fertilizers

Many kinds of fertilizers are added to soils. It is important to distinguish among “natural” nitrate fertilizers (e.g., guano, desert nitrate salts), “synthetic” nitrate produced by oxidation of ammonia produced via the Haber-Bosch process, and “microbial” nitrate derived from ammonium fertilizers. Inorganic fertilizers have $\delta^{15}\text{N}$ values that are uniformly low reflecting an origin from atmospheric N_2 (Figure 12.1), generally in the range of -4 to $+4$ ‰; however, some fertilizer samples have shown a total range of -8 to $+7$ ‰ (see compilations by Hübner 1986; Macko & Ostrom 1994; Vitoria et al. 2004). Nitrate fertilizers often have slightly higher $\delta^{15}\text{N}$ values than NH_4^+ fertilizers. Organic fertilizers, including cover crops and plant composts,

and liquid and solid animal waste, generally have higher $\delta^{15}\text{N}$ values and a much wider range of compositions (generally +2 to +30‰) than inorganic fertilizers, reflecting their more diverse origins. Note that the $\delta^{15}\text{N}$ of NO_3^- in soils fertilized with NH_4^+ may not be the same as the fertilizer. During nitrification of applied ammonium, residual NH_4^+ and the NO_3^- formed in the soil can show $\delta^{15}\text{N}$ values that change by 20‰ during the process (Feigin et al. 1974), although ultimately the $\delta^{15}\text{N}$ of the total resulting nitrate may only be a few permil higher than the original NH_4^+ .

Amberger & Schmidt (1987) determined that nitrate fertilizers have distinctive $\delta^{18}\text{O}_{\text{NO}_3}$ values. Synthetic fertilizers where the O is chiefly derived from atmospheric O_2 (ca. +23.5‰), have $\delta^{18}\text{O}$ values ranging from +17 to +25‰ whereas natural fertilizers derived from Chilean deposits have $\delta^{18}\text{O}$ values of +46 to +58‰ (Böhlke et al. 2003; Vitoria et al. 2004). Nitrate derived from nitrification of ammonium fertilizers has lower $\delta^{18}\text{O}$ values, usually in the range of -5 to +15‰. This range of $\delta^{18}\text{O}$ values (shown in the area labeled " NH_4^+ in fertilizer or precipitation" on Figure 12.1) reflects the normally observed range of $\delta^{18}\text{O}$ values for microbially produced nitrate in well-oxygenated soils. See the section on nitrification for more information on the controls on the $\delta^{18}\text{O}$ of microbial nitrate.

Animal and human waste

It has often been observed that consumers (microbes to invertebrates) are 2–3‰ enriched in ^{15}N relative to their diet. The increase in $\delta^{15}\text{N}$ in animal tissue and solid waste relative to diet is due mainly to the excretion of low $\delta^{15}\text{N}$ organics in urine or its equivalent (Wolterink et al. 1979). Animal waste products may be further enriched in ^{15}N because of volatilization of ^{15}N -depleted ammonia, and subsequent oxidation of much of the residual waste material may result in nitrate with a high $\delta^{15}\text{N}$. By this process, when animal waste with a typical $\delta^{15}\text{N}$ value of about +5‰ is converted to nitrate, the $\delta^{15}\text{N}$ values are generally in the range of +10 to +20‰ (Kreitler 1975, 1979). Nitrate derived from human and other animal waste becomes isotopically indistinguishable using $\delta^{15}\text{N}$ under most circumstances (an exception is Fogg et al. 1998). However, with a multi-isotope approach, some recent studies show that it is possible to distinguish waste from different animal sources (Spruill et al. 2002; Curt et al. 2004; Widory et al. 2004, 2005), and this is discussed further below.

Soils (organic N and dissolved inorganic nitrogen)

The $\delta^{15}\text{N}$ of total soil N ranges from about -10 to +15‰. Cultivated soils have slightly lower $\delta^{15}\text{N}$ values ($+0.65 \pm 2.6\%$) than uncultivated soils ($+2.73 \pm 3.4\%$), according to a major soil survey by Broadbent et al. (1980). Most of the N in soils is bound in organic forms and not readily available to plants;

hence, the $\delta^{15}\text{N}$ of total soil N is generally not a good approximation of the $\delta^{15}\text{N}$ of N available for plant growth.

Soluble dissolved inorganic nitrogen (DIN; mainly NO_3^-) constitutes about 1% of the N in soils, and represents a very small pool which can be much more sensitive to change than the larger organic pool. The $\delta^{15}\text{N}$ of soil nitrate ranges from about -10 to $+15\text{‰}$, with most soils having $\delta^{15}\text{N}_{\text{NO}_3}$ values in the range of $+2$ to $+5\text{‰}$ (Kendall 1998). The $\delta^{15}\text{N}$ of soil nitrate and organic matter is strongly affected by drainage, topographic position, vegetation, plant litter, land use, temperature, and rain amount (Shearer & Kohl 1988; Amundson et al. 2003). Depending on land use and proximity to possible atmospheric sources of anthropogenic contaminants, the soil nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ may reflect "natural conditions" or the effects of various anthropogenic activities. The discussion below is intended to provide some background on this important source of N to ecosystems. For a more detailed discussion of the $\delta^{15}\text{N}$ of soil N and DIN, see the literature review in Kendall & Aravena (2000).

There have been several investigations of the $\delta^{15}\text{N}_{\text{NO}_3}$ values of soils from different environments (i.e., "natural" soils (tilled and untilled), soils fertilized with synthetic fertilizers or manure, soils contaminated with septic waste, etc). For example, in a study of variability in the $\delta^{15}\text{N}$ of soil water in lysimeters, Ostrom et al. (1998) found low $\delta^{15}\text{N}_{\text{NO}_3}$ in the spring and fall related to mineralization of soil organic matter (OM), and high $\delta^{15}\text{N}_{\text{NO}_3}$ values in late summer because of denitrification. The $\delta^{15}\text{N}_{\text{NO}_3}$ of soil water in non-tilled land was ca. 1.5‰ higher than in tilled land, and both were $>4\text{‰}$ lower than soil OM (Ostrom et al. 1998).

In general, the soil NO_3^- produced from fertilizer (average $\delta^{15}\text{N}$ value = $+4.7 \pm 5.4\text{‰}$) and animal waste (average $\delta^{15}\text{N} = +14.0 \pm 8.8\text{‰}$) are isotopically distinguishable but they both overlap with the $\delta^{15}\text{N}_{\text{NO}_3}$ of precipitation and natural soils. However, given the large range of $\delta^{15}\text{N}$ values of the NO_3^- sources, the average values of sources from one site cannot be automatically applied to another. This is vividly illustrated by a compilation of nitrate $\delta^{15}\text{N}$ data (Fogg et al. 1998).

The higher $\delta^{15}\text{N}_{\text{NO}_3}$ values in soils on lower slopes or valley bottoms are usually attributed to either greater denitrification in more boggy areas (Karamanos et al. 1981), or higher relative rates of immobilization and nitrification in these bottom soils (Shearer et al. 1974). Soil nitrate is preferentially assimilated by tree roots relative to soil ammonium (Nadelhoffer & Fry. 1994). Surface soils beneath bushes and trees often have lower $\delta^{15}\text{N}$ values than those in open areas, presumably as the result of litter deposition (Nadelhoffer & Fry 1988; Shearer & Kohl 1988); the $\delta^{15}\text{N}$ of soil N and DIN generally increases with depth.

Plants

Plants, a major reservoir of organic N, can utilize a variety of materials from purely inorganic compounds (NH_4^+ , NO_3^- , NO_2^- , N_2) to more complex forms

of organic N, and can have a wide range in $\delta^{15}\text{N}$ values depending on environmental conditions. Plants fixing N_2 from the atmosphere have $\delta^{15}\text{N}$ values of ca. 0 to +2‰, close to the $\delta^{15}\text{N}$ value of atmospheric N_2 (= 0‰). Most terrestrial plants have $\delta^{15}\text{N}$ in the range of -5 to +2‰ (Fry 1991). Algae and other aquatic plants have a much larger range of $\delta^{15}\text{N}$ values (-15 to +20‰), with values typically in the range of -1 to +7‰ (Kendall, unpublished data). For more information about the isotopic compositions of plants, see (for terrestrial plants) Garten et al. (this volume, pp. 61–82) and Evans (this volume, pp. 83–98), and (for aquatic plants) Finlay & Kendall (this volume, pp. 283–333).

The agronomy literature is full of studies showing that plants grown on anthropogenic ammonium or nitrate fertilizers usually have lower $\delta^{15}\text{N}$ values than plants grown on natural soils or “green manure”. Some of the spatial variability in foliar $\delta^{15}\text{N}$ that is commonly observed within forested catchments may be due to anthropogenic effects. For example, the lower foliar $\delta^{15}\text{N}$ values on ridgetops compared with valley bottom foliage in Tennessee (USA) may reflect the greater uptake of low- $\delta^{15}\text{N}$ atmospheric ammonium on ridges where soil dissolved inorganic N (DIN) is more limited, and the greater uptake of high- $\delta^{15}\text{N}$ soil NO_3^- , resulting from denitrification, by plants in the valleys (Garten 1993). Plants near busy roads have $\delta^{15}\text{N}$ values that are ca. 10‰ higher than in rural areas (Pearson et al. 2000).

There is considerable literature on using the $\delta^{15}\text{N}$ of aquatic plants and consumers to trace anthropogenic sources of N; these are reviewed by Finlay & Kendall (this volume, pp. 283–333) and briefly described in a later section on isotope **biomonitoring**. Perhaps the most generally useful observation is that in nutrient-rich environments, the $\delta^{15}\text{N}$ of the algae can closely track the $\delta^{15}\text{N}$ of the nitrate; studies in the Mississippi Basin (Battaglin et al. 2001a, 2001b) and San Joaquin Rivers (Kratzer et al. 2004) have shown an isotope fractionation of 4–5‰, offering considerable support for the usefulness of “isotope biomonitoring” of anthropogenic inputs.

Processes affecting the isotopic composition of DIN

In order for isotopes to be useful as tracers of various N sources to aquatic systems, an understanding of how biogeochemical cycling affects the isotopic composition of various inorganic forms of N is critical. It is impossible to do justice to this complex topic in the space allowed. However, here we briefly present the state of our understanding regarding major factors that can affect isotopic composition of N species in environmental systems, with an emphasis on processes with relevance to studying nitrate in watersheds and, to a lesser degree, coastal areas.

The basic fundamentals of isotope fractionation are discussed by Sulzman (this volume, pp. 1–21). Irreversible (unidirectional) kinetic fractionation effects involving metabolic nitrogen transformations are generally more

important than equilibrium fractionation effects in low temperature environments. Many biological processes consist of a number of steps (e.g., nitrification: $\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$). Each step has the potential for fractionation, and the overall fractionation for the reaction is often dependent on environmental conditions, including the number and type of intermediate steps, sizes of reservoirs (pools) of various compounds involved in the reactions (e.g., O_2 , NH_4^+), soil pH, species of the organism, etc. Hence, estimation of fractionations in natural systems can be very complex.

Generally, most of the fractionation is caused by the rate-determining or slowest step. This step commonly involves a large pool of substrate where the amount reacting is small compared with the size of the reservoir. In contrast, a step that is not rate-determining generally involves a small pool of a compound that is rapidly converted from reactant to product. When the compound is converted to product as soon as it appears, there is little net fractionation at this step. The isotopic compositions of reactant and product pools during a multi-step reaction where the net fractionation is controlled by a single rate-determining step can be successfully modeled either by a Rayleigh closed-system approach or by a "steady-state" open-system approach. For more details, see reviews by L  tolle (1980), H  bner (1986), Kendall (1998), Kendall & Aravena (2000), and B  hlke (2002).

The main biologically mediated reactions that control nitrogen dynamics in ecosystems are fixation, assimilation, mineralization, nitrification, and denitrification. These reactions commonly result in increases in the $\delta^{15}\text{N}$ of the substrate and decreases in the $\delta^{15}\text{N}$ of the product, unless the reactions go to completion. Physical processes, specifically ammonia volatilization, also significantly influence the $\delta^{15}\text{N}$ of the released ammonia, residual NH_4^+ , and any subsequently formed NO_3^- . Processes that consume NO_3^- (primarily denitrification and assimilation by phytoplankton and/or prokaryotes) generally cause the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in the remaining pool of NO_3^- to increase in a relatively predictable pattern. These processes and their impact on isotopic compositions of selected N-bearing compounds are discussed below.

Fixation

The term **N-fixation** refers to processes that convert unreactive atmospheric N_2 into other forms of nitrogen (Cleveland et al. 1999). Although the term is usually used to mean fixation by bacteria, it has also been used to include fixation by lightning and, more importantly, by human activities (energy production, fertilizer production, and crop cultivation) that produce reactive N (NO_x , NH_y , and organic N). Nitrogen fixation by human activity (industrial and agricultural) was estimated to be ca. 160TgNyr^{-1} in 1995, which is ca. 45% of all the nitrogen fixed on land and in the oceans (Galloway et al. 1995, 2004). These authors predict that the anthropogenic fixation rate will increase by 60% by the year 2020, mainly due to increased fossil-fuel

combustion and fertilizer use, especially in the developing countries of India and Asia. This increase in N loading and N saturation is causing critical ecosystem changes on both the local and global scale (Galloway et al. 1995). Concern about the impact of these changes on human activities is the main reason for the increased interest in uses of nitrogen isotopes in environmental studies.

Bacterial fixation of atmospheric N_2 by the enzyme nitrogenase commonly produces organic materials with $\delta^{15}N$ values slightly less than 0‰. A compilation by Fogel & Cifuentes (1993) indicated measured fractionations ranging from -3 to +1‰. Because these values are generally lower than the values for organic materials produced by other mechanisms, low $\delta^{15}N$ values in organic matter are often cited as evidence for N_2 fixation (though other process can also give rise to low values). The isotopic compositions of N-bearing materials produced by anthropogenic fixation (atmospheric gases produced during fossil fuel combustion, and artificial fertilizers produced from atmospheric gases) are discussed in detail below.

Assimilation

Assimilation refers to the transformation of inorganic N-bearing compounds into an organic form during biosynthesis by living organisms. Generally, oxidized forms of N are initially reduced to NH_4^+ and then assimilated into organic matter. Assimilation, like other biological reactions, discriminates between isotopes and generally favors the incorporation of the isotope with the lower mass. A large range of N fractionations (-30 to 0‰) has been measured in field studies (Cifuentes et al. 1989; Montoya et al. 1991), and in laboratory experiments for nitrate and ammonium assimilation by algae (Pennock et al. 1996; Waser et al. 1998; Altabet et al. 1999; Granger et al. 2004), and bacteria (Hoch et al. 1992) in aquatic environments. While there have been few studies of the effects of O fractionations during assimilation on the $\delta^{18}O$ of the residual NO_3^- , nitrate assimilation by marine phytoplankton seems to cause ca. 1:1 changes in the $\delta^{15}N$ and $\delta^{18}O$ of nitrate, regardless of species or the magnitude of the isotope effect; hence, these fractionations can be viewed as strongly "coupled" (Granger et al. 2004).

Fogel & Cifuentes (1993) present an elegant model for ammonium assimilation in aquatic algae that predicts total fractionations of -4, -14, or -27‰ depending on whether algae cells are nitrogen limited, enzyme limited, or diffusion limited, respectively. However, for the low pH values and low NH_4^+ concentrations common to soils and many aquatic environments, the model predicts that availability of N is the limiting condition and the transport of ammonium across cell walls is probably rapid, resulting in a small (<-4‰) overall fractionation.

More recently, Needoba et al. (2004) demonstrated that the isotopic effects or "apparent fractionation" imparted by phytoplankton during NO_3^-

assimilation on external NO_3^- pool results from several physiological factors. Phytoplankton cells transport and store high concentrations of internal NO_3^- in vacuoles, from which an internal enzyme (nitrate reductase) reduces NO_3^- for assimilation. While the transport step does not fractionate, the enzymatic reduction exhibits a large fractionation (-13 to -6%) depending on growth rate and conditions. Interestingly, the only means by which the external nitrate pool is affected by this fractionation is through the efflux of internal, highly fractionated (i.e., ^{15}N -enriched) nitrate.

Mineralization

Mineralization is usually defined as the production of ammonium from organic matter and is also referred to as **remineralization** or **ammonification**. Mineralization usually causes only a small fractionation ($\pm 1\%$) between soil organic matter and soil NH_4^+ . Many other workers use the term **mineralization** for the overall production of nitrate from organic matter by several reaction steps. This usage results in literature that gives fractionations for mineralization that can range from -35 to 0% , depending on which step is rate limiting (Delwiche & Steyn 1970; Feigin et al. 1974; Létolle 1980; Macko & Estep 1984). The large fractionations are caused by the nitrification of NH_4^+ , not the conversion of organic N to NH_4^+ . In general, the $\delta^{15}\text{N}$ of soil NH_4^+ is usually within a few permil of the $\delta^{15}\text{N}$ of total organic N in the soil.

Volatilization

Volatilization, the loss of ammonia gas to the atmosphere, is a highly fractionating process in which the ammonia gas produced has a lower $\delta^{15}\text{N}$ value than the residual NH_4^+ . Volatilization involves several steps that can cause fractionation, including (i) the equilibrium fractionations between ammonium and ammonia in solution, and between aqueous and gaseous ammonia, and (ii) the kinetic fractionation caused by the diffusive loss of ^{15}N -depleted ammonia. The overall process causes a fractionation of ca. 25% , but the actual fractionation depends on the pH, temperature, humidity and other factors (Hübner 1986).

Volatilization in farmlands results from applications of urea and manure to fields, and occurs within piles of manure. Ammonium produced from this organic N may have $\delta^{15}\text{N}$ values $>20\%$ due to ammonia losses. While there is little information about the $\delta^{15}\text{N}$ of the volatilized ammonia, it may reach values as low as -20% . The downwind transport of this ^{15}N -depleted N may be a significant source of atmospheric ammonium and (when oxidized) nitrate to adjacent areas (W. Showers, pers. comm.). In a survey of fertilized soils in Texas, Kreitler (1975) attributed a 2 – 3% increase in $\delta^{15}\text{N}_{\text{NO}_3}$ in underlying groundwater relative to the applied fertilizer to volatilization, and noted

that losses of ammonia in alkaline soils can be very large and cause dramatic shifts in $\delta^{15}\text{N}$ of the resulting nitrate.

Nitrification

Nitrification is the two-step process of NH_4^+ oxidation to NO_3^- mediated by several different autotrophic bacteria or archaea for the purpose of deriving metabolic energy. Various byproducts or intermediates can also be produced during nitrification and released into the environment including aqueous compounds (e.g., NH_2OH and NO_2^-) as well as gaseous compounds (e.g., NO and N_2O). In contrast to the “coupled” nature of isotope effects for nitrate-consuming processes such as assimilation and denitrification, nitrification can be considered “decoupled” because the sources of N and O atoms are unrelated. During nitrification, N atoms originate from NH_4^+ and/or NO_2^- molecules, while O atoms originate from O_2 and/or H_2O . Hence, the processes that control the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values during nitrification are discussed separately below.

Controls on $\delta^{15}\text{N}$

The total fractionation associated with nitrification depends on which step is rate determining. Because the oxidation of NO_2^- to NO_3^- is generally rapid in natural systems, this is generally not the rate-determining step, and most of the N fractionation is caused by the slower oxidation of NH_4^+ to NO_2^- . This first step of nitrification has been well studied in cultures of ammonium-oxidizing bacteria and been shown to have a large N isotope effect ranging from -38 to -14% (Mariotti et al. 1981; Casciotti et al. 2003). Similar to the effect of substrate concentration on NH_4^+ fractionation during assimilation, nitrification is expected to be diffusion limited at low NH_4^+ concentrations, and thus the isotope effect smaller (Casciotti et al. 2003). In diffusion-limited environments where nitrification is closely coupled with denitrification (e.g., benthic sediments), almost all the microbial nitrate may be rapidly denitrified, resulting in minimal efflux of nitrate with low $\delta^{15}\text{N}$ values to the water column (Lehmann et al. 2004).

The recent discovery of ammonium-oxidizing archaea may raise new questions about the role of archaea in N cycling (Francis et al. 2005; Könneke et al. 2005; Schleper et al. 2005). While it is generally believed that N isotope effects will be similar to those found in cultures of nitrifying bacteria, ammonium-oxidizing archaea have only recently been isolated in pure culture (Könneke et al. 2005) and the isotope effects are still unknown.

In general, the extent of fractionation during nitrification is dependent on the fraction of the substrate pool (reservoir) that is consumed. In N-limited systems, the fractionations are minimal. Hence, in soils where NH_4^+ is rapidly converted to NO_3^- , the $\delta^{15}\text{N}$ of soil NO_3^- is usually within a few permil of the

$\delta^{15}\text{N}$ of total organic N in the soil. If there is a large amount of NH_4^+ available (e.g., artificial fertilizer recently applied), nitrification is stimulated, and the oxidation of fertilizer NH_4^+ becomes the rate-determining step; this would result in a large fractionation. The $\delta^{15}\text{N}$ value of the first-formed NO_3^- would be quite low, but as the NH_4^+ pool is consumed, nitrification rate decreases, oxidation of NH_4^+ is no longer the rate-determining step, and the $\delta^{15}\text{N}$ of the total NO_3^- increases towards pre-fertilization values (Feigin et al. 1974).

As a result of the fractionations during transformation from NH_4^+ fertilizer to soil NO_3^- , one cannot accurately estimate the $\delta^{15}\text{N}$ value of NO_3^- being leaked to surface water or groundwater from an agricultural field from simple measurement of the average $\delta^{15}\text{N}$ of the NH_4^+ fertilizers. Even if the fertilizer applied were 100% synthetic KNO_3 , there would still be a possibility of post-depositional increases in $\delta^{15}\text{N}$ caused by denitrification as the nitrate was slowly transported to the sampling point. Increases in $\delta^{15}\text{N}$ (and $\delta^{18}\text{O}$) of NO_3^- caused by denitrification are less likely in coarse-grained soils where waters percolate rapidly (and have higher concentrations of dissolved oxygen) than in finer-grained soils (Gormly & Spalding 1979). Hence, the best way to assess the "effective" $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ value of the fertilizer or manure **end-member** is to collect samples from beneath the field where the materials are applied, avoiding sample collection soon after application since the fractionations are greatest then.

Controls on $\delta^{18}\text{O}$

While the fractionation of N during NH_4^+ oxidation is relatively well understood (Mariotti et al. 1981; Casciotti et al. 2003), the source of O atoms ultimately incorporated into the NO_3^- molecule during nitrification remains somewhat unresolved. The $\delta^{18}\text{O}$ resulting from nitrification is controlled by the composition of the oxidant sources (i.e., H_2O and/or O_2). During the first step, NH_4^+ is oxidized to hydroxylamine (NH_2OH) via an ammonium monooxygenase; this reaction has been shown to incorporate O atoms from dissolved O_2 (Hollocher et al. 1981). It is currently unknown whether this process causes a kinetic fractionation and consequently preferential incorporation of $^{16}\text{O}_2$. Andersson & Hooper (1983) demonstrated that the resulting NO_2^- contains one O atom from dissolved O_2 and one from H_2O . However, they also revealed that during NH_4^+ oxidation to NO_2^- there can be considerable isotopic exchange between the O in H_2O and NO_2^- . The oxidation of NO_2^- to NO_3^- has been shown to incorporate O atoms from H_2O only (Aleem et al. 1965; Kumar et al. 1983; Hollocher 1984; Dispirito & Hooper 1986). Thus, $\delta^{18}\text{O}_{\text{NO}_3}$ largely has been interpreted as a mixture of two oxygen atoms from H_2O and one from O_2 . Hence,

$$\delta^{18}\text{O}_{\text{NO}_3} = 2/3(\delta^{18}\text{O}_{\text{H}_2\text{O}}) + 1/3(\delta^{18}\text{O}_{\text{O}_2}) \quad (12.1)$$

where the $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ is assumed to be that of ambient H_2O , and the $\delta^{18}\text{O}_{\text{O}_2}$ is assumed to be that of ambient O_2 . For waters with $\delta^{18}\text{O}$ values in the normal range of -25 to $+4\text{‰}$, and soil O_2 with the $\delta^{18}\text{O}$ of atmospheric O_2 (ca. $+23.5\text{‰}$), soil NO_3^- formed from *in situ* nitrification, should be in the range of -10 to $+10\text{‰}$, respectively (Figure 12.1).

The simple equation above for calculation of the $\delta^{18}\text{O}_{\text{NO}_3}$ makes four critical assumptions:

- 1** the proportions of O from water and O_2 are the same in soils as observed in laboratory cultures;
- 2** there are no fractionations resulting from the incorporation of oxygen from water or O_2 during nitrification;
- 3** the $\delta^{18}\text{O}$ of water used by the microbes is equal to that of the bulk soil water;
- 4** the $\delta^{18}\text{O}$ of the O_2 used by the microbes is equal to that of atmospheric O_2 .

However, the $\delta^{18}\text{O}$ of dissolved O_2 in aquatic systems reflects the effects of three primary processes:

- 1** diffusion of atmospheric O_2 (ca. $+23.5\text{‰}$) in the subsurface;
- 2** photosynthesis – resulting in the addition of O_2 with a low $\delta^{18}\text{O}$ similar to that of water;
- 3** respiration by microbes – resulting in isotopic fractionation and higher $\delta^{18}\text{O}$ values for the residual O_2 .

Many studies have used measurement of $\delta^{18}\text{O}_{\text{NO}_3}$ in freshwater systems for assessing sources and cycling (see below). Often it has been found that the $\delta^{18}\text{O}$ of microbial NO_3^- is a few permil higher than expected for the equation and the assumptions above (e.g., Kendall 1998). A variety of explanations have been offered for these high $\delta^{18}\text{O}_{\text{NO}_3}$ values including:

- 1** nitrification in soil waters with higher than expected $\delta^{18}\text{O}$ values because of evaporation (Böhlke et al. 1997) or seasonal changes in rain $\delta^{18}\text{O}$ (Wassenaar 1995);
- 2** changes in the proportion of O from H_2O and O_2 sources (i.e., $>1/3$ from O_2) (Aravena et al. 1993);
- 3** nitrification using O_2 that has a high $\delta^{18}\text{O}$ due to respiration (Kendall 1998);
- 4** nitrification that occurs simultaneously via both heterotrophic and autotrophic pathways (Mayer et al. 2001).

At this time, it is still unresolved how each of these mechanisms affects $\delta^{18}\text{O}_{\text{NO}_3}$ during nitrification reactions.

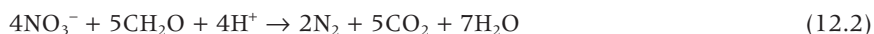
Open ocean settings, because of the broader nature of the chemical gradients and relative isolation from interfering sources of contamination, may provide a simpler conceptual background in which to understand $\delta^{18}\text{O}_{\text{NO}_3}$.

Recent data from oceanic settings (Casciotti et al. 2002; Lehmann et al. 2004; Sigman et al. 2005; Wankel et al. 2007) indicate that NO_3^- formed in the deep ocean ultimately assumes a $\delta^{18}\text{O}_{\text{NO}_3}$ only slightly higher (ca. +3‰) than that of the $\delta^{18}\text{O}$ of seawater (ca. 0‰). It has been postulated that this low $\delta^{18}\text{O}_{\text{NO}_3}$ value is caused by water–nitrite O isotopic exchange which may be catalyzed by nitrifying bacteria (Andersson & Hooper 1983; Casciotti et al. 2002). Additionally, in a study of $\delta^{18}\text{O}_{\text{NO}_3}$ along an estuarine gradient (where $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ ranged from –10 to ca. 0‰), it was postulated that rapid cycling of NO_3^- (assimilation → decomposition → nitrification) at low nitrate concentrations (~15 μM) increased the degree to which O from H_2O was incorporated into the NO_3^- molecule, potentially making $\delta^{18}\text{O}_{\text{NO}_3}$ a useful indicator of N recycling in such environments (Wankel et al. 2006).

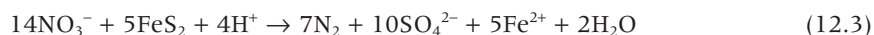
Denitrification

Denitrification refers to the dissimilatory reduction of NO_3^- to gaseous products (N_2 , N_2O , or NO) and usually occurs only where O_2 concentrations are less than 20 μM. Although denitrification does not generally occur in the presence of significant amounts of oxygen, it has been hypothesized that it can occur in anaerobic pockets within an otherwise oxygenated sediment or water body (Brandes & Devol 1997; Koba et al. 1997). Denitrification causes the $\delta^{15}\text{N}$ of the residual nitrate to increase exponentially as nitrate concentrations decrease; values >100‰ are not unusual. For example, denitrification of fertilizer NO_3^- with a $\delta^{15}\text{N}$ value of +0‰ can yield residual nitrate with much higher $\delta^{15}\text{N}$ values (e.g., +15 to +30‰) that are within the range of compositions expected for NO_3^- derived from a manure or septic-tank source (Figure 12.1). Additionally, denitrification causes the $\delta^{18}\text{O}$ values to increase in the residual NO_3^- pool. Thus, the effects of denitrification on the dual isotopic composition of NO_3^- are considered coupled since both the N and O atoms originate in the same molecule.

Nitrate reduction by heterotrophic microbes and the simultaneous respiration of CO_2 from the oxidation of organic matter is the generalized pathway of heterotrophic denitrification:



Most denitrifying heterotrophic microorganisms are actually facultatively anaerobic, switching from oxygen to nitrate respiration at O_2 levels of less than about 0.5 mg L^{-1} (Hübner 1986). However, chemo-autotrophic denitrification by bacteria such as *Thiobacillus denitrificans*, which oxidizes sulfur, can also be important (Batchelor & Lawrence 1978). The stoichiometry of the denitrification reaction mediated by *Thiobacillus denitrificans* is:



Measured enrichment factors for (apparent fractionation, or ϵ) associated with denitrification ($\epsilon_{\text{N}_2\text{-NO}_3^-}$) range from -40 to -5% (Mariotti et al. 1981, 1982; Böttcher et al. 1990; Aravena & Robertson, 1998; Granger 2006) with the $\delta^{15}\text{N}$ of the N_2 lower than that of the NO_3^- . The N_2 produced by denitrification results in excess N_2 dissolved in groundwater; the $\delta^{15}\text{N}$ of this N_2 can provide useful information about sources and processes (Böhlke & Denver 1995).

The extent of fractionation is highly dependent on environmental conditions. Authors have distinguished between “benthic” denitrification in which NO_3^- diffuses into the anaerobic groundwater from a surficial aerobic environment before denitrification can occur (Brandes & Devol 1997), and “riparian” denitrification where there is partial conversion of the nitrate in the anaerobic groundwater (Sebilo et al. 2003). The apparent fractionation associated with benthic denitrification is small (ranging from -1.5 to -3.6%), because NO_3^- diffusion through the water–sediment interface, which causes minimal fractionation, is the rate-determining step (Sebilo et al. 2003; Lehman et al. 2004). In contrast, riparian denitrification causes a much larger apparent fractionation (about -18% ; Sebilo et al. 2003). Similarly, one can distinguish between benthic and “pelagic” (or “water column”) denitrification (Brandes & Devol 2002; Lehmann et al. 2004; Sigman et al. 2005), where again diffusion limits the effects of fractionations in the sediments on the $\delta^{15}\text{N}_{\text{NO}_3^-}$ in the overlying water column.

It is important to remember that even though benthic (sedimentary) denitrification has a minimal “isotope effect” on the overlying water column (i.e., does not cause a significant increase in the $\delta^{15}\text{N}$ or $\delta^{18}\text{O}$ of the NO_3^- in the water column), pore-water NO_3^- probably shows about the same isotopic fractionations observed in open-water environments (Sigman et al. 2001; Lehmann et al. 2004). The lack of isotope effect observed in the overlying water is simply the result of a lack of “communication” of fractionated (i.e., ^{15}N -enriched) pore-water with overlying water across the sediment–water interface. Hence, the potential large fractionation for denitrification is not “expressed” in the water column.

There are several methods for determining the presence, extent and/or rate of denitrification, including various enzyme-block methods (e.g., the acetylene block method) and ^{15}N tracer methods (Nielsen 1992; Mosier & Schimel 1993). Natural abundance isotope methods include comparison of decreasing NO_3^- concentrations with increases in (i) $\delta^{15}\text{N}_{\text{NO}_3^-}$, (ii) concentration and $\delta^{15}\text{N}$ of total N_2 , or (iii) relative $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of residual nitrate (see the section on “Fractionation due to denitrification” below).

It has been recognized for several decades that denitrification causes the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in the remaining pool of NO_3^- to increase in a relatively predictable pattern. (e.g., Olleros 1983; Amberger & Schmidt 1987; Voerkelius & Schmidt 1990). With the development of larger datasets, primarily from groundwater studies, relatively consistent patterns emerged that suggested

denitrification causes $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ to increase in roughly a 2:1 ratio; thus, $^{15}\epsilon$ was approximately twice as large as $^{18}\epsilon$ (e.g., Böttcher et al. 1990; Aravena & Robertson 1998; Mengis et al. 1999; Cey et al. 1999; Panno et al. 2006). While no clear mechanism was suggested for this phenomenon, it generally has been accepted that when nitrate isotope data from natural freshwater settings plot along a slope of ca. 0.5 ($\delta^{18}\text{O}/\delta^{15}\text{N}$), the pattern was consistent with an interpretation of denitrification.

There is currently some uncertainty about the expected fractionation caused by nondiffusion-limited denitrification. If earlier $\delta^{18}\text{O}_{\text{NO}_3}$ data generated using sealed-tube combustions (Revesz & Böhlke 2002) or pyrolysis were potentially biased because of exchange of O with the glass and/or contamination by O-bearing contaminants in the silver nitrate, then earlier $\delta^{18}\text{O}_{\text{NO}_3}$ data may have been subject to a “permil-scale contraction”. Hence, if we expand the $\delta^{18}\text{O}_{\text{NO}_3}$ scale, a trend that previously had a slope of ca. 0.5 on plots such as Figure 12.1, would now have a higher slope. However, it is interesting to note that data from many studies, using a variety of different versions of the sealed tube and pyrolysis methods (e.g., Olleros 1983; Amberger & Schmidt 1987; Böttcher et al. 1990; Voerkelius & Schmidt 1990; Aravena & Robertson 1998; Mengis et al. 1999; Cey et al. 1999; Panno et al. 2006), show nitrate $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values in areas where denitrification is likely plotting along slopes of 0.5 to 0.7. Evidently, there is more to be learned about controls on NO_3^- isotopic composition by denitrification in the environment.

Recent work with pure cultures of denitrifying bacteria indicate a slope equal to 1 for the respiratory process of denitrification (Sigman et al. 2005; Granger 2006), similar to the effects seen in NO_3^- assimilation by marine phytoplankton (Granger et al. 2004). Nevertheless, even with newer methods (e.g., the denitrifier method) which avoid the potential interferences of O isotope exchange with the glass (e.g., sealed tube combustion) and/or contamination by other oxygen-bearing compounds (e.g., pyrolysis), nitrate isotopic compositions in groundwater where denitrification occurs still give rise to slopes <1 (Wankel & Kendall, unpublished data) and thus may require additional explanation. Such alternate explanations might include the co-occurrence of respiratory denitrification with: (i) other nitrate consuming pathways such as bacterial nitrate assimilation (for which fractionation systematics are poorly characterized); (ii) anaerobic nitrification involving oxidants other than O_2 ; (iii) enzymatically catalyzed O exchange between NO_2^- and water (Andersson & Hooper 1983) and re-oxidation of NO_2^- to NO_3^- ; or (iv) the process of “aerobic denitrification” or “auxiliary denitrification” (Granger 2006).

Interestingly, the results of Granger (2006) also indicate that while the truly respiratory process of denitrification gives rise to a slope of 1 (through isotope effects imparted by the NO_3^- reductase enzyme NAR, an additional nitrate reducing enzyme used by some bacteria for maintaining cellular redox balance), denitrification by the enzyme NAP (used for

“auxiliary denitrification”, which is sometimes referred to as “aerobic denitrification”) results in a consistent slope of ca. 0.6. Thus, slopes <1 for nitrate samples from anaerobic environments may arise from a larger proportion of this NAP or “auxiliary denitrification” pathway. This might include conditions in which electron donor concentrations (i.e., organic C) are abundant relative to NO_3^- (Granger 2006). The ranges of observed denitrification slopes are bracketed by the two different lines on Figure 12.1. Clearly, further work is needed.

The coupled nature of N and O isotope effects during denitrification (or assimilation) offers a means for constraining other biogeochemical processes. In environments where the mixing of multiple sources of nitrate (sewage, fertilizer, precipitation, etc.) can be disregarded or well-constrained by other tracers, deviations from a coupled 1:1 pattern of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (by nitrate consuming processes) suggest the influence of additional processes such as nitrification. Sigman et al. (2005) used this approach in the oxygen minimum zone in the eastern tropical North Pacific Ocean where denitrification results in a net loss of NO_3^- . While denitrification was expected to cause both the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ to increase equally, there were deviations from this pattern (denoted by the term “ $\Delta(15,18)$ ”), which arose from the combined effects of denitrification and nitrification of organic N from N-fixation. Use of a steady-state dual isotope model suggested that up to 65% of the geochemical evidence for inputs by N fixation had been “erased” by denitrification (Sigman et al. 2005). Hence, the combined use of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ provided some constraint on N cycling processes in this region.

Other dissimilatory N transformations

In strongly reducing environments with high sulfide concentrations, such as coastal marshes (e.g., Tobias et al. 2001) and estuarine sediments, the dissimilatory reduction of NO_3^- to ammonium (DNRA) has been shown to be as important, if not more so, than denitrification (Jorgensen 1989; Trimmer et al. 1998; An & Gardener 2002; Ma & Aelion 2005), which is inhibited by the high sulfide concentrations (Joye & Hollibaugh 1995). While direct reports on the N isotope fractionation occurring during DNRA are lacking, McCready et al. (1983) demonstrated that NH_4^+ produced from DNRA has a much lower $\delta^{15}\text{N}$ than the NO_3^- , which is consistent with a kinetic fractionation.

Similarly, there are no existing data for the fractionation occurring during the anaerobic oxidation of NH_4^+ to N_2 (e.g., anammox; Dalsgaard et al. 2003; Kuypers et al. 2003). This process has been recently attributed to a wide range of environments including the Black Sea (Kuypers et al. 2003), upwelling oceanic regions (Kuypers et al. 2005), Arctic marine sediments (Rysgaard et al. 2004), and even Arctic sea ice (Rysgaard & Glud 2004). While the extent of anammox research has focused mostly on estuarine and marine

environments, very little is known regarding its existence or importance in terrestrial groundwater or surface water biogeochemistry.

Atmospheric reactions involving oxidized N species

Atmospheric chemistry of oxidized nitrogen species is complex, and closely coupled with ozone chemistry, hydroxyl radical chemistry, sulfate chemistry, and aerosol dynamics. Because of this complexity, and because we are in the early stages of being able to characterize the isotopic composition of small concentrations of NO_3^- and other N compounds, our understanding of the isotopic fractionations associated with atmospheric chemical reactions is limited. The section "Atmospheric N" (above) contained a brief discussion of whether different anthropogenic sources of atmospheric NO_3^- (e.g., power plant, vehicle, and agricultural emissions) may have distinguishable isotopic signatures. The section below discusses the current understanding of the atmospheric processes and **causes of variability** in the isotopic compositions of atmospheric NO_3^- . Specifically, we present the current understanding of the oxidation chemistry of NO_x in the atmosphere, as well as what is known about how these various pathways influence $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ compositions in atmospheric deposition.

NO_x is released to the atmosphere from human activities (e.g., fossil fuel combustion) and natural process (e.g., biogenic soil emissions, lightning, biomass burning). Once in the atmosphere, NO_x generally has a short lifetime, 1–3 days (Seinfeld & Pandis 1998, and references therein). During the daytime, O atoms are rapidly exchanged between ozone (O_3) and atmospheric NO_x (reactions R1 and R2). The major sink for NO_x in the atmosphere is the oxidation to nitric acid (HNO_3) which occurs via both daytime:



and nighttime reactions:



A highly soluble strong acid, HNO_3 readily dissociates to NO_3^- where it can be deposited as wet deposition. In addition, nitrogenous species can be deposited as dry gases (HNO_3 vapor, NH_3 , NO_2 , HONO , NO , peroxyacetyl nitrate

(PAN)), dry aerosols (particulate NO_3^- , particulate NH_4^+), or in fogwater. Each of these forms can contribute significant loads of atmospherically derived N to ecosystems, and the relative importance of wet/dry deposition is spatially variable.

Atmospheric processes causing variations in $\delta^{15}\text{N}$

The question of whether the seasonal and spatial variability in the $\delta^{15}\text{N}$ of atmospheric NO_3^- is controlled mainly by mixing of sources with different $\delta^{15}\text{N}$ values or by variability in atmospheric processes – or some combination of the two – is a topic of active debate. As discussed previously, there is ample evidence that different NO_x sources have different isotopic signatures; however, it is not yet clear whether these signatures can be substantially overprinted by the effects of chemical reactions during long-range transport and deposition as nitrate. Therefore, it is important to consider several mechanisms that have been proposed in the literature as significant controls on the $\delta^{15}\text{N}$ of wet and dry precipitation. Freyer (1991) provides a thorough review of potential fractionating factors. Briefly, these potential factors include the following:

1 Isotope shifts of several permil can occur between and within storms because of selective washout of N-bearing materials (Heaton 1986). Long-term transport and progressive rainout of ^{15}N has been suggested as a potential factor in low $\delta^{15}\text{N}$ values ($<-20\text{‰}$) observed in polar regions (Wada et al., 1981; Heaton et al. 2004). However, Michalski et al. (2005) point out that because of advances in our understanding of HNO_3 production pathways, that NO_2 equilibrium with water droplets is not a viable explanation for low $\delta^{15}\text{N}$ values observed in polar regions. Further, Michalski et al. (2005) suggest that if kinetic or equilibrium fractionations associated with transport are responsible for low $\delta^{15}\text{N}$ values, that these fractionations are mass dependent and would therefore be accompanied by corresponding low $\delta^{18}\text{O}$ values. Due to the fact that very low $\delta^{18}\text{O}$ values have not been observed in high latitudes, Michalski et al. (2005) suggest that stratospheric NO_3^- characterized by low $\delta^{15}\text{N}$ values is the source of low $\delta^{15}\text{N}$ values observed in polar regions. Hastings et al. (2004) also suggest that interactions between NO_x and PAN (peroxyacetyl nitrate) over long distances may alter the isotopic composition of NO_x .

2 Freyer et al. (1993) reported that at an urban, polluted site in Germany, equilibrium reactions between NO and NO_2 can result in higher $\delta^{15}\text{N}$ values in NO_2 (and resulting HNO_3). High NO_2 concentrations at this site result in the incomplete oxidation of NO , particularly during the winter when O_3 concentrations are lowest. This equilibrium fractionation during the winter is suggested to be the cause of the seasonal patterns observed in $\delta^{15}\text{N}$ (higher in winter, lower in summer). However, subsequent studies have been able to rule out this potential effect as a factor in $\delta^{15}\text{N}$ seasonality by considering

mixing ratios of NO_2 , NO , and O_3 . In particular, Hastings et al. (2003, 2004) determined that given abundant concentrations of O_3 relative to NO_2 , equilibrium reactions between oxidized N species cannot fully account for variability observed in $\delta^{15}\text{N}_{\text{NO}_3}$ in Bermuda and Greenland (Hastings et al. 2003, 2004).

3 The preferential evaporation of ^{14}N from the dissociation of NH_4NO_3 has been suggested as a mechanism for causing higher $\delta^{15}\text{N}$ in residual nitrate (Freyer 1991).

4 Heaton et al. (1997) attributed seasonal variations in $\delta^{15}\text{N}$ to humidity, which can affect the $\delta^{15}\text{N}$ of NO_3^- and NH_4^+ by equilibrium exchange of N with gaseous HNO_3 and NH_3 , respectively, and produce higher $\delta^{15}\text{N}$ values for NO_3^- and NH_4^+ when humidities are low.

Atmospheric processes causing variations in $\delta^{18}\text{O}$

A survey of the nitrate isotope literature in the late 1990s observed that there appeared to be a bimodal distribution of $\delta^{18}\text{O}_{\text{NO}_3}$ values in precipitation in North America, with mode values of +25‰ and +60‰ (Kendall 1998). Furthermore, precipitation in Europe showed a somewhat similar pattern, with $\delta^{18}\text{O}_{\text{NO}_3} > 50\text{‰}$ in Bavaria (Germany), which has high concentrations of NO_3^- in precipitation, many acid-rain damaged forests, and is downwind of the highly industrialized parts of central Europe (Voerkelius & Schmidt 1990; Durka et al. 1994). However, $\delta^{18}\text{O}_{\text{NO}_3}$ values were lower in Muensterland, which is farther from the pollution sources in central Europe (Mayer et al. 2001). Because of the seasonality in $\delta^{18}\text{O}$, the bimodal $\delta^{18}\text{O}_{\text{NO}_3}$ distribution in North America, and the trends in Europe (Kendall 1998) speculated that these patterns might reflect the presence of at least two sources and/or processes affecting $\delta^{18}\text{O}_{\text{NO}_3}$; specifically, that the higher $\delta^{18}\text{O}_{\text{NO}_3}$ values (i.e., $> 50\text{‰}$) may be associated with anthropogenic NO_x pollution.

However, most new investigations using the microbial denitrifier method to analyze nitrate samples have concluded that the temporal variations in $\delta^{18}\text{O}_{\text{NO}_3}$ of precipitation are likely due to seasonal changes in atmospheric oxidation chemistry rather than source contributions. In particular, $\delta^{18}\text{O}_{\text{NO}_3}$ values are thought to vary according to the relative contributions of O_3 to HNO_3 molecule formation caused by seasonal shifts in HNO_3 production pathways. In a study of nitrate aerosols collected in southern California (USA), Michalski et al. (2004) report that seasonal variations in $\delta^{18}\text{O}$ and mass-independent $\Delta^{17}\text{O}$ result from seasonal shifts in temperature, hours of sunlight, and oxidant concentrations. Using $\Delta^{17}\text{O}$ coupled with a isotopic-photochemical box model, Michalski et al. estimate that during the spring, HNO_3 production is dominated (ca. 50%) by the homogeneous reactions R3 and R4; however, during the winter months, HNO_3 production ($> 90\%$) is driven by heterogeneous reactions R5 and R6.

Several studies in Bermuda (Hastings et al. 2003) and Greenland (Hastings et al. 2004, 2005) report that $\delta^{18}\text{O}$ seasonality depends on the reaction pathway for HNO_3 formation. In particular, during the daytime and summer months, reactions R1–R3 result in HNO_3 molecules where 2/3 of oxygen molecules are derived from O_3 . In comparison, during the nighttime and winter months, reactions R4–R6 produce HNO_3 with up to 5/6 of the oxygen molecules from O_3 (Hastings et al. 2004). Because the $\delta^{18}\text{O}$ of O_3 in the troposphere generally is very high (>90‰; Johnston & Thiemens 1997) relative to OH and H_2O vapor (generally <0‰; Dubey et al. 1997), the resulting wintertime HNO_3 has higher $\delta^{18}\text{O}$ values.

A study of pre-industrial nitrate isotopes in ice cores from Summit, Greenland shows that $\delta^{18}\text{O}$ values ranged from +52‰ to +87‰ throughout the Holocene interglacial and preceding glacial period of the past 25,000 years (Hastings et al. 2005). This range in values is similar to what is reported in contemporary studies (e.g., Hastings et al. 2003, 2004; Elliott et al. 2006; in preparation) and further suggests that $\delta^{18}\text{O}$ values are reflective of HNO_3 production pathways.

Atmospheric processes causing variations in $\Delta^{17}\text{O}$

Seasonal variations in atmospheric $\Delta^{17}\text{O}$ (from +20 to +30‰) observed in Southern California (USA) were explained by a shift from nitric acid production by the $\text{OH}^* + \text{NO}_2$ reaction, which is predominant in the spring and summer, to N_2O_5 hydrolysis reactions that dominate in the winter (Michalski et al. 2004). Figure 12.5 illustrates seasonal patterns in $\Delta^{17}\text{O}$ of bimonthly volume-weighted precipitation samples across New England (USA) and the strong correlation of $\Delta^{17}\text{O}$ with $\delta^{18}\text{O}$ (Wankel 2006), presumably due to a seasonality in the relative proportions of NO_x oxidation by OH^* or O_3 (Michalski et al. 2003; Hastings et al. 2003). Ice-core samples from a glacier in Greenland show higher $\Delta^{17}\text{O}$ values during 1880s due to the effects of large biomass burning events in North America on NO_x (Alexander et al. 2004).

Separating mixing of sources from the effects of cycling

Under ideal circumstances, nitrate isotopes offer a direct means of source identification because the two major sources of NO_3^- in many agricultural areas, fertilizer and manure, generally have isotopically distinct $\delta^{15}\text{N}_{\text{NO}_3}$ values. In contrast, the two major sources of NO_3^- to more pristine watersheds, atmospheric NO_3^- and microbial NO_3^- , have isotopically distinct $\delta^{18}\text{O}_{\text{NO}_3}$ values (Figure 12.1). Hence, the relative contributions of these two sources to groundwater or surface water can be estimated by simple mass balance.

Figure 12.1 shows the normal range of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values for the dominant sources of nitrate. Nitrate derived from ammonium fertilizer, soil organic matter, and animal manure has overlapping $\delta^{18}\text{O}$ values; for these sources,

$\delta^{15}\text{N}$ is a better discriminator. In contrast, NO_3^- derived from nitrate fertilizer or atmospheric sources is readily separable from microbial NO_3^- using $\delta^{18}\text{O}$, even though the $\delta^{15}\text{N}$ values are overlapping. While these general ranges of isotopic values are useful starting points for distinguishing among various sources, because nitrogen is a major nutrient and thus undergoes significant amounts of cycling in most ecosystems, the actual isotopic values of the sources can be outside these ranges. Nitrogen cycling, as discussed above, imparts a wide variety of isotopic fractionations which tend to obscure the original source signal, whether there is a single source or a mixture of two or more sources. The following section discusses how isotopes can be used to determine the relative contributions of different sources to a mixed pool, as well as methods for recognizing and accounting for the impact of cycling (i.e., fractionation due to denitrification, assimilation, nitrification, etc.) on isotopic composition and water chemistry. Applications to different environmental settings are briefly discussed in a later section.

Mixing

If nitrate in groundwater or surface water derives from the mixing of two different sources that are known to have distinctive $\delta^{15}\text{N}_{\text{NO}_3^-}$ values, in the absence of any subsequent fractionations, the relative contributions of each can readily be calculated. Many studies have illustrated this point using $\delta^{15}\text{N}$ versus NO_3^- concentration plots, showing that mixtures must plot on a line between the two “end-member” compositions. However, such mixing lines are straight lines only when the nitrate concentrations of the two end-members are identical; otherwise, mixing lines are hyperbolic on such plots. Hence, a good test of whether $\delta^{15}\text{N}$ or $\delta^{18}\text{O}$ data can be explained by simple mixing is to plot the δ values vs. $1/\text{NO}_3^-$. An example of this is given in Figure 12.6 (modified from Mariotti et al. 1988), where two waters with NO_3^- concentrations of 200 and $1\ \mu\text{M}$ mix together. Note that the curvature of the mixing line is very slight for some concentrations (e.g., 50–200 μM) where NO_3^- concentrations of the end-members are very different.

Unfortunately, real-life studies are rarely this simple. The multiple potential sources of nitrate in various ecosystems rarely have constant isotopic compositions, and the initial compositions may be altered by various fractionating processes before, during, or after mixing. Hence, estimates of relative contributions will often be only qualitative (see Bedard-Haughn et al. 2003). In particular, denitrification can greatly complicate the interpretation of $\delta^{15}\text{N}$ values because the exponential increase in $\delta^{15}\text{N}$ of residual nitrate with decreasing NO_3^- content caused by denitrification can sometimes be confused with mixing of NO_3^- sources. For example, on Figure 12.6a, all three curves are almost linear for nitrate concentrations 100–200 μM . Thus, an incautious worker could try to interpret all three as mixing lines. However, as shown on Figure 12.6b, two of these curves are exponential relations resulting from denitrification, not mixing lines. Figure 12.6c illustrates that

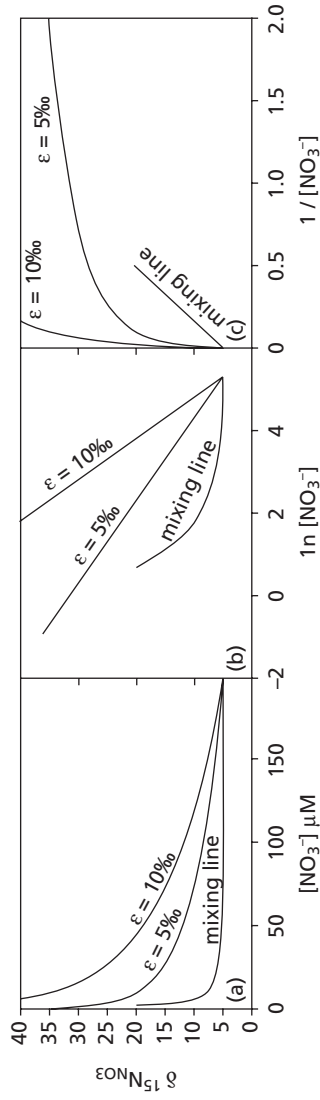


Figure 12.6 The curves on a plot of $\delta^{15}\text{N}$ vs. NO_3^- (a) resulting from mixing of two sources of nitrate with different concentrations, can be distinguished from the curves resulting from denitrification with two different fractionations by plotting $\delta^{15}\text{N}$ vs. $\ln[\text{NO}_3^-]$ (b), where different denitrification fractionations yield straight lines whereas mixing yields a curve, and by plotting $\delta^{15}\text{N}$ vs. $1/[\text{NO}_3^-]$ (c), where different denitrification fractionations yield curves whereas mixing yields a straight line.

true mixing will only be straight when $\delta^{15}\text{N}$ is plotted against the inverse of concentration.

Mixing of NO_3^- sources can sometimes be resolved by analysis of both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (or of other semi-conservative isotopic and/or chemical tracers). This dual-isotope approach has three main potential benefits:

- 1 $\delta^{18}\text{O}_{\text{NO}_3}$ separation of some sources is greater than for $\delta^{15}\text{N}_{\text{NO}_3}$, allowing better source resolution by having two tracers;
- 2 some nitrate sources that are usually indistinguishable with $\delta^{15}\text{N}$ alone (e.g., fertilizer vs. soil nitrate, or atmospheric vs. soil nitrate) may be identified only when the $\delta^{18}\text{O}$ is analyzed;
- 3 $\delta^{18}\text{O}$ values vary systematically with $\delta^{15}\text{N}$ during denitrification (as illustrated in Figure 12.1) and assimilation.

Thus, in systems where the dominant sources of nitrate are isotopically distinctive, source contributions can – in theory – be determined despite significant fractionation.

The greatest problems for using isotopes to determine mixing proportions are (i) that different sources can have partially overlapping isotopic compositions, (ii) sources can have considerable spatial and temporal variation in isotopic composition, and (iii) isotope fractionations can blur initially distinctive isotopic compositions. These problems can often be minimized or eliminated by a multi-isotope, multi-tracer approach – with a lot of hydrologic and chemical data. It is important to keep in mind that the successful solution of the mixing algebra does not ensure that the source determinations are accurate. Improvements in statistical analyses of mixing models offer increasingly sophisticated means to separate sources in situations of variable or partially overlapping isotope ratios (Phillips & Gregg 2001, 2003; Phillips & Koch 2002), and to make better use of multiple tracers. Furthermore, sometimes the isotopic variability may prove to be a useful natural “signal” that may actually enhance the power of isotope methods in these situations.

Fractionation due to denitrification

Denitrification is the process that poses most difficulties for simple applications of nitrate isotopes, because of both the large fractionations and its ubiquity in many landscape types. Hence, for successful applications of nitrate isotopes for tracing sources, it is critical to (i) determine if denitrification has occurred, and, if so (ii) determine the initial isotopic composition of the nitrate (which is a necessary prerequisite for later attempts to define sources).

There are several geochemical approaches for identifying and quantifying denitrification, and distinguishing it from mixing.

- 1 First, unless there is evidence of reducing conditions (e.g., low dissolved O_2 , high H_2S or CH_4 , etc) denitrification is unlikely. However, denitrification

may also take place in anoxic microsites in an otherwise oxygenated sediment (Brandes & Devol 1997).

2 Geochemical modeling using chemical data and perhaps $\delta^{13}\text{C}_{\text{DIC}}$, $\delta^{34}\text{S}_{\text{SO}_4}$, $^{87}\text{Sr}/^{86}\text{Sr}$, and other isotopes may also be useful (Böhlke & Denver 1995) for evaluating whether mixing or denitrification best explains chemical and isotopic compositions.

3 Analysis of dissolved N_2 produced by denitrification for $\delta^{15}\text{N}$ can indicate whether there are systematic increases in $\delta^{15}\text{N}_{\text{N}_2}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ with decreases in NO_3^- concentration (e.g., a “Rayleigh equation” relationship) that are consistent with denitrification. This method also requires estimation of recharge temperature (usually accomplished by analysis of dissolved inert gases such as Ar and Ne), verification that the groundwater samples analyzed represent closed N systems, calculation of the amount of excess N_2 produced by denitrification, and correction of $\delta^{15}\text{N}_{\text{NO}_3}$ for the amount of fractionation produced by denitrification (Vogel et al. 1981; Böhlke & Denver 1995).

4 Analysis of the NO_3^- for $\delta^{18}\text{O}$, as well as $\delta^{15}\text{N}$, can also be useful since systematic increases in $\delta^{18}\text{O}$ due to denitrification (or assimilation) will accompany increases in $\delta^{15}\text{N}$; however, $\delta^{18}\text{O}_{\text{NO}_3}$ usually is not as useful for determining extent of denitrification as $\delta^{15}\text{N}_{\text{N}_2}$.

5 Plotting $\delta^{18}\text{O}_{\text{NO}_3}$ vs. $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ can also be useful because nitrification in contact with the ambient water is likely to result in $\delta^{18}\text{O}_{\text{NO}_3}$ values that show a strong correlation with $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (Wankel et al. 2006; McMahon & Bohlke 2006). These data can then be compared with the theoretical nitrification line defined in equation 12.1, or the $\delta^{18}\text{O}_{\text{NO}_3} = \delta^{18}\text{O}_{\text{H}_2\text{O}}$ line observed in recent marine studies (Casciotti et al. 2002). However, if denitrification is the main process affecting the $\delta^{18}\text{O}_{\text{NO}_3}$, there will be no correlation with $\delta^{18}\text{O}_{\text{H}_2\text{O}}$.

6 In special cases with significant atmospheric nitrate contributions, $\Delta^{17}\text{O} > 0$ and processes leading to increased $\delta^{18}\text{O}_{\text{NO}_3}$ (and $\delta^{15}\text{N}_{\text{NO}_3}$) will have no effect on the $\Delta^{17}\text{O}$ value (Figure 12.4), allowing separation of mixing of sources from fractionation.

7 Perhaps most importantly, mixing of sources will follow a hyperbolic relationship while fractionation is an exponential process. Hence, if mixing of two sources is responsible for the curvilinear relationship on a plot of $\delta^{15}\text{N}_{\text{NO}_3}$ (or $\delta^{18}\text{O}_{\text{NO}_3}$) and NO_3^- , plotting $\delta^{15}\text{N}_{\text{NO}_3}$ (or $\delta^{18}\text{O}_{\text{NO}_3}$) vs. $1/\text{NO}_3^-$ will result in a straight line (Figure 12.6c). In contrast, if denitrification (or assimilation) is responsible for the relationship, plotting $\delta^{15}\text{N}_{\text{NO}_3}$ (or $\delta^{18}\text{O}_{\text{NO}_3}$) vs. $\ln \text{NO}_3^-$ will produce a straight line.

Seasonal or storm-related cycles of denitrification and nitrification pose a considerable challenge to the use of isotope techniques for identifying nitrate sources and mixing proportions (Koba et al. 1997). A multi-isotope approach using triple isotopes of nitrate, combined with analysis of the concentrations and isotopic compositions of gases produced and consumed during denitrification and nitrification (e.g., $\delta^{15}\text{N}$ of N_2 , $\delta^{18}\text{O}$ of O_2 , $\delta^{13}\text{C}$ of CO_2), may allow

determination of temporal changes in these processes if waters and soil gases can be sampled with sufficient temporal resolution.

Cycling of DIN through organic matter

The other main process that complicates NO_3^- source identification and apportionment is partial recycling of the NO_3^- through an organic matter pool. There are several geochemical approaches for identifying and quantifying assimilation in soil water or streams, and distinguishing it from mixing. For example, in aquatic systems chlorophyll is a sensitive indicator of productivity and can be used to approximate algal growth and N assimilation rates. Measurement of the concentration and $\delta^{13}\text{C}$ of DIC and/or concentration and $\delta^{18}\text{O}$ of O_2 can also be used to constrain photosynthesis (Parker et al. 2005). As with denitrification, analysis of the NO_3^- for $\delta^{18}\text{O}$ as well as $\delta^{15}\text{N}$, analysis of $\Delta^{17}\text{O}$, comparisons with $\delta^{18}\text{O}_{\text{H}_2\text{O}}$, and/or plotting $\delta^{15}\text{N}$ vs. $\ln \text{NO}_3^-$ can sometimes help distinguish fractionation vs. mixing.

Source identification and quantification becomes more complicated when NO_3^- assimilation co-occurs with nitrification. In this case, not only are the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the residual NO_3^- pool fractionated because of assimilation, but an additional source of NO_3^- has now been added (see the section on "Nitrification"). This kind of complicated mixture of processes is to be expected in biogeochemically and/or hydrologically active "hotspots" (McClain et al. 2003), and is probably quite common. Two good examples of this kind of complicated environmental setting are agricultural fields after fertilizer application (e.g., Feigin et al. 1974) and small forested catchments where atmospheric NO_3^- is a major source of N to the ecosystem (e.g., Burns & Kendall 2002). In both cases, the microbial cycling of the newly applied DIN is complicated by flushing of the soil by rain events and/or snowmelt. Ultimately, the isotopic composition of the fertilizer or atmospheric DIN is partially or totally overprinted in the soil zone. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the new NO_3^- are probably best assessed by analyzing waters that leach past the soil zone, or by leaching soil samples. Laboratory incubations may not be representative because disturbing the soil might cause changes in soil respiration and the resulting $\delta^{18}\text{O}$ of the ambient O_2 . Hence, the NO_3^- produced during incubations might have a different $\delta^{18}\text{O}$ than the natural microbial NO_3^- .

Other good recent examples of using dual isotope approaches to "deconvolute" coupled biogeochemical processes include coupled denitrification–nitrification studies in lacustrine (Lehmann et al. 2003) and marine (Lehmann et al. 2004) environments, and coupled nitrification–assimilation studies in estuarine and marine environments (Wankel et al. 2007). This latter study investigated N cycling in surface waters of Monterey Bay (California, USA) to constrain nitrification occurring in the euphotic zone. While the NO_3^- in surface waters showed the expected pattern of increasing $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ due to phytoplankton assimilation, deviations from the 1:1 pattern were used to

estimate the degree of rapid organic matter remineralization and nitrification occurring in the euphotic zone. The authors suggested that, on average, ca. 30% of the NO_3^- in surface waters had been cycled through organic matter and regenerated via nitrification.

Applications to different environmental settings

Small forested catchment studies

One of the main applications of $\delta^{18}\text{O}_{\text{NO}_3}$ has been for determination of the relative contributions of atmospheric and soil-derived sources of NO_3^- to shallow groundwater and small streams. This problem is intractable using just $\delta^{15}\text{N}_{\text{NO}_3}$, because of overlapping compositions of soil and atmospherically derived NO_3^- , whereas these sources have very distinctive $\delta^{18}\text{O}_{\text{NO}_3}$ values (Figure 12.1). The $\delta^{18}\text{O}_{\text{NO}_3}$ values are such a sensitive indicator of NO_3^- sources that even diel changes in snowmelt, and consequently contributions of snowmelt-derived nitrate to streams, can be detected as diel oscillations in $\delta^{18}\text{O}_{\text{NO}_3}$ in streamwater (Ohte et al. 2004).

A number of studies have found that much of the NO_3^- in runoff from small catchments is microbial (i.e. from nitrification) instead of atmospheric (e.g., Burns & Kendall 2002; Campbell et al. 2002; Sickman et al. 2003; Pardo et al. 2004; Ohte et al. 2004; Piatek et al. 2005). For example, a multi-year investigation at the Loch Vale watershed in Colorado (USA), showed that half or more of the NO_3^- in the stream during the snowmelt period was microbial in origin (Kendall et al. 1995a,b), and probably originated from shallow groundwater in talus deposits (Campbell et al. 2002). Therefore, the NO_3^- eluted from the snowpack appears to go into storage, and most of the NO_3^- in streamflow during the period of potential acidification is apparently derived from pre-melt sources. Much of this NO_3^- was probably originally of atmospheric origin but had lost its atmospheric signature during microbial recycling in the talus (Campbell et al. 2002).

We expect that analysis of $\Delta^{17}\text{O}_{\text{NO}_3}$ will also be valuable in such studies because it is an even less ambiguous tracer of atmospheric NO_3^- than $\delta^{18}\text{O}_{\text{NO}_3}$ (e.g., Michalski et al. 2004). In theory, because all non-atmospheric sources have $\Delta^{17}\text{O} = 0$ and biogeochemical processes do not affect $\Delta^{17}\text{O}$ values, in the absence of any recycling of atmospheric nitrate in the watershed (admittedly a large caveat), the 0.1‰ analytical resolution of $\Delta^{17}\text{O}$ leads to a detection limit for atmospheric NO_3^- of 0.5% of total nitrate (Michalski et al. 2004). The few studies that have compared $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ of NO_3^- in streamwater during storm events indicate that $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ have different responses to discharge changes (Michalski et al. 2004; Showers & DeMasters 2005). Furthermore, both studies found that $\delta^{18}\text{O}$ significantly underestimated the contributions of atmospheric NO_3^- to runoff (Figure 12.7). Michalski

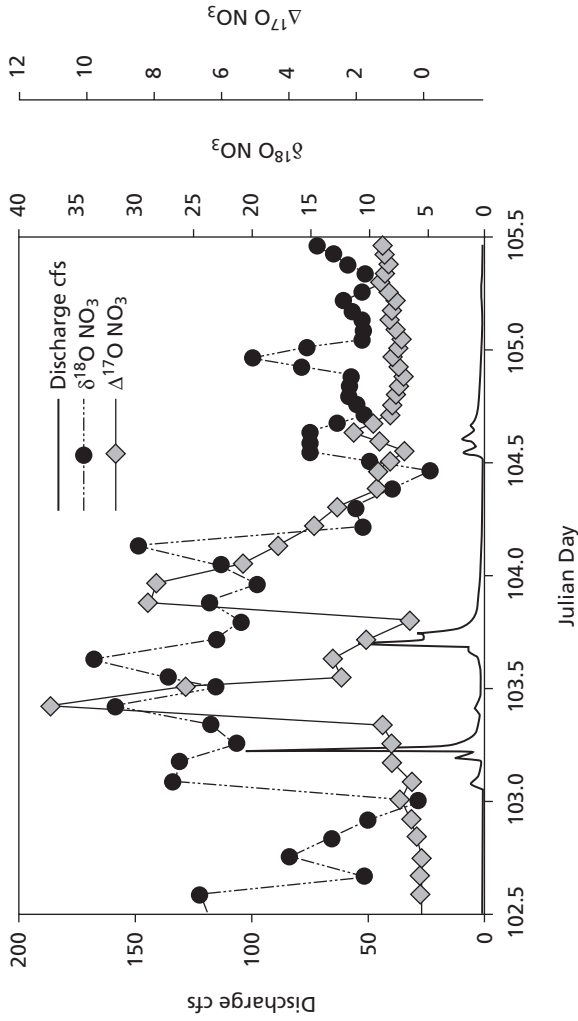


Figure 12.7 Temporal changes in $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ of nitrate and discharge in an urban watershed in the Neuse River Basin, NC, USA. Note that the $\delta^{18}\text{O}$ shows a broad peak over the discharge event while the $\Delta^{17}\text{O}$ nitrate has distinct sharp peaks on the falling hydrograph portion of the storm event. (Plot courtesy of W.J. Showers, modified from Showers & DeMasters 2005.)

et al. (2004) noted that the larger amounts of atmospheric NO_3^- in runoff determined using $\Delta^{17}\text{O}$ implies that previous estimates for streams made using $\delta^{18}\text{O}$ may be too conservative. The temporal variability in stream $\Delta^{17}\text{O}$ at a small urban watershed in the Neuse River Basin (USA; Figure 12.7) suggests that calculations of atmospheric N flux in streams from $\Delta^{17}\text{O}$ measurements must be integrated over an entire event and not from discrete measurements (Showers & DeMasters 2005).

Urban stream studies

Several studies suggest that atmospheric NO_3^- may be a major contributor to streamflow in urban catchments. For example, a pilot study of NO_3^- sources in storm runoff in suburban watersheds in Austin, Texas (USA) found high $\delta^{15}\text{N}$ and low $\delta^{18}\text{O}$ values during baseflow (when Cl^- was high), and low $\delta^{15}\text{N}$ and high $\delta^{18}\text{O}$ values during storms (when Cl^- was low; Ging et al. 1996; Silva et al. 2002). The strong correspondence of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values during changing flow conditions, and the positive correlation of the percentage of impervious land-cover and the $\delta^{18}\text{O}_{\text{NO}_3^-}$, suggests that the stream composition can be explained by varying proportions of two end-member compositions (Figure 12.8), one dominated by atmospheric NO_3^- or nitrate fertilizer, that is the major source of water during storms, and the other a well-mixed combination of sewage and other NO_3^- sources, that contributes to baseflow (Silva et al. 2002). Analysis of stream NO_3^- samples for $\Delta^{17}\text{O}$ would provide more

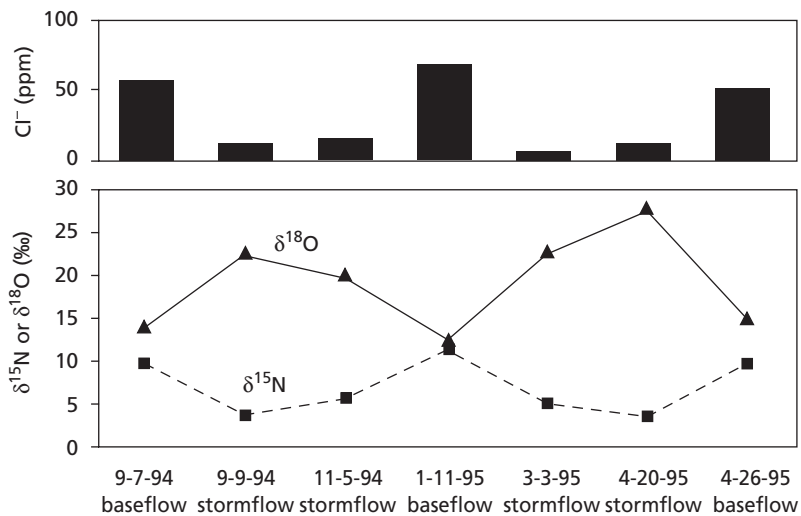


Figure 12.8 Average $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of NO_3^- during stormflow (low Cl^-) and baseflow (high Cl^-) conditions in urban streams in Austin, Texas (USA). (Modified from Silva et al. 2002.)

definitive evidence of an atmospheric source, as is shown in another urban watershed (Figure 12.7).

Several studies have shown that the $\delta^{15}\text{N}$ of nitrate, or “proxies” for nitrate (e.g., particulate organic matter (POM), plants, algae), are correlated with the percentage of wastewater inputs from urban areas. In a large-scale study of 16 large watersheds in the northeastern and mid-Atlantic, USA, Mayer et al. (2002) demonstrated that riverine $\delta^{15}\text{N}_{\text{NO}_3}$ values were positively correlated with wastewater inputs. Elliott & Brush (2006) report similar correlations at lower population densities by comparing historical reconstructions of watershed wastewater N loads and stratigraphic organic nitrogen in wetland sediments (Figure 12.9). Similarly, in a study of groundwater nitrate isotopes on Cape Cod, Massachusetts (USA), Cole et al. (2006) observed positive correlations between wastewater N loads and $\delta^{15}\text{N}$ in groundwater. The positive correlations between $\delta^{15}\text{N}$ and wastewater inputs in these and other studies suggest that the subsurface delivery of wastewater inputs make $\delta^{15}\text{N}$ a particularly effective indicator of wastewater contamination source; boron isotopes are also a useful tracer of wastewater (Widory et al. 2004), as will be described in a later section.

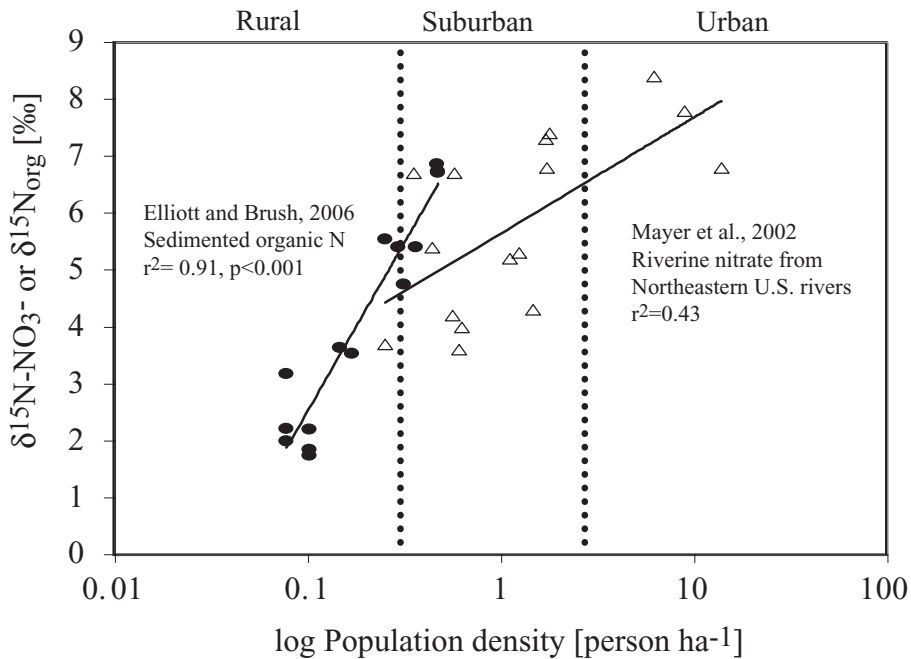


Figure 12.9 $\delta^{15}\text{N}$ vs. population density for nitrate (modified from Mayer et al. 2002) and organic matter (modified from Elliott & Brush 2006). Land use classifications from Theobald (2004).

Small agricultural rivers

There have been fewer nitrate isotope studies in small agricultural rivers than in small forested watersheds, perhaps because of the critical response elicited by the first such study by Kohl et al. (1971). However, several recent studies using combined tracer techniques have provided much insight on the dynamics of NO_3^- in agricultural systems. Surface-water NO_3^- studies by Böhlke & Denver (1995) in an agricultural watershed in Maryland (USA) and McMahon & Böhlke (1996) in the Platte River in Colorado (USA) used $\delta^{15}\text{N}$ data mainly to quantify the effects of denitrification and mixing between the river and aquifer, not to assess contributions from specific NO_3^- sources (although the $\delta^{15}\text{N}$ values did suggest the NO_3^- derived from animal waste and fertilizer). Denitrification was quantified by measurement of the excess N_2 and the $\delta^{15}\text{N}$ of dissolved N_2 , and mixing relations and flowpaths were established using chlorofluorocarbon (CFC) and hydraulic data. Substantial denitrification was found in reducing zones within the aquifer in Maryland. However, there was probably limited denitrification in the wetlands and shallow organic soils adjacent to the streams because the deeper groundwater flowpaths avoided these buffer strips and converged directly beneath the streambeds and rapidly discharged upwards (Böhlke & Denver 1995). The hyporheic zone was found to be a major site for denitrification for the Platte River (McMahon & Böhlke 1996).

Böhlke et al. (2004) conducted an in-stream tracer experiment using Br^- and ^{15}N -enriched NO_3^- to determine the rates of denitrification and other processes in a high-nitrate gaining stream (Sugar Creek, Indiana) in the upper Mississippi Basin. The systematic downstream increase in $\delta^{15}\text{N}_{\text{N}_2}$ indicated high rates of in-stream denitrification. However, while N losses by processes other than denitrification were probably less than the denitrification rate, the overall mass fluxes of N_2 were dominated by discharge of denitrified groundwater and air–water gas exchange in response to changing temperature (Böhlke et al. 2004). The study concluded that the in-stream isotope tracer experiment provided a sensitive measurement of denitrification and related processes where other mass-balance methods were not suitable.

Large river basin studies

Several recent studies in North America evaluated whether the combination of nitrate $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ would allow discrimination of watershed sources of N and provide evidence for denitrification. A pilot study in the Mississippi Basin (Battaglin et al. 2001a,b; Chang et al. 2002) showed that large watersheds with different land uses (crops, animals, urban, and undeveloped) had overlapping but moderately distinguishable differences in nutrient isotopic compositions (e.g., $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of NO_3^- , and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of POM). Atmospheric

NO_3^- was found to be a significant source of NO_3^- to large undeveloped and urban watersheds (Battaglin et al. 2001a, 2001b; Chang et al. 2002). A study in 16 large rivers in the northeastern USA found strong positive correlations between $\delta^{15}\text{N}$ and the calculated wastewater N contributions (Mayer et al. 2002); their $\delta^{15}\text{N}$ data are plotted relative to population density, a surrogate for wastewater, in Figure 12.9. Groundwater NO_3^- , probably derived from near-stream dairies, was found to be a significant source of NO_3^- to the San Joaquin River, the major agricultural basin in the Central Valley of California (USA; Kratzer et al. 2004). A study in the Oldman River Basin (Alberta, Canada) showed that the main source of nitrate in western tributaries draining a relatively pristine forested part of the basin was soil nitrate, whereas the main source in eastern tributaries draining agricultural and urban land uses was manure and/or sewage (Rock & Mayer 2004).

Prior N mass balance studies in the Mississippi Basin and in the northeastern USA rivers suggested appreciable losses of N via denitrification, especially in the headwaters. However, these studies did not find isotopic evidence for denitrification, perhaps because of continuous mixing with new nitrate, the small extent of denitrification, or the low fractionations resulting from diffusion-controlled (i.e., benthic) denitrification (Brandes & Devol 1997; Sebilo et al. 2003). A recent study in the Mississippi River in Illinois (Panno et al. 2006) concluded that the 1:2 relationship between $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ observed in both river and tile drain samples suggests that most of the denitrification probably occurred before discharge into the Mississippi River. They found that most of the NO_3^- in the river is primarily derived from synthetic fertilizers and soil organic N, consistent with published estimates of N inputs to the Mississippi River. Depending on sample location and season, NO_3^- in the river and tile drains has undergone significant denitrification, ranging from about 0 to 55% (Panno et al. 2006).

Investigations of N sources and sinks in the Seine River Basin (France) have also shown denitrification to be a major sink for NO_3^- , especially in the summer (Sebilo et al. 2003, 2006). An investigation of riparian denitrification in various stream orders in the Seine River system during summer low-flow conditions concluded that riparian denitrification removed up to 50% of the N exported from agricultural soils; however, the extent of denitrification determined by shifts in the $\delta^{15}\text{N}$ of residual nitrate provided only a minimum estimate of denitrification (Sebilo et al. 2003). A subsequent study used $\delta^{15}\text{N}_{\text{NH}_4^+}$, $\delta^{15}\text{N}_{\text{NO}_3^-}$, and $\delta^{18}\text{O}_{\text{NO}_3^-}$ to assess the extent of nitrification and denitrification in the water column resulting from export of waste water treatment plant (WWTP) effluent from Paris into the Seine River. During summer low-flow conditions it was found that most of the NH_4^+ released from the WWTP was nitrified in the lower Seine River and its upper estuary, but there was no evidence for water-column denitrification (Sebilo et al. 2006).

A useful adjunct to tracing N sources and sinks in aquatic systems with nitrate isotopes is the analysis of POM for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$. In many river

systems, much of the POM is derived from *in situ* production of algae. Even if an appreciable percent of the POM is terrestrial detritus, the C:N value of the POM and the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ can, under favorable conditions, be used to estimate the percent of POM that is algae, and its isotopic composition (Kendall et al. 2001). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (and $\delta^{34}\text{S}$) of the POM reflect the isotopic compositions of dissolved inorganic N, C, and S in the water column. In turn, these compositions reflect the sources of N, C, and S to the system, and the biogeochemical processes (e.g., photosynthesis, respiration, denitrification, sulfate reduction) that alter the isotopic compositions of the dissolved species; see Finlay & Kendall (this volume, pp. 283–333) for more details. Hence, the changes in the isotopic composition can be used to evaluate a variety of in-stream processes that might affect the interpretation of nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (Kendall et al. 2001).

The $\delta^{15}\text{N}$ of POM may even serve as an integrator for $\delta^{15}\text{N}_{\text{NO}_3}$, when the POM is dominated by *in situ* production of algae; the $\delta^{15}\text{N}$ of POM appears to be ca. 4‰ lower than the associated nitrate in both the Mississippi Basin (Battaglin et al. 2001) and in the San Joaquin River (Kratzer et al. 2004). In the latter river, downstream changes in the $\delta^{15}\text{N}_{\text{NO}_3}$ in response to changes in NO_3^- sources are reflected, albeit shifted about 4‰ because of assimilatory fractionation, in the downstream changes in $\delta^{15}\text{N}_{\text{POM}}$ (see Figure 10.8).

Wetlands studies

The accumulation of organic matter in wetlands affected by human activities provides a historical archive of information about temporal changes in land uses and biogeochemical processes. In addition, due to the large fractionations associated with denitrification and sulfate reduction, the coupled use of N and S isotopes can be useful for examining redox reactions in wetland environments. For example, a study in Florida (USA) showed that export of high-sulfate waters from the agricultural areas around Lake Okechobee into canals and marshes in the Everglades caused increases in the $\delta^{34}\text{S}_{\text{SO}_4}$ as the sulfate (originally about +16‰) is progressively reduced to H_2S (Bates et al. 2002). These high concentrations of sulfate have apparently stimulated the proliferation of sulfate reducers in the marshes, causing the algae and fish in hypoxic marshes to be “labeled” with $\delta^{34}\text{S}$ values as high as +33‰; the biota in these “hot spots” of anoxic conditions are also labeled with low $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ values (Kendall et al. 2000). The high $\delta^{34}\text{S}$ values of organic matter in hypoxic zones in the Everglades are unusual. Sulfate reduction in anoxic marshes and sediments usually results in low $\delta^{34}\text{S}$ of biota because of assimilation of a portion of ^{34}S -depleted H_2S (Trust & Fry 1992).

Wetland environments can also be used to extend the temporal span of our environmental observations. Elliott & Brush (2006) demonstrated that sediment accumulation in wetland environments provides a rich archive of historical information about redox conditions (inferred from coupled $\delta^{34}\text{S}$ and

$\delta^{15}\text{N}$ of organic matter), inorganic N sources to wetland plants ($\delta^{15}\text{N}$ of organic N), plant distributions (inferred from palynological records), and land-use changes (inferred from changes in sedimentation rates and palynology). Using this approach, they concluded that stratigraphic changes in $\delta^{15}\text{N}$ were most likely due to changes in N sources to wetland plants, rather than changes in redox status or diagenesis. In particular, as population density increased in the watershed over 300 years, increasing wastewater N contributions resulted in higher $\delta^{15}\text{N}$ values in sedimented organic N (Figure 12.9).

Groundwater studies

Most natural abundance nitrate $\delta^{15}\text{N}$ studies focus on attempts to trace relative contributions of fertilizer and animal waste to groundwater. This topic was discussed in detail in Kendall & Aravena (2000). Applications of $\delta^{15}\text{N}$ to trace relative contributions of fertilizer and animal waste to groundwater are complicated by a number of biogeochemical reactions, especially ammonia volatilization, nitrification, and denitrification. These processes can modify the $\delta^{15}\text{N}$ values of sources before and/or after mixing, causing estimations of the relative contributions of the sources of nitrate to be inaccurate. The combined use of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ allows better resolution of these issues (e.g. Böttcher et al. 1990; Aravena & Robertson 1998; McMahon & Böhlke 2006). Analysis of the $\delta^{15}\text{N}$ of both nitrate and N_2 (e.g., Böhlke & Denver 1995) provides an effective means for investigating denitrification. Since it is likely that more studies in the future will utilize a multi-isotope or multi-tracer approach, the discussion below will concentrate on multi-isotope studies.

The first dual isotope investigation of groundwater investigated N cycling in municipal wells downgradient from heavily fertilized agricultural areas near Hannover (Germany). This study found that low concentrations of nitrate in groundwater were associated with high $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values, and concluded that the decreases in nitrate away from the fields was caused by microbial denitrification, not mixing with more dilute waters from nearby forests (Böttcher et al. 1990). Furthermore, changes in $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values along the flowpath were linearly related, with a slope of ca. 0.5. The linear relation between the isotope values and the logarithm of the fraction of residual nitrate (Figure 12.6) indicated that denitrification with constant enrichment factors was responsible for the increases in $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$. Many subsequent studies of denitrification in freshwater systems showed slopes ranging from 0.5 to ca. 0.7 (e.g., Aravena & Robertson 1998; Mengis et al. 1999; Cey et al. 1999; Lehmann et al. 2004; Panno et al. 2006; Wassenaar et al. 2006).

One important use of nitrate isotopes is to assess the impact of temporal changes in agricultural practices on groundwater NO_3^- concentrations. For example, an early dual isotope study in British Columbia (Canada) attributed the high NO_3^- in groundwater to nitrification of poultry manure, with lesser

amounts of ammonium fertilizers (Wassenaar 1995). A recent reappraisal of decadal trends in NO_3^- sources at this site, after implementation of best management practices (BMPs) aimed at reducing aquifer-scale NO_3^- contamination, showed increasing nitrate concentrations in young groundwater (<ca. 5 years), suggesting that voluntary BMPs were not having a positive impact in achieving groundwater nutrient reduction goals (Wassenaar et al. 2006). While the stable isotope data showed that animal manure is still the main source of nitrate in the aquifer, a recent decrease in $\delta^{15}\text{N}_{\text{NO}_3}$ suggests a BMP-driven shift away from animal wastes towards inorganic fertilizers. This study and others showed that when the extent of denitrification can be quantified, analyses of dated, denitrification-corrected groundwaters can provide a valuable record of past fertilizer loads in the recharge zone (e.g., Böhlke & Denver 1995). This type of record can be valuable in localities where data on long-term fertilizer-use are missing or unreliable.

The applicability of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of nitrate and other tracers to delineate contaminant plumes derived from domestic septic systems was evaluated by Aravena et al. (1993), in a study within an unconfined aquifer beneath an agricultural area in Ontario (Canada). They found that $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{H}_2\text{O}}$, and water chemistry (especially Na^+) were effective for differentiating between the plume and native groundwater. The lack of a significant difference between the $\delta^{18}\text{O}_{\text{NO}_3}$ in the plume and in local groundwater suggests that nitrification of ammonium, from either human waste or agricultural sources, is the source of the NO_3^- . Another study of a septic plume determined that use of a multi-isotope approach (using $\delta^{13}\text{C}_{\text{DIC}}$, and $\delta^{18}\text{O}$ and $\delta^{34}\text{S}$ of sulfate in addition to nitrate $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$) provided valuable insight into the details of the processes affecting nitrate attenuation in groundwater (Aravena & Robertson 1998).

Coastal and estuarine studies

Coastal and estuarine systems are extremely dynamic, highly productive, and abound with biogeochemical gradients (salinity, temperature, primary productivity, etc.). Over the past few decades, many studies have used the $\delta^{15}\text{N}$ of NO_3^- and NH_4^+ in estuarine systems to investigate sources of N pollution (McClelland & Valiela 1998; Kroeger et al. 2006).

With the advent of the denitrifier method, $\delta^{18}\text{O}_{\text{NO}_3}$ may now be used in estuarine systems to investigate the influence of both cycling and mixing of multiple sources. In their study in the San Francisco Bay estuary, a branched two-arm estuary, Wankel et al. (2006) showed that mixing of sources could explain most of the isotopic variability in the estuary. In particular, $\delta^{15}\text{N}_{\text{NO}_3}$ served as a useful tracer of sewage effluent from the southern arm, while $\delta^{18}\text{O}_{\text{NO}_3}$ was used to separate marine vs. riverine sources along the northern arm. Furthermore, the range of $\delta^{18}\text{O}_{\text{NO}_3}$ was more than twice that of $\delta^{15}\text{N}_{\text{NO}_3}$ and correlated strongly with the gradient in $\delta^{18}\text{O}_{\text{H}_2\text{O}}$. It was hypothesized that

where concentrations of NO_3^- were relatively low, and thus turnover of the pool was relatively rapid, cycling of NO_3^- resulted in the incorporation of water O into the $\delta^{18}\text{O}_{\text{NO}_3}$ along this gradient of $\delta^{18}\text{O}_{\text{H}_2\text{O}}$. While the effects of cycling could not be independently verified, this study nonetheless demonstrated that $\delta^{18}\text{O}_{\text{NO}_3}$ may serve as a more useful tracer of cycling than $\delta^{15}\text{N}_{\text{NO}_3}$, especially in estuaries where large gradients in $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ occur (Wankel et al. 2006). A recent estuarine study in the Seine River estuary (France) found that, despite the apparent conservative behavior of nitrate concentrations, coupled nitrification and denitrification in the water column was probably responsible for the significant shift of $\delta^{15}\text{N}_{\text{NO}_3}$ values from the trend expected for simple mixing of marine and riverine sources (Sebilo et al. 2006).

What sources of agricultural and urban sources of nitrate can be distinguished using isotopes?

Most of the interest in uses of nitrate isotopes centers on how to differentiate:

- 1 fertilizer versus animal waste contributions to groundwater or surface water;
- 2 septic tank leakages (or WWTPs) versus animal waste;
- 3 natural soil N from fertilizer and/or wastes.

Below is a brief discussion of what isotope tools seem to “work” or “not work”, written in a format that we hope will be useful for readers skimming through the chapter and looking for specific answers.

For the purposes of this chapter, we have defined what “works” as follows. Given the usual case where there is a question of which of two main sources of NO_3^- is the dominant source of NO_3^- to groundwater or a stream, can the measurement of $\delta^{15}\text{N}_{\text{NO}_3}$ values (or $\delta^{15}\text{N}$ combined with other isotope and chemical tracers) along with the NO_3^- concentrations of the mixed source allow confident determination of the dominant NO_3^- source? Can the relative contributions be estimated within approximately $\pm 20\%$? If so, we interpret this as a successful “quantitative” tool. A first step in such studies should be analysis of $\delta^{15}\text{N}_{\text{NO}_3}$ (and other isotope and chemical tracers) from near the two potential sources, to insure that the sources have distinctive compositions. Attention should be given to possible temporal and spatial variability in the end-member compositions. Despite the emphasis on the usefulness of isotopes, it is expected that the isotope data are accompanied by appropriate information on hydrology and water chemistry.

Fertilizer vs. animal waste source of nitrate

These sources usually can be differentiated successfully using $\delta^{15}\text{N}$ alone (Figure 12.1), especially in groundwater studies in sandy soils (where the

effects of denitrification should be minimal). In contrast, studies in rivers, poorly drained soils, and poorly oxygenated groundwater are often more complicated, requiring the use of more tracers.

Groundwater and soil water studies

Quantification of the relative contributions of NO_3^- in groundwater or soil water (derived from fertilizer vs. animal waste, which includes human waste from septic systems and WWTPs) using only $\delta^{15}\text{N}_{\text{NO}_3}$ is usually successful if:

- 1** the groundwater is well-oxygenated and soils are sandy instead of clayey, so that denitrification can be (more-or-less) ruled out (Gormly & Spalding, 1979);
- 2** the NO_3^- from soil organic matter is insignificant (or its $\delta^{15}\text{N}$ is similar to fertilizer);
- 3** the fertilizer is nitrate, ammonium, or urea (not manure or green manure, which often has a high $\delta^{15}\text{N}$).

Quantification is much more difficult if the soils are clayey or the groundwater is not well-oxygenated, because the possibility of denitrification would have to be considered (see "Denitrification" section).

$\delta^{18}\text{O}_{\text{NO}_3}$ data are not essential for such determinations because of the large $\delta^{15}\text{N}$ differences between these sources (Figure 12.1). Furthermore, because most applied fertilizer is dominated by ammonium, analysis of $\delta^{18}\text{O}_{\text{NO}_3}$ may not add much additional information; however, this is not always true (see discussion below of Ging et al. 1996). $\delta^{18}\text{O}_{\text{NO}_3}$ could help improve the quantification if there is some difference in the $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ of soil water vs. groundwater or lagoon water, or in the $\delta^{18}\text{O}_{\text{O}_2}$ in the soil zone or groundwater (these instances are not uncommon) where nitrification occurs. In these cases, measuring $\delta^{18}\text{O}_{\text{NO}_3}$ is recommended.

Quantification can be improved if other isotope (B, Sr, S, C, Li, U) or chemical tracers (caffeine, pharmaceuticals, rare earth elements (REEs), heavy metals, etc) specific to the different sources are used (Curt et al. 2004; Vitoria 2004; Vitoria et al. 2004; Widory et al. 2004). Improved quantification of waste sources of nitrate using a multi-isotope approach will be discussed in more detail below.

Surface water studies

Quantification of NO_3^- contributions in drains and streams using only $\delta^{15}\text{N}_{\text{NO}_3}$ (no $\delta^{18}\text{O}_{\text{NO}_3}$) can provide semi-quantitative to quantitative estimates of contributions if:

- 1** denitrification in the riparian zone and/or hyporheic zone can be ruled out or quantified (as above);
- 2** NO_3^- from soil organic matter is insignificant (or its $\delta^{15}\text{N}$ is similar to fertilizer);

3 uptake of nitrate by phytoplankton is minimal (or data are adjusted for this fractionation);

4 nitrification in the stream is minimal (or data are adjusted for this fractionation) (McMahon & Böhlke 1996; Sebilo et al. 2003, 2006).

Quantification can be improved if other chemical and/or isotopic tracers (e.g., $\delta^{18}\text{O}_{\text{NO}_3}$, or $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and/or $\delta^{34}\text{S}$ of POM) are used in addition to $\delta^{15}\text{N}_{\text{NO}_3}$, (e.g., Ging et al. 1996; Battaglin et al. 2001a,b; Silva et al. 2002; Kratzer et al. 2004; Panno et al. 2006; Sebilo et al. 2006).

Soil vs. animal waste source of nitrate

These sources can often be differentiated successfully using $^{15}\text{N}_{\text{NO}_3}$ alone. However, quantification may not be possible because soil nitrates often have variable $\delta^{15}\text{N}$ values, and often these values overlap the $\delta^{15}\text{N}$ values of nitrate derived from animal manure and human waste (see Fogg et al. 1998; Bedard-Haughn et al. 2003).

Quantification of the relative contributions of NO_3^- in soil or groundwater (derived from soil organic matter vs. animal waste) using only $\delta^{15}\text{N}_{\text{NO}_3}$ can be successful if:

1 the groundwater is well-oxygenated and the soils are sandy instead of clayey, so denitrification in the mixing zone can be ruled out;

2 NO_3^- from fertilizer is insignificant (or its $\delta^{15}\text{N}$ is similar to NO_3^- from soil organic matter).

There are many application studies (most not very quantitative), including Kreitler (1975), Kreitler & Browning (1983), Aravena et al. (1993), Komor & Anderson (1993), and Karr et al. (2001, 2003).

$\delta^{18}\text{O}_{\text{NO}_3}$ data are not essential. However, they could help improve the quantification if there is some difference in the $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ of soil water vs. groundwater or lagoon water, or in the $\delta^{18}\text{O}_{\text{O}_2}$ in the soil zone or groundwater (these instances are not uncommon). Therefore, obtaining $\delta^{18}\text{O}_{\text{NO}_3}$ is recommended. For example, NO_3^- from manure piles or lagoons may have a high $\delta^{18}\text{O}$ due to water evaporation or high $\delta^{18}\text{O}_{\text{O}_2}$ due to respiratory fractionation. The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of the lagoon water may even be affected, with higher $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values if water has been reduced via methanogenesis, and lower $\delta^{18}\text{O}$ values if there has been O exchange with CO_2 . Analysis of the water for chemical constituents (animal waste has high chloride and other solutes), $\delta^{13}\text{C}$ of DIC, $\delta^{34}\text{S}$ of sulfate, or DOM for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, might also help distinguish a waste lagoon source of nitrate because these other parameters are often sensitive to biogeochemical reactions in the highly reducing lagoon.

Soil vs. fertilizer source of nitrate

These sources usually cannot be differentiated using $\delta^{15}\text{N}$ alone because soil nitrates are often variable in $\delta^{15}\text{N}$ and usually overlap the $\delta^{15}\text{N}$ values of

fertilizer-derived NO_3^- . Quantification of the relative contributions of NO_3^- derived from soil vs. fertilizer can be successful if:

- 1 it can be demonstrated that the $\delta^{15}\text{N}$ and/or $\delta^{18}\text{O}$ of different sources are sufficiently distinctive;
- 2 the system is well-oxygenated and the soils are sandy instead of clayey, so denitrification in the mixing zone can be ruled out;
- 3 other tracers that are specific for the fertilizer, including different isotopes (B, Sr, S, C, Li, U) or REEs are used.

Good examples include: Kohl et al. (1971, 1972), Hauck et al. (1972), Spruill et al. (2002), and Vitoria et al. (2004).

Septic waste vs. animal manure source of nitrate

These sources almost never can be differentiated using $\delta^{15}\text{N}_{\text{NO}_3}$ alone because the sources have overlapping $\delta^{15}\text{N}_{\text{NO}_3}$ values. However, see Fogg et al. (1998) for an example where the $\delta^{15}\text{N}$ values of the sources are significantly different.

Using a multi-isotope and/or multi-tracer approach, these types of waste can sometimes be distinguished if: (i) the average diets of the humans and animals are at different trophic levels (i.e., one group is herbivorous), causing the $\delta^{15}\text{N}$ of the diets and resulting waste, and hence the NO_3^- resulting from oxidation of the organic matter, to be several permil different; (ii) $\delta^{18}\text{O}_{\text{NO}_3}$ values are different (which could occur if there is some difference in the $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ of public supply water vs. soil water or lagoon water, or in the $\delta^{18}\text{O}_{\text{O}_2}$ in the two environments); (iii) other isotope tracers (B, Sr, S, C, Li, U) or chemical tracers (caffeine, pharmaceuticals, hormones, DNA, K, REEs, heavy metals, etc.) that are specific for the different sources are used (see section on "Other tools"); or (iv) the average diets of the humans and animals have different $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ values (e.g., humans eat a marine-fish-rich diet, or the animals are fed mainly C4 plants, etc.), resulting in waste and resulting dissolved organic matter (DOM) with isotope signatures that are distinctive for the different waste sources. The combination of nitrate $\delta^{15}\text{N}$ and borate $\delta^{11}\text{B}$ can be particularly useful for distinguishing human vs. animal waste (see discussion of **boron isotopes** below). Good multi-tracer examples include: Aravena et al. (1993), Spruill et al. (2002), Curt et al. (2004), Vitoria (2004), and Widory et al. (2004, 2005). Sometimes a more sophisticated statistical or modeling approach can substitute for a lot of additional tracers (e.g., Spruill et al. 2002; Phillips & Gregg 2003; Otero 2004). Now that several studies have shown a multi-isotope approach can be successful, there is need for many more investigations of which kinds of tracers are most useful for which kinds of animals, land uses, and types of human waste.

Nitrate produced from waste from different kinds of farm animals

These sources almost never can be differentiated using $\delta^{15}\text{N}_{\text{NO}_3}$ alone because the sources have overlapping $\delta^{15}\text{N}_{\text{NO}_3}$ values. However, some recent

studies suggest that a multi-isotope and/or multi-tracer approach may be successful in distinguishing between nitrates derived from different sources of manure.

Semi-quantification of contributions of NO_3^- derived from different kinds of farm animals is possible if: (i) $\delta^{18}\text{O}_{\text{NO}_3}$ values are different (which could occur if there is some difference in the $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ of soil or lagoon water, or in the $\delta^{18}\text{O}_{\text{O}_2}$ in the two environments); (ii) other isotope tracers (B, Sr, S, C, Li) or chemical tracers (caffeine, pharmaceuticals, hormones, REEs, K, heavy metals, etc.) that are specific for the different sources are used; or (iii) the average diets of the different animals have different $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ values (e.g., one animal group is fed a diet rich in marine fish or C4 plants), resulting in waste and resulting DOM with isotope signatures that are distinctive for the different animal types. Good examples include: Spruill et al. (2002), Vitoria (2004), and Widory et al. (2004). Sometimes a more sophisticated statistical or modeling approach can substitute for a lot of additional tracers (eg, Spruill et al. 2002; Phillips & Gregg 2003; Otero 2004). Now that several studies have shown a multi-isotope approach can be successful, there is need for many more investigations of which kinds of tracers are most useful for which kinds of animals and land uses.

Organic matter from animal waste vs. human waste vs. natural organic matter

These sources rarely can be differentiated using $\delta^{15}\text{N}_{\text{NO}_3}$ alone because the $\delta^{15}\text{N}_{\text{NO}_3}$ values overlap. However, the organic matter component of animal waste is often distinguishable from human waste or natural organic matter using a multi-isotope approach (e.g., the organic matter is also analyzed for $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$). Quantification of waste from humans vs. animals can be successful if: (i) the average diets of the humans and animals are at different trophic levels (i.e., one group is herbivorous), causing the $\delta^{15}\text{N}$ of the diets and resulting waste, and hence the NO_3^- resulting from oxidation of the organic matter, to be several permil different; and/or (ii) one group eats a diet primarily composed of C3 plants (typical human diet in Asia, but not in Brazil; diets in the USA are intermediate), and the other eats a diet primarily composed of C4 plants (corn and sugar cane are the main C4 crops). $\delta^{34}\text{S}$ can also be useful for differentiating sources, especially when one source is marine (e.g., contamination of coastal waters with WWTP or animal farming operations (AFO) waste). Good examples for differentiating waste from different animals include: DeNiro & Epstein (1981), Minagawa (1992), and Bol & Pflieger (2002). Good examples for differentiating human/animal waste from natural organic matter include: Van Dover et al. (1992), McClelland et al. (1997), McClelland & Valiela (1998), Tucker et al. (1999), Hebert & Wassenaar (2001), and DeBruyn & Rasmussen (2002). For good discussions of the general topic of tracing sources of organic matter with isotopes see

Peterson & Fry (1987), Kendall et al. (2001), and Finlay & Kendall (this volume, pp. 283–333).

Nitrate derived from animal waste ammonium vs. other sources

There has been very little research on the question of whether different sources of NH_4^+ can be distinguished using the isotopic compositions of the resulting NO_3^- . Quantification of ammonium from AFOs vs. other sources (car exhaust, power plant exhaust, fertilizer volatilization) might be successful if:

- 1 there is a lot of volatilization of NH_3 from the waste (like hog lagoons), resulting in $\delta^{15}\text{N}_{\text{NH}_4}$ values that are very low;
- 2 the other sources of NH_4^+ have $\delta^{15}\text{N}$ values that are significantly higher;
- 3 when the NH_4^+ is nitrified, the two sources still have distinctive $\delta^{15}\text{N}$ values;
- 4 the sources show relatively little temporal and spatial variability relative to the difference between the mean $\delta^{15}\text{N}$ values.

Good examples include: Heaton (1986) and Karr et al. (2003).

Atmospheric nitrate derived from anthropogenic vs. natural sources

Although more extensive characterization of $\delta^{15}\text{N}_{\text{NO}_3}$ from individual NO_x sources is required, existing data suggest that $\delta^{15}\text{N}_{\text{NO}_3}$ of wet and dry atmospheric deposition can be used to help distinguish NO_x sources to deposition (see “Isotopic composition of NO_x sources” above). At the regional scale, $\delta^{15}\text{N}$ in wet deposition is strongly correlated with NO_x emissions from stationary sources surrounding precipitation monitoring sites (Figure 12.2; Elliott et al., in press). Several road gradient studies (e.g., Ammann et al. 1999; Pearson et al. 2000; Saurer et al. 2004) have illustrated how $\delta^{15}\text{N}$ isotopes in vegetation and NO_2 can be used to help assess the relative influence of vehicle emission in near-road environments. $\delta^{15}\text{N}$ can also be used to help distinguish sources of bulk particulate matter (Widory 2007), as well as aerosol NO_3^- and NH_4^+ (Yeatman et al. 2001).

Other tools for tracing anthropogenic contaminants

There are a variety of other isotope and geochemical “tools” that can help identify and (possibly) quantify anthropogenic sources of N (or contaminants related to N) to ecosystems. Some of these are described below.

Isotope biomonitoring

There is considerable literature on using the $\delta^{15}\text{N}$ of algae, terrestrial plants, and animals as “proxies” for the isotopic compositions of nitrate and/or ammonium – and hence for sources and the land uses that are specific to the N source. Some good examples include: Harrington et al. (1998), Hebert & Wassenaar (2001), Sauer et al. (2004), Anderson & Cabana (2005; 2006), Elliott & Brush (2006). See Finlay & Kendall (this volume, pp. 283–333) for more details.

Sulfur isotopes

The general terrestrial range of stable S isotope ($\delta^{34}\text{S}$) values is -50 to $+50\%$, with rare values much higher or lower. Analysis of sulfate for $\delta^{34}\text{S}$ and/or $\delta^{18}\text{O}$, or organic matter for $\delta^{34}\text{S}$, can provide information about:

- 1 atmospheric sources of acidic rain (Wadleigh et al. 1996; Wadleigh & Blake 1999);
 - 2 fertilizer sources (Bates et al. 2002; Vitoria et al. 2004);
 - 3 sources of animal/human waste (Bol & Pflieger 2002);
 - 4 use of detergents in WWTP or animal waste caused by animal washing (Vitoria et al. 2004; Widory et al. 2004);
 - 5 biogeochemical reactions occurring in the sediments or water column.
- Boron isotopes can be useful for these purposes as well (Widory et al. 2004).

Much of the literature on using $\delta^{34}\text{S}$ in ecosystems has been driven by the need to understand the effects of atmospheric deposition on S cycling in the natural environment, particularly in forest ecosystems. This is in response to increased S loadings to terrestrial ecosystems from anthropogenic S emissions, as sulfur is a dominant component of acid rain. Since nitrate is another major component of acid rain, it is obvious that linked studies of sulfate and nitrate isotopes are likely to be useful in tracing anthropogenic N sources.

Atmospheric $\delta^{34}\text{S}$ values are typically in the range of -5 to $+25\%$ (Krouse & Mayer 2000). Sulfate in precipitation derived from sea spray has a $\delta^{34}\text{S}$ value of $+21\%$ and $\delta^{18}\text{O}$ of $+9.5\%$, whereas rainwater sulfate derived from long-range transport of continental sources has a $\delta^{34}\text{S}$ of approximately $+4\%$ and $\delta^{18}\text{O}$ of approximately $+11\%$ (Wadleigh et al. 1996). Nriagu & Coker (1978) report that the $\delta^{34}\text{S}$ of precipitation in central Canada varies seasonally from about $+2$ to $+9\%$, with the low values caused mainly by biological S whereas the high values reflect S from fossil fuels.

The spatial distribution of $\delta^{34}\text{S}$ and the relative contributions from marine versus continental (including anthropogenic combustion) sources in Newfoundland (Canada) have been monitored by analyzing the $\delta^{34}\text{S}$ of rainfall and of epiphytic lichens that obtain their entire S content from the atmosphere (Wadleigh et al. 1996; Wadleigh & Blake 1999; Wadleigh 2003).

The lichen study (Wadleigh & Blake 1999) yielded a wonderful “bullseye” $\delta^{34}\text{S}$ contour plot showing low values in the interior of the island that are probably related to anthropogenic point sources, and progressively higher (more marine) values towards the coasts. These studies suggest that the study area is influenced by both marine (high $\delta^{34}\text{S}$ values) and continental sources (lower $\delta^{34}\text{S}$ values), with the possibility of anthropogenic influence from fossil-fuel powered plants.

Atmospheric sulfate (aerosol and rainfall) has recently been found to have a mass independent isotopic composition, with excess ^{17}O over what would have been expected based on the $\delta^{18}\text{O}$ of sulfate (Lee et al. 2002). For sulfate, the mass independent fractionation is described by: $\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O}$ (Lee et al. 2002). The $\Delta^{17}\text{O}$ values of wet and dry precipitation are generally $<+2\%$ (Lee et al. 2002; Johnson et al. 2001). Hence, $\Delta^{17}\text{O}$ can be used to identify the relative contributions of atmospheric sulfate versus terrestrial biological or geologic sources of sulfate to streams.

Sulfur-35 (^{35}S) is a naturally produced radioactive tracer (half-life = 87 days) that can be used to trace the movement of atmospherically derived sulfate in the environment. It is formed in the atmosphere from cosmic ray spallation of argon-40, and deposits on the Earth's surface in precipitation or as dryfall. It can be used both to trace the time scales for movement of atmospheric sulfate through the hydrosphere and, in ideal cases, to trace the movement of young (<1 year) water. It is an especially useful tracer in regions away from the ocean where sulfate concentrations are relatively low. Watershed application studies include: Cooper et al. (1991), (Michel et al. (2000), Novak et al. (2003), and Shanley et al. (2005).

Water isotopes

Analysis of water for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ can provide extremely useful information about the sources of the nitrate and other solutes in water (Aravena & Robertson 1998; McMahon & Böhlke 2006). Very often, different sources of nitrate in rivers and groundwater are associated with different water $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values because of evaporation or because the waters are derived from very different geographic areas. See Kendall & McDonnell (1998) for a review of the general topic, Kendall & Coplen (2001) for data on surface waters in the USA, and Dutton et al. (2005) for data on precipitation in the USA.

Boron isotopes

Boron (B) has two naturally occurring stable isotopes, ^{10}B and ^{11}B . The large relative mass difference between the boron isotopes leads to a wide (ca. 90%) natural range of $\delta^{11}\text{B}$ values (Barth 1993). Because B is widely used in industrial, agricultural, cosmetics, and household products, $\delta^{11}\text{B}$ is a useful tool for

determining sources of pollutants including nitrate (Eisenhut et al. 1996; Barth 2000). The main industrial source of B to waters is sodium perborate (NaBO_3), which is used in laundry detergents (primarily as a bleaching agent) and in household cleaners; consequently, B is commonly found in household sewage. Purification of waters in sewage treatment plants generally removes little or no B (Barth 2000); hence, $\delta^{11}\text{B}$ is a conservative tracer of a wastewater source. While B isotopes are not affected by denitrification, they are fractionated through processes such as adsorption on clays.

Boron isotopes have been shown to be useful for identifying anthropogenic B sources in surface water and shallow groundwater systems:

- 1 municipal wastewater and sewage (Bassett 1990; Vengosh et al. 1994, 1999; Basset et al. 1995; Eisenhut et al. 1996; Barth 1998; Vengosh 1998; Seiler 2005);
- 2 irrigation return flows (Bassett et al. 1995);
- 3 fertilizer-affected irrigation waters from various agricultural settings (Komor 1997);
- 4 domestic solid waste deposit leachates from landfills (Eisenhut & Heumann 1997; Barth 2000);
- 5 mixed agricultural sources dominated by animal waste (Widory et al. 2004);
- 6 fly ash deposit leachates from a coal-fired power plant (Davidson & Bassett 1993).

Use of $\delta^{11}\text{B}$ coupled with $\delta^{15}\text{N}$ has proved to be an effective means for tracing agricultural nitrate sources (e.g., hog manure, cattle feedlot runoff, synthetic fertilizers) in surface and groundwaters (Basset et al. 1995; Komor 1997; Widory et al. 2004). In a recent study (Widory et al. 2004), $\delta^{11}\text{B}$ was used to distinguish between two types of sewage that were indistinguishable using $\delta^{15}\text{N}$ alone: a high-B/low- NO_3 /low- $\delta^{11}\text{B}$ type that is derived from washing powders, and a moderate-B/moderate- NO_3 type with $\delta^{11}\text{B}$ values close to animal (probably human) manure. Some separation of different animal sources of B (e.g., sewage, cattle, hogs, poultry) is seen on plots of $\delta^{11}\text{B}$ vs. $1/\text{B}$ (Widory et al. 2004).

Strontium isotopes

Strontium (Sr) isotopes (^{87}Sr and ^{86}Sr) are another potentially useful tracer of anthropogenic sources of contaminants related to nitrate. Strontium isotopes can be used to distinguish between a phosphorite and carbonatite origin of phosphate fertilizers (Vitoria et al. 2004). Several studies have shown significant differences between the Sr isotopic composition (denoted as $\delta^{87}\text{Sr}$ or as $^{87}\text{Sr}/^{86}\text{Sr}$) of natural groundwater and human inputs (e.g., Négrel & Deschamps 1996; Négrel 1999; Böhlke & Horan 2000). In contrast to N and B isotopes, Sr isotopes will not fractionate through natural processes because

of the low mass contrast between the ^{87}Sr and ^{86}Sr . Therefore Sr can, in theory, be used to identify mixing processes and water–rock interaction within the aquifer. Unfortunately, the flux coming from water–rock interaction is often large compared with the anthropogenic flux, and thus totally overprints the isotope signal (Widory et al. 2004).

Widory et al. (2004) tested whether the combined use of geochemical and isotopic tracers (N, B, and Sr) would provide a sensitive method for tracing sources of NO_3^- in contaminated groundwater. The basic idea was that N isotopes, as an intrinsic tracer of the NO_3^- molecule, will reflect both the sources and the fate (i.e. denitrification) of NO_3^- in groundwater. In contrast, B isotopes, because they are not affected by denitrification, will be isotopically labeled with the signature of the sources. Nitrogen and B isotopes proved extremely useful for distinguishing among agricultural sources. However, their study showed that differentiation between the different animals (hog, poultry, cattle manure; and sewage) was not possible using Sr alone due to the similarity of the isotope signatures; however, fertilizer is distinctive.

Lithium isotopes

Lithium (Li) has two naturally occurring stable isotopes, ^6Li and ^7Li . Considerable variability of $\delta^7\text{Li}$ has been reported for natural materials, with marine-derived waters and minerals having much higher $\delta^7\text{Li}$ values than minerals derived from igneous and metamorphic rocks and their associated waters (Bullen & Kendall 1998). Additionally, as a consequence of the processing of lithium to produce highly ^6Li -enriched materials for nuclear power plants, highly purified Li with a high $\delta^7\text{Li}$ value (often in the range of +200 to +400‰) is readily available for industrial, agricultural, and pharmaceutical use (Qi et al. 1997). Hence, $\delta^7\text{Li}$ is potentially a very valuable tracer of an anthropogenic source of wastewater.

A study in Pennsylvania, USA (Bullen & Senior 1992) showed that $\delta^7\text{Li}$ values in streams that are affected by discharges from lithium-processing plants and in groundwater downgradient from the processing plants are significantly higher than natural background $\delta^7\text{Li}$ values. As an example of how pharmaceuticals might have distinctive $\delta^7\text{Li}$ values, groundwater downgradient from a mental health facility in Pennsylvania (USA) was also found to have a substantially greater $\delta^7\text{Li}$ value than those found in natural materials. Because lithium is commonly used to treat manic depressive behavior, the elevated $\delta^7\text{Li}$ value was attributed to the transfer of the Li from pharmaceuticals into wastewater in this area of unsewered residential development (Thomas D. Bullen, pers. comm.).

Phosphate isotopes

Phosphorous (P) is an essential macronutrient in aquatic ecosystems. Excess anthropogenic inputs of P in the form of orthophosphate (PO_4^{3-}) can cause

eutrophic conditions. Although phosphate has only one stable isotope, recent advances in analytical methods have made it possible to use the $\delta^{18}\text{O}$ of inorganic phosphate (McLaughlin et al. 2004) or organic phosphate (McLaughlin et al. 2006a) as a tracer of the phosphate source. Because the $\delta^{18}\text{O}$ of phosphate rapidly equilibrates with the O in water, $\text{PO}_4\text{-}\delta^{18}\text{O}$ usually is a usable tracer of phosphate sources only in waters that are not P-limiting and where the waters associated with the two sources (waste vs. natural) have water- $\delta^{18}\text{O}$ values that are different by several permil. Such conditions are likely in estuaries or near the coast (McLaughlin et al. 2006b). Other places where the water- $\delta^{18}\text{O}$ values might be sufficiently different to “label” the $\text{PO}_4\text{-}\delta^{18}\text{O}$ include WWTPs or waste lagoons where there is evaporative enrichment of water- $\delta^{18}\text{O}$, or locations where the public supply water is from a much different geographic location or elevation than the local soil water or groundwater (McLaughlin et al. 2006b). Under such conditions, it might be possible to distinguish phosphate from fertilizer vs. animal waste/septic waste vs. soil organic matter (Gruau et al. 2005; McLaughlin et al. 2006b).

A recent survey of the $\delta^{18}\text{O}$ values generated using the silver phosphate method of McLaughlin et al. (2004) for phosphate from many anthropogenic sources, reports ranges of values for sewage (+7 to +12‰), detergents (+13 to +19‰), and fertilizers (+16 to +23‰; Young et al. 2006). These sewage $\delta^{18}\text{O}$ values are considerably lower than the values (+16 to +19‰) previously reported by Gruau et al. (2005) generated by a different method, suggesting that in certain geographic locations $\delta^{18}\text{O}$ might be able to distinguish between waste and detergent or fertilizer sources of phosphate (Young et al. 2006), while in other areas the values may be indistinguishable.

Other isotopes can also be useful for tracing phosphate sources. For example, uranium is a trace constituent of geologic sources of phosphate. Hence, analysis of ^{234}U and ^{238}U can distinguish between “natural” and “geologic” sources (Zielinski et al. 1997, 2000). In addition, strontium is a trace constituent of geologic sources of phosphate. Different sources of geologic phosphate (phosphorites vs. carbonatites) appear to have different concentrations of trace metals and REEs; hence, these can be used as tracers of phosphate and/or fertilizer source and the different geologic units that different source waters traveled through (Böhlke & Horan 2000; Vitoria et al. 2004; Widory et al. 2004).

Age-dating nitrate contamination of groundwater

One powerful potential application of technological advances in the age-dating of young groundwater (Plummer et al. 1993; Dunkle et al. 1993) is to evaluate the impact of changes in agricultural management practices on water quality (e.g., Böhlke & Denver 1995; McMahon & Böhlke 1996). By combining nitrate isotope analyses with ground-water dating, it is possible to estimate the timing of nitrate-related events including:

- 1 the rate of natural denitrification;
- 2 when contaminated or remediated groundwaters will reach the streams (Böhlke & Denver 1995);
- 3 changes in contamination loads in the recharge zone over time (Böhlke & Denver 1995; Böhlke et al. 2006; McMahon & Böhlke 2006);
- 4 success (or otherwise) of implementation of BMPs (Wassenaar et al. 2006).

Böhlke & Denver (1995) showed that in places where the extent of denitrification can be quantified, analyses of dated, denitrification-corrected groundwaters can provide a valuable record of past contamination loads in the recharge zone. This type of record can be valuable in localities where long-term fertilizer-use data are missing or unreliable.

Statistical, geochemical, and hydrologic modeling

New interpretative techniques beyond simple bivariate plots and linear regressions can be used for isotopic data interpretation. One approach is End Member Mixing Analysis (EMMA: see Christophersen et al. 1990, 1993; Hooper & Christopherson 1992). Other approaches using isotopes and chemical data include: geochemical reaction path modeling (Plummer et al. 1983, 1991; Böhlke & Denver 1995); principal components analysis (Otero 2004); classification trees (Spruill et al. 2002); and uncertainty analysis (Phillips & Gregg 2001).

Pharmaceuticals, hormones, DNA, and other chemical constituents

Additional information for tracing N sources can be gained from: various chemicals specific to different animal types (including humans); trace and rare earth elements for tracing fertilizers; DNA and other molecular markers in the dissolved and particulate organic matter associated with the nitrate; and basic chemistry. For example, simple Cl^- concentrations can be used to identify animal waste contamination of rivers and groundwaters (Aravena & Robertson 1998; Silva et al. 2002; Karr et al. 2003; Seiler 2005).

Conclusions

Despite the initial controversy over 30 years ago regarding the use of nitrate isotopes in agricultural systems (e.g., Hauck et al. 1972; Kohl et al. 1972), there have been many very dramatic examples where isotope data have been critical in advancing our understanding of ecosystems. Although source estimates using isotope data are usually only qualitative to semi-quantitative, isotope hydrologists and biogeochemists have found that stable isotope data,

collected at the appropriate temporal and spatial scale, often usefully integrate the natural patterns in complex systems. With the increasing automation of isotope techniques, it is becoming ever easier to acquire the large sets of isotope data required to distinguish the environmental patterns within the “noise”.

This trend is expected to continue into the future, as new analytical advances allow isotope techniques to address an even wider range of important questions regarding both N biogeochemistry and human effects on N sources and cycling. For example, here we briefly list a few “cutting edge” questions:

- 1** What are the spatial and temporal patterns in $\Delta^{17}\text{O}$ (and $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$) of nitrate in wet and dry deposition – and what causes them?
- 2** Will $\Delta^{17}\text{O}$ of nitrate verify or refute the estimates of atmospheric nitrate in small watersheds determined with $\delta^{18}\text{O}$?
- 3** Can different types of atmospheric N sources (e.g., vehicle exhaust vs. power plants vs. agricultural emissions) be distinguished isotopically and quantified?
- 4** What are the controls on the $\delta^{18}\text{O}$ of nitrate during nitrification, denitrification, and assimilation in different environments?
- 5** Do the $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of nitrate get partially reset in the unsaturated zone (and shallow saturated zone) due to rapid oscillations in nitrification and denitrification as wetting fronts move through the profile?
- 6** What happens to atmospheric ammonium in watersheds?
- 7** What causes the $\delta^{15}\text{N}$ of biota in streams to increase with increasing watershed scale and human utilization of the land (regardless of the specific land use)?
- 8** To what extent does the nitrate in the stream only reflect molecules of nitrate from near-stream environments?

The challenges facing those who use natural abundance isotopic approaches to examine issues involving N biogeochemistry are:

- 1** that different sources of N can have partially overlapping isotopic compositions;
- 2** sources can have considerable spatial and temporal variation in isotopic composition;
- 3** isotope fractionations can overprint or blur initially distinctive isotopic compositions.

However, these problems can often be minimized or eliminated by using a multi-isotope, multi-tracer approach – with a lot of hydrologic and chemical data. In addition, it is hoped that as N isotope biogeochemistry moves forward, we will be able to improve our ability to quantify, rather than qualitatively estimate, contributions from multiple sources.

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