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A GUIDE TO THE USE OF ^{14}N AND ^{15}N IN ENVIRONMENTAL RESEARCH

Arthur P. Edwards

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The fate of the mineral nitrogen in wastewater can be established only through natural or artificial stable isotopic labeling. This report assesses the possibilities and problems associated with such tracer techniques applied to the small amounts of nitrogen normally present after secondary waste treatment. The methods outlined for sample processing to minimize analytical errors are applicable to other types of environmental research involving isotope ratio analysis as a means of tracing nitrogen in the biosphere.

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PREFACE

This report was prepared by Dr. Arthur P. Edwards, Senior Research Scientist, University of New Hampshire, Durham, New Hampshire, in fulfillment of the requirement of P.O. DACA 89-78-0340. The project officer for the study was Dr. I.K. Iskandar, Research Chemist, Earth Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory, Hanover, New Hampshire.

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SUMMARY

Nitrogen as ammonium and/or nitrate is present in varying amounts in all wastewaters, even after secondary treatment. These same ions are also present in rainfall, and are produced naturally in all soils through mineralization of organic nitrogen. In designing or altering systems for land disposal of wastewater one of the primary objectives is to keep the efflux of the very mobile nitrate anion into ground and surface waters, from all sources, down to a level acceptable from a public health standpoint.

For proper evaluation of any disposal technique it is desirable to know exactly how the addition of wastewater affects the nitrogen balance of the immediate biosphere. The system is complex because the added N, water and organic energy sources can markedly influence the mineralization of soil organic nitrogen, the nitrification of ammonium and the denitrification of nitrate-N. The use of stable isotopic labeling, either natural or artificial, is the key to identifying the added wastewater nitrogen wherever it may appear.

The concentrations of mineral N in soils and wastewaters are normally low, making necessary special techniques and unusual precautions for successful utilization of $^{15}N/^{14}N$ ratios in wastewater research. This Special Report was prepared in an effort to anticipate as many of the potential sources of error as possible, and to suggest ways of circumventing or minimizing the major problems. It is the author's

conclusion, after some 22 years of intermittent involvement with research involving ^{15}N, that failure to account for all of the extra isotope introduced into any system is largely the result of cumulative sampling, sample preparation, and instrumentation errors, rather than to a loss of N from the system through an assumed mechanism such as biological denitrification. Until such time as careful nitrogen balance-sheet studies are carried out in prototype systems, there will be no really reliable modeling data available.

The major points emphasized in this report are as follows: 1) Practical methods are available for minimizing the errors involved in sampling, sample preparation and analysis of N-containing organic and inorganic materials, and for carrying out isotope ratio analysis on relatively small amounts of sample nitrogen. 2) There is no substitute for mass spectrometry in isotope ratio analysis of the natural-abundance, ^{15}N-enriched and ^{15}N-depleted samples collected during research on the fate of wastewater-N. 3) More research is needed, employing modern gas chromatographic techniques, to study the hypobromite oxidation of ammonium to dinitrogen gas. All of the reaction products need to be identified quantitatively, the completeness of the reaction needs to be established, and the importance of any errors due to isotopic segregation should be studied. 4) Compounds depleted in ^{15}N will have very little direct application in wastewater research because of the low concentrations of mineral nitrogen present in

effluent after secondary treatment. There is the possibility, however, of using the depleted materials in prototype systems where the soils in columns and lysimeters have had the organic-N labeled through prior use of ^{15}N-enriched compounds. 5) There is a very definite possibility that natural-abundance differences between wastewater and soil-derived mineral-N can be of use in quantitatively separating sources of the NO_3-N reaching the groundwater in specific, long-established land treatment systems. 6) Where significant amounts of ammonium nitrogen are present in wastewater after secondary treatment, and disposal is by spraying or snowmaking, the loss of effluent-nitrogen to the atmosphere should be measurable through the change in the ^{15}N-concentration of the residual ammonium-N.

INTRODUCTION

The element N occurs in nature as a mixture of the two stable isotopes ^{14}N and ^{15}N. Relative to ^{12}C = 12.00000 amu the exact atomic mass of ^{14}N = 14.00307 amu and of ^{15}N = 15.00011 amu (Handbook of Chemistry and Physics, recent editions). The percentage of the heavy isotope in atmospheric N_2 gas is rather constant at 0.3663 $\pm$ 0.0004% of the total N atoms (Junk and Svec 1958), and the accepted value for the atomic mass of N (14.0067 amu) was calculated for the nitrogen in air. If we exclude the artificially high values for ^{15}N concentrations found in N_2 gas occluded in certain radioactive ores (White and Yagoda 1950), the proportions of the two isotopes in naturally occurring, N-containing substances in the biosphere can vary enough to produce uncertainties in the atomic mass of N amounting to about 2×10^{-4} amu. Although dinitrogen gas constitutes only about 2% of the N in planet earth (Stevenson 1965), atmospheric N_2 can be considered as the primordial source of the nitrogen in the biosphere, and its isotopic composition is therefore a good reference standard in investigations of natural variations in the N isotopes.

The small natural variations in the relative abundances of stable isotopic pairs such as $^{15}N/^{14}N$, $^{13}C/^{12}C$, $^{18}O/^{16}O$, $^{1}H/^{2}H$ and $^{34}S/^{32}S$ result from the difference in mass between molecules containing the heavy vs the light isotope. These mass differences produce differences in physical properties such as equilibrium vapor pressures, rates of migration under a thermal gradient, rates of diffusion, and rates of

chemical and biochemical reactions. Therefore the small variations in the $^{15}N/^{14}N$ ratios within and between naturally occurring nitrogenous compounds represent the net result of the isotope discrimination effects which have occurred during formation and degradation of the substance at the point of sampling. For materials which are still labile and reactive in the environment in which they occur, the manner and rate of recycling of the nitrogenous constituents are important in determining the isotopic composition at any point in time. The organic nitrogen in soils, for example, contains both labile and non-labile fractions which can exhibit differences in ^{15}N concentrations (Cheng et al. 1964).

In order to be useful as tracers in environmental research, substances must be labeled either with *long*-lived radioactive isotopes or must contain substantially more or less of the scarce heavy isotope of a stable pair. In the case of the element nitrogen, there are four radionuclides which can be prepared, but only the ^{13}N isotope has a half-life long enough to make it useful in biological research ($t_{1/2}$ = 10.05 minutes) and its use is restricted to short-term kinetic studies at a location where the isotope is being produced (Table I).

Table I. Stable and radioactive isotopes of nitrogen.

Mass number	Natural abundance (%)*	Half-life†
12		0.0125 s
13		10.05 min
14	99.6337 ± 0.0004	-
15	0.3663 ± 0.0004	-
16		7.36 s
17		4.14 s

*Data from Junk and Svec (1958) for atmospheric N_2.

†From Friedlander and Kennedy (1955).

Therefore enrichment or depletion with respect to ^{15}N is necessary to produce tracer materials satisfactory for long-term experimentation and for all studies where very little of the N from the labeled compound is likely to appear at final assay.

The same differences in physical behavior which result in natural variations in ^{15}N abundance can be used as the basis for preparing compounds containing up to 99.9 atom % ^{15}N, and ^{15}N-depleted materials containing less than 100 ppm of the heavy isotope, i.e. less than 0.01 atom % ^{15}N (Matwiyoff et al. 1975; Edmunds and Lockhart 1975). Labeled compounds are expensive, especially when highly enriched (Hauck and Bremner 1976). However, the only alternative to their use in tracing N sources in the biosphere is to establish the significance of differences in natural abundance of ^{15}N and utilize these where practical. No expensive artificial labeling is involved, but much detailed sampling and analysis will be required to establish cause and effect, and to confirm the uniqueness of the $^{15}N/^{14}N$ ratios of a specific source of nitrogen (Rennie and Paul 1975; Hauck and Bremner 1976).

It is the purpose of this review to assess the current state-of-the-art relevant to tracer techniques (^{15}N-enriched and ^{15}N-depleted tracers and natural-abundance variations) applicable to studies of N-sources in surface- and groundwaters, with particular emphasis on the ecological impact of systems for land treatment of wastewater.

DEFINITIONS AND INTERRELATIONSHIPS

1. $\underline{\text{Atom \% } {}^{15}N} = \dfrac{\text{number of } {}^{15}N \text{ atoms} \times 100}{\text{number of } {}^{15}N + \text{number of } {}^{14}N \text{ atoms}}$

$\underline{\text{Atom \% } {}^{14}N} = 100 - \text{atom \% } {}^{15}N$

2. $\underline{\text{Isotope ratio}} = \dfrac{\text{number of } {}^{15}N \text{ atoms}}{\text{number of } {}^{14}N \text{ atoms}}$, or reciprocal.

The ratio $\dfrac{{}^{14}N}{{}^{15}N} = \dfrac{99.6337}{0.3663} = 272{:}1$ in atmospheric N_2.

The ratio $\dfrac{{}^{15}N}{{}^{14}N} = \dfrac{0.3663}{99.6337} = 0.003676{:}1$ in atmospheric N_2.

3. Mass to charge ratio (m/e).

Where positive ions are produced by electron bombardment in a mass spectrometer

$$m/e = \frac{\text{mass of atom or molecule}}{\text{number of electrons lost in ionization}}$$

During analysis of pure N_2 gas, the predominant ions are $[{}^{14}N{}^{14}N]^+$, $[{}^{14}N{}^{15}N]^+$ and $[{}^{15}N{}^{15}N]^+$ with m/e ratios of 28, 29 and 30. Substantial amounts of $[{}^{14}N{}^{14}N]^{2+}$, $[{}^{14}N{}^{15}N]^{2+}$ and $[{}^{15}N{}^{15}N]^{2+}$ are also produced, with m/e ratios of 14, 14.5 and 15.

4. The $\dfrac{m/e\ 29}{m/e\ 28}$ ratio (R), or reciprocal (R')

The ratio measured during mass spectrometric analysis of N_2 gas to determine the atom % ^{15}N. What is actually measured is the i_{29}/i_{28} ratio (ratio of ion currents produced by collection of the mass 29^+ and mass 28^+ ion species).

$$\text{atom \% } {}^{15}N = \frac{100\ R}{2 + R} = \frac{100}{2R' + 1}.$$

(See Appendix for mathematical development of these relationships.)

5. Percent deviation in ^{15}N

$$\frac{\text{atom \% } {}^{15}N \text{ in sample} - \text{atom \% } {}^{15}N \text{ in standard} \times 100}{\text{atom \% } {}^{15}N \text{ in standard}}$$

6. Per mil (°/oo) deviation in ^{15}N. Simply 10 x the percent deviation in ^{15}N.

This is the $\Delta^{15}N$ term as defined by Cheng et al. (1964).

7. Per mil deviation in isotope ratio (δ°/oo).

This is the term used in geochemistry to express small deviations in isotopic abundances.

$$\delta^{15}N = \frac{{}^{15}N/{}^{14}N \text{ in sample} - {}^{15}N/{}^{14}N \text{ in standard} \times 1000}{{}^{15}N/{}^{14}N \text{ in standard}}$$

The $\Delta^{15}N$ and $\delta^{15}N$ terms are not absolutely identical at higher deviations because the atom % ^{15}N is not a simple multiple of the isotope ratio.

8. Atom % deviation in ^{15}N (atom % excess ^{15}N)

The difference found by subtracting the atom % ^{15}N in the reference material from the atom % ^{15}N in the material under investigation.

The use of "atom % excess ^{15}N" is unfortunate, because the term assumes 1) that the tracer material being used always contains more of

scarce isotope than does the reference material (this was true when ^{15}N-enriched tracers were the only ones available) and 2) that the atom % ^{15}N in the reference material, against which changes are measured, is a constant (usually assumed to be the atom % ^{15}N found in atmospheric N_2). Neither assumption is valid in many applications.

Table II. The relationship between measured and calculated expressions of ^{15}N concentrations in the natural abundance range.

A. Where i_{29}/i_{28} current ratios are measured.

	i_{29}/i_{28}	$^{15}N/^{14}N$	At. % ^{15}N	At. % Dev.	$\Delta^{15}N$	$\delta^{15}N$
Sample 1	0.007400	0.003700	0.3686	+0.0023	+6.3	+6.3
Standard	0.007353	0.003676	0.3663	-		
Sample 2	0.007300	0.003650	0.3636	-0.0027	-7.4	-7.3

B. Where i_{28}/i_{29} current ratios are measured.

	i_{29}/i_{28}	$^{14}N/^{15}N$	At. % ^{15}N	At. % Dev.	$\Delta^{15}N$	$\delta^{15}N$
Sample 1	135.14	270.28	0.3686	+0.0023	+6.3	+6.3
Standard	135.99	271.98	0.3663	-	-	-
Sample 2	136.99	273.98	0.3636	-0.0027	-7.4	-7.3

9. A ^{15}N-enriched tracer. One which contains a higher atom % ^{15}N than natural sources of N.

10. A ^{15}N-depleted tracer. One which contains less ^{15}N than naturally occurring substances.

11. Isotope effects. The segregation of isotopic species due to differences in the behavior of molecules, ions or compounds containing the heavy vs the lighter isotope.

12. Isotope dilution. The decrease in ^{15}N concentration which results from mixing of a ^{15}N-enriched tracer with N containing less of the heavy isotope (usually natural N-sources) or the decrease in ^{14}N concentration produced by the mixing of a ^{15}N-depleted tracer with N sources containing more ^{15}N (less ^{14}N).

13. Isotope dilution analysis. As originally defined, a special indirect method for determining the concentration of an element in a complex mixture. A compound containing a radioactive isotope of the element at known concentration is added to the system. A pure sample of the compound is then isolated from the system. From the decrease in activity of the tracer element the original concentration of the element in the system can be computed.

In studies involving determination of $^{15}N/^{14}N$ ratios, a special version of isotope dilution analysis can be used to establish the concentration of the heavy isotope in a sample containing too little N for direct mass spectrometric analysis. To the unknown sample is added a known amount of N with a known (usually higher) ^{15}N concentration, as the ion or compound under investigation. By determining the total N and ^{15}N concentrations for the appropriate moiety after mixing, the nitrogen and ^{15}N concentrations of the fraction of interest can be calculated for the unknown sample (see Appendix).

14. Isotope exchange. The exchange of one isotope of an element for a different stable or radioactive isotope of the same element in a different position in the same molecule or in a different molecule of the same or chemically different compound.

$$\text{e.g. } [^{14}N^{14}N] + [^{15}N^{15}N] \rightleftarrows 2[^{14}N^{15}N]. \quad K_{eq} = 4$$

METHODOLOGY

It is proposed to discuss analytical methods in general terms in this section, and to present the most important options currently available for carrying out the various stages of sample preparation and analysis. In the "Assumptions and Errors" section to follow, more detail will be given on the combination of procedures of choice for natural-abundance, ^{15}N-depleted and ^{15}N-enriched tracer studies which are applicable to investigations of N-cycling in the environment.

Three-Stage Analytical Scheme for Nitrogen Isotope-Ratio Analysis

The analytical procedures most commonly employed are modifications of those developed by Rittenberg and Sprinson (Rittenberg et al. 1939, Rittenberg 1948, Sprinson and Rittenberg 1948, 1949) for tracer investigations involving use of ^{15}N-enriched compounds. The following steps are involved:

1. Conversion of sample nitrogen to ammonium.

2. Conversion of the ammonium to N_2 gas by oxidation with alkaline sodium or lithium hypobromite in the complete absence of air.

3. Determination of the isotopic composition of the N_2 gas.

Diatomic nitrogen is the gas of choice for mass spectrometric and optical emission isotope-ratio analysis for several reasons: it is easier to convert a variety of N-containing compounds to N_2 than to NO, NO_2 or NH_3; N_2 is inert and noncorrosive; only one element is present and interpretation of spectra is therefore easier.

1. Conversion of sample nitrogen to ammonium.

(a) Total N: There are really only two options available for converting all of the sample nitrogen to ammonium; some modification of the procedure due to Kjeldahl (1883) involving digestion in concentrated H_2SO_4 in the presence of catalysts or a recently developed reductive pyrolysis technique involving use of H_2 and a heated nickel catalyst (Walker et al. 1975) to produce NH_3 rather than $(NH_4)_2\ SO_4$ as in the Kjeldahl technique. The pyrolysis method will be discussed later as an interesting option of future applicability, but to date most total N determinations have been done using the Kjeldahl approach.

Recovery of total sample N by digestion in H_2SO_4, followed by distillation to recover the NH_4^+ produced during digestion, is not easy, contrary to popular misconceptions. The many procedural variations developed to improve N recovery from a variety of compounds are testimony to the degree of difficulty involved (Bremner 1960, 1965). Some organic compounds are refractory and resist oxidation, e.g. nicotinic acid. Procedures designed to recover NO_3^- - and NO_2^- - N may work with one material but not with another. For example, the Olsen (1929)

procedure, involving pretreatment with $KMnO_4$ (to oxidize NO_2^- to NO_3^-) and reduced Fe (as iron powder) to convert NO_3^- to NH_4^+ works well for plant material but not for all soils (Edwards 1971). Failure to include all of the N can affect the interpretation of the results of a tracer study, particularly N-balance investigations, since both the answers for the quantity of N and the ^{15}N content of this nitrogen may be affected. The only safe course to follow is to check two or more likely procedures for maximum recovery, reproducibility and ^{15}N concentrations before proceeding to later stages of ^{15}N analysis. Where clay-fixed ammonium-N is present or suspected, the procedure normally used for soil materials should be checked against a procedure involving pretreatment with HF (Silva and Bremner 1966), and the influence of particle size or the answers obtained should also be evaluated.

Both the Kjeldahl digestion and distillation steps can be accomplished on a macro to a micro scale. Where ^{15}N analysis is the objective, the semi-micro scale to give N recovery in the 1 mg range is desirable. Where reproducibility because of non-uniformity of sample is a problem, an aliquot from a macro-digest may be preferable.

The distillation of ammonium must be accomplished from an alkaline system, either by boiling using direct heating, or more rapidly through the use of steam (Bremner 1960, 1965).

(b) Other forms of N.

Until fairly recently, few attempts were made to determine isotope ratios of various forms of nitrogen because the necessary quantitative

procedures for recovering specific fractions of soil, water and plant nitrogen as ammonium were not available. However, the situation has changed recently, largely through the efforts of J.M. Bremner of Iowa State University and associates (Bremner 1965, Bremner and Edwards 1965, Bremner and Keeney 1966, Keeney and Bremner 1966, Silva and Bremner 1966, Bundy and Bremner 1973). The procedures all involve the use of a simple, semimicro steam distillation unit (Bremner and Edwards 1965) which permits rapid recovery of ammonium (3.5 min) without hydrolysis of any organic N present. The same units can be used for recovery of total N as NH_4^+ from Kjeldahl digests. The list of N-compounds and fractions recoverable quantitatively from soils now includes extractable, exchangeable and fixed ammonium, nitrate-N, nitrite-N (by difference), urea-N, hydrolyzable and non-hydrolyzable organic-N, α-amino acid-N, hexosamine-N, and serine + threonine-N. The procedures are all highly specific, quantitative and rapid.

2. Conversion of the ammonium to N_2 by oxidation with alkaline sodium or lithium hypobromite.

The general progression in this area has been from unnecessarily complex, detached systems for preparing one to many samples simultaneously, to simple, single-sample systems attached directly to the mass spectrometer. Automated sample systems are also available for optical emission ^{15}N analyzers. Most commonly, however, the preparation and introduction of samples is completely manual for both types of instrumentation. In the Appendix (Fig 1 and 2) are sketches of two examples of current

sample preparation-introduction units. The first (Fig. 1) employs greaseless modifications of Rittenberg tubes as sample-hypobromite vessels, and mixing is accomplished by rotation of the assembly (Bremner 1965, Fiedler and Proksch 1975). In the second approach (Fig. 2), disposable glass sample vials are used, and previously degassed hypobromite is introduced through a needle valve after evacuation of the vial (Ross and Martin 1970).

The basic requirements in any acceptable system are: 1) Complete removal of air must be accomplished from sample and hypobromite solutions, as well as from the sample vessel, before the oxidant and sample are mixed to convert sample NH_4^+ to N_2 gas. 2) All water and CO_2 must be removed from this N_2 before introduction into a mass spectrometer, and the partial pressure of any oxides of nitrogen must be kept low. 3) The system must be capable of handling sufficient NH_4^+-N to meet the minimum needs of the instrument to be used for isotope ratio analysis. 4) The N_2 sample which is finally introduced into the mass spectrometer must have the same isotopic concentration as the NH_4^+-N in the salt prior to oxidation with hypobromite. Many systems can be designed to meet these requirements but unfortunately a number currently in use fail to do so for reasons to be discussed under "Sources of Error."

3. Determination of the isotopic composition of the N_2 gas by mass spectrometric analysis.

The principles of operation of a magnetic-deflection mass spectrometer for isotope assay of N_2 gas can be described briefly as follows:

A small amount of sample gas is allowed to leak, at a constant rate, through a suitable small orifice into the ion source of the instrument, which is maintained under a high vacuum; here the N_2 molecules are bombarded by an electron beam, and some acquire a single positive charge; a large potential at the front of the ion source accelerates these positive ions into the flight tube where they pass through a high-intensity magnetic field; the ions containing the lighter isotope, $(^{14}N^{14}N)^+$ of mass 28, are deflected more than the ions containing the heavier isotope, $(^{14}N^{15}N)^+$, mass 29 and $(^{15}N^{15}N)^+$, mass 30; the magnetic separation permits the two major ions (28^+ and 29^+) to be distinguished on the basis of their momentum, and hence on the basis of the mass difference between them.

There are two basic approaches to measurement of the relative abundance of the mass 28^+ and 29^+ ions in a magnetic-deflection mass spectrometer. They are illustrated in Figure 3 (Appendix). In the first method, which is the one normally used in so-called isotope-ratio mass spectrometers, separate collector plates are positioned so that the two types of ions can be collected independently and simultaneously. In the second method, a single collector (in this case, an electron multiplier, but more often a Faraday cup) can be used to measure the ion intensities in an alternating mode. In a static magnetic field this accelerating voltage alternation switches the ion focus from light to heavy and back. With the advent of precise ratiometers, rapid sweeping over both peaks can produce an integrated figure

for relative peak heights or area, and the ratio of the two peaks of interest is then displayed digitally, printed on tape, or fed directly into a computer.

Both systems have advantages and disadvantages. The dual collector system means duplication of amplifier circuitry, usually requires larger samples, and permits only a rather restricted mass separation between the two ions of interest. Fluctuation in ion source characteristics is not as critical with dual collectors since production of both ion species will be affected simultaneously. Accelerating voltage alternation makes for a much more versatile system which is adaptable, for example, to monitoring isotope ratios on gas chromatographic fractions where actual quantities of sample N are in the microgram range.

With recent improvements in mass spectrometers, especially those resulting from the development of more efficient ion sources and more sensitive ion detectors, mass spectrometry is now approaching, or surpassing, radioactive counting techniques in sensitivity (Matwiyoff et al. 1975). The limit of detection of small differences in isotopic ratios of the lighter elements is now 0.01% relative. This means that sampling and sample preparation become the limiting factors in studies of natural isotope abundances (McMullen and Thode 1963) when a suitable mass spectrometer is available. The measurement of absolute isotopic abundances is much more difficult, and for the stable isotopes of nitrogen the accuracy is limited, by the Kjeldahl determination of total N, to no better than 1 part per 1000 (Junk and Svec 1958). Uncertainties in corrections for discrimination effects within the

mass spectrometer itself would limit the accuracy to this order of magnitude if the total N determination did not (McMullen and Thode 1963).

In addition to magnetic-deflection instruments, other types of mass spectrometers have been used in isotope-ratio work where semi-quantitative or qualitative answers only were needed, e.g. time-of-flight and quadrupole instruments.

The rationale behind the use of i_{29}/i_{28} ratios and the conversion of this measurement to ^{15}N concentration in the sample nitrogen is given in the Appendix. For enriched material containing more than about 5 at. % ^{15}N, the i_{30} current can be measured and used to obtain the ^{15}N concentration (see Appendix), providing that contaminants with peaks in the m/e 30 position are not a problem, e.g. $^{14}N^{16}O$ generated in the hypobromite reaction and not completely removed by the liquid N_2 trap.

ALTERNATIVES TO USE OF THE THREE-STAGE PROCEDURE

A. For Stages 1 and 2.

There has been considerable interest, particularly in Europe, in using a Dumas dry-combustion technique (Dumas 1834) for converting N-containing materials directly to N_2 gas, thus bypassing the conversion to ammonium (Barsdate and Dugdale 1965, Faust 1967, Bremner and Tabatabai 1971, Proksch 1972, Fiedler and Proksch 1975). However, there are many problems associated with this direct approach, including memory effects (Bremner et al. 1966) and probably isotopic segregation, because quantitative recovery of both oxidized and reduced forms of N is frequently not achieved, especially in a closed system (Fiedler and

Proksch 1975). An approach which avoids some of the difficulties is to use a Kjeldahl digestion to convert total N to NH_4^+, followed by conversion of this ammonium to N_2 by Dumas combustion. However, the Dumas conversion as recommended by Fiedler and Proksch (1975), carried out in small quartz capillaries, is never complete and therefore unacceptable for truly quantitative studies.

Another disadvantage to Dumas technique, where the N_2 gas must be recovered for isotope-ratio analysis, is the fact that two sets of samples must be analyzed — one for percentage total N, the other for ratio analysis. Sampling problems are difficult enough without introducing two different populations which may not be identical unless large numbers of samples are processed for each determination. The errors could be large if only one sample is processed for total N and another single sample for the isotope-ratio measurement. The system for doing total N on the same sample processed for ^{15}N analysis devised by Fiedler and Proksch (1975), and described as an "automated nitrogen analyzer," has never been used satisfactorily even by the designers (personal observation during 2 years with IAEA, Vienna, 1975-77).

Walker et al. (1975) have described a novel procedure for total N and ^{15}N analysis of plant tissue, water and soil samples. In this procedure the nitrogen is converted to NH_3 by a reductive pyrolysis technique requiring use of H_2 and a heated nickel catalyst. To estimate the total nitrogen concentration in the sample pyrolyzed, a portion of the ammonia is collected in water and determined by a conductivity detector.

The remainder is collected in a cold-finger reaction vessel and subsequently converted to N_2 by a thermal decomposition technique, again requiring H_2 and a rhenium filament heated to 1050°C. The filament must be replaced after analysis of 10-15 samples, and after each sample heating to 1600°C is required to avoid memory effects. The minimum amount of N is 20 µg for a complete analysis. The cost and analytical complexity of this system will, in all likelihood, prevent it from becoming popular in very many laboratories, especially those interested in analyzing for a range of N fractions and materials.

B. For Isotope Ratio Analysis.

(1) Optical Emission Spectrometry: Optical emission spectrometry involves the analysis of the UV spectrum of light emitted by N_2 gas when excited in an electrodeless discharge tube by a high-frequency generator (Broida and Chapman 1958). Excited $^{14}N^{14}N$, $^{14}N^{15}N$ and $^{15}N^{15}N$ molecules emit light at 2977, 2983 and 2989 Å, respectively, and quantitative measurement of the three masses is possible through the use of a crystal or grating monochrometer, a photomultiplier and amplifier.

Instruments specific for determining $^{15}N/^{14}N$ ratios have been produced commercially by two manufacturers: Statron, 124 Fürstenwalde, Ehrenfried-Jopp Strasse 59, DDR, and Japan Spectroscopic Co., Ltd., Hachioji City, Tokyo. The advantages claimed over mass spectrometry include lower cost, simplicity (no high vacuum required), small sample size (0.2 - 2 µg N) and automation in some recent models. However, instrument prices have increased sharply in the last few years, small

sample size is a source of error in quantitative applications, and many problems, both optical and electronic, have been encountered in using the instruments (Keeney and Tedesco 1973, Meyer et al. 1974). The main restriction in environmental research is that the technique cannot be used at or below natural abundance levels of the N isotopes.

Two additional methods can be used for locating and assaying the N isotopes — infrared and NMR spectroscopy. Table III summarizes the capabilities of all four methods and clearly illustrates why there is no alternative to mass spectrometry for isotope-ratio analysis at or below natural abundance levels of the N isotopes.

Table III. Common techniques for isotope analysis of nitrogen.

Technique	Sample size	Sample form	Info obtained	Rel. sensitivity*
Mass spectrometry	A few µg 0.5-1 mg preferred	N_2	m/e 29/28 ratio	Highly sensitive†
Optical emission spectroscopy	0.2-2 µg	N_2	"	Insensitive
Infrared spectroscopy	mg - µg	NO_x	Isotope ratio	Insensitive
NMR spectroscopy	mg - µg	Simple molecules to complex mixtures	^{15}N concentration, label location	Insensitive

*For N isotopes at natural abundance levels of ^{15}N or below.

†Assuming a magnetic deflection mass spectrometer, equipped for isotope ratio comparisons, is used.

BASIC CONCEPTS AND ASSUMPTIONS IN NITROGEN-15 TRACER RESEARCH

A. Where ^{15}N-depleted and ^{15}N-enriched Compounds are Introduced to Trace the Element N.

The only assumption that needs to be made here is that the N in the depleted or enriched compound will behave exactly the same as the N in identical chemical form but containing ^{15}N and ^{14}N at a concentration in the natural abundance range (Edwards 1978). This assumption implies that the ^{14}N and ^{15}N, in every physical, chemical and biological event involving applied nitrogen, will participate in proportion to their relative concentrations in the reacting, N-containing moieties.

This assumption is valid if isotope discrimination effects are small or cancel out, and are therefore relatively insignificant as a source of experimental error. The assumption is valid without reservation if net isotope discrimination effects are measured, and commensurate corrections applied, through the use of both ^{15}N-enriched and a comparable ^{15}N-depleted material in the same experiment (Edwards 1975). There are many investigations where this correction procedure is not practical, however, since depleted tracers cannot be used where dilution of the applied N is large, and only a few depleted compounds are available commercially at present (Monsanto Research Corporation, Stable Isotope Sales, P.O. Box 32, Miamisburg, Ohio 45342).

B. For Kinetic Studies where a Nitrogen Molecule or Ion or Reaction Product is to be Traced.

Here the objective may be to measure kinetic isotope effects, and no labeling of the added N-containing ion or compound will normally be required. One must assume for this situation that the only nitrogen involved comes from the added source, and that only the specific reaction of interest is responsible for the disappearance or transformation of the source.

This assumption is valid for many pure systems (Delwiche and Steyn 1970), but very seldom in the soil situation. However, denitrification of NO_3^--N added to soils under strictly anaerobic conditions is one transformation for which the assumption is valid, at least for a short period, as the N-balance data of Blackmer and Bremner (1977) show. Validation through the use of a comparable ^{15}N-labeled source can obviate the need for the single-source assumption. If the labeled N does not appear anywhere in the system during the experiment except in reactant, intermediate and final product(s), the kinetic data will be valid for the conditions of the investigation.

Extreme caution must be observed in attempting to extrapolate the data on isotope segregation found in a specific soil for a specific transformation to other soils and situations. There is not even uniformity within a given soil mapping unit for many parameters, unlike the situation in sea water or the atmosphere, where the present can definitely be the key to the past in applications of stable isotope data to problems in geochemistry and hydrology.

C. Where Small Differences in the Natural Abundance of the N-isotopes are Used for Quantitative Separation of Sources in Field Studies.

There have been two reported attempts at quantitative separation of sources contributing to the nitrate load of surface water and groundwater using this technique (Kohl et al. 1971, Kreitler and Jones 1975).

There are several preconditions which must be satisfied before this type of investigation can be justified: 1) There must be a difference in the $\Delta^{15}N$ values of the N from the sources to be separated, with little or no overlapping in the range of values established for each source prior to mixing. 2) The $\Delta^{15}N$ values of the natural source or sources must be established in the field for the specific site where mixing is to be studied. 3) There must be a suitable experimental control available (or established). Where separation of sources of nitrate in groundwater is the objective, the amounts and ^{15}N concentration of the natural nitrate (all sources except the fertilizer or human and animal wastes under investigation) can be established on the proposed experimental area beforehand. This means monitoring the groundwater for both parameters for at least 1 year, and preferably 2-3 years, prior to initiation of the experiment proper (Edwards 1973). Alternatively, an adjacent area not influenced by surface water or groundwater from the treated plots can be monitored simultaneously with the latter.

Proper controls are essential for all quantitative research with stable isotopes in the soil system (Edwards 1978). They are particularly vital for tracer work at natural ^{15}N-abundance levels, since

changes in ^{15}N concentration can occur during and after movement to the point of mixing where final isotope assay is to be carried out (Edwards 1973, 1975). Attempting to conduct a tracer experiment at natural levels of ^{15}N-abundance without appropriate controls would be comparable to initiating a radiotracer experiment where final activity levels would be barely above background, then ignoring the background radioactivity (Edwards 1971).

In the presence of a suitable control, the only assumption that needs to be made in this type of investigation is that the applied source of N has no influence on the ^{15}N concentration of the nitrate derived from natural sources. This is a safe assumption for the small amount of mineral and organic nitrogen introduced in wastewater, but not necessarily so for the soil-fertilizer system (Edwards 1975).

SOURCE OF ERROR

The principal errors in N-tracer studies will arise during sampling, sample preparation and isotope-ratio analysis, provided that 1) proper controls are included, 2) overall isotope discrimination effects are negligible, or have been corrected for, 3) the degree of natural or artificial labeling is sufficient for the experimental objective.

The sampling and analytical errors could be greatly reduced in most laboratories engaged in N-isotope studies if the variability inherent in biological systems was more fully appreciated. In this section consideration will be given to the major problems associated with all phases

of experiments involving the stable isotopes of N as tracers, where the usual 3-stage approach discussed under methodology is applied.

A. Sampling and Handling Prior to Chemical Analysis.

Soils are far from uniform with respect to physical and chemical properties, either vertically or horizontally even within units classified as the same soil type on a detailed soil map (e.g., Nielsen et al. 1973, Biggar et al. 1975, Keeney and Walsh 1978). This variability holds for the N-containing organic soil materials also, and for the isotopic composition of the organic and mineralized nitrogen (Cheng et al. 1964, Delwiche and Steyn 1970, Hauck 1973, Rennie and Paul 1975). This is not surprising, since isotopic segregation takes place in the N of plant material both positionally (Moore and Craswell 1976) and in plant proteins (Hoering 1955, Gaebler et al. 1963). The uptake of mineralized N by soil organisms is also selective with respect to the N isotopes (Delwiche and Steyn 1971). To the writer's knowledge, the variability in a number of soil parameters within a restricted area, e.g. within a circle of 2-m radius on a level, apparently uniform site, has not as yet been assessed, perhaps out of fear that the results of such a study might have an inhibiting effect on field investigations!

For investigations involving measurement of $^{15}N/^{14}N$ ratios, the variability inherent in soil and plant material can be handled only through a statistical approach (see "Methods of Soil Analysis," Part I, 1965, for a general treatment of the statistics of measurement and

sampling). The objective should be to select a representative sample whose analysis (if accurate) will yield the true scientific value for any parameter of interest. The requirements for sample selection with N-isotope ratio determinations in mind are not different from those needed where any exacting chemical analysis is contemplated. This means composited, well-mixed bulk samples; very careful sub-sampling of the bulk sample to make the sub-samples as representative as possible; selection of several sub-samples; freezing of samples to be extracted later for mineral-N, and rapid air-drying of those destined for other chemical determinations; fine grinding (to <100 mesh) for chemical procedures, and to <30 mesh for biological incubations; thorough mixing of dried and ground samples (in the case of plant material, grinding to a fine flour is required); storage in tightly sealed containers; handling of all samples in a manner which prevents any cross-contamination between them (particularly important if ^{15}N-enriched or ^{15}N-depleted nitrogen sources have been used as tracers); at least three replicate samples carried through every chemical determination, and for incubation studies where soil microorganisms are involved and grinding is coarse (<30 mesh), eight to ten replicates should be considered. Tables IV and V (from Edwards 1973) illustrate the need for such a high degree of replication, especially when working at natural abundance levels. The data reported in the two tables are for NO_3^--N produced during a 2 1/2-week incubation at 30°C.

Table IV. Replicate variability in amount and ^{15}N-content of NO_3-N produced by 10 g of Webster soil fertilized with labeled vs. unlabeled $(NH_4)_2SO_4$

	Meq NO_3-N produced	Atom % ^{15}N in NO_3^-	% enrichment in ^{15}N	% NO_3^--N from fertilizer*
		Unlabeled Fertilizer		
	0.1026	0.3677	+0.38	+10.5
	0.0981	0.3644	-0.52	+97.4
	0.1044	0.3669	+0.16	+39.0
	0.1241	0.3688	+0.68	-18.4
	0.1122	0.3667	+0.11	+36.8
	0.1123	0.3673	+0.27	+21.1
	0.1180	0.3671	+0.22	+26.3
	0.1114	0.3673	+0.27	+21.1
	0.1100	0.3668	+0.14	+34.2
	0.1119	0.3699	+0.98	-47.3
Mean	0.1105	0.3673	+0.27	+21.1
Maximum difference	0.0199	0.0055	1.5	144.7
		^{15}N-Enriched Fertilizer		
	0.1104	0.5622	+53.5	52.4
	0.1109	0.5608	+53.1	52.0
	0.1127	0.5612	+53.2	52.1
	0.1059	0.5616	+53.3	52.2
	0.1064	0.5570	+52.1	51.0
	0.1103	0.5602	+52.9	51.9
	0.1141	0.5631	+53.7	52.6
	0.1122	0.5618	+53.4	52.3
	0.1074	0.5575	+52.2	51.1
	0.1098	0.5633	+53.8	52.6
Mean	0.1100	0.5608	+53.1	52.0
Maximum difference	0.0082	0.0063	1.7	1.6

*Applied fertilizer contained 0.3643 at. % ^{15}N (unenriched) and 0.7388 at. % ^{15}N (enriched).

Table V. Statistical analysis of average differences between the atom percent ^{15}N of the nitrate-N produced in Webster soil fertilized with unlabeled materials or left unfertilized.

	No fertilizer	Ammonium sulfate	Aqua ammonia	Aqua ammonia + bermudagrass
	0.3677	0.3677	0.3676	0.3673
	0.3674	0.3644	0.3654	0.3691
	0.3681	0.3669	0.3678	0.3688
	0.3696	0.3688	0.3676	0.3661
	0.3672	0.3667	0.3680	0.3680
	0.3679	0.3673	0.3668	0.3688
	0.3697	0.3671	0.3670	0.3686
	0.3680	0.3673	0.3676	0.3683
	0.3673	0.3668	0.3687	0.3688
	0.3683	0.3699	0.3669	0.3673
Average	0.3681	0.3673	0.3673	0.3681
Standard Error (SE)	2.79×10^{-4}	4.08×10^{-4}	2.80×10^{-4}	2.98×10^{-4}
Mean difference (D)	--	0.0008	0.0008	0.0000
Standard error of mean difference (E_D)	--	4.94×10^{-4}	3.91×10^{-4}	0
D/E_D	--	1.619	2.046	0
D/E_D for significance*		2.101	2.101	2.101
Significance		ns**	ns	ns

* From Fisher's Table of t for n = 18, p = 0.05.

**ns = not significant.

Recoveries of labeled N applied as fertilizers in field studies are usually low and are frequently in the range of 50-80% of that applied (Hauck and Bystrom 1970). The N not recovered is then assumed to have been lost by leaching or denitrification. However, until really careful balance-sheet studies are conducted, with much more attention paid to better sampling, sample processing, and isotope-ratio analysis techniques, losses arrived at by difference will always be open to question.

B. Chemical Preparation of Samples for Mass Spectrometric Analysis.

There are four stages involved for samples containing too little N per unit volume for direct conversion to ammonium and mass spectrometric analysis, but only the last three apply to samples of acceptable N-content. The four stages are:

(1) Concentration of the sample to bring the volume and N concentration to a level suitable for subsequent distillation as NH_4^+. Alternatively, ^{15}N-enriched, natural abundance or depleted N may be added so that a suitable aliquot may be distilled directly.

(2) The complete conversion of sample N to ammonium.

(3) Conversion of the ammonium to N_2 gas by oxidation with NaOBr or LiOBr in an alkaline medium and in the complete absence of air.

$$2\ NH_3 + 3\ NaOBr \rightarrow 3\ NaBr + 3\ H_2O + N_2 \uparrow \qquad (1)$$

(4) Determination of the isotopic composition of the N_2 gas by mass spectrometric analysis.

Table VI summarizes the sources of error which must be avoided during all four stages of sample preparation and analysis.

The sources of error listed in Table VI will be discussed in detail for each step in sample preparation and analysis.

1(a)(b) Concentration of sample or addition of labeled N.

Semimicro distillation units for quantitative recovery (as ammonium) of the N in a variety of N-containing compounds can handle only a limited volume of solution, especially if successive distillation of NH_4^+ - and NO_3^--N in the same sample is involved (Bremner 1965). Soil extracts, runoff, leachate, and wastewater samples may contain only a few ppm of NH_4^+ or NO_3^--N, and dissolved organic N-containing materials are usually present. Some means of sample concentration prior to distillation or of augmenting the small amount of N, without coagulation of organic matter, is therefore a necessary first step in sample preparation.

Three methods were recently evaluated by Hauck (1978) for use on runoff and leachate samples from experiments where ^{15}N-depleted fertilizers had been used. They included 1) evaporation of slightly acidified solution samples using warm, dry, ammonia-free air directed onto the surfaces of the solutions, 2) extraction of NH_4^+ and NO_3^- with cation and anion exchange resins, followed by direct distillation of the exchangeable ions from a KCl solution, and 3) an isotope dilution technique involving addition of a known amount of NH_4^+ and/or NO_3^- of known ^{15}N concentration to an aliquot of the unknown sample. The dilution method was found to require much less time and effort, and as

Table VI. Sources of error during sample preparation and isotope-ratio analysis

STEP	SOURCE OF ERROR
1(a) Concentration of sample	Loss of NH_3-N. Pickup of atmospheric NH_3.
(b) Isotope dilution technique	Volumetric errors in dispensing labeled N. Addition of too much N relative to sample N.
2(a) Conversion of sample N to ammonium during, or prior to, steam distillation from an alkaline medium for recovery of N as NH_4^+	Losses of N during Kjeldahl digestion. Incomplete conversion of N to ammonium. Use of non-specific methods for separating N-containing fractions. Incomplete distillation of ammonium. Cross-contamination during distillation of a series of samples. Extraneous NH_4^+-N from chemicals and water.
2(b) Concentration of distillate containing sample ammonium	Loss of ammonium by volatilization. Contamination of sample by atmospheric ammonia.
3. Conversion of ammonium to N_2 by hypobromite oxidation.	Incomplete removal of dissolved and free gaseous N_2 from reaction vessel before conversion. Incomplete conversion of ammonium to N_2. Failure to recover dissolved N_2 after conversion. Air leakage prior to analysis. Contamination of N_2 by N_2O, O_2 and other gaseous impurities evolved during oxidation of NH_4^+.
4. Determination of the isotopic composition of the N_2 gas by mass spectrometric analysis.	Memory effect in cold trap. Isotopic segregation due to leak type and differential pumping from analyzer section. Application of corrections for air leakage. Application of corrections for instrumental background. Zero-enrichment factors and peak-overlap in double-collector instruments. Mixing of sample and standard gases during switching.

long as the ratio of 1 part sample N to 5 parts labeled N was not exceeded, the error involved was acceptably low, in spite of the additional opportunity for error which the additional step represented.

Knowing the exact amount and ^{15}N concentration of the N added, the amount of sample N in the mixture is determined by difference following steam distillation of the mixture. Similarly the ^{15}N concentration of the unknown can be calculated following isotope ratio analysis of the N in the mixed sample.

The error inherent in this isotope dilution procedure is low (<1% relative error) if the 1:5 ratio of sample to added N is not exceeded, and precisely calibrated volumetric glassware is used to add the labeled solution. Forcing the technique by increasing the ratio to 1:10 or 1:20 results in a sharp error increase (to 5 to 10%).

2(a) Conversion of sample N to ammonium.

For determining total N in soils, organic wastes and soil-derived soluble-N, some variation of the Kjeldahl procedure (Kjeldahl 1883), involving high-temperature sample oxidation in concentrated sulfuric acid in the presence of salt and catalysts (Bremner 1960, 1965), is the standard approach against which all other techniques are judged. It is not an easy procedure in practice, in spite of popularly held notions to the contrary. Complete recovery of sample N is necessary if isotope discrimination effects are to be avoided. Failure to recover all of the NO_3^-- and NO_2^--N in samples can lead to quantitative errors and can

affect the isotope ratio values of the total N under certain conditions (Edwards 1971). The only safe practice is to check out several recommended procedures and use the technique giving the highest and most consistent value for total N.

Losses of N because the digestion temperature is too high (too much salt) can occur, and if the necks of Kjeldahl flasks are exposed to too much heat the same thing can happen. Since $(^{14}NH_4)_2\ SO_4$ will be lost selectively, both total N and isotope ratio values will be affected. McKenzie and Wallace (1954) have shown that a temperature of 422°C can be tolerated under certain conditions, without loss of N, but losses at 410°C have been shown to occur (Lake et al. 1951). Much attention has been paid to catalysts to permit rapid digestion at lower temperatures to avoid N loss. Copper, selenium and mercury are most common, and two in combination are frequently used. Consumption of acid by the material being digested can also increase the temperature of the digest by increasing the ratio of acid to salt, and an excess of acid must always be added to prevent too high a temperature towards the end of the digestion period. The work of Bremner (1960, 1965) is the best critical review for use in sorting out the Kjeldahl maze.

Sources of error which were of much concern to researchers in the past, e.g. contamination of ammonium by amines (Sprinson and Rittenberg 1948, Rittenberg 1948), have not turned out to be a problem with current techniques (Hauck and Bremner 1976).

The ammonium from Kjeldahl digest must be distilled for quantitative estimation and isotope ratio analysis of the total nitrogen. Steam distillation is also the key to the determination of a range of N-containing mineral and organic N fractions (Bremner 1965). The sources of error listed opposite Step 2(a) in Table VI are associated for the most part with the distillation of ammonium from an alkaline system.

Complete recovery of total sample N, and of each specific fraction, is necessary for truly quantitative work with nitrogen tracers. The fact that isotopic fractionation can occur when recovery is incomplete is an additional source of error. Specificity is also necessary when several N-fractions are to be separated in succession by the steam-distillation route. The distillation time, the dimensions of the distillation units, the temperature of the condensate and procedural details are all critical and must be adhered to for satisfactory use of the methods developed by Dr. J.M. Bremner and Associates (references already cited under Methodology). These methods have all been checked for specificity using mixtures of compounds, with one N-source labeled.

Cross-contamination between successively distilled samples has been a common source of error where ^{15}N-enriched or depleted materials were used as tracers (Bremner et al. 1966). Repeated checks by the author using a typical steam distillation unit and samples containing 2 mg N have demonstrated a holdup of N, on the cool (condenser) portion

of the distillation apparatus, of from 1 to 2 μg. The procedure used was to distill two ^{15}N-enriched samples, of known ^{15}N concentration, in succession, and follow with a sample with known ^{15}N concentration in the natural abundance range. The increase in ^{15}N concentration in this latter sample gives a direct measure of cross-contamination. The most practical way of overcoming, or at least minimizing, such errors is to arrange samples in groups with similar ^{15}N concentrations, and distill these in ascending order of ^{15}N level. In between groups, the apparatus can be thoroughly cleaned by distillation of 15 ml of 95% ethanol (Bremner and Edwards 1965). Newman (1966) and Martin and Ross (1968) have also suggested methods of overcoming cross-contamination. However, silver condensers, or complete stainless steel distillation units, are expensive alternatives to a simple and satisfactory cure, as outlined above.

The distillations for conversion of a range of N fractions to NH_4^+ all require the use of chemicals for the conversion, for raising the pH prior to distillation, and to provide an acid medium for collection of the ammonia distilled. Practically all chemicals, especially acids, will contain some ammonium-N, with ^{15}N concentration in the natural abundance range, which will measurably affect the isotope ratio values for ^{15}N-enriched and -depleted sample nitrogen. The error can be large if no correction is applied and total sample N is low.

Since a blank titration does not represent NH_4^+-N only, it cannot be used as a correction. However, an accurate figure for the amount

and ^{15}N content of the N actually present in the chemicals required for a given N determination is easily obtainable. All that is needed is a ^{15}N-enriched $(NH_4)_2 SO_4$ standard, an aliquot of which can be carried through the same procedure as the unknown. The difference between the ^{15}N concentration found in this standard sample and the value obtained when the same standard is subjected to isotope ratio analysis directly reflects dilution by a precise amount of N at any natural-abundance value assumed for it. The same correction is applicable to all samples affected by the same amounts of the same batch of chemicals. An example of the technique for obtaining and applying such a correction is given in the Appendix.

2(b) Concentration of distillate containing sample ammonium.

For hypobromite oxidation of ammonia to N_2, where Rittenberg "Y" tubes or small disposable glass vials are used as reaction vessels (Bremner 1965), approximately 2 ml each of hypobromite and sample solution are the optimum amounts for carrying out the reaction. This means that the ammonium distillates, which normally have a volume of 35-40 ml after titration, must be concentrated prior to conversion to N_2.

The sources of error possible in this step are loss of sample ammonium by volatilization, and absorption of atmospheric ammonia by the acid sample. To avoid loss of sample, the distilled samples must be acidified after titration, whether collected in mineral acid or in boric acid, and H_2SO_4 rather than HCl should be used for sample collection, titration and for the additional acidification, since NH_4Cl is

much more volatile than $(NH_4)_2SO_4$, especially when heated in the dry state. Too great an addition of acid is undesirable, since Br_2 can be evolved during the hypobromite oxidation of NH_3 in the presence of excess acid.

$$Br^- + OBr^- + 2H^+ \rightarrow H_2O + Br_2\uparrow \qquad (2)$$

A safe figure, to avoid Br_2 formation, is the addition of no more than 5 ml of 0.1 $\underline{N}$ H_2SO_4 before concentration. The author has conducted isotope ratio analysis on a number of samples containing much more excess acid than recommended above, but in spite of the presence of large amounts of bromine (frozen out in liquid N_2 traps) no effect on the answers for sample ^{15}N concentration has been observed. The suggestion by Fiedler and Proksch (1975) that the acidified sample must be added to the hypobromite, rather than the reverse, to maintain an alkaline medium for the reaction is not a valid one, in the author's experience.

Evaporation must take place in open containers to be practical and these are usually 50-ml Erlenmeyer flasks. Ambient air, particularly in a laboratory located in a building with central air conditioning and heating where cleaning solutions containing ammonia are used, will always contain ammonia in the vapor phase. Acidified samples in open containers, particularly if the evaporation is carried out in fume hoods which draw air from the laboratory over the samples, will absorb some of this ammonia, especially if evaporation takes several hours.

The absorption error can be avoided by using a simple system to generate hot, dry, ammonia-free air, and directing this onto the surfaces of individual samples through a manifold. The heat source may be heat lamps or a hot plate to warm not only the air stream but also the sample solutions. Between batches of samples the tips delivering the air to the samples must be dipped in NaOBr to destroy any of the previous sample with which the tip may have come in contact. The hypobromite treatment should be followed by dipping in ethanol to destroy any residual NaOBr.

3. Conversion of ammonium to N_2 gas by hypobromite oxidation.

Ideally, all of the dissolved and ambient N_2 and O_2 should be removed from both hypobromite and sample before mixing. This is not easily accomplished, even though small volumes of solution are involved ca. 1-2 ml each of hypobromite solution and sample, or dry salt in the case of the sample in many laboratories. Failure to properly degas, and to completely remove ambient N_2, and failure to achieve identical isotopic concentration in the dissolved and ambient N_2 generated by oxidation of sample NH_4^+ can combine to produce relative errors of several percent in the measured isotopic ratios (Edwards 1975).

The author's cure for this problem, as given in the reference above, is (1) to have both sample and hypobromite as solutions during initial evacuation, using a combination of mechanical and oil diffusion pumps capable of 10^{-6} torr. The reaction vessel is separated from the vacuum system by a single toggle valve which is either fully open

or fully closed. (2) A liquid N_2 trap is present between pumps and reaction vessel. (3) Two solid 5 to 6 mm diameter Pyrex glass beads are placed in both sample and hypobromite solutions. (4) Violent agitation of both sample and hypobromite solutions, accomplished externally through the use of a plastic-coated metal stirring rod, is provided immediately after the toggle valve to the vacuum line is opened, and is continued for about 30 seconds. (5) The hypobromite and sample solutions are not permitted to freeze during the 2 1/2-minute evacuation. This means continuous cryogenic pumping of water vapor from the reaction vessel and complete displacement of air. Timing rather than the system pressure gauge is used since the gauge is unreliable as a true pressure indicator after a few samples have been evacuated, because of partial pressures of condensed gases pumping from the trap. (6) Violent agitation is also applied to the mixed hypobromite and sample solutions to avoid isotopic segregation (Edwards 1975).

Although the bulk of the NH_4^+-N is converted to N_2 gas by reaction with strongly alkaline hypobromite, the reaction is not clean and there are gaseous products other than nitrogen. Hypobromite tends to decompose, with formation of O_2.

$$2\ NaOBr \rightarrow 2\ NaBr + O_2 \uparrow \qquad (3)$$

The amount of O_2 formed seems to be a complex function of the age of the hypobromite solution, the amount of ammonium being oxidized, and the concentration of the sample solution. Large, dry samples result in the highest O_2 production.

It is questionable whether production of O_2 should be a matter of concern, since O_2 level should not be used as a measure of air contamination (Hauck and Bremner 1976). In the author's experience, the level of O_2 in sample N_2 never approaches the 5% limit above which the isotope ratio can be affected (Junk and Svec 1958). Furthermore, O_2 production seems impossible to prevent. The use of KI in NaOBr (Sims and Cocking 1958) or LiOBr instead of NaOBr (Martin and Ross 1968) made no difference in the level of O_2 produced during experiments conducted by the author.

More serious perhaps is the finding that 1.5 to 3.0% of the gas produced in the reaction of ammonium with hypobromite is in the form of N_2O (Clusius and Rechnitz 1953), and that some of the ammonium oxidized is converted to nitrate (Clusius and Buhler 1954). Capindale and Tomlin (1957) showed that the amount of N_2O produced depended upon the age of the hypobromite, being greater with a freshly prepared solution of the oxidizing agent. These same authors also concluded that nitrous oxide interfered with isotope ratio analysis of N_2 gas samples prepared by the hypobromite method. However, nitrous oxide is removed by liquid N_2 trapping applied to the prepared sample gas before it is introduced into the mass spectrometer (Bremner et al. 1966). More recent experience by the author (Edwards and Bremner 1971) would suggest that, using a more dilute hypobromite and a modified preparation procedure, there was no interference even when a dry ice-alcohol slurry was substituted for liquid N_2. These data, shown in Table VII, also confirm the completeness of removal of sample and ambient N_2 gas using the procedures outlined

in the previous section. The answers where a small amount of sample N was present would not have been the same as those for a large sample if contamination by residual N_2 was significant.

Table VII. Freezing temperature and quantity of N oxidized by 2 ml NaOBr vs atom % ^{15}N found in a $(^{15}NH_4)_2SO_4$ sample.

	Mg NH_4-N/2 ml Solution					
Refrigerant*	10	1	0.5	0.25	0.1	0.05
A	2.0208	2.0209	2.0206	1.8675	1.6973	1.4489
B	2.0207	2.0208	2.0208	2.0207	2.0206	2.0208
C	2.0206	2.0207	2.0208	2.0208	2.0207	2.0206

A: Mixed NaOBr (2 ml)-$(^{15}NH_4)_2SO_4$ (2 ml) frozen with liquid N_2

B: 10 mg-1 mg samples incl. frozen with liquid N_2
-.5 - 0.05 mg samples incl. frozen with dry ice-ethanol

C: Dry ice-ethanol used on all samples.

* Liquid N_2 used around "U"-tube cold trap in all cases.

It would seem desirable to employ modern gas chromatographic techniques (Blackmer and Bremner 1977) to check on the hypobromite reaction, and to use a sensitive isotope ratio mass spectrometer to investigate the possibility of isotopic segregation by determining i_{45}/i_{44} ratios of any N_2O produced. The production of NO_3^- during the hypobromite oxidation also needs investigating.

Where reference standards of $(NH_4)_2SO_4$ are oxidized in the same way as unknowns, errors due to gases other than N_2 are minimized. This is another argument for using a sample of $(NH_4)_2SO_4$ as a standard rather than a tank of prepurified N_2 gas.

Air leakage into sample vessels used to be a problem where preparation was carried out on detached manifolds, and some means of sealing the sample vessels against 1 atmosphere of external pressure had to be devised. This problem has been essentially eliminated by using a system attached directly to the mass spectrometer and vessels shown to be leak-free by testing with a Tesla coil. Vessels which do leak are easily spotted, and usually the sample can be saved and transferred to another sample container.

4. Determination of the isotopic composition of the N_2 gas by mass spectrometric analysis.

Martin and Ross (1968) reported a memory effect due to entrapment of N_2 gas in the liquid N_2 cold trap between the mass spectrometer and the reaction vessel. Their remedy was to clean out this trap (small diameter stainless steel tubing) after every sample. Using an all-glass system with an expansion where the sample first encounters the liquid N_2 temperature, the author has found no memory effect even after analyzing 50-60 samples of various ^{15}N concentrations.

It is necessary to avoid isotopic segregation in the N_2 gas in the batch inlet system of the mass spectrometer. Such segregation may occur due to temperature differences, as illustrated in Table VII for small samples.

Segregation of isotopic species may also occur if a fine needle valve is used to bleed N_2 gas slowly into an expansion volume. Use of toggle valves, or stopcocks which are either completely open or shut, will avoid this difficulty.

The most important segregation takes place due to the arrangement used to bleed gas at relatively high pressure in the inlet system into the high vacuum of the analyzer section where pumping is continuous. In the case of mass spectrometers with molecular leaks (usually a few tiny perforations in a gold foil) the changes in ^{15}N concentration across the leak are compensated for by the more rapid pumping of the molecules of lower mass from the analyzer section. This means that the analysis will reflect the exact isotopic composition of the gas getting through the molecular leak, but the concentration of ^{15}N in the gas in the expansion volume will be increasing slightly during the course of the analysis. Following the same time schedule for reference standards and unknowns can eliminate this potential source of error. In a system with a truly viscous leak ("slug" flow through a restricted capillary), no segregation takes place during sample introduction, but more rapid pumping of the $[^{14}N^{14}N]^{+}$ ions from the analyzer section leads to high i_{29}/i_{28} ratios, which must be corrected downwards in studies where absolute abundance values are important. The magnitude of the correction will vary with every mass spectrometer having a viscous leak.

In the summary of sources of error (Table VI) corrections for air leakage and for instrumental background have been listed as sources of error. This seeming anomaly arises because corrections based on the amount of oxygen present during analysis of N_2 have been applied. This O_2 unfortunately may be derived almost exclusively from the decomposition of hypobromite. The use of the m/e 40 peak for argon as the basis for a

correction may also increase rather than decrease the error, because the nitrogen/argon ratio in air is high and the i_{40} peak is very small where argon-free N_2 makes up the bulk of the gas in the mass spectrometer. The presence of air can be accounted for without ever having to be measured, and can be considered simply as part of instrumental background.

The usual procedure for applying corrections for background is to measure the absolute peak heights at the m/e 29 and 28 positions, after a specified pumping time following removal of the last sample. These values are then subtracted from the sample i_{29} and i_{28} current readings before the ratio is calculated. Application of a background correction in this manner assumes that the contribution of background to the ion currents of interest is the same in the presence as in the absence of a sample of N_2 gas in the analyzer section at the recommended pressure for isotope ratio analysis. This assumption is not necessarily valid, and application of a correction on this basis could also be a source of error (Edwards and Bremner 1971). A much simpler and more logical procedure which does not require measurement of background peaks is to use an amplifier baseline setting (usually not zero) which gives the accepted isotope ratio on reference standards. This setting can then be used to give corrected isotope ratios directly on the unknowns, provided that the same inlet pressures and instrumental settings are used.

When isotope ratio mass spectrometry is mentioned the automatic reaction is to think of a magnetic deflection instrument with dual collectors and inlet systems, a single or matched viscous leaks and

provision for switching from standard to unknown for measurement of small differences in natural isotopic abundances. The tacit assumption is that such instruments are more accurate and precise than single collector instruments (Hauck and Bremner 1976). The author challenges this notion since the dual-collector instruments have inherent sources of error built in which are not found in the better single-collector models with comparable electronic and vacuum systems.

Double-collector instruments require two amplification systems and usually matched viscous leaks and the two inlet and amplification systems never behave exactly the same, leading to the necessity to apply a correction called a "zero-enrichment" factor to equate analyses run on opposite sides. The valves for switching from standard to unknown are never completely leak-proof. The resulting mixing precludes the use of the instrument in its most sensitive mode for studies involving ^{15}N-enriched and depleted materials.

It is significant that a single-collector of a dual-collector instrument was used to obtain the most generally accepted value for the absolute abundances of ^{15}N and ^{14}N in atmospheric N_2 (Junk and Svec 1958). The fact that simultaneous collection of ion species eliminates errors due to fluctuations in the ion source is more than offset by the inherent errors in the double-collector approach. A single-collector system in which the standards are introduced and analyzed in exactly the same way as the unknowns, and employing an accurate ratiometer for repetitive rapid scanning of the two peaks

of interest has, in the writer's opinion, many inherent advantages. The production of such an instrument, particularly one equipped with a perfect-focusing cycloidal analyzer (no peak overlap), should be investigated.

NITROGEN-15-DEPLETED vs NITROGEN-15-ENRICHED COMPOUNDS AS TRACERS

Materials enriched in ^{15}N have been used in most tracer studies, largely because a range of enriched compounds, with ^{15}N concentrations up to 99 atom %, have been available for the past 25 years, and common salts with lower enrichments were available as early as 1936. Furthermore, there are many obvious advantages in using materials enriched in the least abundant isotope. The degree of enrichment can be selected to ensure that an excess of ^{15}N will be present in the N-fraction of interest at the final assay of isotope abundances. Use of compounds with substantial ^{15}N-enrichments also relaxes the instrumentation requirements, and ratio analysis by optical emission spectroscopy becomes adequate in many applications. Long-term studies of residual nitrogen become feasible, even where dilution by natural nitrogen in the system may be many thousand fold.

The use of ^{15}N-depleted materials as tracers is a much more recent phenomenon. Recovery of the large amount of salt or gas from which the ^{15}N has been extracted is practical in at least two processes used for producing ^{15}N-enriched compounds. Spedding and co-workers (1955) recovered about 100 kg of ammonium sulfate containing about .03 atom %

^{15}N from the cation exchange process they developed for ^{15}N-enrichment. In the early 1970's a drive to produce large quantities of stable isotopes was launched under USAEC auspices at the Los Alamos Laboratory (USAEC 1971), largely to meet the needs of medical research, and to eliminate the use of such long-lived radioactive isotopes as ^{14}C. The so-called ICONS program was the result (Isotopes of Carbon, Oxygen, Nitrogen and Sulfur, now shorteneed to ICON program, since S isotopes are not currently being produced). Separations are based on cryogenic distillation of nitric oxide (for ^{16}O, ^{17}O, ^{18}O, ^{14}N and ^{15}N) and of carbon monoxide (for ^{13}C and ^{12}C). According to Matwiyoff et al. (1975) the production capacity at Los Alamos in late 1974 for the ^{14}N isotope was 240 kg/year (about 1200 kg $(^{14}NH_4)_2SO_4$) and the salt contained < .01 at. % ^{15}N.

The first demonstration of the usefulness of ^{14}N was as a tracer to plant uptake studies conducted by Edwards and Hauck (1968, 1974), in which $(^{14}NH_4)_2SO_4$ was compared with several levels of ^{15}N-enrichment in the same fertilizer salt. The depleted material used in this study was obtained by the principal author from D. F.H. Spedding of the USAEC Ames Laboratory, and after purification it contained 0.031 atom % ^{15}N. The ICON's program began to recover ^{14}N in 1973, and Hauck and Bremner (1976) state that some 1500 kg of $(^{14}NH_4)_2SO_4$ had been applied during the subsequent 2-year period in larger fields and lysimeter plots by USDA and land grant universities in cooperative research with the Tennessee Valley Authority, Muscle Shoals, Alabama. The TVA has been obtaining the bulk of the depleted ammonium sulfate (as a 40%

water solution) for fertilizer-related environmental research. The rest, along with the ^{15}N, ^{13}C, ^{12}C, ^{18}O and ^{16}O, are marketed through the Mound Laboratory of Monsanto Research Corporation, P.O. Box 32, Miamisburg, Ohio 45342, a laboratory operated for the USAEC (now ERDA) by Monsanto.

The recovery of ^{14}N, a very useful tracer material previously discarded or lost, represents a further step towards lower costs for all stable isotopes. Although only two ^{15}N-depleted compounds are available at present (ammonium sulfate and doubly labeled ammonium nitrate) they are available in relatively large quantities, making possible experiments of a more extensive nature. However, the cost advantage is not as large as claimed for the depleted materials (Matwiyoff et al. 1975). The present price of ^{14}N as ammonium sulfate is 17¢/g to a non-government purchaser. Therefore 1 kg of $(^{14}NH_4)_2SO_4$ (21% N) will contain 210 g N, essentially all of which is ^{14}N, so the total cost will be roughly $36.00 for the kilogram of salt. The tracer value of a ^{15}N-depleted compound containing 0.01 at. % ^{15}N is equivalent to that of an enriched compound containing 0.722 at. % ^{15}N [(0.366 - 0.01) + 0.366] as illustrated in Fig. 4, Appendix. The cost of ^{15}N/g as 10 at. % ^{15}N ammonium sulfate is roughly $56.00 from a European source. The amount of ^{15}N required to prepare 1 kg of ammonium sulfate containing 0.722 at. % ^{15}N is 0.777 g, or $43.50 worth of isotope, a differential of about $7.00 in favor of the depleted salt, assuming 1 kg of fertilizer-grade ammonium sulfate will cost 10¢. The enriched

and natural-abundance ammonium sulfate must be dissolved to distribute the isotopes uniformly, but the $(^{14}NH_4)_2SO_4$ is also sold as a 40% solution so cost of preparing a dry salt would be the same for the enriched as for the depleted material. In addition, the $(^{14}NH_4)_2SO_4$ solution is relatively impure, and the disadvantages of having to determine isotope ratios in the range between 0.366 and .01 atom % ^{15}N, as opposed to 0.366 to 0.722 atom % ^{15}N, probably far outweigh the slight cost differential. If a compound like labeled urea was required, the cost differential would be in favor of the ^{15}N-enriched tracer, since ^{15}N-urea is readily available, but ^{14}N-urea is not.

Use of ^{15}N-depleted tracers requires use of a mass spectrometer for isotope ratio analysis, and the instrument may be called upon to accurately measure 50-60 ppm ^{15}N in ^{14}N. Not all magnetic-deflection mass spectrometers are capable of operating satisfactorily in this range. There are also a limited number of situations in which the depleted materials can be used, since the acceptable dilution between application of the tracer and final assay is rather limited. For example, if a depleted fertilizer containing 0.006 at. % ^{15}N is diluted 180-fold with natural nitrogen (0.366 at. % ^{15}N), the resulting mixture will contain 0.364 at. % ^{15}N, a value which is in the natural abundance range of many soil-N fractions.

The usefulness of depleted materials as tracers is therefore limited to short-term plant uptake studies, and to following direct

movement of applied fertilizer or wastewater-N to groundwater. Since the amount of mineral N as NH_4^+ and/or NO_3^- is usually low and in the range from a few to 30 ppm in wastewater after secondary treatment, there is no way that this N could be tagged with ^{14}N short of overwhelming the wastewater nitrogen with an unnaturally large slug of depleted salt. The subsequent tracing of N through the soil would be more a reflection of the fate of an applied fertilizer than the fate of wastewater-N at normal concentrations. This would also apply to the use of depleted compounds in overland flow investigations.

For prototype systems designed to study the various parameters involved in recovery of N from wastewater, the use of ^{15}N-enriched materials as tracers is not only desirable but necessary if the amount of N retained in the soil from one or two specific applications is to be measured. For example, if 10 kg soil containing 0.3 % N are present in a leaching column, the total N present will be 30 g. If effluent is applied a liter at a time, and contains 30 mg N/l, 10 mg of N may be taken up by the crop while 15 mg leaches through, leaving 5 mg to be retained. This represents a dilution of 30,000 times if the effluent N retained is uniformly distributed. Since the surface horizon will pick up most of the N retained, the dilution will probably range from several thousand here to several hundred thousand in lower horizons. Such figures suggest that the 30 mg of effluent N applied should be essentially all ^{15}N if a proper N-balance is to be achieved for the

single application. More than one application, or an increase in the amount of N in the labeled addition, may be advisable to ensure quantitative recovery of the applied ^{15}N in the zone of lowest retention.

For investigating the fate of N in full-scale land treatment systems, the use of labeled compounds is not practical. The only option therefore is to investigate differences in natural isotopic abundances between wastewater-N and soil-derived NH_4^+ - and NO_3^--N.

USE OF VARIATIONS IN NATURAL ISOTOPIC ABUNDANCES

As indicated previously, only two attempts to use small differences in natural abundance of the N isotopes as tracers in environmental research have come to the writer's attention. The first of these claimed separation of soil-derived from fertilizer-derived nitrate-nitrogen (Kohl et al. 1971); the second involved separation of organic waste sources of the anion from natural soil nitrate (Kreitler and Jones 1975). Only the Kreitler-Jones study can be considered a legitimate application of this approach: proper controls were used; attention was paid to the range of $\delta\ ^{15}N$ values in soil-derived vs waste-derived NO_3^--N, and there was no overlapping of the figures in the two ranges; the investigation covered the well waters of a whole county, and included a number affected by septic tanks, livestock feedlots, barnyards, pastures and cultivated fields. The conclusion reached was that, with rare exceptions, the accumulation of nitrate affecting groundwaters was derived from the microbial mineralization

of soil organic matter which had been going on since farming began in the county about 1900. Natural soil nitrogen was found to be contributing as much as 1000 times more nitrate to the groundwater than animal wastes. A contributing factor to the pollution was a rise in the water table following terracing in the 1950's.

It is proposed to apply this technique to separate wastewater-N from soil-N at the Manteca, California, site. Here a proper control area, with wells unaffected by wastewater, is available adjacent to the wastewater-treated fields. It is also proposed to use changes in ^{15}N concentration of wastewater ammonium as a measure of the amount of nitrogen being lost to the atmosphere during spraying, e.g. at West Dover, Vermont.

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APPENDIX

1. Relationship between atom % ^{15}N and R, R', and R" ($29^+/28^+$, $28^+/29^+$ and $30^+/28^+$ ratios, resp.)

$$\text{by definition, atom \% } ^{15}N = \frac{\text{no. of } ^{15}N \text{ atoms} \times 100}{\text{no. of } ^{14}N \text{ atoms} + \text{no. of } ^{15}N \text{ atoms}}$$

adding up all of the ^{15}N and ^{14}N atoms as they occur in molecular combination as diatomic N_2, i.e. the binomial expansion (2 atoms taken 2 at a time)

$$(^{14}N + ^{15}N)^2 = ^{14}N^{14}N + 2\ ^{14}N^{15}N + ^{15}N^{15}N$$

$$\text{atom \% } ^{15}N = \frac{[^{14}N^{15}N] + 2\,[^{15}N^{15}N] \times 100}{2\,[^{14}N^{14}N] + 2\,[^{14}N^{15}N] + 2\,[^{15}N^{15}N]} \qquad (1)$$

To simplify this equation, and in the process to eliminate the need to measure the m/e 30 peak, advantage can be taken of the equilibrium distribution which pertains when N_2 gases made up entirely of 1 isotopic species are mixed at room temperature.

$$[^{14}N^{14}N] + [^{15}N^{15}N] \rightleftarrows 2\,[^{14}N^{15}N]$$

From the general rule of the Law of Mass Action,

$$K_{eq} = \frac{[^{14}N^{15}N]^2}{[^{14}N^{14}N]\ [^{15}N^{15}N]} = \frac{2^2}{1 \times 1} = 4$$

$$\therefore\ [^{15}N^{15}N] = \frac{[^{14}N^{15}N]^2}{4\,[^{14}N^{14}N]} \qquad (2)$$

Substituting for $[^{15}N^{15}N]$ in equation (1),

$$\text{atom \% } ^{15}N = \frac{(2[^{14}N^{15}N]\,[^{14}N^{14}N] + [^{14}N^{15}N]^2) \times 100}{4\,[^{14}N^{14}N]\,[^{14}N^{14}N] + 4\,[^{14}N^{15}N\,[^{14}N^{14}N] + [^{14}N^{15}N]^2}$$

Dividing top and bottom by $[^{14}N^{15}N]^2$,

$$\text{atom \% } ^{15}N = \frac{\left(2\,\frac{[^{14}N^{14}N]}{[^{14}N^{15}N]} + 1\right) \times 100}{4\,\frac{[^{14}N^{14}N]^2}{[^{14}N^{15}N]^2} + 4\,\frac{[^{14}N^{14}N]}{[^{14}N^{15}N]} + 1}$$

$$\text{and } R' = \frac{[^{14}N^{14}N]}{[^{14}N^{15}N]}$$

$$\underline{\text{so th atom \% } ^{15}N} = \frac{(2R' + 1) \times 100}{4R'^2 + 4R' + 1} =$$

$$\frac{(2R' + 1) \times 100}{(2R' + 1)\,(2R' + 1)} = \frac{100}{2R'+1}$$

and since $R' = \frac{1}{R}$

$$\underline{\text{the atom \% } ^{15}N} = \frac{100}{\frac{2}{R} + 1} = \frac{100\,R}{2 + R}$$

For samples containing high concentrations of ^{15}N it may be advantageous to measure R", the i_{30}/i_{28} ratio. For this situation

$$\text{Atom \% } ^{15}N = \frac{100\,(R'' + \sqrt{R''})}{(1 + \sqrt{R''})^2} \quad .$$

as R" → 1, the atom % ^{15}N → 50

compared to R → 1, atom % ^{15}N → 33 $\frac{1}{3}$

2. Isotope Dilution Analysis, where unknown sample contains too little N/ml to provide enough in an aliquot of maximum size for distillation and subsequent isotope ratio analysis:

To 20 ml of unknown sample is added .01 meq. of NH_4^+ as 1 at. % ^{15}N $(NH_4)_2SO_4$.

Distillation of ammonium from the mixture shows the presence of 0.012 meq, and isotope-ratio-analysis of the N in the distillate gives an at. % ^{15}N = 0.900.

if x = at. % ^{15}N in the unknown,

$.01(1) + .002\ (x) = .012\ (0.9000)$

$.002\ x = .008$

$x = 0.4000$ at. % ^{15}N.

Previous knowledge of the approximate amount of N present in the unknown sample, obtained by a quick colorimetric test, for example, is desirable as a guide to the amount of ^{15}N-enriched nitrogen to add.

3. Milliequivalents or equivalents vs. mass in ^{15}N research.

In environmental research involving nitrogen, the tracers used are normally salts rather than gases, although the salts are frequently administered as solutions. In most cases the need is to equate the meq or eq of NO_3^--, NH_4^+- or -NH_2-N in the labeled and unlabeled treatments. Because of the greater mass of the ^{15}N atoms it is therefore

necessary to weigh out more of the labeled than of the comparable unlabeled salt. Calculation of salt requirements on the basis of contained mass of N would suggest the reverse, since the percentage of N by weight in any compound increases as the ^{15}N concentration increases. The following calculations (Table AI) for "natural abundance" vs. 10 and 50 atom % ^{15}N-urea illustrate this point. The assumptions made in deriving the tabular data are that (a) the salts are pure and moisture free, (b) the ^{15}N concentration in the unlabeled urea is 0.366 at. %, (c) the C, H and O isotopes are present in the proportions needed to give the accepted atomic mass of each element, and (d) the mass of the ^{15}N atom = 15.00011 amu, the ^{14}N atom = 14.00307 amu.

Table AI. Potential errors from ignoring the effect of ^{15}N on equivalent mass and from calculating N requirements on the basis of the percentage by weight of contained N.

	Nat. Abundance	10 at. % ^{15}N	50 at. % ^{15}N
Molec. wt. urea (g)	61.05583	61.24808	62.04561
gN/molec. wt.	28.01340	28.20555	29.00318
% N by wt.	45.88	46.05	46.75
wt. salt for 2 eq $-NH_2-N$	61.05583	61.24808	62.04561
wt. salt for 28.0134 gN	61.05583	60.83083	59.92820
Weighing error through % N calculation (g)		0.41725	2.01741
% error		0.68	3.25
Weighing error (g) if no allowance made for additional ^{15}N		0.22500	1.13063
% error		0.37	1.82

4. Purchasing ^{15}N-labeled materials.

As an example, assume that you wish to order 10 g ^{15}N as 10 at. % ^{15}N urea. How much dry salt will such an order bring you?

From the previous example, the m.w. of 10 at. % urea = 61.24808 g, and 1 m.w. contains 28.20555 g of N.

The temptation is to calculate as follows: 10 g N will be present in $\frac{10 \times 61.24808}{28.20555}$ g salt, and, since only 10% of the N will be ^{15}N, the amount of salt involved will be $\frac{100 \times 61.24808}{28.20555} = 217.1490$ g. Actually, 10 at. % ^{15}N means $\frac{10\ (15.00011\ amu)}{10(15.00011) + 90(14.00307)} = \frac{150.0011\ g\ ^{15}N}{1410.2774\ gN}$. So the correct calculation is

$$\frac{10 \times 61.24808}{28.20555} \times \frac{1410.2774}{150.0011} = 204.1588 \text{ g urea,}$$

or about 13 g less than anticipated. And the purchaser pays full (enriched) price for the 0.366 at. % ^{15}N (approx.) present in the urea before enrichment.

5. Estimating the level of ^{15}N-enrichment required in a tracer material to meet a specific experimental objective.

Since artificially labeled materials are expensive, a few simple calculations may serve to avoid the waste associated with use of too little or too much ^{15}N. There are several points to consider and assumptions which can be made in arriving at a satisfactory approximation. The most important factors are:

1. The total amount of labeled N to be introduced into the system relative to the amount of N already present.

2. The amount of dilution of the excess ^{15}N to be expected in the experimentally significant N-fraction that is likely to receive the smallest portion of the label.

3. Probable removal of added labeled N from the system, by all mechanisms, during the course of the experiment.

4. The duration of the experiment.

5. The least significant difference in $\Delta^{15}N$ (above or below "background" values) required to establish the quantitative contribution of the labeled source using the number of replications envisioned in the sampling program.

For field and greenhouse studies, the author's experience would suggest that changes in isotope content will have to be $\pm$ 1% to be significant. If the natural abundance of ^{15}N is assumed to be 0.366 at. %, this means that a significant change must amount to $\pm$ 0.004 at. % ^{15}N.

To calculate the allowable dilution where 10 at. % ^{15}N is available in a tracer material,

let x = fraction of N in the assay material derived from the labeled source, and

let y = fraction of N in the assay material coming from natural sources.

then $x + y = 1$

and $10.000\ x + .366\ y = 0.370\ (x+y) = 0.370$

$$0.366\,x + .366\,y = .366$$

$$9.634\,x = .004$$

$$x = .000415$$

$$y = 0.999585$$

So the allowable dilution amounts to approximately 1 part labeled N to 2,500 parts natural N.

The foregoing calculation assumes that a mass spectrometer suitable for precise isotope ratio measurements is available. If an optical emission instrument must be used, the degree of difference required between atom percentages for statistical significance will be much greater than $\pm$ 1%, e.g. $\pm$10-30% of the background value.

6. Dilution of a highly enriched material to produce a specified quantity of salt with a lower ^{15}N concentration.

An investigation calls for 1 equivalent weight of NH_4^+-N as 10 at. % ^{15}N $(NH_4)_2SO_4$, and the only enriched salt on hand contains 99.0 at. % ^{15}N. What proportions of reagent-grade ammonium sulfate (measured ^{15}N concentration = 0.366 at. %) and the highly enriched salt must be mixed to meet the need?

Table AII. Molecular and equivalent weights of 0.366, 10 and 99 atom % ^{15}N $(NH_4)_2SO_4$.

	Mass Units		
Element	Reagent Grade	10 at. % ^{15}N	99 at. % ^{15}N
2xN	28.01340	28.20555	29.98028
8xH	8.06376	8.06376	8.06376
1xS	32.06400	32.06400	32.06400
4xO	63.99760	63.99760	63.99760
Molec. wt.	132.13876	132.33091	134.10564
Equiv. wt.	66.06938	66.16546	67.05282

1 equiv. wt. of NH_4^+-N in 10 at. % ^{15}N A.S. = 14.10278 g, and is contained in 66.1654 g of pure salt.

Let x = the fraction of 1 equiv. wt. of NH_4^+-N required as natural-abundance A.S.

Then 1-x = the fraction of 1 equiv. wt. of NH_4^+-N required as 99 at. % A.S. and $0.366x + 99(1-x) = 10(1)$

$$98.634\ x = 89$$

$$x = 0.9023 \text{ eq.}$$

$$1-x = 0.0977 \text{ eq.}$$

0.9023 eq. of NH_4^+-N will be contained in

$$.9023 \times 66.06938 = 59.61440 \text{ g Reagent Grade Salt}$$

and 0.0977 eq. NH_4^+-N will be contained in

$$0.0977 \times 67.05282 = \underline{6.55106 \text{ g}} \quad 99 \text{ at. \% } ^{15}N \text{ A.S.}$$

$$\text{TOTAL} = 66.16546 \text{ g}$$

and the total is the equivalent weight of 10 at. % ^{15}N-$(NH_4)_2SO_4$.

Satisfactory mixing of labeled and unlabeled salts, as required in the foregoing example, can be accomplished only by dissolution and re-crystallization.

The figures generated in Table AII assumed 100% pure, dry salts and the accepted atomic weights for H, S and O.

7. Measurement and correction of the dilution error from extraneous N accumulated during sample preparation.

The usual final step in the quantitative determination of total N, or a specific N fraction, is titration of distilled NH_4^+ collected in

boric or mineral acid. Titration of comparably treated "blanks" cannot be used as a measure of the amount of extraneous N accumulated during processing since colorimetric and potentiometric end-points are affected by substances other than distilled NH_3, e.g. CO_2 and water. However, an accurate quantitative figure for the N from sources other than the sample itself can be arrived at by comparing the isotopic composition of a ^{15}N-enriched sample of $(NH_4)_2SO_4$ when converted directly to N_2 gas against the atom % ^{15}N in a known quantity of nitrogen as the same enriched salt which has been carried through the identical series of sample preparation procedures to which sample unknowns were subjected.

e.g. atom % ^{15}N in $(^{15}NH_4)_2SO_4$ (direct analysis) = 5.000
and the atom % ^{15}N in the same enriched sample when 0.1 meq of NH_4^+-N was carried through all prep. procedures = 4.951.

Assuming 0.366 atom % ^{15}N is present in the extraneous N accumulated, and x = meq of N involved, then

$$(0.1)(5) + x(0.366) = (0.1 + x)\ 4.951$$

$$0.5 + 0.366x = 0.4951 + 4.951x$$

$$4.585x = .0049$$

$$x = .00107 \text{ meq } NH_4^+\text{-N}$$

or approx. 15 µg NH_4^+-N from extraneous sources.

An unknown sample analyzed using the same chemicals and procedures requires 0.60 meq H^+ to neutralize distilled ammonia (blanks subtracted). The atom % ^{15}N in this sample = 1.542. The corrected value (x) is calculated as follows:

$$.060x + .00107\ (0.366) = .06107\ (1.542)$$

$$.060x = .0937783$$

$$x = 1.563 \text{ at. \% } {}^{15}N.$$

The actual at. % ^{15}N in the extraneous N need not be determined to make the correction valid. Once the amount of N being derived from outside sources (at an assumed natural abundance figure for ^{15}N concentration) has been determined, it can be applied to all samples analyzed using the same batches and quantities of chemicals.

8. Calculating the fractional contribution of naturally or artificially labeled materials to a mixture.

A. Assuming a natural label is used or that the net isotope discrimination effects have a negligible influence on the ^{15}N concentration of the N applied as an artificially labeled source.

Let x = the fraction of the N in the assay material derived from the labeled source. Then 1-x = fraction of N in the same material derived from all other sources.

and a = at. % ^{15}N in assay material containing N from all sources

b = at. % ^{15}N in controls (all sources except the labeled material)

c = at. % ^{15}N in fraction x.

then $c(x) + b(1-x) = a(1)$

$$cx + b - bx = a$$

$$x(c - b) = a - b$$

$$x = \frac{a - b}{c - b}$$

B. The situation where isotope discrimination effects can be measured through simultaneous use of ^{15}N-enriched and ^{15}N-depleted materials as tracers.

Let x' = the experimentally determined fraction of N in the assay material calculated as in A above from the use of a ^{15}N-depleted tracer.

and let x" = the same fraction determined using a ^{15}N-enriched tracer.

Then the true x value = $\frac{x' + x''}{2}$ (Edwards 1975)

e.g. x' = .490

x' = .510

x = .500

The actual figures determined (or assumed in the case of c = at. % ^{15}N in enriched material at time of application) are as follows:

$$x'' = \frac{2.366 - 0.366}{4.288 - 0.366} = 0.510$$

The true C is then determined by substituting x = 0.500 for

$$x'' = 0.510, \text{ i.e. } \frac{2.366 - 0.366}{C - 0.366} = 0.500$$

$$C = 4.366 \text{ at. \% } ^{15}N.$$

This means that the at. % ^{15}N in the added tracer, at the time of mixing with other sources of N in the assay material, had increased from the 4.288 at. % ^{15}N at application to 4.366 at. %, an increase of .078 at. % ^{15}N.

9. Balance-sheet calculations to assess the fate of applied N.

The figures required for direct quantitative tracing of applied N are:

1) The amount and ^{15}N-concentration of the N in the added tracer.

2) The same figures for the N in all fractions (solid, liquid and gaseous) to which the applied N has contributed during the course of the experiment.

3) Accurate average values for the at. % ^{15}N in the nitrogen diluting the tracer-N in each fraction.

The third set of figures is the most difficult to obtain directly, especially where the applied N has an influence in determining the ^{15}N concentration of the background nitrogen. Where ^{15}N-enriched or -depleted tracers are used, the assumption of the atmospheric concentration of the N-isotopes for the non-tracer nitrogen will not lead to appreciable error. However, such an assumption cannot be made when working at natural-abundance levels and proper controls are an absolute necessity.

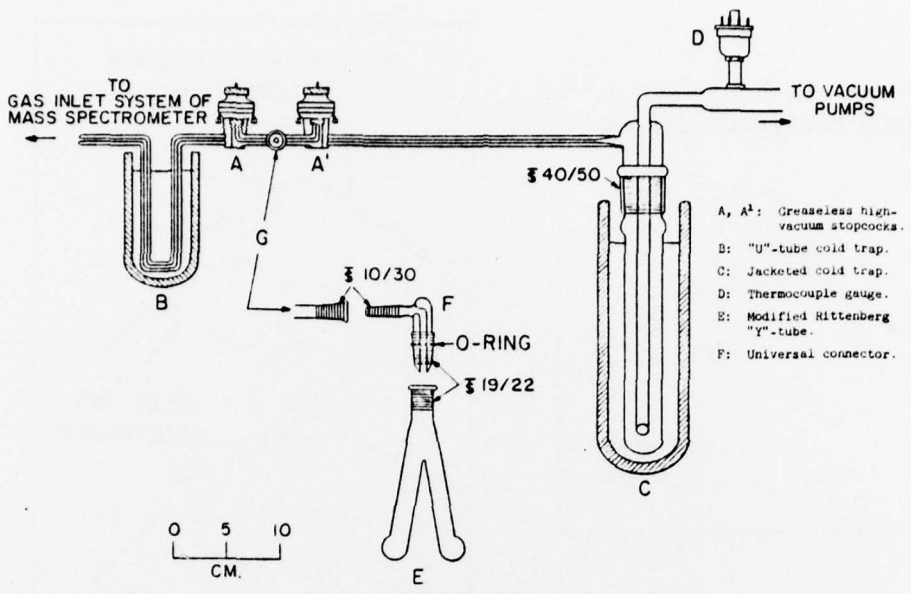

Figure 1. Sample preparation system designed by the author and in use at Iowa State University, TVA, Muscle Shoals, AL and Louisiana State University.

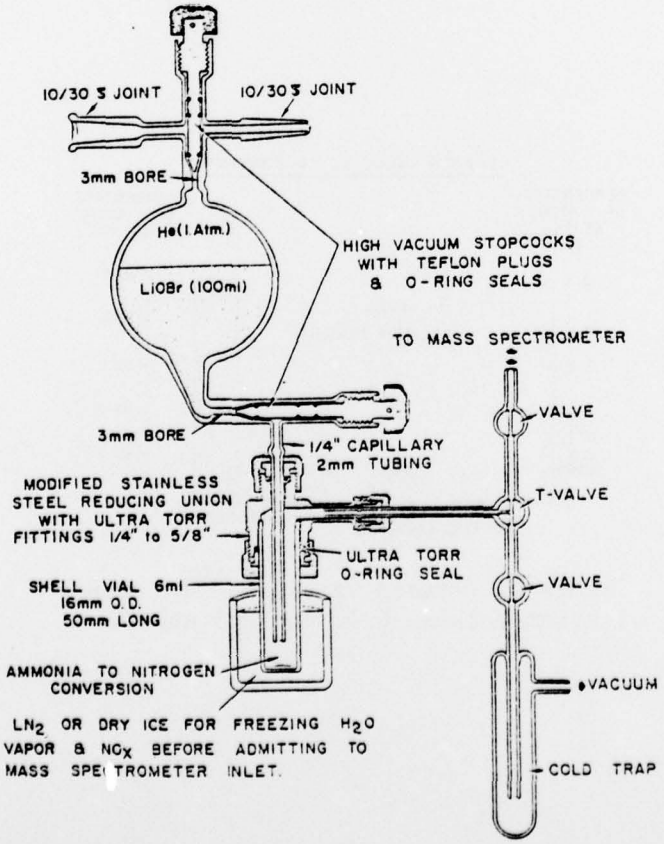

Figure 2. Sample preparation system designed by Dr. L.K. Porter, USDA, Fort Collins, Colorado, and a modification of the design of Martin and Ross (1968).

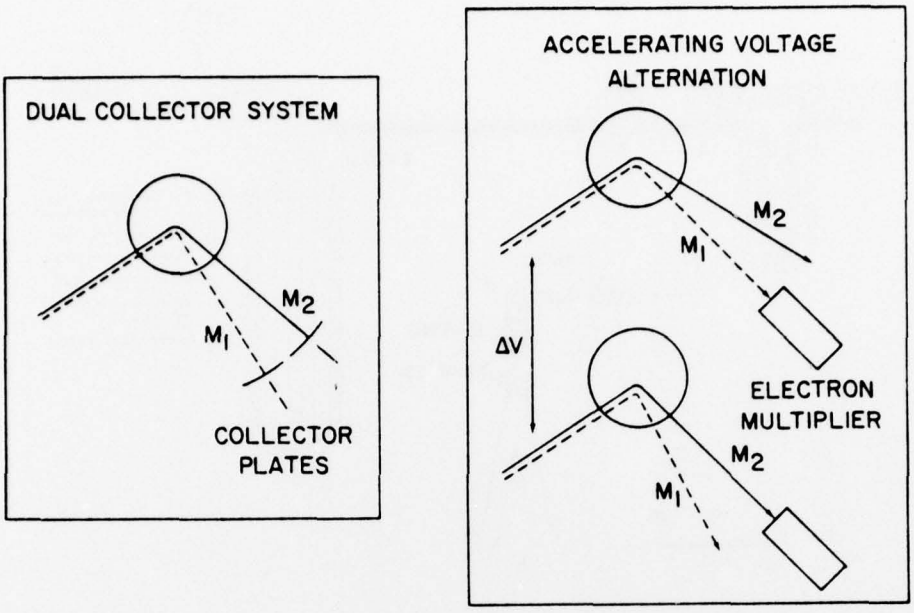

Figure 3. Two principles for the measurement of isotopic abundances.

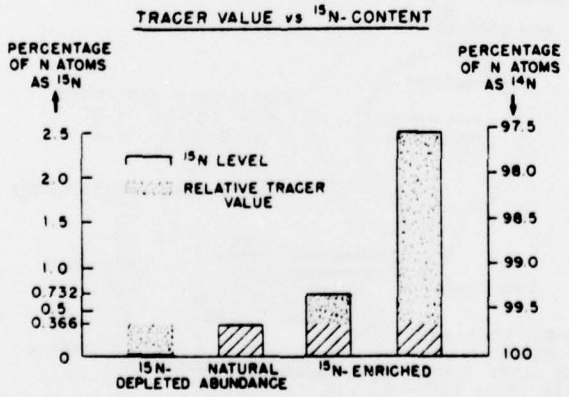

Figure 4. Tracer value vs ^{15}N concentration between 0.01 and 2.5 at. %.