

In Reply Refer To:
Mail Stop 412

July 1, 2003

Office of Water Quality Technical Memorandum 2003.07

Subject: Nitrogen Isotope Analyses-- Changes in Laboratory Capabilities and Field Procedures

PURPOSE:

The purpose of this memo is to announce expansion of and changes in analytical capabilities for nitrogen isotopic analysis of total nitrogen in solids and of nitrate and ammonium in water and soils. The National Water Quality Laboratory (NWQL) has arranged for the Reston Stable Isotope Laboratory (RSIL) in Reston, Virginia, to carry out these analyses, which were performed by a contract laboratory in the past. The RSIL will also provide new analytical capability for oxygen isotope ratios of nitrate in water and soil and nitrogen and carbon isotope ratios of solids samples. NWQL lab codes and Analytical Services Request (ASR) forms will be used and billing and data release will be done through the NWQL, but samples will be shipped directly to the RSIL. The new lab codes and schedule follow:

DESCRIPTION	OLD LC/ SCHEDULE	NEW LC/ SCHEDULE
¹⁵ N/ ¹⁴ N of nitrate dissolved in water	LC1718	LC 2899
¹⁵ N/ ¹⁴ N and ¹⁸ O/ ¹⁶ O of nitrate dissolved in water	--	LC 2900
¹⁵ N/ ¹⁴ N of ammonium dissolved in water	LC1717	LC 2898
¹⁵ N/ ¹⁴ N of nitrate in soil	LC1719	LC 2894
¹⁵ N/ ¹⁴ N and ¹⁸ O/ ¹⁶ O of nitrate in soil	--	LC 2897
¹⁵ N/ ¹⁴ N of ammonium in soil	LC1720	LC 2895
¹⁵ N/ ¹⁴ N and N concentration of solids	--	LC 2893
¹⁵ N/ ¹⁴ N, ¹³ C/ ¹² C, N concentration, and C concentration of solids	--	S 1832

BACKGROUND:

Introduction—nitrogen isotopic variation

Molecules, atoms, and ions in their natural occurrences contain isotopes in varying proportions, whereby they possess slightly different physical and chemical properties. This gives rise to partitioning of isotopes (isotope fractionation) during physical or chemical processes, and these fractionations commonly are proportional to differences in their relevant isotope masses. Nitrogen has two isotopes, ¹⁴N and ¹⁵N with mole fractions of approximately 0.9963 and 0.0037, respectively. The compounds of nitrogen possess isotopic variability, and this variation in isotopic composition (see Figure 1) allows researchers to “tag” nitrogen-bearing sources and investigate the fate of nitrogen in the environment.

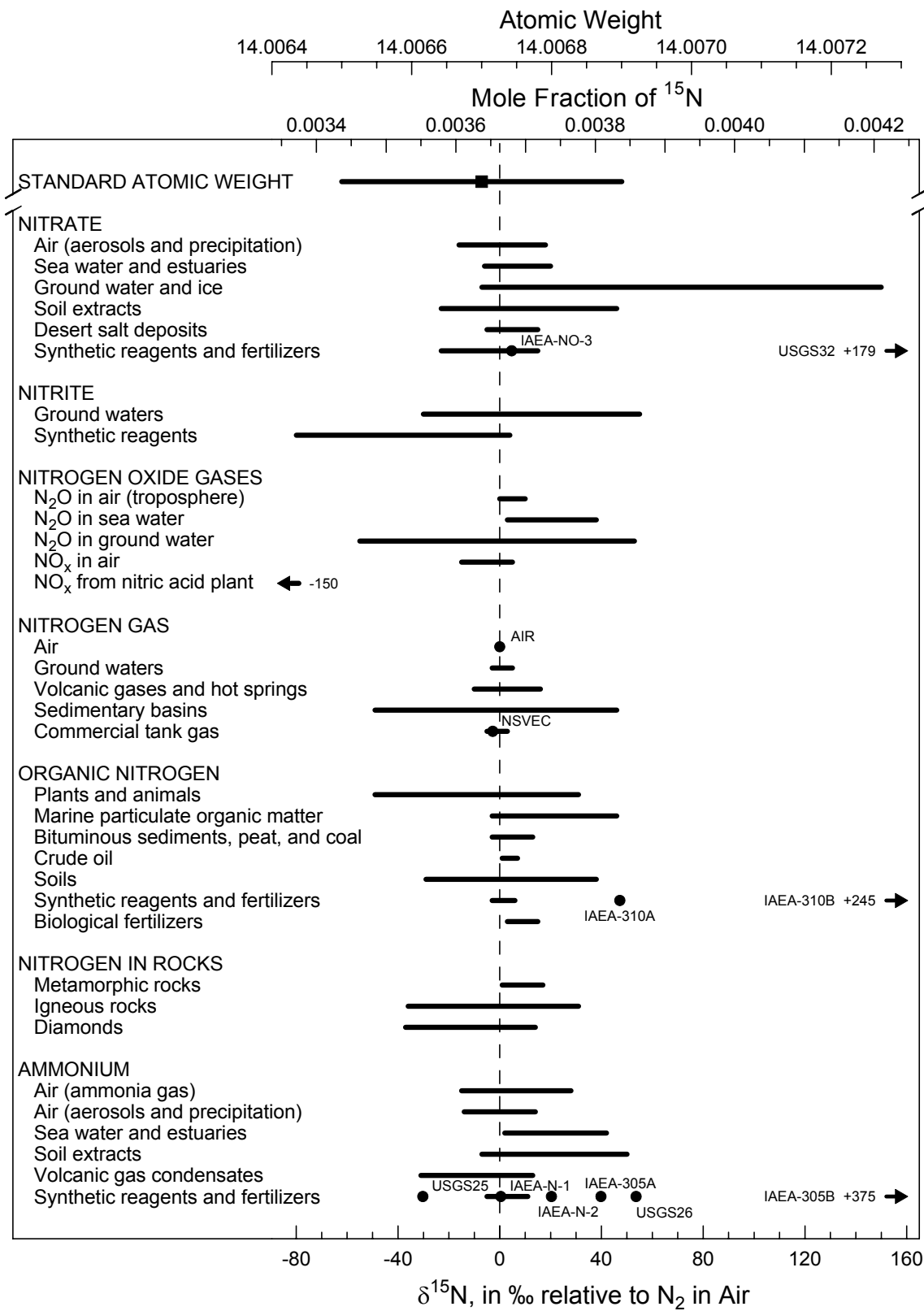


Figure 1. Nitrogen isotopic composition and atomic weight of selected nitrogen-bearing materials (Coplen and others, 2002).

Reporting units, ‰

Variations in stable isotope abundance ratios typically are small. Nitrogen stable isotope ratios commonly are expressed as relative isotope ratios in $\delta^{15}\text{N}$ notation (pronounced delta) according to the relation

$$\delta^{15}\text{N} = \left[\frac{n_{\text{X}}(^{15}\text{N})/n_{\text{X}}(^{14}\text{N})}{n_{\text{air}}(^{15}\text{N})/n_{\text{air}}(^{14}\text{N})} - 1 \right],$$

where $\delta^{15}\text{N}$ refers to the delta value of sample X relative to N_2 in air, and $n_{\text{X}}(^{15}\text{N})/n_{\text{X}}(^{14}\text{N})$ and $n_{\text{air}}(^{15}\text{N})/n_{\text{air}}(^{14}\text{N})$ are the ratios of the isotope amounts in unknown X and in N_2 in air (Böhlke and Coplen, 1995). A positive $\delta^{15}\text{N}$ value indicates that the unknown is more enriched in ^{15}N than is the reference. A negative $\delta^{15}\text{N}$ value indicates that the unknown is depleted in ^{15}N relative to the reference. In the literature, $\delta^{15}\text{N}$ values commonly are reported in parts per thousand (‰ or per mil). Thus, the expression above can be written

$$\delta^{15}\text{N} = \left[\frac{n_{\text{X}}(^{15}\text{N})/n_{\text{X}}(^{14}\text{N})}{n_{\text{air}}(^{15}\text{N})/n_{\text{air}}(^{14}\text{N})} - 1 \right] \cdot 1000 \text{ ‰}$$

because one per mil is 1/1000, and $1000 \cdot 1/1000 = 1$. Correspondingly, for oxygen isotope ratios, for example in nitrate, the expression is

$$\delta^{18}\text{O} = \left[\frac{n_{\text{X}}(^{18}\text{O})/n_{\text{X}}(^{16}\text{O})}{n_{\text{VSMOW}}(^{18}\text{O})/n_{\text{VSMOW}}(^{16}\text{O})} - 1 \right] \cdot 1000 \text{ ‰}$$

and the reference is now VSMOW reference water instead of nitrogen in air. Nitrogen and oxygen isotope data are calibrated by analyzing isotopic reference materials. Descriptions of reference materials used in the analyses listed below are given in Böhlke and Coplen (1995) and Böhlke and others (2003).

CHANGES:

New Procedures

For several years, the National Water Quality Laboratory (NWQL) has offered nitrogen isotope ratio analyses in support of District projects that require isotopic analysis of nitrate and ammonium in hydrologic and geologic systems (see <http://nwql.cr.usgs.gov/usgs/catalog/index.cfm>). These isotopic analyses have been provided by commercial contract with Zymax of San Luis Obispo, California. The NWQL has now arranged for the Reston Stable Isotope Laboratory (RSIL) to provide isotopic analyses of nitrogen-bearing materials to the NWQL. This new analytical capability will provide nitrogen isotopic analysis of water and soil samples with lower nitrate concentrations than that offered by Zymax. For the first time, oxygen isotope ratios of nitrate in water and soil will be available to NWQL district customers, and

nitrogen and carbon isotope ratios of solid samples will be available. There is no replacement for LC 1921 (nitrogen isotope ratio of total dissolved nitrate and ammonium in water) because users should determine the nitrogen isotopic composition of each of these species by using the new lab codes if these species both occur in their samples.

Users should be aware that atmospheric nitrate is enriched in ^{17}O by mass-independent processes (Michalski and Thiemens, 2000; Galanter and others, 2000) and that this new method for nitrate isotope measurements may overestimate the $\delta^{15}\text{N}$ values of atmospheric nitrate samples by as much as 1 to 2 per mil (Sigman and others, 2001). For samples that users suspect may contain more than about 20 percent atmospheric nitrate, users should contact RSIL about methods to resolve this problem. Methods are currently being developed to quantify the mass-independent ^{17}O enrichment, and this independent oxygen isotope ratio may be of use in investigating processes forming nitrate.

New Shipping Address

One primary difference between the new analytical services and the old services is that samples must now be shipped, with their Analytical Services Request (ASR) form (the Electronic ASR can be used; RSIL has access to the information), to the RSIL at the following address:

**U.S. Geological Survey
Isotope Laboratory
431 National Center
12201 Sunrise Valley Drive
Reston, VA 20192**

When the samples are received in Reston, the RSIL will assign the NWQL sample identification number for tracking samples. The NWQL will provide analytical results to Districts and will carry out the billing. Additional information may be found on the website of the RSIL, which is at <http://isotopes.usgs.gov/>.

Sampling Requirements

Lab Code/ Schedule	Description	Bottle	Treatment and Preservation
LC2899	$^{15}\text{N}/^{14}\text{N}$ of nitrate dissolved in water	4 oz (125 mL) amber polyethylene bottle, untreated (Q33FLD or Q405FLD in One-Stop Shopping)	Filter sample thru 0.45-micron filter; chill and maintain at 4 deg C; ship overnight. Submitter must provide concentration of nitrate (LC1975 or 1979) in sample to Reston Stable Isotope Laboratory (RSIL) before sample can be analyzed. Samples should be refrigerated and held until the nitrate concentration is available.
LC2900	$^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ of nitrate dissolved in water	4 oz (125 mL) amber polyethylene bottle, untreated (Q33FLD or Q405FLD in One-Stop Shopping)	Filter sample thru 0.45-micron filter, chill and maintain at 4 deg C; ship overnight. Submitter must provide concentration of nitrate (LC1975 or 1979) in sample to Reston Stable Isotope Laboratory (RSIL) before sample can be analyzed. Samples should be refrigerated and held until the nitrate concentration is available.
LC2898	$^{15}\text{N}/^{14}\text{N}$ of ammonium dissolved in water	32 oz (1 L) Polyethylene bottle, plain (Q35FLD in One-Stop Shopping)	Filter sample thru 0.45-micron filter, chill and maintain at 4 deg C, ship overnight. Submitter must provide concentration of ammonium (LC1976 or 1980) in sample to Reston Stable Isotope Laboratory (RSIL) before sample can be analyzed. Samples should be refrigerated and held until the ammonium concentration is available.
LC2894	$^{15}\text{N}/^{14}\text{N}$ of nitrate in soil	HDPE liquid scintillation vial with Polyseal cap, Wheaton 986706, available from RSIL	Sample should be dried (or frozen) to prevent biological reaction of nitrogen-bearing species during storage or transport to laboratory. No special shipping is required.
LC2897	$^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ of nitrate in soil	HDPE liquid scintillation vial with Polyseal cap, Wheaton 986706, available from RSIL	Sample should be dried (or frozen) to prevent biological reaction of nitrogen-bearing species during storage or transport to laboratory. No special shipping is required.
LC2895	$^{15}\text{N}/^{14}\text{N}$ of ammonium in soil	16 oz (500 mL) polypropylene, plain bottle, 89 mm cap, Q413FLD (bottle) and Q422FLD (cap) in One-Stop Shopping	Sample should be dried (or frozen) to prevent biological reaction of nitrogen-bearing species during storage or transport to laboratory. No special shipping is required.
LC2893	$^{15}\text{N}/^{14}\text{N}$ and N concentration of solids	HDPE liquid scintillation vial with Polyseal cap, Wheaton 986706, available from RSIL	Sample should be dried and powdered to 100 to 200 μm to ensure isotopic homogeneity. No treatment, preservation, or special shipping requirements.
S1832	$^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$, N concentration, and C concentration of solids	HDPE liquid scintillation vial with Polyseal cap, Wheaton 986706, available from RSIL	Sample should be dried and powdered to 100 to 200 μm to ensure isotopic homogeneity. No treatment, preservation, or special shipping requirements.

ANALYSES

A discussion of the new analyses and associated lab codes follows.

$^{15}\text{N}/^{14}\text{N}$ of nitrate dissolved in water, New LC 2899

This is a new analytical capability for the NWQL—it does not replace any existing lab code or schedule. Concentration of nitrate in the sample can be as low as 0.03 mg/kg as N. The sample amount can be as low as 0.002 mg as N. Thus, users can submit milliliter quantities of nitrate-rich water or 125 mL of water with a concentration as low as 0.03 mg/kg as N. The new analytical method involves the bacterial conversion of nitrate to nitrous oxide and nitrogen isotopic analysis by continuous flow isotope ratio mass spectrometry (Sigman and others, 2001). The 1- σ uncertainties of the nitrogen and oxygen isotope ratios are $\pm 0.25\text{‰}$ and $\pm 0.5\text{‰}$, respectively. The sample collection, preservation, and shipping requirements are to filter the sample thru 0.45-micron filter, chill and maintain at 4 °C to prevent biological reaction of any nitrogen-bearing species during storage or transport to laboratory; ship overnight after nitrate concentration is known. The user must provide to the RSIL the concentration of nitrate in the sample. Submitting a sample to NWQL for LC 1975 or LC 1979 analysis of nitrate concentration and providing this result to RSIL is fully satisfactory. The analytical result should be written on the ASR form, which is shipped with the sample, overnight delivery, to RSIL in Reston.

$^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ of nitrate dissolved in water, New LC 2900

This is a new analytical capability for the NWQL—it does not replace any existing lab code or schedule. Concentration of nitrate in the sample can be as low as 0.03 mg/kg as N. The sample amount can be as low as 0.002 mg as N. Thus, users can submit milliliter quantities of nitrate-rich water or 125 mL of water with a concentration as low as 0.03 mg/kg as N. The new analytical method involves the bacterial conversion of nitrate to nitrous oxide and nitrogen isotopic analysis by continuous flow isotope ratio mass spectrometry (Casciotti and others, 2002). The 1- σ uncertainties of the nitrogen and oxygen isotope ratios are $\pm 0.25\text{‰}$ and $\pm 0.5\text{‰}$, respectively. The sample collection, preservation, and shipping requirements are the same as LC 2899 above (Filter sample thru 0.45-micron filter, chill and maintain at 4 °C to prevent biological reaction of any nitrogen-bearing species during storage or transport to laboratory; ship overnight when nitrate concentration is known). The user must provide to the RSIL the concentration of nitrate in the sample. Submitting a sample to NWQL for LC 1975 or LC 1979 analysis of nitrate concentration and providing this result to RSIL is fully satisfactory. The analytical result should be written on the ASR form, which is shipped with the sample, overnight delivery, to RSIL in Reston.

$^{15}\text{N}/^{14}\text{N}$ of ammonium dissolved in water, New LC 2898

This isotope ratio analysis replaces LC 1717. This analytical method involves the collection of ammonium in ion exchanger, combustion by elemental analyzer, purification of N_2 by GC, and isotopic determination by continuous flow isotope ratio mass spectrometry (Brand, 1996). The 1- σ uncertainty of the method is $\pm 0.3\text{‰}$. The sample collection, preservation, and shipping requirements are to filter the sample thru

0.45-micron filter, chill and maintain at 4 °C to prevent biological reaction of any nitrogen-bearing species during storage or transport to laboratory; ship overnight when ammonium concentration is known. The user must provide to the RSIL the concentration of ammonium in the sample. Submitting a sample to NWQL for LC 1976 or LC 1980 analysis of ammonium concentration and providing this result to RSIL is fully satisfactory. The analytical result should be written on the ASR form, which is shipped with the sample, overnight delivery, to RSIL in Reston.

$^{15}\text{N}/^{14}\text{N}$ of nitrate in soil, New LC 2894

This isotope ratio analysis replaces LC 1719. The advantage of this analysis over LC 1719 is that the concentration of nitrate in the sample is reduced and can be as low as 1 mg/kg as N. The sample amount can be as low as 0.02 mg as N. This analytical method involves the aqueous dissolution of nitrate, bacterial conversion of nitrate to nitrous oxide, and nitrogen isotopic analysis by continuous flow isotope ratio mass spectrometry (Sigman and others, 2001). The 1- σ uncertainty of the method is ± 0.25 ‰. The sample should be dried (or frozen) to prevent biological conversion of any nitrogen-bearing material to nitrate during storage or transport to laboratory. The sample and ASR form are sent to the RSIL in Reston.

$^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ of nitrate in soil, New LC 2897

This is a new analytical capability for the NWQL—it does not replace any existing lab code or schedule. The concentration of nitrate in the sample can be as low as 0.2 mg/kg as N. The sample amount can be as low as 0.02 mg as N. This analytical method involves the aqueous dissolution of nitrate, bacterial conversion of nitrate to nitrous oxide, and isotopic analysis by continuous flow isotope ratio mass spectrometry (Casciotti and others, 2002). The 1- σ uncertainties of the nitrogen and oxygen isotope ratios are ± 0.25 ‰ and ± 0.5 ‰, respectively. The sample should be dried (or frozen) to prevent biological reaction of nitrogen-bearing species during storage or transport to laboratory. The sample and ASR form are sent to the RSIL in Reston.

$^{15}\text{N}/^{14}\text{N}$ of ammonium in soil, New LC 2895

This isotope ratio analysis replaces LC 1720. The amount of ammonium in the sample must be at least 0.2 mg as N. This analytical method involves aqueous dissolution of ammonium in KCl solution, distillation and collection of ammonium in ion exchanger, combustion by elemental analyzer, purification of N_2 by GC, and isotopic analysis by continuous flow isotope ratio mass spectrometry (Brand, 1996). The 1- σ uncertainty of the method is ± 0.3 ‰. The sample should be dried (or frozen) to prevent biological reaction of nitrogen-bearing species during storage or transport to laboratory. The sample and ASR form are sent to the RSIL in Reston.

$^{15}\text{N}/^{14}\text{N}$ and N concentration of solids, New LC 2893

This is a new analytical capability for the NWQL—it does not replace any existing lab code or schedule. The concentration of nitrogen in the sample can be as low as 1 mg/g. The amount of nitrogen in the sample can be as low as 0.15 mg. This analytical method involves combustion by elemental analyzer, purification of N_2 by GC, and isotopic determination by continuous flow isotope ratio mass spectrometry (Brand,

1996). The 1- σ uncertainty of the nitrogen isotope ratio value is ± 0.25 ‰. The 1- σ uncertainty of the nitrogen concentration is ± 4 %. The sample must be dried and powdered to 100 to 200 μm by the user to ensure isotopic homogeneity, and a maximum sample amount of 1 g should be submitted. The sample and ASR form are sent to the RSIL in Reston.

$^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$, N content, and C content of solids, New Schedule 1832

This is a new analytical capability for the NWQL—it does not replace any existing lab code or schedule. The concentration of nitrogen in the sample can be as low as 1 mg/g and the concentration of carbon in the sample can be as low as 1 mg/g. The amount of nitrogen in the sample can be as low as 0.15 mg, and the amount of carbon in the sample can be as low as 0.15 mg. This analytical method involves combustion by elemental analyzer, purification of N_2 and CO_2 by GC, and isotopic determination by continuous flow isotope ratio mass spectrometry (Brand, 1996). The 1- σ uncertainties of the nitrogen and carbon isotope ratio values are both ± 0.25 ‰. The 1- σ uncertainties of the nitrogen and carbon concentrations are both ± 4 %. The sample must be dried and powdered to 100 to 200 μm by the user to ensure isotopic homogeneity and a maximum sample amount of 1 g should be submitted. The sample and ASR form are sent to the RSIL in Reston.

References

- Böhlke, J.K., and Coplen, T.B., 1995**, Interlaboratory comparison of secondary reference materials for nitrogen-isotope-ratio measurements, in Reference and intercomparison materials for stable isotopes of light elements: Vienna, Austria, International Atomic Energy Agency, IAEA-TECDOC-825, p. 51–66.
- Böhlke, J.K., Mroczkowski, S.J., and Coplen, T.B., 2003**, Oxygen isotopes in nitrate: new reference materials For 18O:17O:16O measurements and observations on nitrate-water equilibration: Rapid Communications in Mass Spectrometry, v. 17, p. 1835–1836.
- Brand, W.A., 1996**, High precision isotope ratio monitoring techniques in mass spectrometry: Journal of Mass Spectrometry, v. 31, p. 225-235.
- Casciotti, K.L., Sigman, D.M., Galanter Hastings, M., Böhlke, J.K., and Hilkert, A., 2002**, Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method: Analytical Chemistry, v. 74, p. 4905-4912.
- Coplen, T.B., Hopple, J.A., Böhlke, J.K., Peiser, H.S., Rieder, S.E., Krouse, H.R., Rosman, K.J.R., Ding, T., Vocke, Jr., R.D., Révész, K.M., Lamberty, A., Taylor, P., and De Bièvre, P., 2002**, Compilation of minimum and maximum isotope ratios of selected elements in naturally occurring terrestrial materials and reagents: U.S. Geological Survey Water-Resources Investigations Report 01-4222, 98 p.

Galanter, M., Sigman, D.M., Levy, H., Böhlke, J.K., Lipshultz, F., and Steig, E., 2000, Controls on the oxygen isotopic composition of atmospherically-derived nitrate (abstract): EOS, Transactions of the American Geophysical Union, v. 81, p. F191.

Michalski, G., and Thiemens, H.H., 2000, Mass independent fractionation in nitrate aerosols (abstract): EOS, Transactions of the American Geophysical Union, v. 81, p. F120.

Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M., and Böhlke, J.K., 2001, A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater: Analytical Chemistry, v. 73, p. 4145-4153.

Questions about these nitrogen isotope laboratory methods and associated field procedures should be directed to Tyler Coplen (tbcoplen@usgs.gov).

Timothy L. Miller /s/
Chief, Office of Water Quality

This memorandum does not supersede any other Office of Water Quality Technical Memorandum.

Distribution: All WRD Employees

Timothy L. Miller
Chief, Office of Water Quality
U.S. Geological Survey
412 National Center
Reston, VA 20192
703/648-6868
tmliller@usgs.gov