Report of Investigations No. 83

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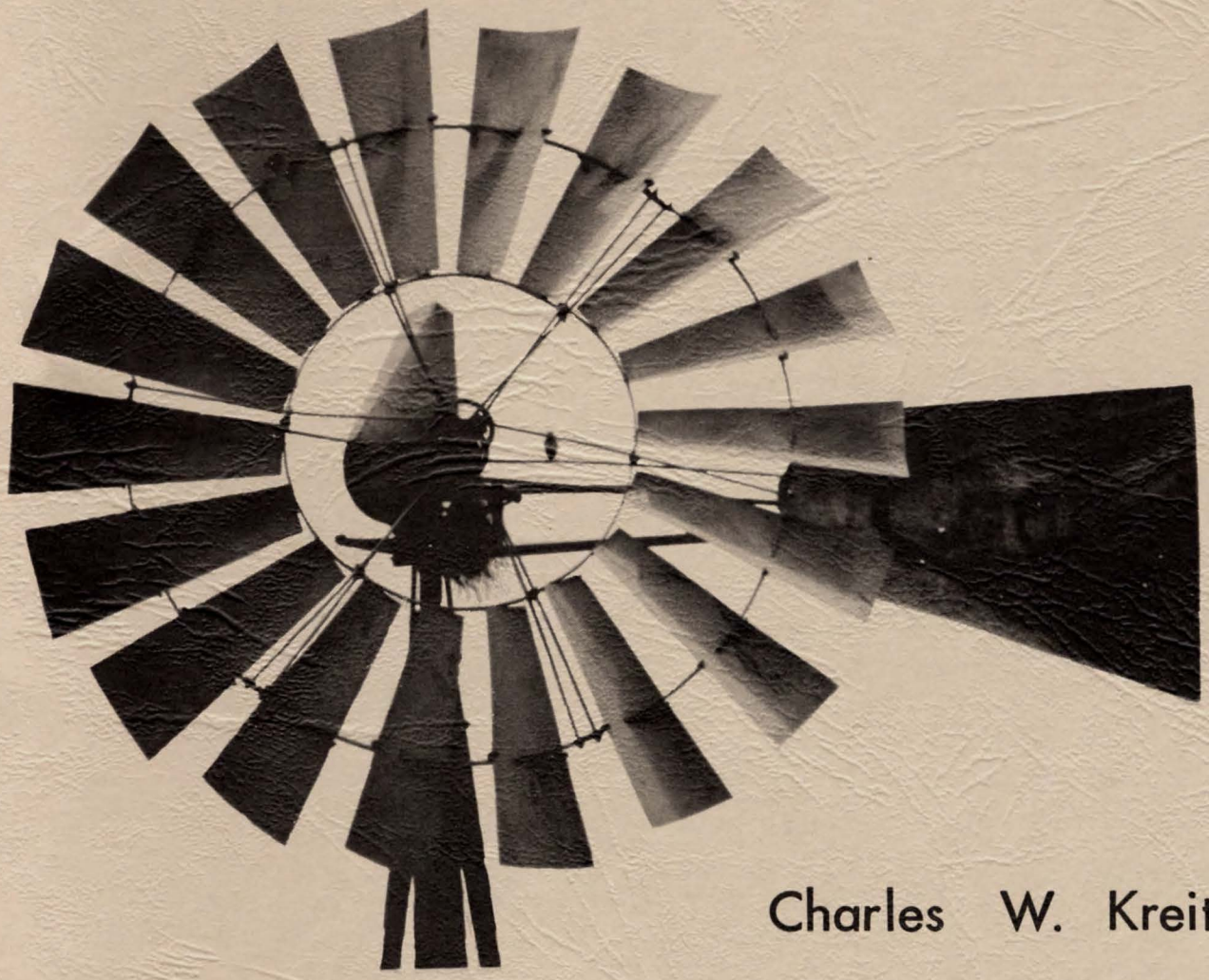

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DETERMINING THE SOURCE OF NITRATE IN GROUND WATER BY NITROGEN ISOTOPE STUDIES

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ABSTRACT

Nitrogen isotope ratios of ammonium and nitrate ions from soil and water samples can be analyzed reproducibly with an experimental error of approximately ±1 parts per thousand (ppt).

Two isotopic ranges of soil nitrate are found in the soils of southern Runnels County, Texas. Nitrate from the decomposition of animal waste nitrogen has a del N15 (δN^{15}) of +10 ppt to +22 ppt. The isotopic ratio is controlled by the volatilization of isotopically light ammonia gas during the decomposition of urea in urine. Nitrate derived from the mineralization of organic nitrogen in cultivated soils has a del N15 of +2 ppt to +8 ppt. In southern Runnels County, the major source of nitrate in ground water is natural soil nitrate. The isotopic composition of ground-water nitrate beneath cultivated fields corresponds with del N15 of natural soil nitrate. Ground waters beneath farmhouse-barnyard complexes have a higher average del N15, indicating the addition of animal waste nitrate.

Eleven samples of ground water from Macon County, Missouri, have del N15 of +10 ppt to +19 ppt, indicating that the waters are contaminated with nitrate from animal wastes. Nitrates in ground waters from the Upper Glacial aquifer in Queens County, New York, appear to be from an animal waste source, whereas nitrates in ground waters from the Magothy aquifer in Nassau County, New York, appear to be from either natural soil nitrogen or artificial fertilizer.

INTRODUCTION

The study of nitrogen isotopes provides a method of monitoring the migration of nitrogenous compounds in biogeochemical cycles. As nitrogen compounds are altered chemically within a system, the stable isotopes, N^{14} and N^{15}, may undergo isotopic fractionation. If such fractionations are unique, then nitrogen isotopes can be used to determine major sources of nitrate in natural systems.

More specifically, nitrogen isotopes can be used to determine sources and pathways of migration of nitrates to natural waters. Since the early 1950's, investigators have tried to trace the sources of nitrate in surface and ground waters. However, many investigators neither understood the complexities of the nitrogen cycle nor the number of nitrogen sources available for oxidation to nitrate. Their results were either indefinite or inaccurate. Viets and Hageman (1971) reviewed much of the literature concerning nitrates in ground water.

The development of nitrogen isotope techniques for tracing nitrates in natural systems is of value for environmental health reasons as well as for strictly scientific considerations. Nitrates have been blamed for clinical and chronic methemoglobinemia (blue babies). Methemoglobinemia occurs when nitrates in the gastrointestinal tract are reduced to nitrite. The nitrite is absorbed into the blood and oxidizes the ferrous iron of hemoglobin to ferric iron. The hemoglobin loses its ability to release oxygen to cells. Cell asphyxiation then occurs (Winton and others, 1971). The U. S. Public Health Service (USPHS) has set a recommended limit of 45 milligrams per liter (mg/l) nitrate for public water supplies to prevent methemoglobinemia. Gruener and Shuval (1970) observed, however, that the continual ingestion of low concentrations of nitrate produces chronic methemoglobinemia, an anemic condition. The USPHS limit of 45 mg/l nitrate for drinking water may be too high if widespread anemia results as large numbers of persons drink water with lower nitrate concentrations.

In laboratory studies with rats, a positive correlation has been made between nitrate and

Table 1. Nitrate in aquifers of Central Texas (data from Texas Water Development Board, Texas Water Oriented Data Bank, Ground Water Quality System, Program WD 500).

Aquifer	Zone	Age	Lithology	County	Average NO_3 (mg/l)	Number of Samples
Edwards	Recharge	Cretaceous	limestone	Williamson	16	40
	Downdip			Williamson	< 4	8
Hensell	Recharge	Cretaceous	sand	Burnet and Williamson	15	12
	Downdip				< 4	35
Leona	Recharge	Pleistocene (?)	sand and gravel	Caldwell and Guadalupe	83	49
Wilcox	Recharge (depths; less than 100 feet)	Tertiary	sand	Bastrop	22	23
	Downdip (depths; 100-700 feet)				0.3	38
Carrizo	Recharge (depths; less than 300 feet)	Tertiary	sand	Zavala	15	36
	Downdip (depths; 300-4,698 feet)				0.8	56

cancer (Wolff and Wasserman, 1972). Nitrite (one oxidation state below nitrate), reacting with a secondary amine, forms a nitrosamine; some nitrosamines are carcinogenic. Alam and others (1971a, b), Asahina and others (1971), Ayanaba and others (1973), Challis (1973), Harington and others (1973), Lijinsky and Epstein (1970), McCormick and others (1973), and Verstraete and Alexander (1971) have studied various aspects of this problem. Some evidence suggests that an epidemiological correlation may exist between nitrate and cancer in humans. Hill and others (1973) found an increased death rate from gastric cancer in English towns with high nitrate water supplies. There appears to be a high incidence of cancer with Virginians who use water from shallow aquifer systems which may contain high nitrates (A. G. Everet, personal communication, November 1973), but no evidence has been presented to substantiate this hypothesis. Because of the potential health problems, the extent of nitrate contamination and the major nitrate contributors to a water supply should be known.

Nitrate concentrations above 1 or 2 mg/l are in the ground waters in many of the geographic areas of Texas (fig. 1), with the highest percentage values in West-Central and North-Central Texas. The following counties have average nitrate concentrations approaching or greater than the U. S. Public Health Service recommended limit of 45 mg/l: Archer (42 mg/l), Baylor (67 mg/l), Borden (39 mg/l),[1] Brown (48 mg/l), Coleman (41 mg/l), Fisher (43 mg/l), Foard (39 mg/l),[1] Garza (65 mg/l),[1] Hardeman (36 mg/l), Haskell (85 mg/l), Jones (60 mg/l), Knox (50 mg/l), Palo Pinto (121 mg/l), Runnels (233 mg/l), Taylor (41 mg/l), Throckmorton (39 mg/l), Wichita (42 mg/l),[1] and Young (42 mg/l). (Original data is from Texas Water Development Board Analysis Max-Min-Mean Report, Texas Water Oriented Data Bank, October 29, 1974.) This nitrate is probably concentrated in the shallow, updip parts of the aquifers and not present in the deeper parts. A compilation of nitrate concentrations at different depths in aquifers in Central Texas shows this to be true. The recharge zones in table 1 all have nitrate concentrations greater than 10 mg/l, whereas the average values from deeper in the aquifer are less than 0.5 mg/l.

[1]Counties with less than 10 recorded water samples.

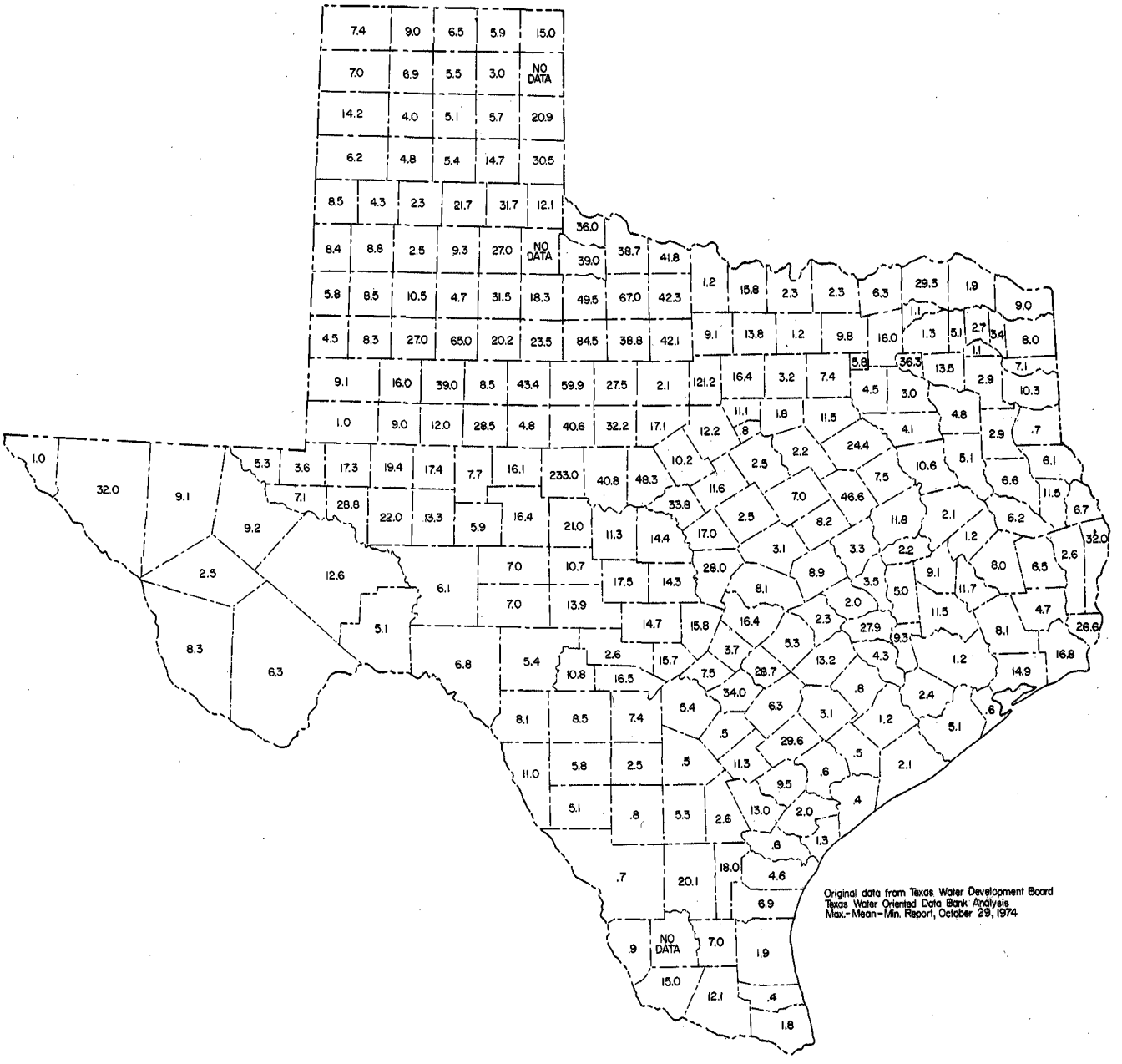

Figure 1. Ground water NO_3 in Texas.

In Runnels County, Texas (the county with the highest average concentration in Texas), the Permian carbonate aquifers and the Tertiary gravel aquifer have no downdip portion so the entire aquifers have been polluted. The residents in this county do not have the option to drill deeper to find better water. The average nitrate concentration in the ground water is 233 mg/l with 90 percent of the waters above the U. S. Public Health Service's recommended limit of 45 mg/l. Nitrates in individual wells were high enough to kill two herds of cattle in the summer of 1969.

Texas is not alone in this contamination problem. Illinois, Nebraska, Colorado, California, New York, and others have or are developing nitrate contamination problems of water supplies. The solution to this contamination problem is to either remove it from the water supply through treatment or to prevent it from entering the water

supply in the first place. The solution by treatment is generally not economically feasible for either the private well owner or the municipality because nitrate, a very soluble, nonreactive anion, is very difficult to remove.

The second solution, preventing the nitrate from ever entering the aquifer, is an equally difficult task because of the multiple sources from which nitrate can originate, Sources vary from completely natural phenomena such as bat guano in cave deposits (Feth, 1966) to entirely man-made phenomena such as leaking sewer lines (Kimmel, 1972). Considering only the sources related to human activity, researchers have attributed nitrate in surface waters and ground waters to high nitrate in rainfall (Junge, 1958), fertilizers (Commoner, 1970b), animal waste material (Smith, 1969; Gillham and Webber, 1969), cultivation (Stout and Burau, 1967), septic tanks (Walker and others, 1973a, b), leaking sewer lines (Kimmel, 1972), and spray irrigation of sewage effluent (Nuhou Kumu Wai, 1972; King, 1973). Most of these studies did not determine the relative importance of a particular source because generally there was more than one source available and classical chemical techniques could not separate them. The extremely soluble nature of nitrate permits easy migration away from its source, which commonly separates it from other identifiable tracers, such as septic tank pollutants migrating in an aquifer. Fecal bacteria may be filtered out and phosphates may be adsorbed, but the nitrate remains in solution and migrates through the aquifer. No conventional chemical technique can trace this nitrate back to the septic tank.

Statistical approaches also have been tried with a similar lack of success. Sylvester (1959), Commoner (1970b), and Jaworski and others (1971) have tried to determine the relative contribution from various sources by studying the amount of fertilizer used, the number of septic tanks per acre, or the per capita flush of a toilet. Their numbers are no more than rough estimates because they have no way to validate their conclusions.

By studying the natural nitrogen isotope ratios of nitrate in ground water and comparing them to the nitrogen isotope ratios of nitrate from different soil environments, the nitrate of certain ground waters can be traced to unique sources. This was accomplished in a four-part program: (1) developing methods and confirming reproducible results in measuring the nitrogen isotope ratio of nitrates, (2) studying the pertinent nitrogen cycles to determine if there are unique isotopic ranges, (3) identifying the isotopic fractionations, and (4) applying this information and these techniques in areas where the waters have been contaminated by nitrates.

By knowing the sources of contamination we can better curtail the movement of nitrate into our water supplies.

The principal area of study for this report was southern Runnels County, Texas. The nitrogen isotope ratios of nitrate from ground waters in Macon County, Missouri, and Long Island, New York, were also evaluated.

Acknowledgments

This report is based on a dissertation at The University of Texas at Austin. Dr. L. J. Turk supervised this work, and Drs. Lynton S. Land and Keith Young of the Department of Geological Sciences, The University of Texas at Austin, and Peter T. Flawn, formerly Director of the Bureau of Economic Geology, The University of Texas at Austin, and Stanley N. Davis of the Geology Department of Indiana University served on the dissertation committee. To these gentlemen I extend a special thanks for their help.

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Several people were helpful in the collection of water and soil samples. I would like to thank Werner Lange and Paul Pieper (Runnels County, Texas) for their generous expenditure of time and effort. In Missouri, Dr. George Smith and Gerald Kerr helped in sample collection. In Long Island, New York, Dr. Olin Braids and Pete Maniscalco of the U. S. Geological Survey aided in sample collection. Dr. Harry Svec, Ames Laboratory, Iowa State University, provided a nitrogen standard gas.

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Finally, I would like to especially thank my wife, Berf. She prepared samples, chased cattle for ammonia gas collection, and drafted, typed, and edited the preliminary drafts of this report.

METHODS OF ANALYSIS

Of the five nitrogen isotopes, N^{13}, N^{14}, N^{15}, N^{16}, and N^{17}, three are extremely unstable and hence do not exist in measurable quantities in natural systems. Half-lives of N^{13}, N^{16}, and N^{17} are ten minutes, seven seconds, and four seconds, respectively (Jansson, 1968). The stable isotopes are N^{14} and N^{15} of which N^{14} predominates; 99.632 ± 0.002 percent of nitrogen in the atmosphere is N^{14} (Junk and Svec, 1958; Nier, 1955). In other nitrogenous compounds, these percentages vary slightly because of isotopic fractionation.

The variations of mass are measured on a gas-source mass spectrometer in which the sample is compared to atmospheric nitrogen, the standard. Another available standard is the tank nitrogen used by Junk and Svec (1958). A sample may be obtained from Dr. Svec at the Ames Laboratory, Ames, Iowa. Ammonium sulfate has also been used as a working standard (Commoner, 1970a; Edwards, 1973). The ratio of sample to standard is expressed in the accepted isotopic terminology as:

$$\delta N^{15}\ (\text{ppt}) = \frac{(N^{15}/N^{14})_{\text{Sample}} - (N^{15}/N^{14})_{\text{Standard}}}{(N^{15}/N^{14})_{\text{Standard}}} \times 1000$$

If N^{15} in the sample is enriched relative to N^{15} in the atmospheric nitrogen standard, the sample has a positive del N15 value; if relatively depleted in N^{15}, the sample has a negative del N15 value.

Nitrogen in natural systems occurs as organic nitrogen (amides, amines, proteins, and amino acids), ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and nitrogen gas (N_2). For isotopic analysis, these compounds must be converted to nitrogen gas. Organic nitrogen is converted to ammonia by Kjedahl distillation. Nitrite and nitrate are reduced to ammonia with Devardas Alloy (50% Cu, 45% Al, and 5% Zn) and then distilled. The ammonia generated from either organic nitrogen or nitrite and nitrate is oxidized to nitrogen gas with sodium hypobromite. The nitrogen gas is then purified by circulation through a liquid nitrogen cold trap, thereby eliminating carbon dioxide, carbon monoxide, nitrous oxides, water vapor, and oxygen. The gas is analyzed by comparing the ratio of mass 29 to mass 28 ($N^{15}N^{14}/N^{14}N^{14}$).

Sample Preparation and Isotopic Analysis of Nitrate

Preparation and analysis of the nitrate samples are accomplished as follows (preparation of reagents, appendix 1):

(a) Determine approximate concentration of nitrate with specific ion electrode (Orion Model No. 92-07). Treat aliquot containing less than 50 mg of nitrate with 5 ml of sulfamic acid to convert any nitrate in solution to nitrogen gas. Transfer sample to 500 ml boiling flask.

The specific ion electrode for nitrate is used to determine the approximate concentration of the samples so that aliquots of the sample solutions have nitrate concentrations equal to or less than the nitrate in the standard $CaNO_3H_2O$ (50 mg NO_2) and NH_4Cl (20 mg NH_4) solutions. The various reactions are then complete, eliminating potential isotopic fractionation. Most aliquots had nitrate concentrations of approximately 30 mg NO_3.

Sulfamic acid reacts with nitrite by the following reaction:

$$NH_2SO_3H + HNO_2 \rightleftharpoons N_2 + H_2SO_4 + H_2O$$

The addition of sulfamic acid to eliminate nitrite was not necessary in this study because Kreitler (1972) found negligible levels of nitrite in the ground waters of southern Runnels County, Texas.

(b) Raise pH of solution to between 9.5 and 10.0 by adding powdered magnesium oxide (MgO). Distill solution until 50 to 75 ml of distillates are collected. Interfering nitrogen species are eliminated.

The distillation procedures are modifications of Bremner and Keeney (1966). Five hundred milliliter, three-necked and two-necked boiling flasks are used instead of the steam distillation apparatus recommended by Bremner and Edwards (1965). The pH of the solution is raised to between 9.5 and 10.0 to convert all NH_4^+ to NH_3 and to break down any labile organic compounds to ammonia. Hydrolyzable organic nitrogen will ammonify at a nonpredictable rate with pH over 12. A weak base, such as MgO, maintains the pH in the desired range. Tests using 69 organic nitrogen compounds, including amino acids, purine, pirinudine derivatives, and alkali labile compounds such as amides and hexosamines, demonstrate that the distillation method has a high specificity (Bremner and Keeney, 1966). For the present study, this means that the nitrate can be separated from the rest of the nitrogen compounds common to soils.

(c) Immerse tip of water-jacketed condenser into beaker containing 50 ml of 0.1 normal HCl. Add 5 grams of Devardas Alloy (75% passing a 200-mesh screen, 100% passing a 100-mesh screen) through the second neck on the flask and immediately restopper. Stir solution for 15 to 20 minutes without heating to reduce nitrate to ammonia.

Devardas Alloy (50% Cu, 45% Al, and 5% Zn) is a reducing agent which reacts with nitrate to form ammonia. If the Devardas Alloy is not ground to a fine powder, reduction of nitrate to ammonia may be incomplete. Without complete reduction, an isotopic fractionation occurs.

Three ground-water samples and three soil samples had negative del N15 values (table 2), whereas all other samples from this study had positive values. Slight variations in the methods of collection or analysis may have caused these negative values. The first analyses of all six negative samples were made using a coarse-grained Devardas Alloy. When resampled and prepared with fine-grained Devardas Alloy, all the soil and water nitrate samples were positive.

The negative del N15 ground-water samples were collected from deserted barnyard wells which had not been pumped for at least one year. Previous sampling showed high nitrate concentrations (Kreitler, 1972). Upon resampling, the wells were pumped from three to four hours, and a fine-grained Devardas Alloy was used in the analyses. The del N15 values as well as the nitrate concentrations drastically increased with the second analysis. The negative del N15 and the lower nitrate concentrations may indicate chemical reactions occurring in the well bore or biologic or nonbiologic denitrification, but more likely they indicate an incomplete reaction caused by using coarse-grained Devardas Alloy. This incomplete reaction would preferentially select isotopically light NO_3.

Upon reanalyzing the three soil samples with negative del N15 values, the same sample or a soil sample one foot above or below the depth of the original sample was used. With the second group of analyses, the soil nitrate of the samples had positive del N15 values. Analyses of the first set of samples were made with a soil slurry of soil and water and with coarse-grained Devardas Alloy. In the analyses of the second samples, the soil slurries were centrifuged to separate the soil and water, and fine-grained Devardas Alloy was used.

Interference of clay minerals in the soil slurry did not cause the observed isotopic fractionations. Keeney and Bremner (1966) analyzed soil slurries for ammonium, nitrate, and nitrite, and found that their direct distillation techniques were within 1 mg/l of their extraction and distillation method. The writer concludes that the negative del N15 values can be attributed to the coarse-grained nature of the Devardas Alloy and not the duration of well pumping nor the use of a soil slurry.

(d) Redistill the solution until 75 ml of distillate are collected in the 0.1 normal HCl solution. Titrate the acidic ammonium solution with 0.1 normal NaOH to determine the amount of nitrate in the original sample. As soon as the end

Table 2. Experimental fractionation of ground-water nitrate and soil nitrate by coarse-grained Devardas Alloy.

Ground water

Well Number[a]	Frequency of Pumping	NO_3 (mg/l)	del N15 (ppt)	Devardas Alloy
141	not pumped	141	- 3.1	coarse grained
141	pumped	211	+7.5	fine grained
388	not pumped	63	- 6.0	coarse grained
388	pumped	978	+10.3	fine grained
1004	not pumped	186	- 1.8	coarse grained
1004	pumped	250	+10.4	fine grained

Soil

Location[b]	Depth (ft)	Owner	Land Use	del N15 (ppt)	Devardas Alloy
o	1	Beimer	turnrow	- 0.2	coarse grained
o	2	Beimer	turnrow	+6.7	fine grained
n	3	Beimer	cottonfield	- 2.0	coarse grained
n	3	Beimer	cottonfield	+8.6	fine grained
n	6	Beimer	cottonfield	- 0.5	coarse grained
n	5	Beimer	cottonfield	+6.2	fine grained

[a]Locations in appendix 2 and figure 8.

[b]Locations in appendix 3 and figure 8.

point is reached, immediately reduce the pH to 2 by adding concentrated HCl to prevent loss of ammonia.

The second distillation is collected in 50 ml of 0.1 normal HCl and is titrated with 50 ml of 0.1 normal NaOH. The distilled ammonia absorbs an equal molar quantity of H^+ to form ammonium by the following reaction:

$$NH_3 + H^+ \rightleftharpoons NH_4^+$$

The NaOH is titrated against the excess of H^+ to determine the distilled ammonia or the original nitrate concentration. The number of moles of NH_3 distilled is calculated by the following equation:

$$mNO_3 \text{ or } mNH_3 = (\text{ml} \times 0.1 \text{ N HCl}) - (\text{ml} \times 0.1 \text{ N NaOH})$$

The solutions of 0.1 normal HCl and 0.1 normal NaOH are too acidic and too basic respectively, causing a loss in precision. The relatively concentrated solution (0.1 normal) of HCl was used to insure that all ammonia was absorbed, but solutions of 0.05 normal would have been adequate. This concentration of H^+ would have covered the range of ammonia concentrations analyzed with a resulting increase in precision. Nitrate concentrations determined by the titration method were used in preference to nitrate activities observed with an ion electrode because of the interference of HCO_3^- and Cl^- in the electrode measurement. The distillation technique is not subject to interference from either organic or inorganic compounds (Bremner and Keeney, 1966).

After the second distillation, the apparatus is disassembled and cleaned with Alconox, distilled water, and reagent-grade ethanol. This cleaning prevents the cross contamination of samples. Cleaning techniques are derived from Bremner and Edwards (1965, p. 507).

(e) Concentrate the solution to 5 to 10 ml by heating on a hot plate at 70°C to 80° C.

To avoid the loss of ammonia, acidic ammonia chloride solutions are never boiled. Similarly, samples are never evaporated to dryness because of the potential for isotopic fractionation.

(f) Add the ammonium chloride solution (less than 25 mg NH_4) to the large volume side of a dog-legged tube and add approximately 5 ml of sodium hypobromite to the small side arm (fig. 2).

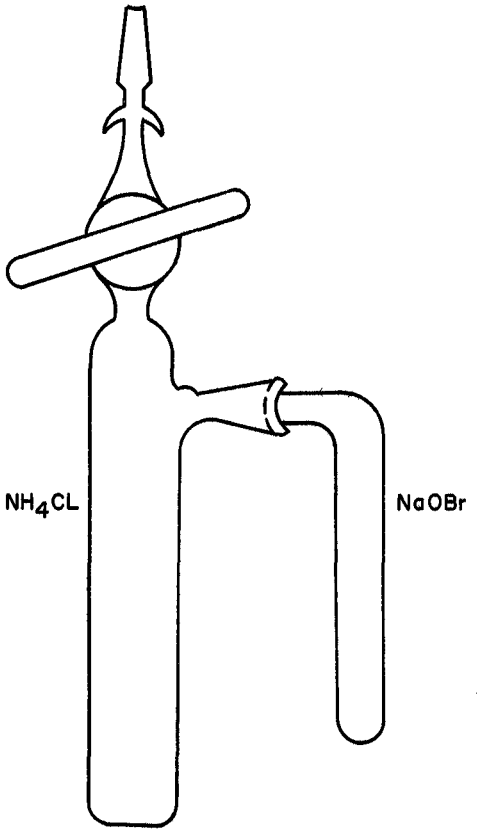


Figure 2. Nitrogen generation tube, modified from Rittenberg (1946) and Balestrieri (1968).

Attach the sample reaction tube to the nitrogen purification vacuum system (fig. 3). Freeze, evacuate, and thaw the two solutions to degas any atmospheric nitrogen from them. After the third thawing, rotate the small side 180° and drain the sodium hypobromite-iodate solution into the ammonium chloride solution. The resulting reaction will liberate nitrogen gas.

The freeze-thaw method for degassing the NH_4Cl solution eliminates atmospheric nitrogen. Table 3 shows the effectiveness of this technique.

Table 3. Effectiveness of degassing sample of atmospheric nitrogen by freeze-thaw technique as analyzed by a mass spectrometer (from Balestrieri, 1968).

Number of Freezing	N_2^{28} [a]	O_2^{16} [a]
1	8381.3	1787.7
	7613.2	1555.1
	7665.7	1586.0
	8224.0	1596.3
	8363.2	1796.6
	8518.0	1735.1
2	4770.6	1777.7
	6790.4	2507.7
	6645.0	2426.6
	2421.4	806.5
	8095.3	2678.3
	6027.1	2178.6
3	215.4	124.2
	193.7	118.2
	255.6	147.6
	213.1	114.7
	124.1	64.0
	187.9	120.7
4	B.L.D. [b]	B.L.D. [b]

[a] arbitrary units

[b] below limits of detection

Atmospheric nitrogen contamination of the sample can be determined by measuring the argon peak with the mass spectrometer. Argon present above background levels is the result of atmospheric contamination. The percentage of contamination can be calculated by comparing the argon 40 peak-nitrogen 28 peak ratio of the sample to the argon 40 peak-nitrogen 28 peak ratio of the standard. The oxygen 32 peak cannot be used for calculating the amount of contamination since part of the oxygen may be derived from the hypobromite reaction (Balestrieri, 1968). Because of the relatively large NH_4 samples used in this study, atmospheric nitrogen contamination was considered insignificant, and the argon peak was not checked. The argon peak should be checked for nitrogen isotope studies where the concentration of the nitrogen species is very low (such as surface water where the NO_3 concentrations rarely exceed 1 mg/l).

A dry-ice and M-17 (organic solvent) cold trap is used for freezing instead of a liquid nitrogen cold

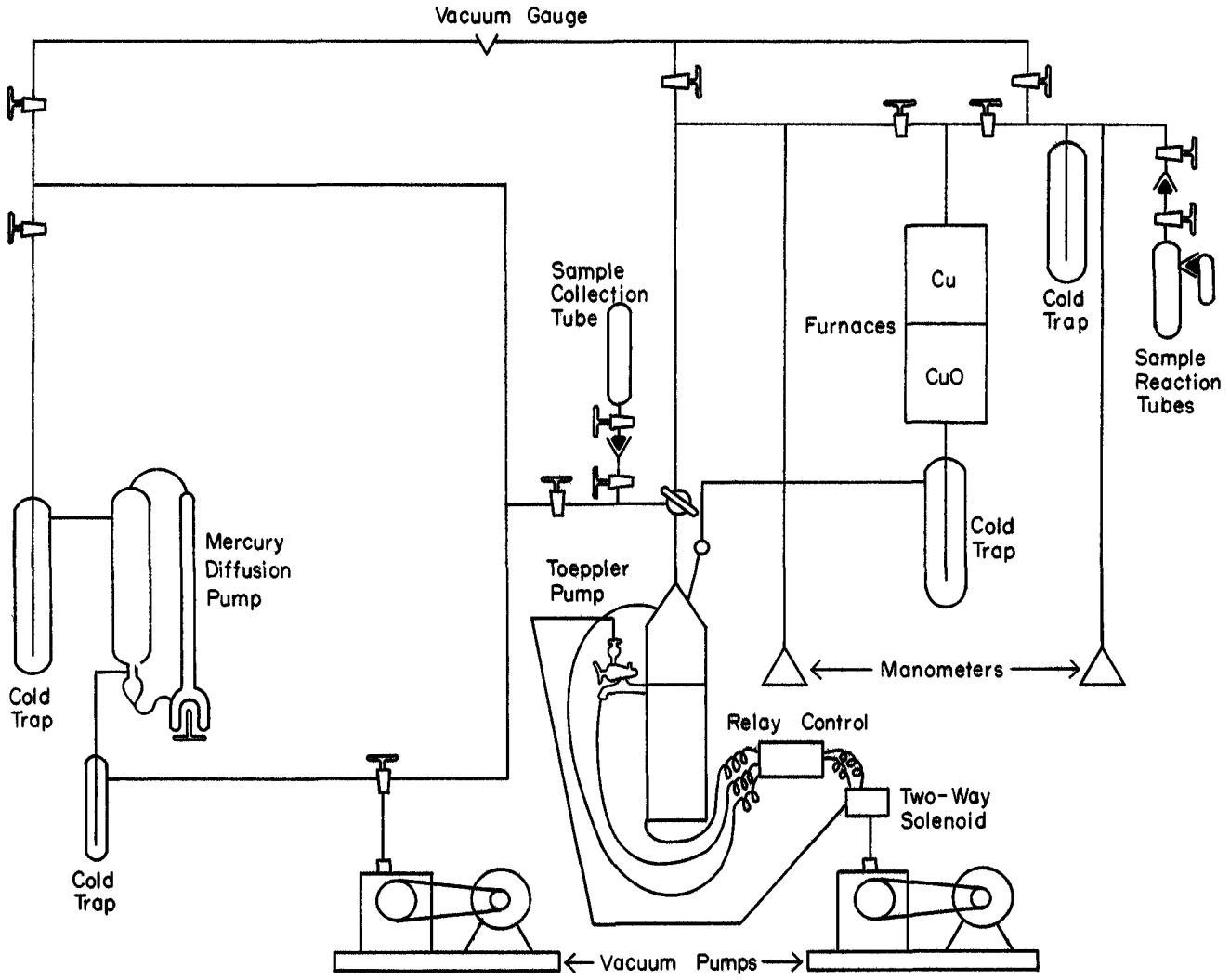


Figure 3. Apparatus for purification of nitrogen gas.

trap because the liquid nitrogen cracks the Pyrex glass of the reaction vessel by the rapid expansion of freezing water. Dry-ice cold traps do not cause this problem.

Sodium hypobromite reacts with ammonium chloride liberating nitrogen gas by the following reaction:

$$2\ NH_3 + 3\ NaOBr \rightleftharpoons 3\ NaBr + 3\ H_2O + N_2$$

The use of sodium hypobromite carries two inherent problems. One of these problems is that sodium hypobromite tends to decompose and liberate oxygen (Bremner and others, 1966):

$$2\ NaOBr \rightleftharpoons 2\ NaBr + O_2$$

This problem is eliminated by using an excess of NaOBr in the oxidation reaction. If the solution remains yellow (the initial color of the NaOBr solution) after the reaction, an excess of NaOBr is present, and the reaction has gone to completion.

The second problem is that the oxidation reaction is not quantitative. Bremner and others (1966), Riley and others (1954), and Capindale and Tomlin (1957) all observed the formation of nitrous oxide as well as nitrogen gas. Nitrous oxide is eliminated by freezing in a liquid nitrogen cold trap. Erratic isotopic fractionation caused by the incomplete oxidation is not present. The repro-

ducibility of standard $CaNO_3H_2O$ and NH_4Cl solutions (table 4) confirms the absence of experimental error.

Table 4. del N15 reproducibility of analyses of replicate $CaNO_3H_2O$ and NH_4Cl solutions.

	$CaNO_3H_2O$ del N15 (ppt)	NH_4Cl del N15 (ppt)
	+1.30	+1.64
	+0.97	+0.55
	+0.58	+0.95
	+0.54	+3.30
	+0.72	+1.17
	+1.49	+0.36
	+0.69	+1.00
	+2.40	
	+1.20	
	+0.47	
Number of samples	10	7
Mean del N15 (ppt)	+1.04	+1.22
Standard deviation (ppt)	0.59	1.06

(g) Circulate the nitrogen gas through the hot Cu (T = 400°C), hot CuO (T = 800°C), liquid nitrogen cold trap, vacuum system for four hours to eliminate any oxygen, carbon monoxide, organic material (carbonaceous and nitrogenous), and any gaseous oxides of nitrogen. Collect the purified N_2 in an evacuated sample tube.

Cu-CuO furnaces are used for gas cleaning because of possible contamination by carbon monoxide (T. Hoering, personal communication, January 1972). Carbon monoxide nominally has the same atomic weight as nitrogen gas and would, therefore, introduce an erratic error. The copper reacts with free oxygen to form a CuO precipitate. The copper oxide oxidizes carbon monoxide to carbon dioxide, which subsequently "freezes out" in the liquid nitrogen cold trap. The furnace temperatures were obtained from Hoering (1955). The circulation time of four hours was based on work of Myaka and Wada (1967). The mass spectrometer trace of the nitrogen samples did not drift, which would have indicated possible contamination. The circulation of the nitrogen gas for one hour would probably be sufficient. Myaka and Wada (1971) circulated nitrogen gas for only one hour using a molecular sieve method of gas transfer.

(h) Determine the nitrogen isotope ratio with a double collector, gas source, mass spectrometer, using atmospheric nitrogen as a standard.

Validity of del N15 Data

The validity of this study depends on both accuracy[2] and precision[3] of the values obtained for del N15. First, the measurement of nitrogen isotopes with the mass spectrometer must be demonstrated to be correct; second, the techniques used for sample preparation must be both accurate and precise.

The precision of the mass spectrometer and thus the precision of the standard is determined by comparing four atmospheric nitrogen samples to another atmospheric sample (table 5). Little isotopic variation was detected among samples collected at different locations and at different times. Little isotopic variation exists among these samples and the atmospheric nitrogen analyzed by Junk and Svec (1958). Kent Murman (personal communication, June 1973) has found only small isotopic variation among atmospheric nitrogen samples.

Accuracy in stable isotope mass spectrometry is only relative because samples are compared to a standard. An interlaboratory correlation of standards must be made because isotopic measurements by one researcher are of reduced value if they cannot be duplicated in another laboratory. If the mass spectrometer used in this study measured the absolute abundance of N^{15} in the atmosphere at any other value than 0.3663 atom-percent, then all other measurements would deviate by a similar amount. Therefore, it is desirable to compare results with another laboratory. This was accomplished by determining the del N15 value of a sample measured on another mass spectrometer. Dr. Harry Svec, Ames Laboratory, University of Iowa, kindly sent the writer a flask of Mathewson prepurified nitrogen tank gas which he had previously analyzed. The average atmospheric nitrogen sample (Ames, Iowa air) of Junk and Svec (1958) was 3.01 ppt heavier than their Mathewson prepurified nitrogen tank gas. The writer found that

[2]Accuracy is deviation of the observed value from the absolute value.

[3]Precision is the degree to which analyses of identical samples can be reproduced.

Table 5. del N15 of atmospheric nitrogen and of Mathewson prepurified tank nitrogen.

Source	Author	Location	Elevation	del N15 (ppt)
Air	Kreitler (this report)	Austin, Texas	surface	+0.05
	Kreitler (this report)	Austin, Texas	surface	- 0.01
	Kreitler (this report)	Austin, Texas	surface	0.00
	Kreitler (this report)	Austin, Texas	surface	- 0.22
	Junk and Svec (1958)	Leavenworth, Kansas	18,000 ft	- 0.27
	Junk and Svec (1958)	Des Moines, Iowa	36,000 ft	- 0.27
	Junk and Svec (1958)	Ames, Iowa	surface	0.00
	Junk and Svec (1958)	Moosonee Bay, Ontario	surface	- 0.14
Mathewson prepurified tank nitrogen	Kreitler (this report)			- 2.92
	Kreitler (this report)			- 2.88
	Junk and Svec (1958)			- 3.01

atmospheric nitrogen from Austin, Texas, was 2.87 ppt to 2.92 ppt heavier than the same Mathewson prepurified nitrogen tank gas from the same tank used by Junk and Svec in 1958 (table 5). This is a difference of only 0.11 ppt which is within the precision of the mass spectrometer. It is assumed, therefore, that the standards used in this study are accurate.

To check the reproducibility of the sample preparation techniques, the writer analyzed ten samples of $NaNO_3$ solution and seven samples of NH_4Cl solution (table 4). The NH_4Cl samples had a mean del N15 of +1.22 ppt with a standard deviation of 1.06 ppt. The $NaNO_3$ samples had a mean del N15 of +1.04 ppt with a standard deviation of 0.59 ppt. Eight samples of $(NH_4)_2SO_4$ analyzed by Commoner (1970a) had a mean del N15 of +0.71 ppt and a standard deviation of 1.21 ppt (table 6). This is in close agreement with the writer's NH_4Cl and $NaNO_3$ data. Experimental error is approximately ± 1 ppt.

Table 6. del N15 reproducibility of analyses of replicate KNO_3 and $(NH_4)_2SO_4$ solutions (from Commoner, 1970a).

	KNO_3 del N15 (ppt)	$(NH_4)_2SO_4$ del N15 (ppt)
	- 2.20	- 1.62
	+0.54	+1.62
	+3.50	+1.62
	+0.54	+0.80
	+1.30	+0.30
	+1.10	+1.90
		0.00
		0.00
Number of samples	6	8
Mean del N15 (ppt)	+0.87	+0.71
Standard deviation (ppt)	1.12	1.21

Collection of Soil Samples

The writer analyzed 54 samples (1 to 2 kg) of 120 soil samples collected every foot to depths of five to ten feet from 19 holes drilled with a rotary air drilling rig. Samples were stored in an air conditioned room (25°C) before the summer of 1973 and in a freezer locker at a meat packing plant (below 0°C) from the summer of 1973 until the fall of 1973. The writer made no attempt to adjust the soil moisture content or to heat the soils as is done in soil incubation studies.

Samples were leached overnight with de-ionized or distilled water. After decanting the clear liquid, the soil slurry was centrifuged for maximum recovery of the nitrate solution.

Edwards (1973) opposed the use of stable nitrogen isotopes in soil nitrogen studies because of the lack of precision among the various authors caused by different times of incubation. Incubation is a method which is used to stimulate the oxidation of soil humus in the laboratory and to generate enough nitrate for nitrogen isotope analysis. With one method of incubation, the water moisture content of the soil is raised to 50 percent of its water-holding capacity, and the soil is heated

to approximately 30° C (Bremner and Tabatabai, 1973). Analyses of 10 replicate soil samples incubated for two weeks by Edwards (1973) had a mean del N15 value of 3.54 ppt with a standard deviation of 0.76 ppt. A 22-week incubation of soils yielded del N15 values of nitrate which, in respect to atmospheric N_2, had both negative and positive results (Bremner and Tabatabai, 1973). Kohl and others (1971) found that NO_3 from virgin soil had a del N15 of +13 ppt. However, neither incubation time nor soil sample location were stated in the Kohl and others (1971) report. Edwards (1973) suggested that if scientists use a constant incubation period, results will be more reproducible.

The lack of reproducibility of data as reported by Edwards (1973), Bremner and Tabatabai (1973), and Kohl and others (1971) has not been a problem in this study because incubation was not used. The consistency of the del N15 values of nitrate from the soils shows that no significant experimental error was introduced.

In most of the del N15 profiles (e.g., fig. 4), the del N15 values remain constant with depth,

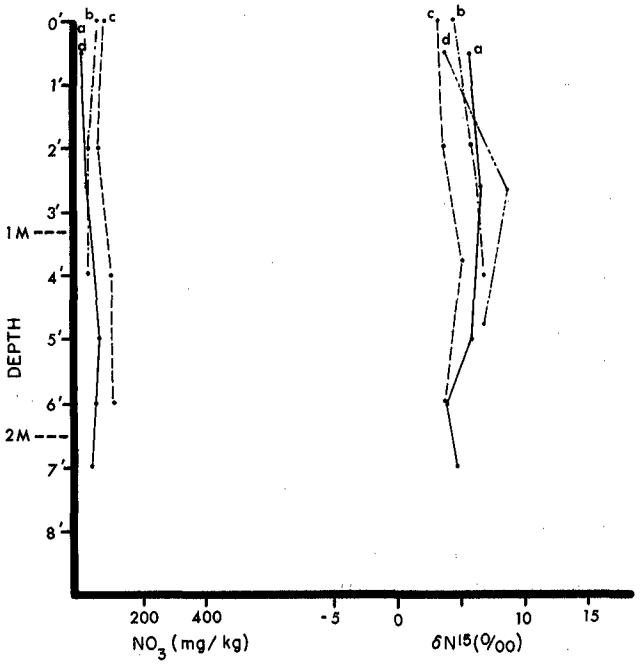


Figure 4. Nitrate and del N15 versus depth beneath a cottonfield with no animal wastes.

indicating that differential incubation has not taken place in the laboratory. In the deeper part of the soil profiles (deeper than one meter), insufficient organic nitrogen and nitrifying bacteria will prevent incubation. In the upper meter, the soils are biologically active and should have adequate concentrations of organic nitrogen for mineralization. Conceivably, accidental incubation by storage at room temperature may have occurred with the shallow samples, but the consistency of del N15 with depth suggests that this has not happened. Owing to the low nitrate concentrations, the entire soil sample from a selected depth was used for the initial analysis. Therefore, replicate samples could not be run.

The del N15 of nitrates from similar soil environments remains in the same range regardless of whether the soil samples were stored frozen or at room temperature. Profile a on figure 4 represents del N15 values of nitrate from nonfrozen soils, whereas profile b on figure 4 represents del N15 values of nitrate from frozen soils. There is no appreciable isotopic difference. Again, incubation of the unfrozen samples has not occurred. The del N15 values for the soil nitrates are considered valid because preparation techniques, collecting techniques, and mass spectrometry show good reproducibility.

Collection of Water Samples

Water samples were collected in collapsible one-quart polyethylene bottles. Most samples were from frequently pumped wells. For this reason, most wells were not pumped more than five to ten minutes prior to the collection of the sample. Infrequently used wells were pumped for at least one hour before samples were collected. Bailed samples were taken from wells without pumps.

Samples were stored untreated at room temperature for periods of a few days to more than one year. No mercurial compounds or acids were added to inhibit algal growth. Table 7 shows the analyses of duplicate samples stored for different intervals of time. Water samples 865b and 865c showed no appreciable isotopic variation within three months. Analysis after storage for one year showed a change in del N15 (e.g., water samples 369, 867, and 1034). Storage of a few months does not cause significant isotopic variation, but storage for long periods of time is not recommended.

Table 7. del N15 reproducibility of analyses of replicate samples of ground-water nitrate.

Sample Number	del N15 (ppt)	Time Interval Between Analyses	Difference (ppt)
865b	+13.0	3 months	0.2
865c	+13.2		
369a	+7.0	1 year	0.7
369b	+7.7		
1034a	+12.0	1 year	3.1
1034b	+8.9		
867a	+10.4	1 year	1.5
867b	+8.9		

Collection of Ammonia Gas Samples

The writer collected and analyzed three gaseous ammonia samples in an attempt to determine the isotopic fractionation controlling the del N15 range of nitrate from animal waste. Ammonia constitutes 94 to 98 percent of the nitrogenous compounds evaporating from barnyards. Certain amines represent the remaining 2 to 6 percent of volatile nitrogen compounds (Mosier and others, 1973). The small addition of volatile amines is assumed to have little effect on the isotopic composition of the gaseous ammonia collected.

The gases were collected by placing a one-gallon plastic bucket over soil freshly saturated with cow urine. A plastic hose ran from the top of the bucket to a flask containing a 0.1-normal hydrochloric acid solution. Another hose ran from the flask to a small vacuum pump (fig. 5). Gases volatilizing from the urine-soaked ground were pumped through the acid solution, and ammonia was trapped in the solution. Gas was collected from each site for approximately two hours. Samples of the urine-soaked soils were then collected, and nitrate leached from these soils was subjected to N^{15}/N^{14} analysis.

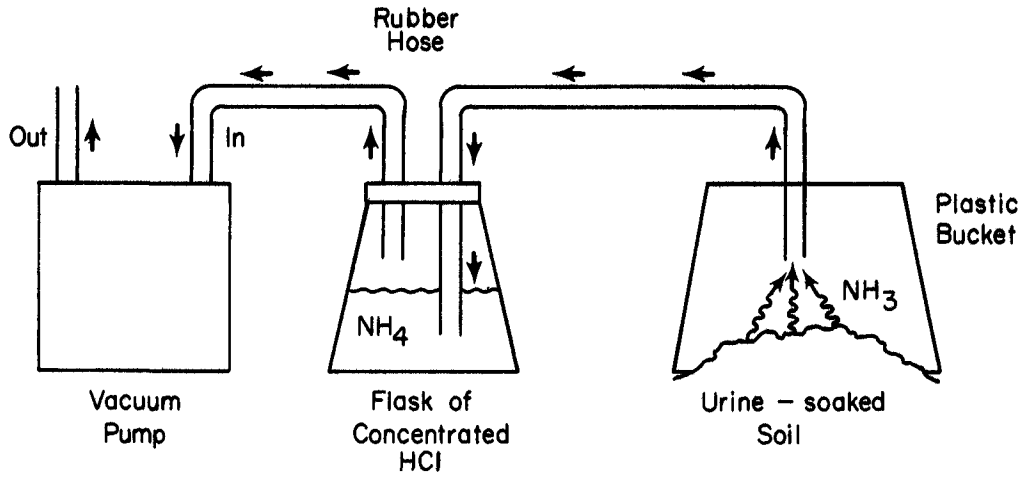


Figure 5. Apparatus for collecting gaseous ammonia.

Alternate Methods of Study

In order to use nitrogen isotopes to trace ground-water nitrate, the nitrogen isotope geochemistry of the soil system needs to be understood. Four approaches can be taken: (1) study the isotopic fractionations of specific reactions, e.g., nitrification; (2) identify the del N15 values of all the nitrogen species in a closed system; (3) analyze the del N15 of nitrogen from different sources, for example, the del N15 of the nitrogen in fertilizer or the nitrogen in animal waste material; and (4) identify the del N15 of NO_3 from different soil environments. Only the fourth approach is considered appropriate for this study, as the other three approaches do not directly help in tracing nitrate in ground water to its source.

The first approach, the study of isotopic fractionation of specific reactions, is needed for a complete understanding of the nitrogen cycle in the soils. The work of Hoering and Ford (1960) on nitrification, Wellman and others (1968) on denitrification, Myaka and Wada (1971) on nitrification and denitrification, and Delwiche and Steyn (1970) on nitrification, nitrogen fixation, nutrient assimilation, and ion-exchange capacity in soils, are all valuable pieces of research. However, these studies were simplified, controlled experiments, and the applicability of their data to natural systems is difficult to evaluate.

The second approach, the identification of the del N15 values of all the nitrogen species in a closed system, has many of the same problems as the first approach. The del N15 values of the nitrogen species cannot be effectively compared to the del N15 of NO_3 in ground water, nor can the multitude of organic nitrogen species, as well as ammonium, nitrite, and nitrate, be easily separated and analyzed isotopically. The advantages of this approach are that a mass balance of the nitrogen in the soil can be calculated and that some of the isotopic fractionations can be determined. Cheng and others (1964) analyzed nine different nitrogenous species and found a wide isotopic variation, but did not calculate a mass balance nor identify any fractionations.

The third approach, the analysis of del N15 of nitrogen from different sources, cannot be used to trace ground-water nitrate because the technique assumes that no isotopic fractionation occurs as the original nitrogen compound is chemically altered to the nitrate form in the ground water. Kohl and others (1971) compared the del N15 of a nitrogen fertilizer to the del N15 of NO_3 in Decatur Reservoir in Illinois. They assumed that there was no isotopic fractionation as the nitrogen fertilizer was integrated into the soil nitrogen cycle and then leached as nitrate. This is one point on which Hauck and others (1972), Hauck (1973), and Edwards (1973) criticized Kohl and others (1971).

The approach taken in this study is to identify the del N15 of nitrate from different natural soil environments. This permits a direct comparison of the del N15 of nitrate in the soils to the del N15 of the nitrate in the ground water. The nitrate in a soil should be analyzed in samples collected from different depths to ascertain whether the del N15 remains constant as the nitrate is leached through the soil profile. This approach also simplifies analytical techniques because only one nitrogen species is analyzed. This approach has two disadvantages: (1) it provides little quantitative information about the nitrogen cycle in a particular environment, and (2) a worker may not know the total history of a natural soil.

Results and Discussion of del N15 of Nitrate in Different Soil Environments

The soil-nitrogen environments studied were barnyards, septic tank drain fields, cultivated fields where cattle had never grazed, cultivated fields where cattle had grazed, and turnrows between cottonfields. Soil samples were collected in southern Runnels County, Texas, and in the feedlot of Capitol Cattle Livestock Commission, Austin, Texas.

Kreitler (1972) reported that all the soil environments listed above, with the exception of cultivated fields, could have high nitrate concentrations. The turnrows, narrow dirt roads between cultivated fields used as turning areas for tractors and to bring farm equipment to the fields, had abnormally high nitrate concentrations but no obvious source of nitrogen. Soils in barnyard and

septic tank drain fields had abnormally high nitrate concentrations with an obvious source. Soils treated with artificial fertilizers were not considered because there is little nitrogen fertilizer used in Runnels County. Fertilizers are used sparingly in dryland farming because of their tendency to burn the crops with insufficient rainfall (C. T. Parker, Runnels County Agricultural Agent, personal communication, June 1970).

Soil samples were collected from sites previously sampled by Kreitler (1972) because the approximate nitrate concentrations and probable source of nitrate were already known. Sampling sites were located in as wide a geographic area as possible and in many different soil associations so that the del N15 ranges would represent the average for a large area. Hauck and others (1972), Hauck (1973), Bremner and Tabatabai (1973), and Edwards (1973) criticized Kohl and others (1971) for having only one datum point in establishing the del N15 of virgin soil covering 900 square miles with 50 different soil series. This type of problem has, hopefully, been avoided in this study. Figure 6 shows the geographic distribution of soil samples and the different soil associations sampled. Appendix 2 lists sample numbers, location, owner, depths of sampling, nitrate concentrations, and del

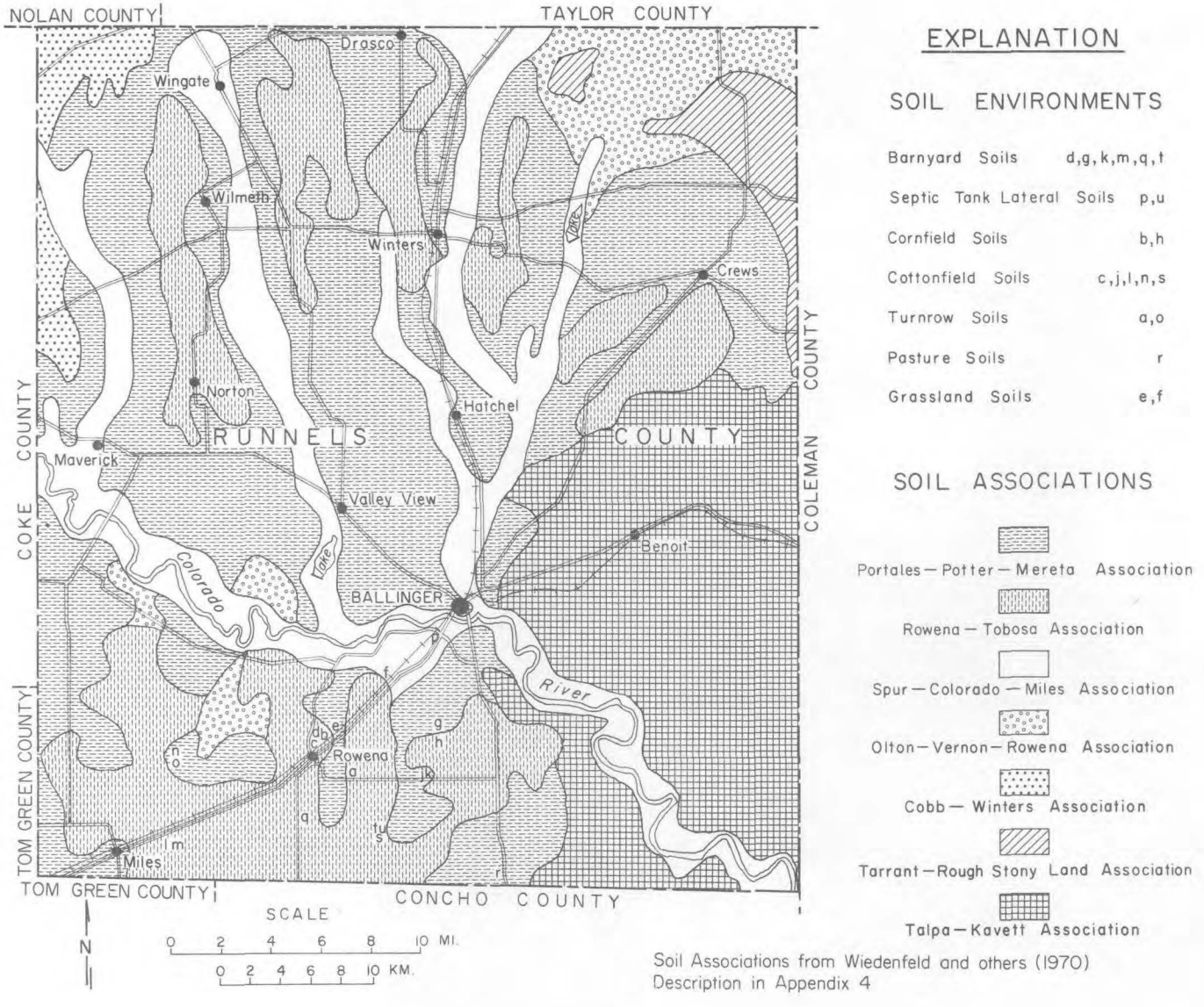


Figure 6. General soil association and soil sample location map, Runnels County, Texas.

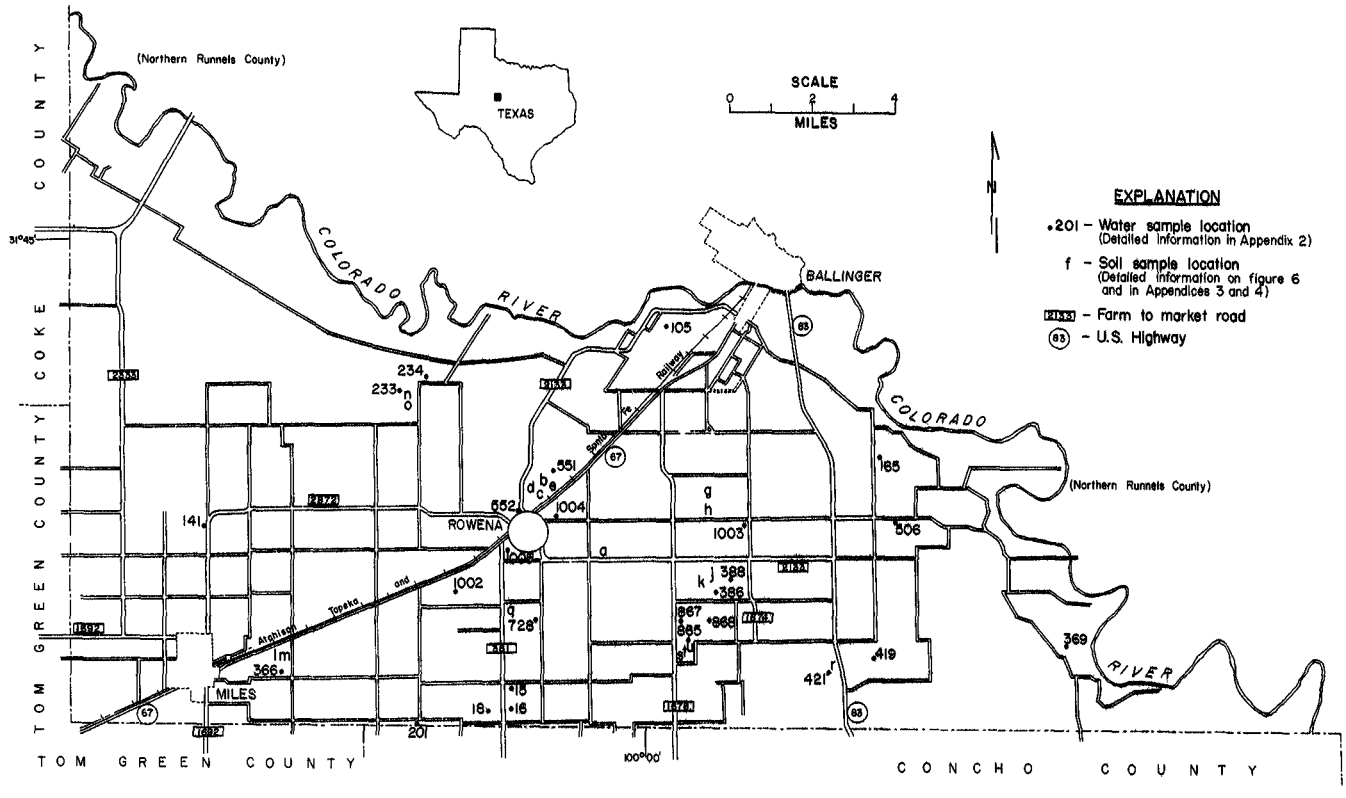


Figure 7. Location map for water samples and soil samples from southern Runnels County, Texas.

N15 values. Soil sample locations are shown on figure 7.

Nitrates in soils from barnyards and septic tank laterals.—Figure 8 compares del N15 and nitrate concentrations of a barnyard soil at various depths. The value of del N15, approximately +14 ppt, remains relatively constant with depth. The nitrate concentrations are high, similar to nitrate profiles of barnyards shown by Kreitler (1972). The higher del N15 at a depth of three feet, which appears to coincide with the nitrate peak at that depth, is not considered significant because the increase is only slightly greater than the experimental error.

Figure 9 shows the del N15 values and nitrate concentrations from five other barnyards. The location and depth of each sample is listed in appendix 3 and on figure 7. The five del N15 values are consistent with the del N15 values of figure 8. Figure 9 also shows the del N15 and nitrate concentrations of soils in septic tank drain fields. The del N15 values of these soils are greater than +10 ppt.

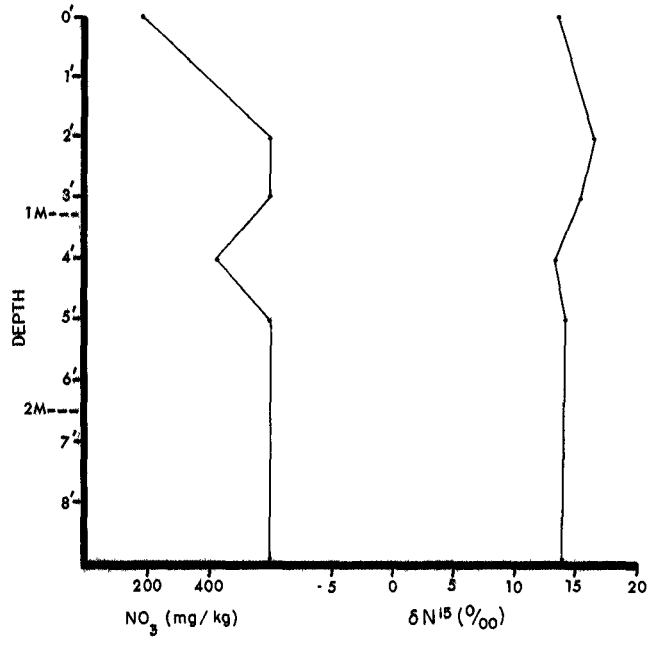


Figure 8. Nitrate and del N15 versus depth beneath barnyard with definite animal waste contribution of N. Soil sample location (d) (figs. 6 and 7).

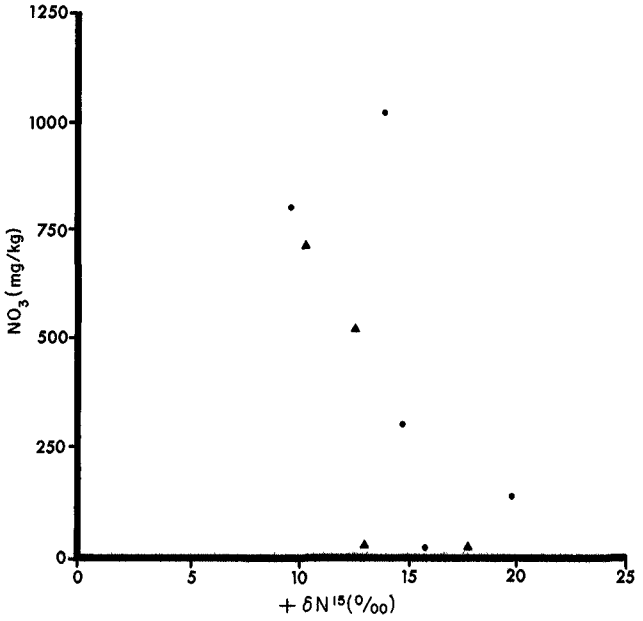


Figure 9. del N15 and NO_3 in soils from barnyards (●) and septic tank laterals (▲). Detailed data are listed in appendix 3.

The right side of figure 10 is a frequency distribution curve of the del N15 values of nitrate from barnyard and septic tank drain field soils in southern Runnels County, Texas.

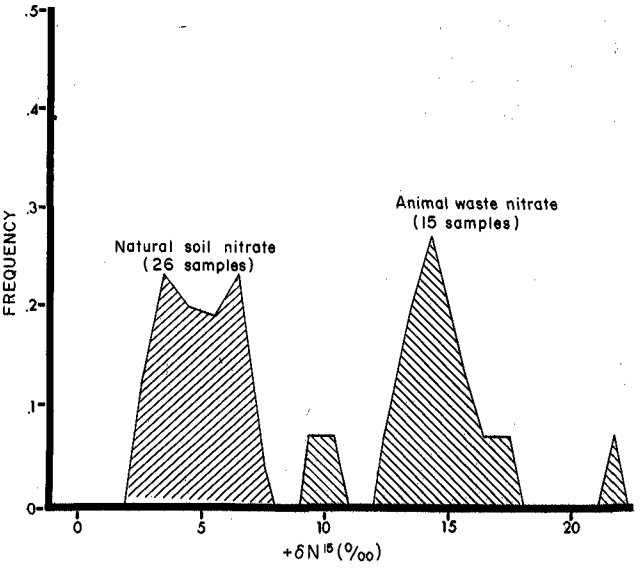


Figure 10. del N15 ranges of natural soil nitrate and animal waste nitrate (Runnels County, Texas). Frequency polygons have a class interval of one del N15 unit. Cumulative frequency of each curve is equal to 1.0.

Nitrates in soils from cultivated fields and turnrows with no history of cattle.—Profiles a, b, c, and d on figure 4 compare the del N15 and nitrate concentrations in soils from various depths in cottonfields where, according to the landowners, livestock have never grazed. These soils have low nitrate concentrations and lower del N15 than the del N15 of barnyard or septic tank nitrate. The del N15 of profiles a, b, and c on figure 4 remains constant with depth. There is no apparent correlation of del N15 with nitrate concentration, soil type, or geographic distribution of cottonfield soils.

Figure 11 shows the del N15 and nitrate concentration in soils from turnrows. Cattle have never grazed along these roads. These soils have similar del N15 values but higher nitrate concentrations in comparison to cultivated fields with no history of cattle. In both turnrow profiles, the del N15 remains constant with depth even though the nitrate concentrations vary greatly. The soils represented in figures 4b and 11b are within 30 feet of each other. The del N15 values in the lower parts of the profiles are similar. The difference in nitrate concentrations of these two soils reflects the complete lack of plant growth on the turnrows, in contrast to the nutrient assimilation by crops in the cultivated fields. In the planted fields, some of the soil humus is annually oxidized to nitrate

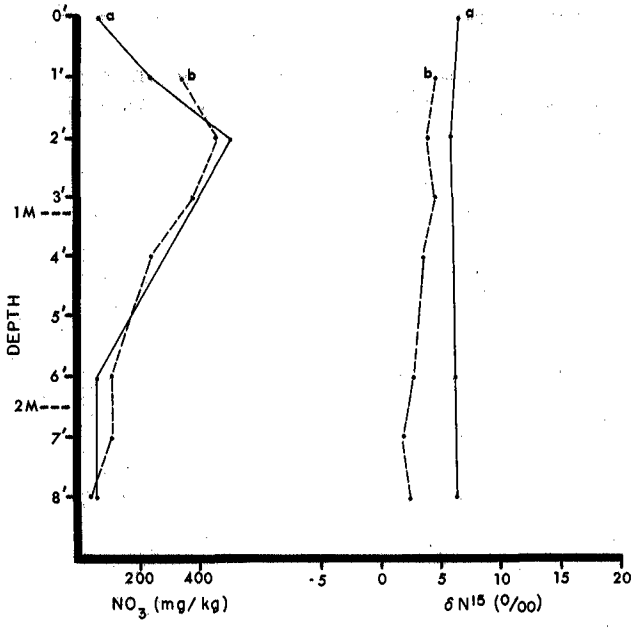


Figure 11. Nitrate and del N15 versus depth beneath turnrow with no animal waste contribution of N.

which is assimilated as plant nutrient. In the turnrow soil there is no utilization of the nitrate by plants, thus nitrate concentrations are high.

The left side of figure 10 is the frequency distribution of del N15 in the cultivated fields with no history of cattle and the turnrows with no history of cattle. These environments produce a lower isotopic range than the barnyard and septic tank soils.

Nitrates in soils from cornfields with grazing cattle.—Figure 12 is a del N15-NO_3 profile of a cornfield where cattle have grazed on the corn stubble. The owners could not remember how long cattle had grazed in these fields, but they had a "feeling" that it was for the past 10 to 20 years.

Figure 12 shows lower del N15 values with depth, but constant nitrate concentrations. The writer's interpretation of this profile is that the dominant source of nitrogen has changed with time. The nitrates deeper in the profile are older than the nitrates shallower in the profile. The del N15 of nitrate from shallow depths is in the range of animal waste material, whereas the del N15 in the deeper portion of the profile is in the range of natural soil nitrogen. The higher values represent nitrate accumulation during the years when cattle grazed on the corn stubble, whereas the lower del N15 values represent nitrate accumulation during the years before the cattle were grazing this field.

The soils which have a known source of nitrate can be divided into two categories: (1) soils with animal waste material as a dominant nitrate source, and (2) soils with a natural soil nitrate source with no contribution from animal waste material (fig. 10). These isotopic ranges do not overlap. Soils from fields with grazing cattle would be expected to have del N15 values between the del N15 of nitrate of animal wastes and the del N15 of natural soil nitrate, reflecting a mixing of nitrates from the two sources.

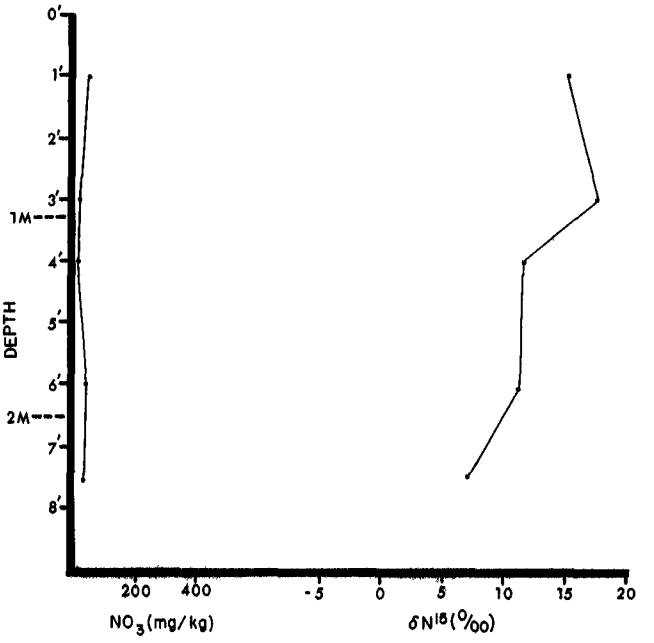


Figure 12. Nitrate and del N15 versus depth beneath cornfield with grazing cattle. Soil sample location (b) (figs. 6 and 7).

ISOTOPIC FRACTIONATION CONTROLLING del N15 OF NITRATE IN SOUTHERN RUNNELS COUNTY, TEXAS

Isotopic Fractionation and Its Role in Natural Systems

The isotopic ratio of a nitrogen compound is the result of (1) physical fractionation, (2) chemical equilibrium fractionation, (3) chemical kinetic fractionation, and (4) the isotopic ratio of the source material.

Physical fractionations occur through diffusion, evaporation, and sublimation (Ingerson, 1953). In diffusion, the lighter isotope will have a higher velocity. In evaporation and sublimation, the lighter isotope will have a higher vapor pressure (Bigeleisen, 1965), which favors the loss of the lighter isotope.

Chemical equilibrium fractionation (isotope exchange equilibrium) is the concentration of an isotope in one species of a chemical equilibrium reaction. The values of the isotope equilibrium constants are dependent on the different energy levels of the molecules (Urey and Greiff, 1935). The isotopic equilibrium constants (K or a)[4] have been determined experimentally and/or calculated from partition functions relevant to surface or near-surface conditions (table 8).

Chemical kinetic fractionations occur in non-equilibrium reactions which are typical of biogeochemical systems. When a reaction does not go to completion, the lighter isotope is often concentrated in the products while the heavier isotope is concentrated in the reactants.

Physical fractionation, chemical equilibrium fractionation, and chemical kinetic fractionation are all important in the isotopic fractionation of nitrogen in natural biogeochemical systems. However, their relative importance is only beginning to be ascertained.

Nitrogen Isotope Fractionations Related to Soils

The original nitrogen source for a soil system is atmospheric nitrogen. As the nitrogen moves through the soil, it can be fractionated by a variety of chemical reactions causing the intermediate nitrogen compounds to have different isotopic

[4]The calculated isotope equilibrium constant is "K", where:

$$K = \frac{(\text{Products})}{(\text{Reactants})}$$

The second reaction of table 8 is written:

$$1.035 = \frac{(N^{15}H_4)_{(aq)}\,(N^{14}H_3)_{(g)}}{(N^{14}H_4)_{(aq)}\,(N^{15}H_3)_{(g)}} = \frac{(N^{15}H_4/N^{14}H_4)}{(N^{15}H_3/N^{14}H_3)}$$

The experimentally determined isotope equilibrium constant is "*a*", where:

$$a = K^{1/n}$$

n is the number of equivalent exchangeable atoms in the reaction. In equations 1 through 6 (table 8), there is only one exchange atom, thus:

$$a = K$$

For reaction 2,

$$a = 1.034 = \frac{N^{15}/N^{14}\,(NH_4)_{\text{reactant}}}{N^{15}/N^{14}\,(NH_3)_{\text{reactant}}}$$

This equation states that there is a 34 ppt enrichment of N^{15} in NH_4 compared to NH_3. Further usage of the term *a* in this study is based on these conventions.

Table 8. Isotope equilibria constants.

Reaction	T°K	Isotope Equilibrium Constant	
		Calculated (K) (author)	Experimental (α) (author)
$N^{14}H_4 + \frac{1}{2}(N_2{}^{15}) = N^{15}H_4 + \frac{1}{2}(N_2{}^{14})$	298.1	1.023 (Urey, 1947)	not determined
$N^{14}H_4 + N^{15}H_3 = N^{15}H_{4(aq)} + N^{14}H_{3(g)}$	298.1	1.035 (Urey, 1947)	1.034 ± 0.002 (Kirshenbaum and others, 1947)
$N^{14}H_4 + N^{15}O = N^{14}O + N^{15}H_4$	298.1	1.038 (Urey, 1947)	not determined
$\frac{1}{2}(N_2{}^{14}) + N^{15}H_3 = \frac{1}{2}(N_2{}^{15}) + N^{14}H_3$	298.1	1.012 (Urey, 1947)	
$N^{14}H_{3(aq)} + N^{15}H_{3(g)} = N^{15}H_{3(aq)} + N^{14}H_{3(g)}$	298.1		1.005 (Ishimori, 1960)
$N^{15}H_{3(aq)} + N^{14}H_{4(aq)} = N^{14}H_{3(aq)} + N^{15}H_{4(aq)}$	298.1		1.029 (Ishimori, 1960)

compositions. The del N15 of soil nitrate may also be influenced by fractionations in animal and plant protein as well as soil reactions.

Nitrogen fixation.—Nitrogen fixation is the process by which organisms break the N_2 triple bond of elemental nitrogen gas and reduce it to ammonia. This requires an organic carbon source, pyruvic acid, for energy to break the N_2 bond and to donate electrons to the nitrogen atoms during reduction (Martin and Goff, 1972). In the soil ecosystems, symbiotic nitrogen fixation and nonsymbiotic nitrogen fixation both occur.

Nonsymbiotic nitrogen fixation is accomplished by a limited number of bacteria genera, e.g., *Azotobacter* and *Clostridium*. Hoering and Ford (1960) observed a kinetic fractionation (B)[5] of 1.0022

$$B = \frac{N^{15}/N^{14}\ (\text{atmospheric } N_2)}{N^{15}/N^{14}\ (\text{fixed nitrogen})}$$

[5]B is the kinetic fractionation factor for nonequilibrium reactions. No single convention for its usage has been established. Because of this, the equation defining B will be given for each kinetic fractionation discussed.

for nitrogen fixation with *Azotobacter vinelandii*. They considered this B factor small enough to mean no fractionation at all. With the same bacteria, Delwiche and Steyn (1970) measured a kinetic fractionation of 1.004

$$B = \frac{N^{15}/N^{14}\ (\text{atmospheric } N_2)}{N^{15}/N^{14}\ (\text{fixed nitrogen})}$$

indicating a slight preference of N^{14} in the fixed nitrogen during nitrogen fixation.

Symbiotic nitrogen fixation is characterized by rhizobium bacteria associated with leguminous plants. No isotope studies have been made of this type of fixation.

Nitrification.—Nitrification is the oxidation of ammonium to nitrate. The reaction is a two-step biological process. *Nitrosomonas* bacteria convert ammonium to nitrite; *Nitrobacter* bacteria then convert nitrite to nitrate. Delwiche and Steyn (1970) measured a kinetic fractionation of 1.026

$$B = \frac{N^{15}/N^{14}\ (NH_3)}{N^{15}/N^{14}\ (NO_2)}$$

for the oxidation of ammonium to nitrite with *Nitrosomonas europeae*. Myaka and Wada (1971) observed the depletion of N^{15} in nitrite during the oxidation of ammonium to nitrite with marine nitrifying bacteria. Studies of the second half of the nitrifying reaction have not been made because of the difficulty of analysis.

Denitrification.—Denitrification is a biochemical reduction of nitrate and nitrite to nitrogen gas or nitrous oxide. Nitrite and nitrate are hydrogen acceptors in this anaerobic reduction (Martin and Goff, 1972). Wellman and others (1968) measured a kinetic fractionation of approximately 1.02

$$B = \frac{N^{15}/N^{14}\ (NO_3)}{N^{15}/N^{14}\ (N_2)}$$

for denitrification with *Pseudomonas denitrificans*. Delwiche and Steyn (1970), using the same species, measured a kinetic fractionation of 1.0173.

Myaka and Wada (1971), using a marine denitrifying bacteria, measured a kinetic fractionation of approximately 1.02.

$$B = \frac{N^{15}/N^{14}\ (NO_3)}{N^{15}/N^{14}\ (N_2)}$$

The nitrogen gas produced during denitrification is depleted in N^{15}

Ion exchange.—Cation exchange resins (Dowex 50) and kaolinitic clay prefer $N^{15}H_4$ to $N^{14}H_4$; and B = 0.99926

$$B = \frac{N^{15}/N^{14}\ (\text{solution } NH_4)}{N^{15}/N^{14}\ (\text{adsorbed } NH_4)}$$

for cation exchange; and B = 0.99922

$$B = \frac{N^{15}/N^{14}\ (\text{solution } NH_4)}{N^{15}/N^{14}\ (\text{adsorbed } NH_4)}$$

for kaolinite.

Anion exchange resins (Dowex 1) prefer isotopically light nitrate; and B = 1.0021

$$B = \frac{N^{15}/N^{14}\ (\text{solution } NO_3)}{N^{15}/N^{14}\ (\text{adsorbed } NO_3)}$$

(Delwiche and Steyn, 1970). None of these reactions shows fractionations as large as nitrification (1.02), denitrification (1.02), or ammonia volatilization (1.034). They are probably not important in the soil nitrogen isotope system.

Ammonia volatilization.—Ammonia is the end product of two biological reactions in soil: nitrogen fixation and ammonification of organic nitrogen. Most soils are neutral or acidic, and the ammonia goes rapidly to ammonium ($NH_3 + H \rightleftharpoons NH_4$; K = 9.34). However, if any gaseous ammonia is lost from the system, an equilibrium isotope fractionation can occur with an α factor of 1.034:

$$N^{15}H_{3\,(gas)} + N^{14}H_{4\,(aq)} \rightleftharpoons N^{14}H_{3\,(gas)} + N^{15}H_{4\,(aq)}$$

$$\alpha = 1.034 = \frac{(N^{15}/N^{14})_{NH_4\,(aq)}}{(N^{15}/N^{14})_{NH_3\,(g)}}$$

The result will be a loss of isotopically light ammonia gas.

Nutrient assimilation.—Plants and some animals, such as a few bacteria, Eumycetes, and flagellates, assimilate most nitrogen as ammonium and nitrate. A few plants have the ability to absorb organic nitrogen. Delwiche and Steyn (1970) showed that bacteria preferentially use $N^{14}H_4$ over $N^{15}H_4$. Studies of the residual nitrogen after plant nutrient assimilation have not been made. Commoner (1970a) studied the fractionation of nitrogen isotope tracers within vascular plants, but there was total assimilation of the nitrogen tracer and no residual could be analyzed.

Fractionations within more complex plants and animals.—Nitrogen passes through plants and animals, thus important isotopic fractionations may occur within the organism. Gaebler and others (1966) observed an enrichment of N^{15} in amino acids from rat liver protein and rat muscle protein, relative to the isotopic composition of the ingested

amino acids (table 9). These variations were not caused by experimental error (Gaebler and others, 1963). The nonessential amino acids, those that animals are capable of generating, were more enriched in N^{15} than the essential amino acids, those that animals cannot generate. The fractionation is attributed to nitrogen transfer in the protein.

Myaka and Wada (1967) observed a gradual enrichment of N^{15} with higher trophic levels of marine organisms. The average del N15 of total nitrogen of phytoplankton and seaweed was +7 ppt, whereas the average del N15 of zooplankton and fish was +10 ppt and +15 ppt, respectively. As the organisms of higher trophic levels ingest the organisms of lower levels, the protein of the higher levels of the food chain become enriched in N^{15}. This suggests a fractionation similar to the N^{15} enrichment in protein observed by Gaebler and others (1966).

Commoner (1970a) found that in tobacco leaves, the alcohol-precipitable nitrogen in proteins and nucleic acids was isotopically lighter than the alcohol-soluble nitrogen in low molecular weight compounds such as amino acids, ammonia, and nitrate. Isotopic fractionations do occur within plants and animals. Their influence on the isotopic composition in the soil nitrogen system is not known.

Mineralization.—Decomposition or mineralization of organic nitrogen is the result of soil humus oxidizing to nitrate. The complex process involves deamination of proteins to amino acids, ammonification of amino acids to ammonia-ammonium, and nitrification of ammonium to nitrate. Laboratory incubation of soils to produce nitrate is supposedly a simulation of field conditions. Cheng and others (1964) found that after a two-week incubation, the del N15 of the nitrate was one-half to one-third more negative than the del N15 of the original total nitrogen. After 22 weeks of incubation, Bremner and Tabatabai (1973) found that half of the samples had negative del N15 values, while the other half were slightly positive. Edwards (1973) incubated replicate

Table 9. Accumulation of del N15 in proteins of rats (from Gaebler and others, 1966).

	del N15 in rat protein minus del N15 in dietary protein (ppt)			
Source of Nitrogen	Protasoy[a] Group		Caesin[a] Group	
Nonessential Amino Acids	Liver	Muscle	Liver	Muscle
Proline	+8.8	+4.1	+8.5	+3.5
Glutamic Acid	+7.7	+6.8	+4.6	+3.3
Alanine	+3.5	+5.7	+4.1	+4.8
Aspartic Acid	+3.0	+3.6	+3.8	+4.4
Arginine	+7.4	+3.6	+7.1	+3.0
Glycine	+3.8	+7.7	+5.8	+6.0
Serine	+9.3	+7.1	+5.2	+2.5
Tyrosine	+1.6	- 0.3	+1.6	- 0.1
Essential Amino Acids				
Phenylalanine	- 1.6	- 0.8	+1.9	0.0
Valine	+2.7	+5.8	+2.7	+5.2
Leucine	+1.3	+6.3	+3.8	+4.1
Lysine	+0.1	0.0	+2.5	- 1.1
Histidine	+3.0	+2.5	+4.1	+2.2
Threonine	- 2.7	- 7.4	- 3.3	- 4.4
Amide Groups	+4.6	+1.1	0.0	0.0
Amidine Groups	+8.8	+4.1	+3.0	- 0.1

[a]Dietary proteins

samples of soils for two weeks and found del N15 of the nitrate to be +4.9 ppt, whereas the del N15 for the original total nitrogen was +11.7 ppt. The results of these studies indicate that mineralization favors N^{14}. However, two other studies complicate the interpretation. Myaka and Wada (1971) measured the del N15 of the residual total nitrogen during the decomposition of *Scenendesmus*, a marine organism. In the first 20 days, the residual material became isotopically lighter, and then became slightly heavier for the duration of the experiment (120 days). In a 42-week incubation study, Feigin and Shearer (1973) found that the del N15 of the nitrate started low but eventually rose to the same value as the total nitrogen. They also found that the del N15 of the nitrate from nonincubated soil samples was significantly lighter than the del N15 values for the nitrate found during incubation. Table 10 lists the available isotopic data on incubation of soils.

Discussion of Isotopic Fractionations In Southern Runnels County, Texas

The isotopic ranges of the two soil nitrate environments observed in Runnels County, Texas, are controlled by the isotopic fractionations previously described. A detailed discussion of the important fractionations will describe which chemical reactions control the isotopic ranges.

Soils contaminated with animal wastes.—The predominant source of the nitrate in soils contaminated with animal waste material is urea from urine. Approximately 80 percent of excreted nitrogen is in urine (Spedding, 1971). Fifty to 85 percent of the urine is in the form of urea (table 11).

Urea is hydrolized to ammonia and carbon dioxide and then nitrified to nitrate:

$$CO(NH_2)_{2(g)} + H_2O_{(g)} \rightleftharpoons CO_{2(g)} + 2\,NH_{3(g)}$$

$$\Delta G^\circ = (-)\ 290 \text{ cal at } 25^\circ C$$

The Gibbs free energy (ΔG°) indicates that reaction will occur spontaneously, but experimentation indicates that the kinetics are very slow. The biochemical hydrolysis is much more rapid and is the normal reaction (Chin and Kroontze, 1963).

The hydrolysis of urea forms a weak acid (CO_2) and a strong base (NH_2). For every mole of carbonic acid formed, two moles of ammonium ion are created. This tremendous drain of H^+ ions causes the pH to rise and ammonia volatilization to occur. Doak (1952) measured pH changes during hydrolysis from an initial 5.5 to a maximum of 9.2. Stewart (1970) and Watkins and others (1972) also observed pH rises during urea hydrolysis. The ammonia-ammonium reaction has a K equilibrium of 9.34; that is, at a pH of 9.34 there will be an equal molar concentration of ammonia and ammonium. A rise in pH will thus cause a conversion of ammonium to ammonia, and subsequent volatilization. Ammonia losses from the hydrolysis of urea also occur at pH levels below 9.34. Ernst and Massey (1960) found 10 percent ammonia loss from a urea fertilizer at a pH of 5.0 and 50 percent ammonia loss of the same fertilizer at a pH of 7.5. A possible explanation for these phenomena is that the hydrolysis of urea creates microenvironments of high pH.

Table 10. Published data on the del N15 of total nitrogen of soils and incubated soil nitrate.

Reference	Total Nitrogen			Soil-incubated Nitrate			
	Number of Samples	Range del N15 (ppt)	Mean del N15 (ppt)	Number of Samples	Incubation Time (wks)	Range del N15 (ppt)	Mean del N15 (ppt)
Cheng and others, 1964	28	- 1 to +17	+6.3	5	2	+1 to +6	+2.2
Bremner and others, 1966	40	- 3 to +18	+6.2				
Delwiche and Steyn, 1970	39	+2 to +11	+5.4				
Kohl and others, 1971							+13
Riga and others, 1971	69	- 7 to +6	+2.6				
Bremner and Tabatabai, 1973	16	- 4 to +3	- 0.2	10	22	- 10 to +6	- 1.1
Edwards, 1973	10		+11.7	10	2.5		+4.9

Table 11. Nitrogen compounds in bovine urine (from Doak, 1952).

Nitrogen Compound	Percent of Total Nitrogen in Urine (%)		
	Calf (1 year old)		Cow
	avg	range	range
Urea - N	76.4	68 - 85	50.3 - 74.2
Alanine - N	4.1	2.9 - 5.2	4.0 - 6.4
Hippuric acid - N	2.6	2.1 - 3.1	1.9 - 6.0
Creatine - N	1.5	1.0 - 1.8	1.3 - 2.0
Amino - N	12.0	10.5 - 18.9	
Ammonia - N	0.7	0.5 - 0.9	
	Percent Total Nitrogen in Urine (%)		
	Calf (1 year old)		Cow
	avg	range	range
Total - N	8.68	5.7 - 12.0	2.5 - 8.3

Ammonia volatilization from soil is affected by temperature, air speed, and soil moisture content as well as by the pH. Higher temperatures cause greater ammonia losses (Volk, 1959; Watkins and others, 1972). Ammonia loss is directly proportional to air speed (Watkins and others, 1972). Soil moisture appears to be important in controlling ammonia losses. In one experiment, 90 percent of urea applied to dry soils was lost through ammonia volatilization, whereas only 25 percent of the urea applied to wet soils was lost through ammonia volatilization (Stewart, 1970). Quantifying the amount of ammonia lost during urea hydrolysis is not practical because of the number of variables involved, but there is no doubt that such loss does occur.

Ammonia volatilization appears to be a reaction controlling the isotopic composition of nitrate from animal waste material. The equilibrium isotope reaction

$$N^{15}H_{3\,(g)} + N^{14}H_{4\,(aq)} \rightleftharpoons N^{14}H_{3\,(g)} + N^{15}H_{4\,(aq)}$$

has an a factor of 1.034; that is, gaseous ammonia should be +34 ppt lighter than aqueous ammonium. The hydrolysis of urea in a barnyard soil should result in the loss of isotopically light ammonia gas. The nitrification of isotopically heavy ammonium will then form an isotopically heavy nitrate.

To check this hypothesis, the writer collected and analyzed three gaseous ammonia samples from urine-soaked soils in barnyards (table 12). The ammonia samples were consistently isotopically light (-21 ppt), and the average isotopic difference between the ammonia and the soil nitrate was approximately 38 ppt, which suggests that the equilibrium isotope reaction between $NH_{3\,(gas)}$ and $NH_{4\,(aq.)}$ is the controlling reaction. A kinetic isotope fractionation would cause a wide range of

Table 12. Ammonia volatilization.

Location	del N15	
	NH_3 (ppt)	NO_3 (ppt)
Barnyard, Runnels County, Texas	- 21.6	+13.8
Feedlot, Austin, Texas	- 21.0	
Feedlot, Austin, Texas	- 21.3	+19.5

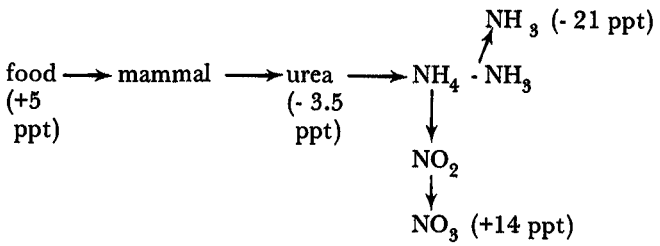
del N15 values for gaseous ammonia. The close correlation of a del N15 of +38 ppt from this study with Kirshenbaum and others (1947) del N15 of +34 ppt for the same reaction suggests that ammonia volatilization is a significant fractionation controlling the del N15 of the nitrate. Fractionation by nitrification or denitrification at the soil surface is not considered important because the del N15 difference would have to be significantly greater than +34 ppt if additional fractionation from nitrification occurs. Fractionation by denitrification deeper in the soil profile is not occurring because the del N15 remains constant with depth (fig. 8). Similarly, fractionations by chromatographic separations (of either NH_4 or NO_3) are not occurring because of the consistency of del N15 with depth.

Fractionations by reactions within mammals excreting urine may also be important in determining the del N15 of the soil nitrate. Gaebler and others (1966) showed that proteins become enriched in N^{15}. Presumably, excreted nitrogen would have to be isotopically lighter to maintain an isotope mass balance:

$$\text{Moles } N^{14} + \text{Moles } N^{15} \text{ (ingested)} =$$

$$(\text{Moles } N^{14} + \text{Moles } N^{15})_{\text{(protein)}} + (\text{Moles } N^{14} + \text{Moles } N^{15})_{\text{(excreted nitrogen)}}$$

A probable nitrogen pathway from ingested protein to nitrate is:



Using this diagram with some simple assumptions, it can be shown that isotopic fractionation is possible within the animal. Assuming that the only fractionation involved in the decomposition of urea to nitrate is ammonia volatilization (ammonia gas-ammonium equilibrium), then the original urea should have a del N15 value of -3.5 ppt:

$$\frac{\delta N^{15}(NH_3) + \delta N^{15}(NO_3)}{2} = \frac{(-21) + (+14)}{2} = -3.5 \text{ ppt.}$$

The ingested protein is probably +5 ppt, the del N15 of ingested protein used by Gaebler and others (1966). If the ingested protein is +5 ppt and the excreted urea is -3.5 ppt, the enrichment of N^{15} may occur within the animal. The assumption that ammonia-ammonium equilibrium (1 molecule NH_3 to 1 molecule NH_4) is the controlling reaction is not entirely arbitrary. The consistency of the del N15 values for gaseous ammonia suggests this and also indicates that a kinetic fractionation is not involved. The del N15 of the original urea then has to be approximately -3 ppt. An 8 ppt fractionation within the animal is only conjecture, however, because the urea accounts for about 50 percent of the excreted nitrogen (70 percent excreted N is in urine, 70 percent urine N is in urea). Nothing is known about the isotopic composition of the other 50 percent of excreted nitrogen. Most of this nitrogen will be in an undigested form in the feces and may not be important to the biochemical and isotope systems of the body.

The isotopic fractionations controlling the del N15 of soils contaminated by animal waste material appear to be ammonia volatilization and a reaction within the animal excreting the nitrogen. Isotopic fractionations caused by nitrification, denitrification, and chromatographic separation do not appear to be important.

Soils from cultivated fields with no animal wastes.—The predominant source of nitrate in cultivated soils with no animal waste or fertilizer is from the oxidation of soil humus turned over by plowing. Cultivated or fallow fields have appreciably higher nitrate concentrations than grasslands such as pastures and virgin prairies (Smith and Vandecaveye, 1946; Thompson and others, 1954; Haas and others, 1957; Kononova, 1961; Vazhenin and Vazhenina, 1969). The enriched N^{15} tracer study of Bartholomew and Clark (1950) showed a faster breakdown of the stable humus in cultivated soils than in uncultivated fields. Figure 13 shows the decrease in organic nitrogen in cultivated soils as it is oxidized to nitrate. The low nitrate concentrations in grasslands have been attributed to toxic effects of grass roots on bacteria (Theron, 1951; Stiven, 1952; Boughey and others, 1964) and to direct competition by grasses for any NH_4 produced (Robinson, 1963). Aerobic bacteria are more numerous in cultivated soils than in grassland soils (Chase and others, 1967). Birch (1959a, b), Soulides and Allison (1961), and Moore and Russel (1970) have measured considerable increases in NH_4 and NO_3 by wetting and drying of aerated agricultural soils. Table 13 shows the much greater concentration of NO_3 in cultivated and fallow fields in comparison to grasslands.

Mineralization of soil humus in cultivated fields should occur throughout the year, but at greater rates in summer because of the higher temperatures. Birch (1959a) found that higher temperatures increased mineralization. However, higher nitrate concentrations occur during the fallow seasons, winter and early spring, because there are no crops to assimilate ammonium and nitrate (Soulides and Allison, 1961).

Nitrogen fixation rapidly increases the total nitrogen in fields planted with white or red clover (legumes), whereas in grasslands the total nitrogen increases very slowly. The increase is also attributed to nitrogen fixation, but the rates must be lower (Cooke, 1967).

Ammonium and nitrate are present in rain, but in low concentrations. Junge (1958) found

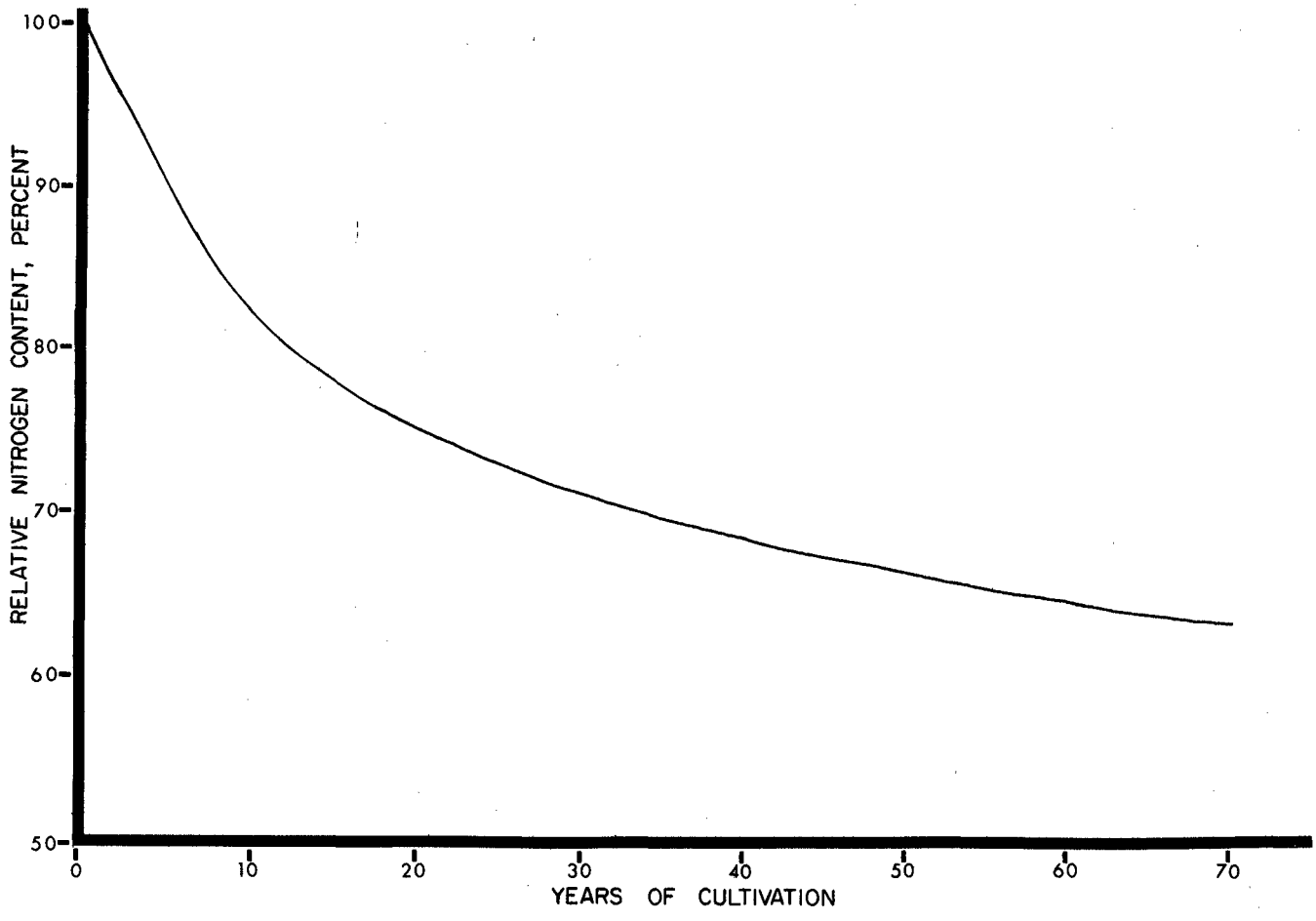


Figure 13. Decrease of total soil nitrogen with years of cultivation (from Millar and others, 1958).

ammonium concentrations of 0.01 to 0.12 mg/l and nitrate concentrations of 0.47 to 1.0 mg/l in rain in Texas in 1956. The source of most of the nitrate and ammonium in the atmosphere probably is wind erosion of cropland. Thus ammonium and nitrate in rainwater are probably only the result of recycling soil nitrogen back to the soil and not the addition of new nitrogen from the atmosphere. The nitrate in the soil is the result of the mineralization of the organic nitrogen of the humus and not the nitrogen produced by nitrogen fixation or precipitation.

The del N15 of nitrate from cultivated fields with no animal waste in Runnels County is +2 ppt to +8 ppt (fig. 10). This is in the same isotopic range as other published del N15 values for natural soil nitrate (Cheng and others, 1964; Bremner and Tabatabai, 1973; Edwards, 1973) (table 10). The nitrate data from these authors are the result of incubation and not field sampling. Cheng and others (1964), Bremner and Tabatabai (1973), and Edwards (1973) observed that total nitrogen of soil humus was isotopically heavier than the incubated nitrate. The close correlation between the data of this study and the incubated-nitrate data suggests that the mechanisms of fractionation are the same. Fractionation during mineralization (incubation) appears to be the mechanism for producing a lighter del N15 of the NO_3 from the heavier del N15 of the total nitrogen of the humus. The reactions of mineralization are nitrification and decomposition of the organic nitrogen of the humus to ammonium. The organic nitrogen in humus is either in protein or amino acid form and is the result of microbial biosynthesis of chlorophyllaceous plants and not residual plant material (Millar and others, 1958). There may be an isotopic fractionation between these metabolic byproducts and ammonia. Tables 13 and 14 indicate considerable mineralization of the nitrate ion with little accumulation of N as NH_4. This

Table 13. Dynamics of Nitrate-N, in chernozems of the Borgoi, Buryat, ASSR (N in mg/kg and NO_3 in mg/kg), 1955 (from Vazhenin and Vazhenina, 1969).

Depth (cm)	8 June		27 June		18 July		5 Aug.		18 Aug.		29 Aug.	
	N	NO_3	N	NO_3	N	NO_3	N	NO_3	N	NO_3	N	NO_3
Plowland, spring rye												
0-10	24.0	108.0	18.0	81.0	14.0	63.0	12.4	51.0	5.5	25.0	2.0	9.0
10-22	6.0	27.0	10.0	45.0	38.0	171.0	22.4	101.0	8.6	39.0	1.5	6.2
22-33	5.5	25.0	0.5	2.2	9.4	42.0	6.8	31.0	3.2	14.0	trace	
33-44	1.0	4.5	trace		0.5	2.2	3.8	16.0	2.8	13.0	trace	
44-67	trace		trace		0.5	2.2	trace		trace		trace	
67-87	trace		trace		trace		trace		trace		trace	
Virgin land (layland for many years)												
0-10	2.0	6.2	7.8	35.0	1.9	8.5	3.6	16.0	6.1	27.0	2.6	12.0
10-20	1.0	4.5	2.4	11.0	2.3	10.0	3.1	14.0	2.5	11.0	1.8	8.1
20-32	trace		2.4	11.0	trace		2.8	13.0	2.9	13.0	1.9	8.5
32-44	trace		2.2	9.9	trace		2.2	10.0	8.9	40.0	1.7	7.6
44-60	trace		2.2	9.9	trace		trace		2.1	9.0	trace	
60-78	trace		2.2	9.9	trace		trace		trace		trace	
Fallow												
0-10	24.0	108.0	26.0	118.0	20.8	94.0	36.0	162.0	6.8	30.0	19.2	87.0
10-22	6.0	27.0	20.0	90.0	52.8	238.0	46.0	207.0	18.0	81.0	13.7	62.0
22-33	5.5	25.0	7.0	31.0	22.0	99.0	22.0	99.0	66.0	298.0	16.9	76.0
33-44	1.0	4.5	0.5	2.0	6.8	31.0	4.8	21.0	18.0	81.0	18.0	81.0
44-67	trace		trace		2.9	13.0	1.0	4.5	13.4	61.0	21.3	96.0

Table 14. Dynamics of water-soluble ammonium-N, in chernozem soils, Borgoi, Buryat, ASSR, 1955, in mg/kg (from Vazhenin and Vazhenina, 1969).

Depth (cm)	8 June	27 June	18 July	5 Aug.	18 Aug.	27 Aug.
Plowland, spring rye						
0-10	2.2	1.2	1.5	1.0	1.3	1.0
10-22	1.8	1.2	1.2	1.0	1.0	1.2
22-33	1.5	1.0	<1.0	<1.0	1.2	1.0
33-44	<1.0	<1.0	<1.0	<1.0	<1.0	1.0
44-67	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
67-87	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Virgin land (layland for many years)						
0-10	2.1	2.9	3.0	2.6	1.8	2.3
10-20	1.8	2.8	2.8	2.5	1.6	1.8
20-32	1.5	1.6	2.4	2.1	1.2	1.3
32-44	1.3	1.1	1.2	1.0	1.0	1.0
44-60	1.2	1.0	1.0	1.2	1.0	1.2
60-78	<1.0	<1.0	1.0	1.0	<1.0	1.0
Clean fallow						
0-10	2.2	1.1	1.9	1.0	1.0	1.2
10-22	1.8	1.0	1.3	1.0	1.2	1.5
22-33	1.5	<1.0	1.0	<1.0	<1.0	1.2
33-44	<1.0	<1.0	<1.0	<1.0	<1.0	1.0
44-67	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

means that the nitrification reaction is going to completion and is thus preventing fractionation. If a nonequilibrium reaction goes to completion, there cannot be a kinetic isotopic fractionation. The fractionation is probably caused by the decomposition of the organic nitrogen to ammonia.

Denitrification is not important in controlling the isotope mass balance if the fractionation is caused by mineralization. Significant denitrification would cause the nitrate to become isotopically heavier rather than becoming lighter as observed. Soils in semiarid regions, such as Runnels County, Texas, probably do not undergo appreciable denitrification because of the low soil moisture (Carter and Allison, 1960). Denitrification does not occur unless soil moisture content is greater than 60 to 70 percent of the water-holding capacity (Cooke, 1967). Denitrification may be important in controlling the del N15 of soil nitrate in wetter climates or in irrigation farming.

Ammonia volatilization is not important because the soil pH is not high enough for adequate ammonia volatilization, and no urea is hydrolyzed to form a strong base. The decomposition of organic nitrogen, other than urea, does not produce a high base/acid ratio, thus the pH would not be expected to rise. Hooker and others (1973) observed no ammonia losses from cultivated fields. If mineralization is the controlling fractionation, then ammonification also would leave nitrate isotopically heavier than observed.

The del N15 profiles beneath cottonfields and adjacent turnrows, where NO_3 concentrations are much higher, cannot be used to determine the importance of nutrient assimilation because all samples were collected in winter and spring. The nitrate in the soils probably was generated during the fallow season and none had as yet been used as plant nutrient. Studying these same soils in late summer or fall would indicate whether assimilation is an important isotopic fractionation.

Chromatographic separations of nitrate do not appear important in controlling the del N15 of these profiles. The del N15 of the nitrate remains relatively constant with depth. In general, soils do not have a high anion exchange capacity. A possible isotopic fractionation exists between aqueous ammonium ions and ammonium adsorbed on clay minerals. A chromatographic separation would result in the $N^{15}H_4$ being preferentially adsorbed (Delwiche and Steyn, 1970). The isotopically lighter NH_4 would then be nitrified to an isotopically light nitrate relative to the heavier organic nitrogen. A comparison of ammonium fixed on clay minerals and exchangeable ammonium (table 15) shows either no fractionation in Grundy, Hayden, and Austin soils or a slight depletion of N^{15} in adsorbed ammonium in Clarion and Glencoe soils.

Cheng and others (1964) and Bremner and Tabatabai (1973) observed that organic soil nitrogen is isotopically heavier than atmospheric nitrogen. This is a result of either the addition of isotopically heavy nitrogen to the soil system or of the removal of isotopically light nitrogen from the soil system. The addition of heavy nitrogen through nitrogen fixation is not likely because of the lack of fractionation (Hoering and Ford, 1960)

Table 15. del N15 of various forms of nitrogen in soils (from Cheng and others, 1964).

Nitrogen Form	Soil Type				
	Grundy	Hayden	Austin	Clarion	Glencoe
			del N15 (ppt)		
Total - N	+16	+7	+5	+3	+2
Hydrolyzable	+18	+10	+7	+5	+4
Ammonium	+7	+7	+3	+6	+5
Hexosamine	+25	+8	0	+2	- 2
Amino acid	+16	+14	+12	+5	+8
Hydroxyamine acid	+19	+11	+8	+7	+3
Nonhydrolyzable	- 3	- 2	- 1	0	+1
N - mineralized	+6	+2	+1	+1	+1
Fixed ammonium	+6	+6	+4	+2	0

or because of a slight fractionation preferring N^{14} (Delwiche and Steyn, 1970). Light nitrogen must be removed from the soil system by either organic nitrogen mineralization, denitrification, or ammonification.

If the mineralization of organic nitrogen in cultivated soils produces an isotopically light nitrate, then the residual organic nitrogen will become heavier. The isotopically lighter NO_3 can be leached below the root zone or adsorbed as a nutrient and harvested as a crop. Light nitrogen is removed from the system permitting the del N15 of the total nitrogen to become heavier. With time, both the del N15 of the nitrate and of the residual total nitrogen will become heavier. If this is true, then grasslands with no animal wastes and no cultivation should have del N15 values of total nitrogen close to 0.0 or slightly negative. The nitrogen added by fixation will be isotopically identical or slightly lighter than atmospheric nitrogen, and there will be little loss of nitrogen through mineralization. This increase in del N15 of total nitrogen in cultivated fields was found by Bremner and Tabatabai (1973), who analyzed three pairs of soils, each pair containing a cultivated soil and a soil in its virgin state. All the cultivated soils were isotopically heavier than their virgin counterparts (table 16).

Table 16. del N15 differences between total nitrogen of cultivated soils and virgin soils (from Bremner and Tabatabai, 1973).

Soil Number	Soil Series	del N15 (ppt)	Difference (ppt)
8A V	Superior	- 1.1	
			+1.4
8A C	Superior	+0.3	
9A V	Nicollet	- 3.3	
			+6.3
9A C	Nicollet	+3.0	
10A V	Webster	- 4.4	
			+4.4
10A C	Webster	0.0	

V - Virgin soil.
C - Cultivated soil.

Denitrification probably is not the controlling reaction for the isotopic composition of the organic nitrogen even though nitrogen is lost from anaerobic soil environments with kinetic isotope fractionation factor of 1.02 (Wellman and others, 1968; Delwiche and Steyn, 1970; Myaka and Wada, 1971). Denitrification reduces the oxidized species of nitrate and nitrite to nitrogen gas and nitrous oxides, but should not affect the organic nitrogen because it is in a more reduced state than the atmospheric nitrogen. Denitrification should not directly affect the del N15 of the organic nitrogen. It may enrich the organic nitrogen if some of the nitrate is lost by reduction leaving a residual heavy nitrate which would then be cycled back to the soil humus by plant nutrient absorption and plant decay. However, no evidence suggests denitrification is occurring in the Runnels County soils.

Soils may be classified into three groups according to nitrogen isotope ranges: soils with animal waste, soils that have been cultivated, and soils still in their virgin grassland state. The isotopic composition of soil with animal waste will be controlled by ammonia volatilization. The isotopic composition of cultivated soils will be controlled by the mineralization of soil humus. The virgin grassland soils will probably be controlled by nitrogen fixation.

ISOTOPIC COMPOSITION OF NITROGEN FERTILIZERS

Synthetic nitrogen fertilizers have a del N15 composition close to the nitrogen isotope ratio of atmospheric nitrogen. The del N15 range of fertilizer overlaps the del N15 range of natural soil nitrate. This may prevent distinguishing by isotopic analysis the nitrate from the mineralization of organic nitrogen in soil from the nitrate of fertilizers (Riga and others, 1971; Hauck and others, 1972; Hauck, 1973; Edwards, 1973).

Commoner (1970a) analyzed four commercial fertilizers having del N15 values of -4.6 ppt to +3.0 ppt (table 17). Kent Murman (personal communication, June 1973) found a similar range for ammonium nitrate fertilizers. Edwards (1973) analyzed $(NH_4)_2SO_4$ and aqua-ammonia and found del N15 values of -5.0 ppt and -6.0 ppt, respectively (table 17).

Edwards (1973) found an average del N15 of +4.9 ppt for ten analyses of samples from the mineralization of organic nitrogen from a soil with no fertilizer. Incubated nitrate from the same soil with $(NH_4)_2SO_4$ or aqua-ammonia had an average del N15 of +2.7 ppt. Total nitrogen from controlled agricultural plots, one with fertilizer and one with no fertilizer, showed a slightly lighter del N15 in the soil without fertilizer, approximately 1.5 ppt (Riga and others, 1971).

Table 17. del N15 of nitrogen in artificial fertilizers.

Author	Fertilizer	del N15 NH_4 (ppt)	del N15 NO_3 (ppt)
Commoner (1970a)	30% N commercial fertilizer (Ammonium nitrate)	- 4.6	+1.6
	8% N commercial fertilizer (Ammonium phosphate)	+3.0	
	12% N commercial fertilizer	+0.5	
	Anhydrous NH_4^+	- 2.7	
Edwards (1973)	$(NH_4)_2SO_4$	- 5.0	
	Aqua-ammonia	- 6.0	

del N15 OF GROUND WATER NO_3 VERSUS del N15 OF SOIL NO_3

High nitrate concentrations in shallow ground waters are generally the result of the activities of man, whether it be excess fertilizer usage, oxidation of the soil humus, or leaching livestock and human waste. The relative importance of each of these sources in a pollution problem is dependent on the land use of the area and the type of aquifer being contaminated.

In areas of intensive cultivation of large tracts of land, the dominant source probably will be fertilizer nitrogen or soil humus nitrogen, with animal wastes contributing minor amounts to the total nitrogen balance. In areas of minimal cultivation but appreciable livestock husbandry, the dominant source would be expected to be animal waste. The type of aquifer is also important in determining relative contributions at any particular point. For example, in a high porosity, moderately permeable aquifer such as sandstone or sand and gravel, the movement of nitrate-polluted waters away from the source of nitrate will be relatively slow in comparison to the movement in a low porosity, high permeability aquifer such as a fractured or cavernous limestone. The groundwater nitrate from a barnyard well, pumping from a shallow sand-and-gravel aquifer is probably originating from animal waste nitrogen, the local source of N, whereas the nitrate from a similar barnyard well, but producing from a shallow cavernous limestone, may originate from sources at appreciable distance from the well.

Three different geographic areas with high nitrate ground waters, different land uses, and different aquifer characteristics were studied to see

if the sources of nitrate within each area would vary and if this variation could be observed isotopically. The nitrate pollution of the limestone aquifers in southern Runnels County was the principal study because of the high levels of contamination, the extensive amount of cultivation, which made natural organic soil nitrogen the probable source of N, and the need for solving this contamination problem for the people of Texas. The second area studied was Macon County, Missouri, where the glacial till aquifers were probably contaminated with nitrate originating from animal waste material. Cultivation in this county was much more limited than in southern Runnels County, Texas. The third study area was Queens and Nassau Counties on Long Island, New York. The shallow glacial aquifers in Queens County appeared to be contaminated by leaky sewer lines, whereas nitrate in the deeper Magothy aquifer in Nassau County had a probable source of fertilizer or natural soil nitrogen. These three areas provided a spectrum of nitrogen sources to see if nitrogen isotopes could be used to determine the sources of nitrate in ground water.

Southern Runnels County, Texas

Levels of nitrate contamination.—Ninety percent of water samples analyzed by Kreitler (1972) had nitrate concentrations greater than 45 mg/l. The highest nitrate concentration measured was 3,100 mg/l. Figure 14 shows the zone of high nitrate in ground water.

Hydrogeology.—South of the Colorado River, ground water is pumped from shallow Permian carbonate aquifers of the Wichita-Albany and Clear

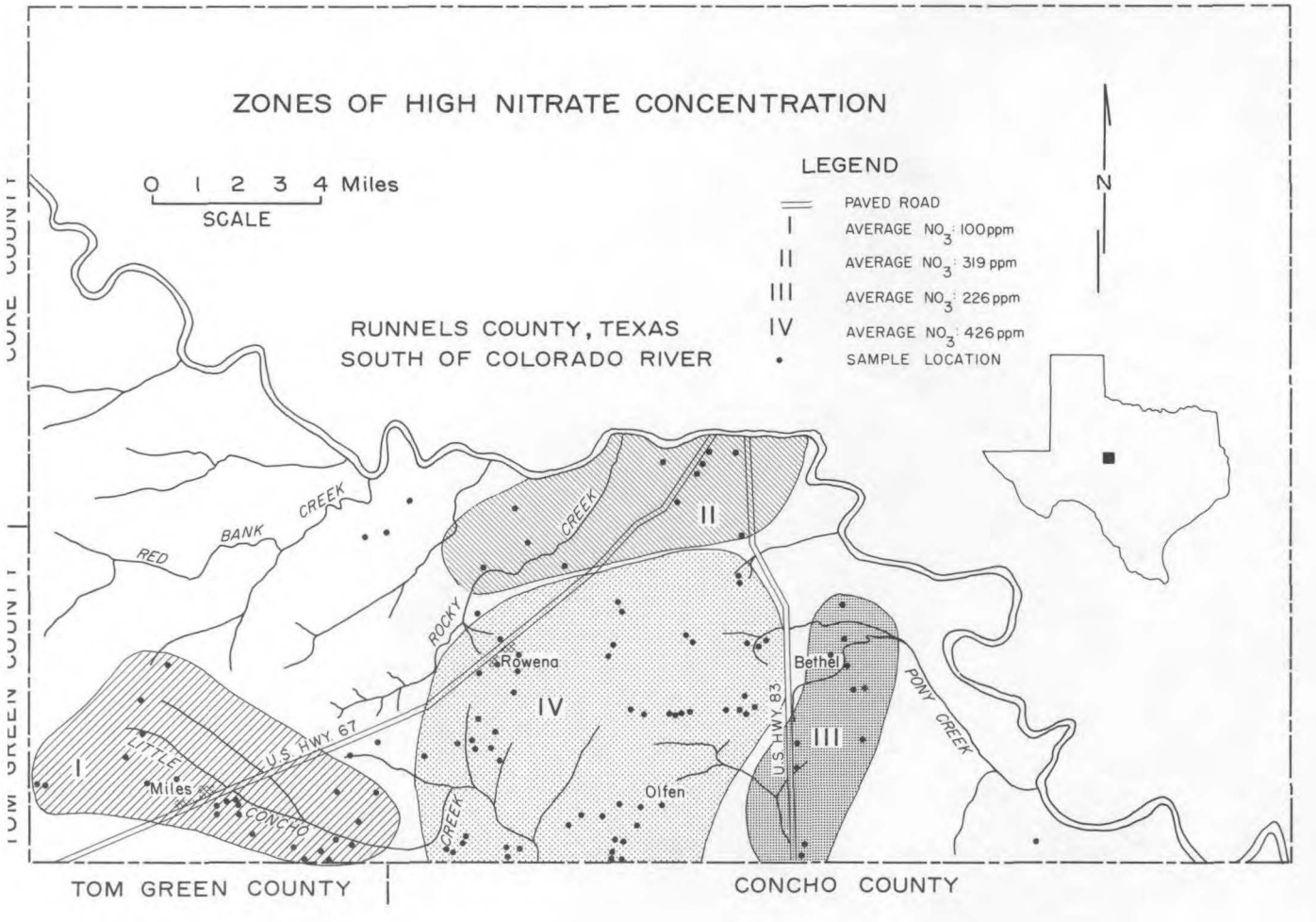


Figure 14. Zone of high nitrate in ground water.

Fork Groups (fig. 15). Water occurs in the Talpa Formation (400 feet of shales and limestones), the Clyde Formation (530 feet of shales and limestones), the Lueders Formation (190 feet of limestone and shales), and the Arroyo Formation (260 feet of shale, limestone, and basal lenses of gypsum). In the Vale Formation (150 feet thick) and Choza Formation (850 feet thick), thin limestone aquifers are found at depths of 100 to 150 feet and are overlain by thick beds of shale. The rocks strike northeast and dip to the northwest approximately 40 feet per mile (7.6 meters per mile). Ground water is also produced from the recent Colorado River deposits.

Significant ground-water flow is restricted to solution cavities along bedding plane surfaces, joint surfaces, and solution cavities in dense limestone beds. Analyses of two aquifer pump-tests on the limestones yielded transmissivities of 670 gallons/day/foot (1.0 cm^2/sec) and 15,000 gallons/day/foot (21.9 cm^2/sec), and coefficients of storage of 4×10^{-4} and 5×10^{-6}, respectively. Well yields are generally less than 100 gallons per minute (6.3 liters/sec). Few wells are deeper than 100 feet (30.4m).

Soils.—The soils in southern Runnels County typically are calichified, clayey soils that have developed on the Permian limestones and shales. They are classified into soils from the Colorado, Kavett, Mereta, Miles, Olton, Portales, Potter, Rowena, Spur, Talpa, Tobosa, and Vernon Series (Wiedenfeld and others, 1970). On figure 6, these series have been grouped into their dominant associations. Appendix 4 characterizes each association. The Kavett, Mereta, Olton, Portales, Rowena, Spur, and Talpa Series are classified in the Ustoll subgroup (long, hot, dry summers) of the Mollisol Order. Mollisols are soils with black organic-rich surface horizons and a high base exchange. The Miles Series is of the Alfisol Order, which is characterized by soils with gray to brown surface horizons and a high base exchange. The Potter Series is an Aridisol characterized by soils with low organic matter and form in semiarid to

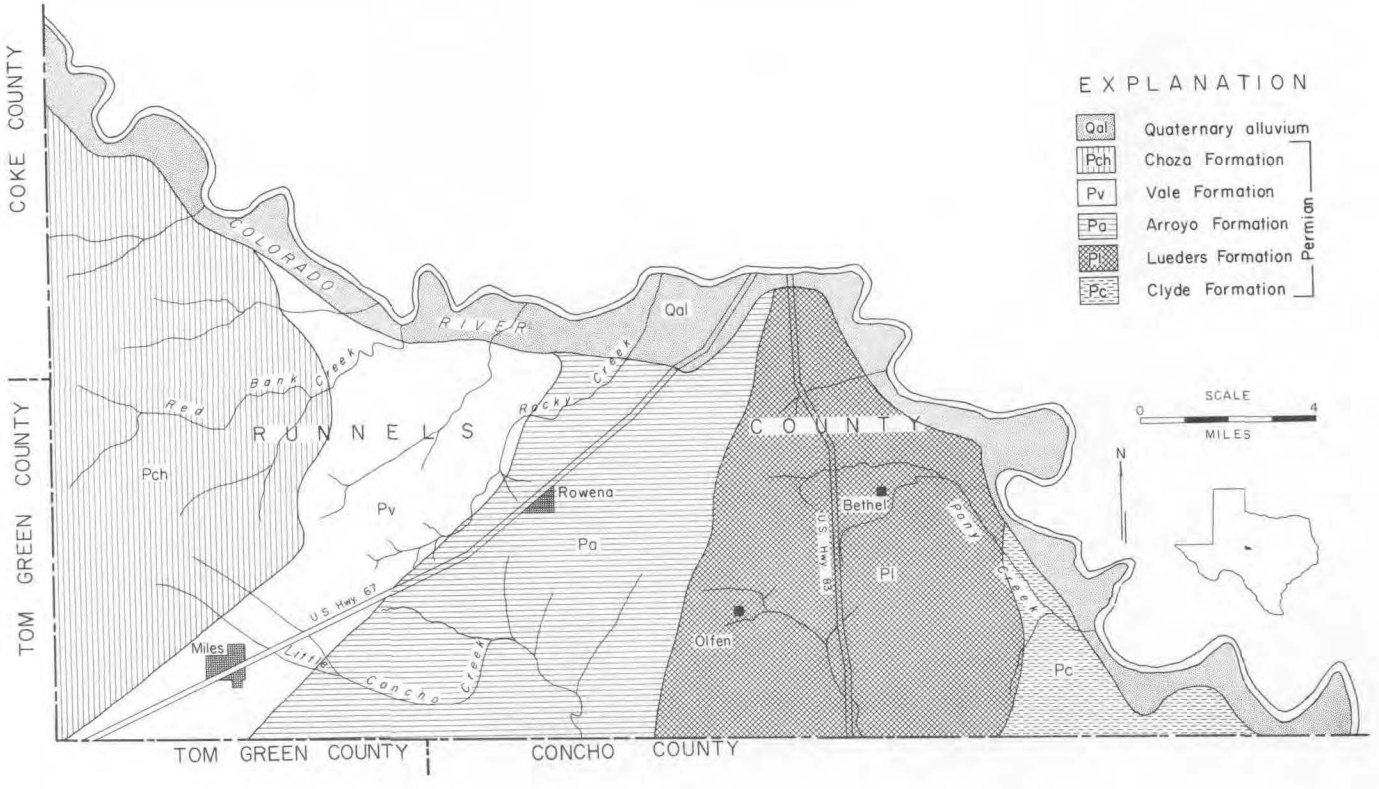


Figure 15. Hydrogeology, southern Runnels County, Texas (modified from Beede and Waite, 1918, and Sellards and others, 1933).

arid climates. The Colorado and Vernon Series are Inceptisols, which are characterized by soils that are usually moist with soil horizon where organic material is decomposing but not accumulating.

The Mollisol soils are the dominant soil type. The General Soil Map of Texas (Godfrey and others, 1973) shows this area to be entirely of the Mollisol Order, with no mention of any Alfisols or Inceptisols in the county. These other soils are present, but cover an insignificant area. The soils with lower organic matter are used for rangeland. Cultivation is limited to the Mollisols.

Land-use practice.—The economy of Runnels County is based on the production of cotton, wheat, sorghum, corn, cattle, hogs, and sheep. In 1970, 27,500 bales of cotton, 513,000 bushels of wheat, and 1,672,800 bushels of sorghum were harvested (Texas Almanac, 1973). Approximately 90 percent of the land (the Mollisol soils) in southern Runnels County is dry-farmed for these crops. Irrigation is rare because of the lack of acceptable quantities of ground water. After the drought of the 1950's, the U. S. Soil Conservation Service, a branch of the U. S. Department of Agriculture, encouraged the terracing of much of the farmland of southern Runnels County for maximum retention of rainfall. According to the farmers of the area, the water table rose appreciably after terracing.

Herds of cattle and flocks of sheep are small, and hog farms are few. More livestock is raised in the northern part of the county, whereas farming is the main occupation in southern Runnels County.

First-generation and second-generation East Europeans still farm most of the land. In the 1920's, there was a family on every 40 acres, whereas the average size farm in 1964 was 551 acres (Texas Almanac, 1973). The farming population has decreased drastically as it has in many other farming communities.

del N15 of nitrate in ground water.—The del N15 of nitrate from 31 different water samples from water wells in southern Runnels County, Texas, was determined. Figure 7 shows the well locations. Water well number, location, owner, del N15 of nitrate, and the nitrate concentrations of samples collected in 1969, 1971, and 1972 are listed in appendix 2. The water wells selected for sampling have large geographic distribution, high and low nitrate concentrations, and different local land use, e.g., wells in cottonfields and wells in barnyards. The most important variable is local land use. Samples were collected from wells in barnyards (samples 15, 18, 388, 867, and 1034), near farmhouses (samples 18, 105, 211, 233, 234, 369, 419, 551, 551a, 865, 1004, and 1005), in pastures (samples 67 and 421), and in cultivated fields away from sources of animal waste (samples 16, 165, 201, 309, 366, 386, 506, 552, 728,1002, and 1003).

A plot of the ground-water nitrate concentration versus del N15 shows a wide spread of del N15 values with only a small correlation of del N15 with the nitrate concentrations (fig. 16). The

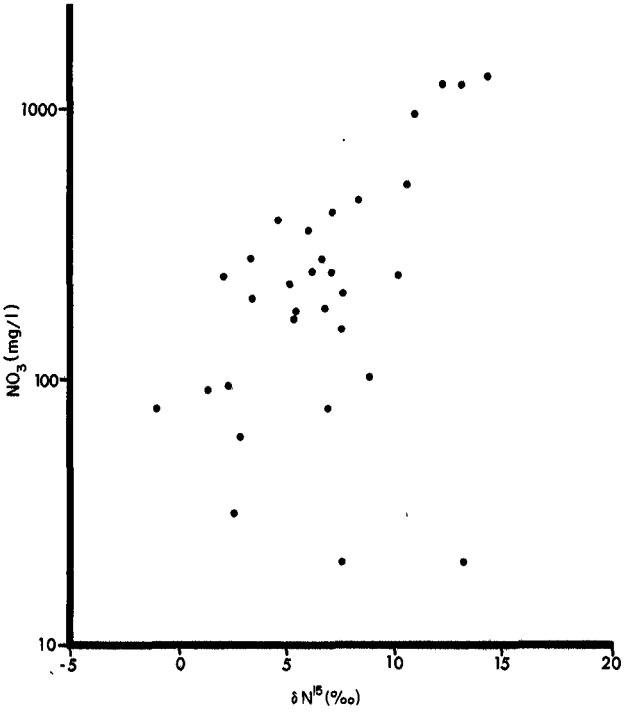


Figure 16. del N15 of nitrate in ground water from Runnels County, Texas.

ground waters with the highest NO_3 concentrations have the most enriched del N15 values. However, even the waters with low del N15 values have nitrate concentrations which exceed the limit recommended by the U. S. Public Health Service. With a linear correlation of nitrate and del N15, one source of nitrate would be predominant. High nitrate concentrations with both high and low del N15 values indicate that there are at least two

sources in the samples analyzed; however, the sources or their relative importance cannot be determined.

A comparison of the del N15 of nitrate in ground waters under specific land-use areas, for example barnyards and cottonfields, with the del N15 of the nitrate from different soil environments identifies the two sources and shows their relative contribution. By overlaying a frequency distribution graph of del N15 in ground waters beneath cultivated fields with no cattle on the graph of the two del N15 soil nitrate ranges, there is a remarkable coincidence between the del N15 of soil nitrate and the del N15 of ground-water nitrate (fig. 17).

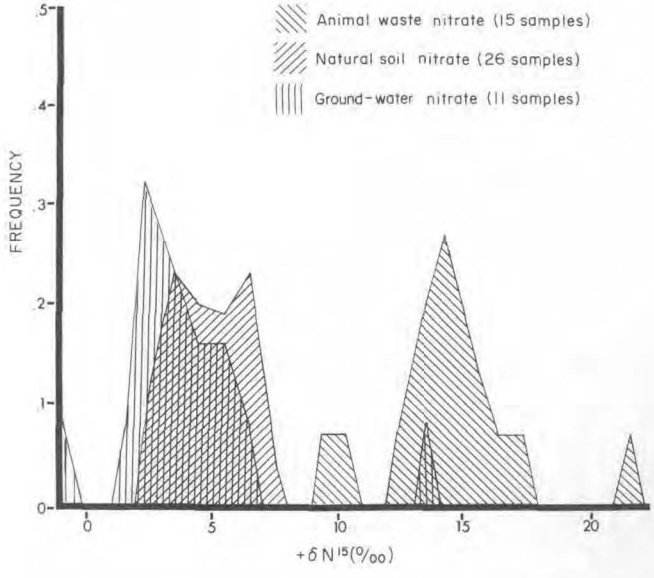


Figure 17. del N15 of nitrate in ground water from wells in cultivated fields compared to the del N15 of natural soil nitrate and animal waste nitrate (Runnels County, Texas). Frequency polygons have class interval of one del N15 unit. Cumulative frequency of each curve is equal to 1.0.

An overlay of the frequency distribution of del N15 of ground-water nitrate from wells near farmhouses, but not in barnyards, on the two del N15 soil nitrate ranges shows an isotopic shift toward the animal waste nitrate (fig. 18). The predominant source in this case is nitrate from cultivated fields, as long as the average del N15 of the ground-water nitrate is below +9 ppt.

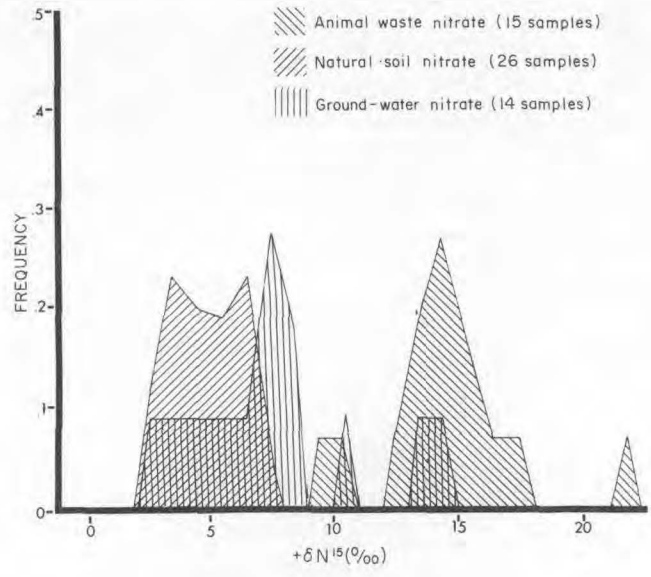


Figure 18. del N15 of nitrate in ground water from wells near farmhouses, but not in barnyards, compared to del N15 of natural soil nitrate and animal waste nitrate (Runnels County, Texas). Frequency polygons have class interval of one del N15 unit. Cumulative frequency of each curve is equal to 1.0.

The frequency distribution of del N15 of ground-water nitrate from barnyard wells shows a wide range of del N15 values, indicating that both natural soil nitrate and animal waste nitrate are contaminating the ground waters beneath barnyards (fig. 19). The predominant source of nitrate in ground waters beneath barnyards may depend on the influence of the regional hydraulic gradient and on the pumping history of the barnyard well.

The three high del N15 samples (388, 867, and 1034) are from barnyard wells located on topographic highs. Kreitler (1972) indicated that the potentiometric surface generally followed the topography. Ground-water flow, and thus nitrate migration, should be away from rather than toward the barnyard. The soil and water samples from the Halfman farm illustrate this point. The farmhouse-barnyard complex is at the top of a slight hill and the direction of ground-water movement should be downslope. The soil and water samples from the barnyard are enriched in del N15. A water sample from a well downslope from the barnyard-farmhouse complex is also enriched in del N15. On the southeast side of the hill, the shallow water table intersects the land surface forming a seep where continual evaporation of ground water

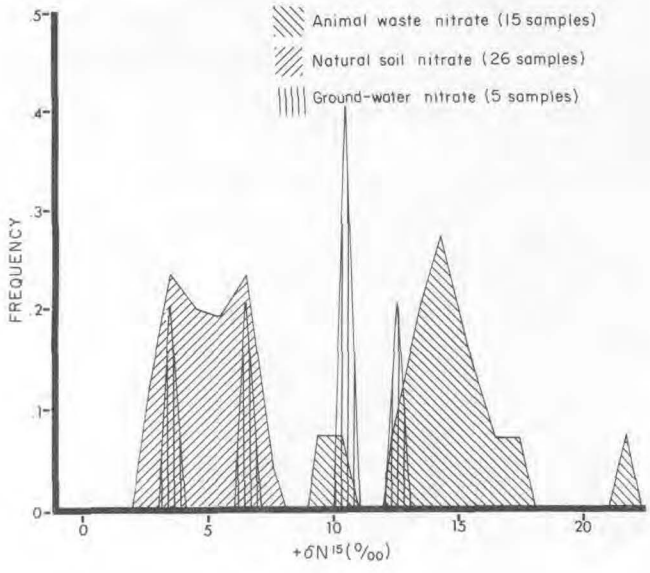


Figure 19. del N15 of nitrate in ground water from barnyard wells compared to del N15 of natural soil nitrate and animal waste nitrate (Runnels County, Texas). Frequency polygons have a class interval of one del N15 unit. Cumulative frequency of each curve is equal to 1.0.

precipitates isotopically heavy nitrate. Movement of nitrate is away from the barnyard and toward the field because of its topographic position.

The two barnyard water samples (numbers 15 and 18) with low del N15 values are on slopes. Water wells would be pumping, in part, ground waters that had been recharged upslope. Therefore, barnyard wells could be pumping ground waters with natural soil nitrate rather than ground waters with animal waste nitrate.

The del N15 of ground-water nitrate from wells in barnyards may be related to how often a well is pumped. If a well is not pumped frequently, the major nitrate source may be animal wastes. Frequently pumped wells will create cones of depression and draw ground water from a more extensive area than just beneath the barnyard. Much of the nitrate in these ground waters may be from natural soil nitrogen. Of the three high del N15 samples, the wells that pumped water samples 388 and 1034 had been used infrequently over a period of one year. However, the well that pumped sample 867 is actively used. The samples with lower del N15 values, 15 and 18, were from wells that are pumped daily.

The del N15 of the nitrate from water wells in farmhouse complexes (fig. 18) indicates some mixing of the two nitrate sources. This mixing is further documented by two samples collected from well 551, listed in appendix 2 as 551 and 551a. Sample 551 was collected from the normal producing horizon of 90 feet (10.9 m), whereas sample 551a was collected from a seep draining into the well at a depth of 16 feet (4.9 m). According to the owner of the well, the seep was a veritable waterfall during the rainy season. The nitrates with higher del N15 from the seep are mixing with the nitrates with lower del N15 deeper in the well.

Causes of high nitrates.—Figures 17, 18, and 19 demonstrate that the del N15 of the ground-water nitrate can be used to identify sources of NO_3 in southern Runnels County, Texas. The two sources are natural soil nitrogen, the predominant source, and animal waste nitrogen. The relative contribution of each source can be calculated by making certain assumptions and by comparing the ratio of acreage for different land uses. The assumptions are: (1) 20 percent of the ground-water nitrate beneath farm complexes originates from animal waste material, whereas 80 percent originates from natural soil nitrogen; (2) the volume of ground water per unit area is the same under farmhouse-barnyard complexes as under cultivated fields; (3) the nitrate concentrations in the ground water are relatively constant under both conditions; (4) the total area of the farm is 400 acres and the farmhouse-barnyard complex occupies two acres. The acreage producing nitrate from natural soil nitrogen is 200 times greater than the acreage producing nitrate from animal wastes, and only 20 percent of nitrate in the ground waters beneath the farmhouse-barnyard complexes is from animal wastes. The estimated contribution of nitrate from soil nitrogen is then 1,000 times greater than the nitrate contribution of animal wastes.

The conclusion that the oxidation of soil humus by cultivation can be a major source of nitrate in ground water is startling and has received little acceptance in the literature. Only Stout and Burau (1967) and Stanford and others (1970) considered organic soil as an important source of nitrogen. To use this model of nitrate contamination for southern Runnels County, three additional problems must be considered: (1) Was there enough organic nitrogen in the original soils to

account for the nitrates in the ground water? (2) When was the organic nitrogen oxidized to nitrate? (3) When was the nitrate leached below the root zone and then leached into the ground water?

The total nitrogen content of a noncultivated soil depends on soil type, type of vegetation on the soil, soil texture, and rainfall regime. Schriener and Brown (1938) found that different soil types had different total nitrogen concentrations (table 18). Chernozem soils and Prairie soils have high concentrations, whereas soils in progressively wetter or drier climates have progressively lower concentrations of nitrogen. In the wet climates, the soil organic matter is rapidly mineralized and leached. In the desert climates, there is inadequate plant growth to develop much soil organic matter. The semiarid climate (20 to 25 inches (510 to 640 mm) of rain per year) provides a happy medium between increased plant growth and minimal decomposition and leaching. The soils in Runnels County, Texas, are Chestnut (or Mollisols) soils which form under average rain of 14 to 24 inches (360 to 610 mm) (Millar and others, 1958). Oxidizing 50 percent of the nitrogen in these soils would create nitrate concentrations of at least 500 mg/kg.

Before 1900, Runnels County, Texas, was covered with buffalograss, which favored a high nitrogen content in the soils. Soils under grasslands develop higher nitrogen concentrations than similar soils beneath forests. As annual grasses die, their roots are rapidly added to the soil humus, whereas in forests, the root systems do not decay annually, nor do they occupy such a large fraction of the soils volume as the grass roots (Villenski, 1957).

The nitrogen content also is related to the clay content. Soils with high clay content can have much higher nitrogen content. This is reflected in the nitrogen content of several New York soils (fig. 20). The texture of the soil in Runnels County is typically clay loam or silty clay loam.

No analyses for total nitrogen in any of the soils were made to confirm original high concentrations. All highly productive soils are now under cultivation and, thus, the total nitrogen would be lower than in the original soils. Likewise, no analyses for total nitrogen of noncultivated soils were made because most noncultivated soils in southern Runnels County are unproductive and would be expected to have lower total nitrogen contents than the productive soils. The few good noncultivated soils have unique land use, such as cemeteries or railroad rights-of-way, and present inherent sampling problems.

The oxidation of soil nitrogen has been occurring since the first days of cultivation. After 1900, there was a steady immigration of East European farmers to southern Runnels County. The population peaked around 1925 with 27,850 inhabitants living on approximately 2,500 farms (Texas Almanac, 1973). In the southern part of the county, the farmers put nearly every acre into crop production. The aerial photography of Runnels County, Texas, shows this extensive cultivation (Wiedenfeld and others, 1970). The high nitrate concentrations of the turnrow profiles are consistent with the amount of nitrate that can be oxidized from the organic nitrogen in a Chestnut soil.

Determining when the nitrates were leached below the root zone and into the ground water is a

Table 18. Average nitrogen content in various soils of the United States (from Schriener and Brown, 1938).

Soil Type	Nitrogen (percent) Surface 15.2 cm	Average to depth of 1 m	Pounds (kg) of N per acre to a depth of 1 m	
Brown Forest	0.05-0.20	0.05	6,700	(3,040)
Red and Yellow	0.05-0.15	0.03	4,000	(1,820)
Prairie	0.10-0.25	0.12	16,000	(7,264)
Chernozem	0.15-0.30	0.12	16,000	(7,260)
Chestnut	0.10-0.20	0.08	10,700	(4,860)
Brown	0.10-0.15	0.06	8,000	(3,630)

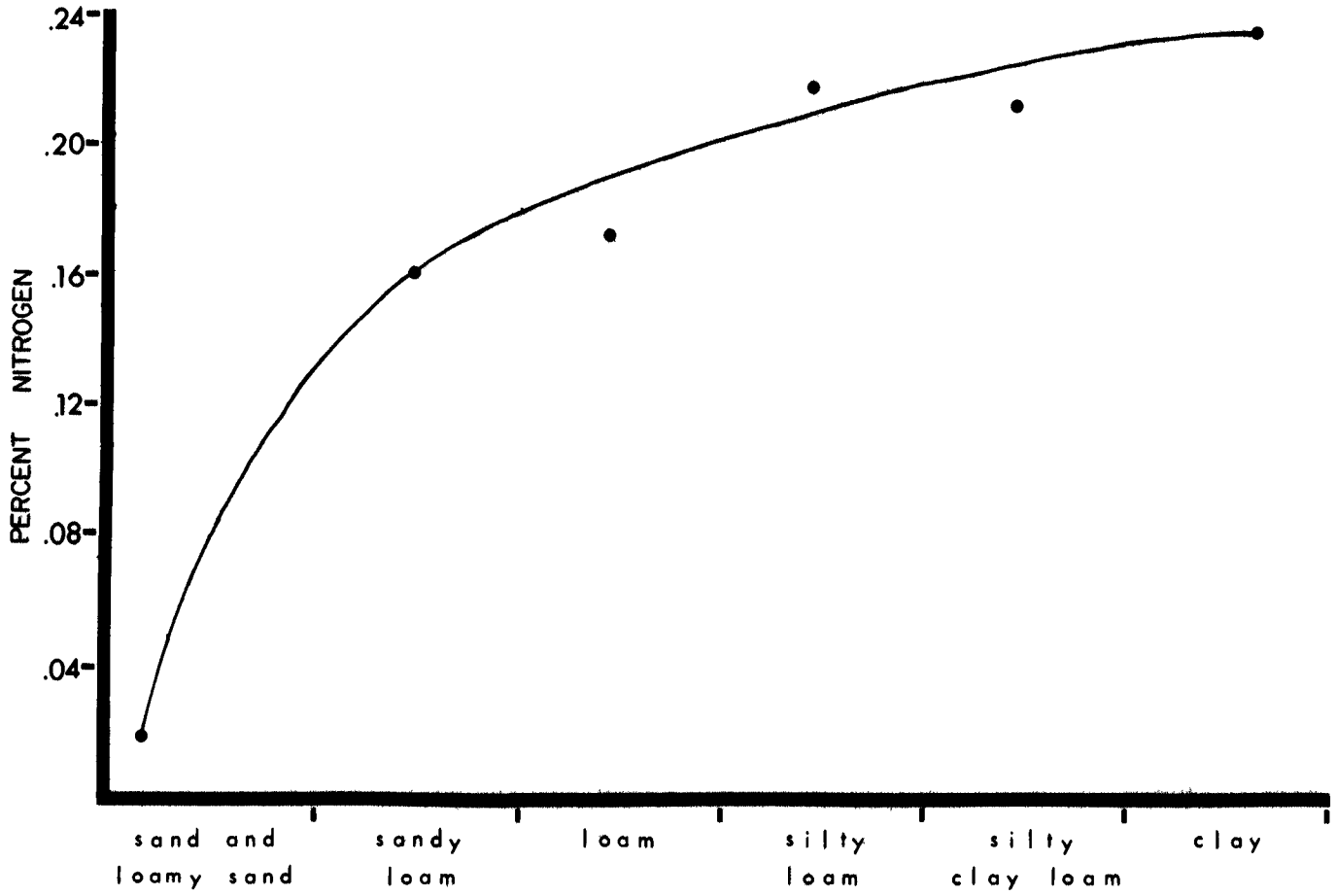


Figure 20. Increase of total soil nitrogen with increased clay content of several New York soils (from Millar and others, 1958).

more difficult problem. Table 13 shows that nitrate at shallow depths in cropland soils will be taken up by plant roots; thus, for nitrate to be a potential ground-water contaminant, it first must be leached below the root zone and then leached to the water table.

Nitrate has been accumulating below the root zone since 1900. The winter fallow season permits both the generation and the leaching of nitrate. The amount of winter rains has been adequate to leach nitrate below the root zone. In the mid-1950's, there was extensive terracing of the fields to improve retention of soil moisture. According to many of the farmers, the water table rose appreciably, more than 20 feet in some places. The increased infiltration of water and the ground water, which rose to near the ground surface, leached the nitrates from the vadose zone into the ground water.

Tritium analyses indicate that the nitrates were leached into the ground water after the drought of the early 1950's. Using the tritium dating techniques of Dincer and Davis (1968), ground waters from wells 1003 and 552, and from the city of Miles were dated at 13 to 17 years old, 8 to 13 years old, and 9 to 12 years old, respectively, relative to 1974. These samples, which represent the deepest ground waters in the area, are all post-terracing, thus the leaching also appears to be post-terracing. A detailed discussion of the ground-water, age-dating techniques is in appendix 5.

The nitrates in the ground waters of southern Runnels County are the result of the oxidation of part of the humus of semiarid grassland soils and the subsequent leaching of the nitrate to the saturated zone by extensive terracing in the 1950's.

This conclusion is disturbing because there are no inexpensive measures which would alleviate the problem. However, most of the nitrate may have been leached away by the rising ground water. Analyses of ground waters over a three-year period show an apparent gradual decrease in the nitrate concentration (appendix 2). Even if this trend continues, it will be many years before the nitrate concentrations are reduced to USPHS recommended limit of 45 mg/l.

Macon County, Missouri

To further test the use of del N15 for tracing nitrate in ground water, ground-water samples were collected from a locality where the major source of nitrate was animal waste and not natural soil nitrogen. The area chosen was Macon County, Missouri, because Smith (1969) believed that many of the high nitrate ground waters in the glacial drifts of northern Missouri were the result of animal waste contamination. More than 50 percent of the wells sampled in Macon County have water with nitrate concentrations above 20 mg/l NO_3 (Smith, 1969).

Hydrogeology.—Macon County is covered by glacial drift of Kansan and Nebraskan age. Maximum thickness is approximately 175 feet (63.5 m). The drift is a heterogeneous mixture of blue-gray clay, sand, and some gravel and boulders. The upper nondissected surface forms a relatively level plain. The major streams cut through the drift to the underlying Pennsylvanian strata (Gentile, 1967).

Ground-water supplies are obtained from shallow wells in the Pleistocene glacial drift and Recent terrace gravels along the larger streams (Gentile, 1967). Wells are typically shallow, hand dug, poorly cased, and have low yields. Many of these wells are a century old and are invariably near houses or livestock feeding areas. Ground water from one dug well 30 feet (10.9 m) deep had a nitrate concentration of 745 mg/l. A deserted barnyard was close to the well (Smith, 1969). Water from the deep wells in Paleozoic formations is highly mineralized and thus is rarely used (Gentile, 1967).

Land-use practice.—Land use is 40 percent cultivated cropland, 50 percent pasture, and 10 percent woodland (Gerald Kerr, personal communication, August 1973). This contrasts with southern Runnels County, Texas, where approximately 90 percent of the land is cultivated. Rainfall in Macon County averages 37 inches (940 mm) per year (Gentile, 1967). Ammonium nitrate is the nitrogen fertilizer most commonly used. The usual rate of application is between 200 and 300 pounds (90 to 140 kg) per acre per year. Little anhydrous ammonia or urea is used (Gerald Kerr, personal communication, August 1973). Nearly all water wells are in farmhouse-barnyard complexes.

del N15 of nitrate in ground water.—Eleven ground-water samples were collected with the help of Gerald Kerr, Area Local Government Specialist of the University of Missouri Cooperative Extension Service. All water samples are from hand-dug or drilled wells or from buried cisterns near active or deserted barnyards or farmhouses. Appendix 6 contains information on well owner, sampling location, nitrate concentration of the water, and del N15 of the NO_3.

Figure 21 shows the relationship of nitrate concentration to del N15 values of the 11 samples (circles). The nitrate concentrations range from 60 mg/l to 330 mg/l. The del N15 values range from +10 ppt to +19 ppt. The low correlation coefficient ($r = -0.11$) indicates no correlation between NO_3 concentrations and del N15 values. An overlay of the frequency distribution of del N15 in Macon County ground-water nitrate on the two isotopic ranges of soil nitrate from Runnels County shows a very good correlation between animal waste nitrate and the ground-water nitrate of Macon County (fig. 22). This should be expected because Macon County is not as extensively cultivated as southern Runnels County. Natural soil nitrogen is greatly diminished as a nitrate source. With rates of ground-water movement probably less than a few feet per year, nitrates could not have migrated far from their source. All the wells sampled are near farmhouses or barnyards, thus the logical source is animal waste nitrate. The del N15 of these nitrates and the del N15 of artificial fertilizers are not in the same range. Fertilizer, therefore, is not considered to be a source of the nitrate in the waters sampled.

The high similarity of the del N15 of the nitrate in ground waters from Macon County and the del N15 of animal waste nitrate supports a number of conclusions. (1) The nitrate in Macon County ground waters is of animal waste origin.

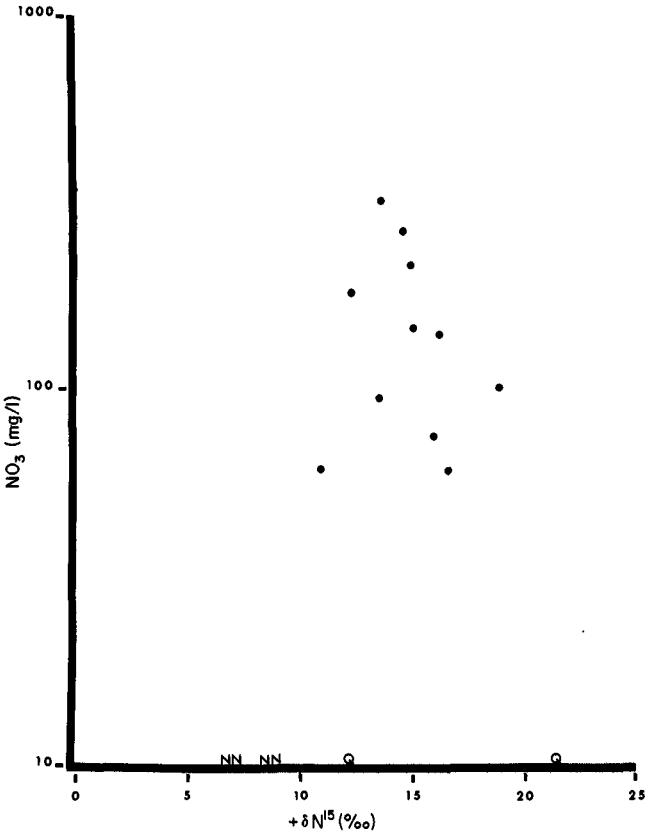


Figure 21. del N15 of ground waters from Macon County, Missouri, and del N15 of ground waters from Long Island, New York.

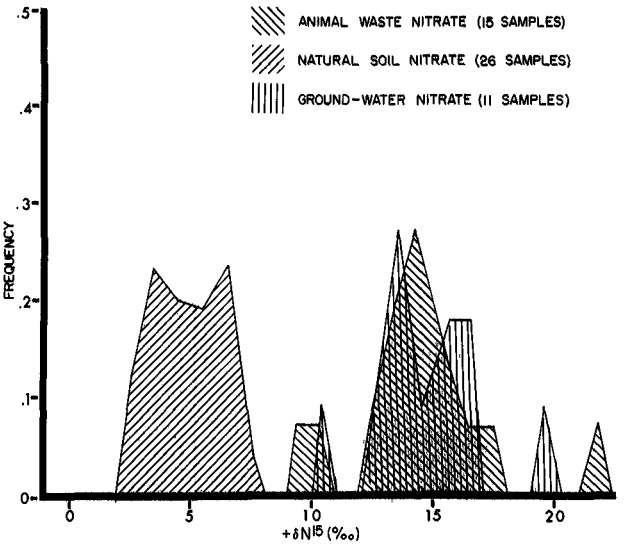


Figure 22. del N15 of nitrate in ground water from Macon County, Missouri, compared to the del N15 ranges of natural soil nitrate and animal waste nitrate from Runnels County, Texas. Frequency polygons have a class interval of one del N15 unit. Cumulative frequency of each curve is equal to 1.0.

This confirms Smith's (1969) hypothesis. (2) The del N15 values show the effectiveness of animal waste nitrate in contaminating ground-water supplies in certain cases. (3) The del N15 techniques can be used to trace animal waste nitrate as well as natural soil nitrate. (4) Finally, the chemical reactions controlling the del N15 of animal waste nitrate are effective in geographic localities other than southern Runnels County, Texas. Differing climate, soil, and geology apparently do not alter the isotope geochemistry of nitrogen.

Long Island, New York

Kimmel (1972) concluded that the high nitrates in ground waters of Kings County, Long Island, New York, were the result of leaking sewers contaminating the Upper Glacial aquifer. Kings County, therefore, seemed a good locale to further test nitrogen isotope techniques, but with a different source of nitrogen, sewage, and a different geographic area, northeastern United States. Upon arrival in Long Island, the writer found it was technically very difficult to collect water samples because the wells were all very old, dating from the early 1900's, and were welded shut with small diameter openings for limited access. The writer decided not to sample ground water in Kings County, but instead to collect ground waters in Queens and Nassau Counties, Long Island, New York, because both counties had high nitrate and sampling was easier. The source of nitrate in Queens County is similar to the probable source in Kings County, old septic tanks and leaking sewer lines (Olin Braids, personal communication, August 1973). The dominant sources of nitrate in Nassau County are septic tank effluent and agricultural sources—both artificial fertilizer and natural soil nitrogen.

Hydrogeology.—The Pleistocene and Cretaceous sediments on Long Island can be divided into six major hydrogeologic units (table 19). Ground-water recharge is derived locally from precipitation. Recharge to the Magothy, Jameco, and Lloyd aquifers is by downward flow from the Upper Glacial aquifer (fig. 23).

Table 19. Major hydrogeologic units in Kings County, Long Island, New York (from Kimmel, 1972).

Hydrogeologic Unit	Approximate maximum thickness in study area (feet)	Description
Upper glacial aquifer	180	Mainly sand and gravel of high hydraulic conductivity; some thin beds of clayey material of low hydraulic conductivity.
Gardiners clay	100	Clay, silty clay, and a little fine sand of low to very low hydraulic conductivity.
Jameco aquifer	200	Mainly medium to coarse sand and some gravel of moderate to high hydraulic conductivity.
Magothy aquifer	200	Mainly very fine sand, silt, and clay of low to very low hydraulic conductivity; some coarse to fine sand of moderate hydraulic conductivity; locally contains gravel of high hydraulic conductivity.
Raritan clay	200	Clay of very low hydraulic conductivity; some silt and fine sand of low hydraulic conductivity.
Lloyd aquifer	200	Sand and gravel of moderate hydraulic conductivity; some clayey material of low hydraulic conductivity.
Bedrock	--	Crystalline rock of very low interstitial hydraulic conductivity.

Land-use practice.—Kings County and Queens County have similar histories of development because of their close proximity to the island of Manhattan (Olin Braids, personal communication, August 1973). Information on Queens County is sparse because no published or open-file reports are available. Development was similar to that of Kings County but slightly later because it is farther from New York City. These urbanized counties have had no agricultural activity and few septic tanks for several years. Kings County, and probably Queens County as well, is underlain by a dense network of sanitary and storm sewers. In Kings County, sewerage began about 1850. There were about 800 miles (1,280 km) of sewer lines in 1908, 1,300 miles (2,080 km) in 1932, and 1,700 miles (2,720 km) in 1962. Leakage from these sewer lines is not only the major source of nitrate in the Upper Glacial aquifer, but may also be the major source of recharge to the aquifer (Kimmel, 1972).

The numerous towns in Nassau County depend on the Upper Glacial, the Magothy, and to a small degree, the Lloyd aquifers for their municipal water supplies. The high nitrate concentration in the Upper Glacial and the Magothy are of great concern because of the public health hazard of using these waters for municipal supplies. Ground water from the Lloyd aquifer is not a problem because of its low nitrate concentration (Harr, 1971). Each municipality pumps a number of wells. The town of Garden City pumps four wells and mixes the waters from these wells to provide drinking water with a nitrate concentration below the recommended limit of 45 mg/l. The nitrate in these aquifers is either from animal waste nitrogen (septic tanks and sewer lines) or from

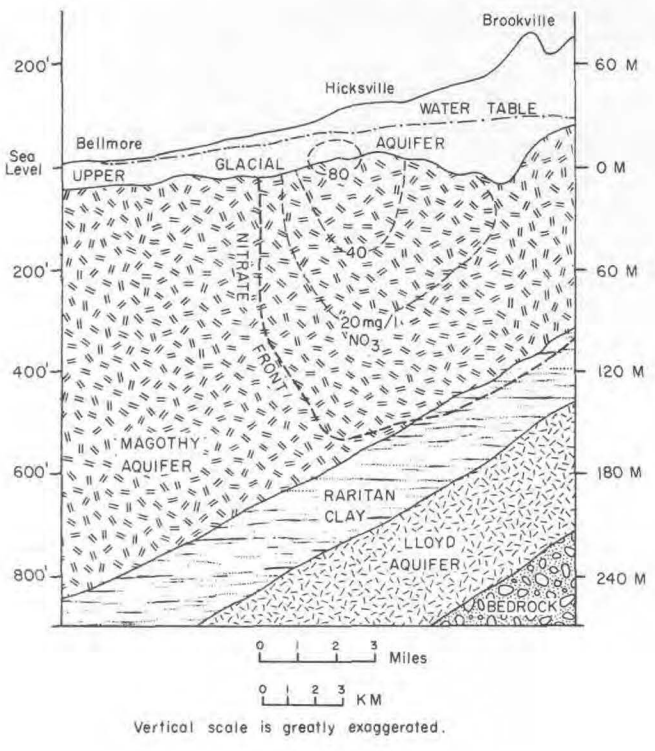


Figure 23. Recharge to Magothy aquifer (from Perlmutter and Koch, 1972).

cultivation (from the oxidation of the soil humus or from the excessive use of nitrogen fertilizers). Nitrate contamination of the Upper Glacial aquifer by septic tanks has been documented by the Nassau-Suffolk Research Task Group (1969). This does not rule out nitrate generated from cultivation as a source, however. Farming began in colonial times and continued to the 1950's. There was heavy fertilizer use from the 1920's to the 1950's (Perlmutter and Koch, 1972). After World War II, the land was gradually converted for residential construction.

High-nitrate waters have migrated through the Upper Glacial aquifer and deep into the Magothy aquifer. Figure 23 shows the nitrate front in the Magothy aquifer. Because there is an average rate of vertical movement of 10 feet per year, the nitrates in the Magothy aquifer probably were added to the ground water in the early days of heavy fertilizer usage and extensive cultivation in the 1920's. This would imply that the fertilizer and expanded cultivation from the 1920's to preresidential days of the 1940's is the cause of the high nitrate concentration. Another parameter which may control the nitrate front is the reduction of the nitrate to ammonium. The reduction of nitrate is implied by the down-gradient loss of dissolved oxygen (Perlmutter and Koch, 1972).

del N15 of nitrate in ground water.—Six samples were collected from the two-county area. In Queens County, two samples were from the Upper Glacial aquifer, one from a shallow well next to the Maryland Pavilion at the deserted site of the New York Worlds Fair (del N15 = +12.1 ppt), the second from a well at the intersection of the Southern Parkway and the Rockway Parkway (del N15 = +21.3 ppt) (table 20).

In Nassau County, four samples were collected from municipal water districts pumping from the Magothy aquifer. The four municipal water districts are Hicksville, Garden City, Westbury, and Eisenhower Park (table 21). Because of circumstances beyond the writer's control, water samples were not taken at the well. Therefore, the samples were collected from randomly chosen water faucets in the four districts. In Hicksville a sample was collected at a Texaco station; in Westbury at a Mobil station; in Eisenhower Park at a public drinking fountain; and in Mineola from a drinking fountain in the U. S. Geological Survey office in the Federal Building which is in the town of Mineola, but in the water district of Garden City. The water samples from the faucets are assumed to be from different wells which are mixed in the distribution system. The del N15 values represent averages for particular well districts. Ground-water nitrate from Hicksville had a del N15 of +9.0 ppt; ground-water nitrate from Westbury had a del N15 of +6.8 ppt; ground-water nitrate from Eisenhower Park had a del N15 of +7.0 ppt; and ground-water nitrate from Garden City had a del N15 of +8.6 ppt (table 20).

Figure 21 shows that all the samples from Nassau County are isotopically lighter than the Queens County samples. They are slightly heavier than the natural soil nitrates from southern Runnels County, Texas, and appreciably lighter than the average del N15 value of animal waste nitrate from southern Runnels County. The del N15 of the Nassau County samples were also lighter than the average del N15 of ground-water nitrate from Macon County, Missouri. The origin of most of the ground-water nitrate in Nassau County appears to be either organic nitrogen of

Table 20. del N15 of nitrate in ground water from Long Island, New York.

Well Number	Location	NO_3 (mg/l)	del N15 (ppt)
Queens County			
Q 2993	Intersection of Southern Parkway and Rockway Parkway	20	+21.3
Q 2417	Maryland Pavilion, New York City Worlds Fair Grounds	30	+12.1
Nassau County			
	Hicksville (Texaco Station)	15	+9.0
	Westbury (Mobil Station, Post Avenue)	17	+6.8
	Eisenhower Park (drinking fountain in Eisenhower Park)	25	+7.0
	Garden City (U.S.G.S., drinking fountain in Federal Building)	15	+8.6

soil humus or nitrogen fertilizer. Animal waste is not the dominant source.

The del N15 values of the ground-water nitrates in Queens County are higher than the del N15 of the ground-water nitrates from Nassau County. These values fall in the range of animal waste nitrate, which would support the leaky sewer hypothesis.

The data presented in this report do not fully prove the source of nitrate in the aquifers of Long Island, but do suggest that some of the sources previously considered by Kimmel (1972) and Perlmutter and Koch (1972) are plausible. Additional analyses of the del N15 of ground-water nitrate would be needed to establish a more definite conclusion.

Table 21. Partial chemical analyses of ground waters from four water districts in Nassau County, Long Island, New York (from Harr, 1971).

Water District	Well Number	Latitude	Longitude	Date	NH_4 as N (mg/l)	NO_2 as N (mg/l)	NO_3 as N (mg/l)	Aquifer	Screened Interval (ft)
Garden City	N94-6	404357N	0733830	5/20/71	0.01	0.00	0.20	M[a]	322-382
	N95-7	404357N	0733830	5/25/71		0.00	0.50	M	474-538
	N3934-10	404357N	0733707	5/25/71	0.01	0.00	5.20	M	377-417
	N8339-14	404320N	0734012	5/20/71	0.01	0.00	6.30	M	358-365
Eisenhower Park	N2923-1	404403N	0733348	5/21/71	0.00	0.00	8.00	M	80-122
	N3243-3	404403N	0733404	5/21/71	0.00	0.00	1.90	M	248-306
	N7500-6	404417N	0733432	5/21/71	0.00	0.00	1.10	M	335-405
	N7561-5-2	404455N	0733249	5/25/71	0.01	0.00	1.10	M	463-550
	N7562-1-4	404639N	0733111	5/25/71	0.01	0.00	0.40	M	458-545
Hicksville	N3488-3-1	404446N	0733057	5/25/71	0.01	0.00	20.00	M	115-168
	N3553-5-1	404455N	0733249	5/25/71	0.16	0.00	17.00	M	99-152
	N3953-6-1	404626N	0733231	5/25/71	0.01	0.00	5.40	M	371-419
	N5336-1-2	404441N	0733209	5/21/71	0.00	0.00	1.00	M	472-523
	N6190-7-1	404706N	0733052	5/25/71	0.01	0.00	0.60	M	550-605
	N8249-1-5	404639N	0733111	5/21/71	0.00	0.00	1.30	M	300-495
Westbury	N6819-12A	404543N	0734543	5/18/71	0.00	0.00	3.80	M	215-260
	N7343-14	404555N	0733411	5/24/71	0.00	0.00	2.50	M	300-390
	N7785-7A	403952N	0733422	5/24/71	0.02	0.00	3.70	M	330-396
	N8007-15	404543N	0733549	5/25/71	0.01	0.00	1.90	M	490-564

[a]M = Magothy aquifer

CONCLUSIONS

(1) Nitrogen isotope ratios of ammonium and nitrate ions from soil and water samples can be analyzed reproducibly with an experimental error of ±1 ppt.

(2) Slight variations from the procedures of analysis can cause isotopic fractionations. Techniques causing fractionations are the use of coarse-grained Devardas Alloy and the incubation of the soil organic nitrogen after collection and before analysis of the sample.

(3) There are two isotopic ranges of soil nitrate in southern Runnels County, Texas. Nitrate derived from the decomposition of animal waste nitrogen yields a del N15 of +10 ppt to +22 ppt. Nitrate derived from the mineralization of organic nitrogen in soil humus has a del N15 of +2 ppt to +8 ppt.

(4) Isotope ratio of animal waste nitrate is controlled in part by the volatilization of isotopically light ammonia gas during the decomposition of urea in urine. Isotopic fractionation may be occurring within an animal.

(5) Isotope ratio of natural soil nitrate may be controlled by the deamination of isotopically light protein material to ammonia and not the nitrification of ammonia to nitrate. Denitrification, ammonia volatilization, and ammonium adsorption do not appear to be controlling reactions for the del N15 of natural soil nitrate.

(6) Nitrogen in artificial fertilizer has a del N15 range which overlaps the del N15 range of natural soil nitrate. Nitrate from fertilizer will be difficult to differentiate from nitrate produced by the oxidation of soil nitrogen.

(7) In southern Runnels County, Texas, the major source of nitrate in the ground water is natural soil nitrate. The del N15 of the ground-water nitrate beneath cultivated fields corresponds well with del N15 of natural soil nitrate. Ground water beneath farmhouse-barnyard complexes has a higher del N15, indicating a contribution of animal waste nitrate. Ground water from wells in barnyards has a wide range of del N15 values.

(8) The nitrates in the ground waters of southern Runnels County, Texas, are the result of cultivation which causes the oxidation of some of the organic nitrogen to nitrate. Natural soil nitrogen may contribute as much as 1,000 times more nitrate to the ground water than animal wastes. Extensive terracing during the 1950's in southern Runnels County caused the water table to rise, allowing the ground water to leach the soil nitrate into the aquifer system.

(9) Ground waters from Macon County, Missouri, are contaminated with nitrate from animal wastes. There is a good correlation between the del N15 range of ground-water nitrate from Macon County, Missouri, and the del N15 range of animal waste nitrate from southern Runnels County, Texas.

(10) The del N15 of nitrates from the Upper Glacial aquifer in Queens County, Long Island, New York, suggests that the source of nitrate is leaky sewer lines. The del N15 of ground-water nitrate from the Magothy aquifer in Nassau County, Long Island, New York, suggests that the source of the nitrate is either natural soil nitrogen or artificial fertilizer. Further research using nitrogen isotopes may positively identify the sources of ground-water nitrate in the Pleistocene and Cretaceous aquifers of Long Island.

(11) The identification of the source of ground-water nitrate in southern Runnels County, Texas, and Macon County, Missouri, indicates that the techniques developed in this study are applicable for identifying sources of nitrate in other natural waters.

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APPENDICES

APPENDIX 1. REAGENTS USED IN del N15 ANALYSIS.

Magnesium oxide.—Finely powdered reagent-grade MgO is used directly from the bottle. Bremner and Keeney (1966) recommended ignited magnesium oxide to remove any carbonate minerals. Distillation of MgO solutions with carbonate contamination can lead to liberation of CO_2 which will interfere with the determination of ammonium by the titration method. This can be a problem for very low ammonium concentrations (in micrograms of N). The concentration of all ammonium analyses of this study were greater than 1 mg of N, thus CO_2 interference was not considered a problem.

Devardas Alloy.—Reagent-grade alloy composed of 50% Cu, 45% Al, 5% Zn and less than 0.05 percent N, is ground with a mortar and pestle until the product will pass a 100-mesh screen and at least 75 percent of it will pass a 200-mesh screen. As previously indicated, the fine-grained Devardas Alloy is absolutely necessary.

Sulfamic acid.—Dissolve 2 grams of crystalline sulfamic acid in 100 ml of water. Store this in a refrigerator.

0.1 HCl solution.—Prepare this from Baker reagent-grade 0.1 N HCl Dilut-it.

0.1 NaOH solution.—Prepare this from Baker reagent-grade 0.1 N NaOH Dilut-it.

Hypobromite-iodide solution.—Bremner (1965, p. 1271) describes in detail the preparation of a hypobromite-iodide solution as follows:

> Dissolve 200 g of NaOH in 300 ml of water and cool the solution in ice. Transfer half of the cooled solution to a 500-ml, wide-mouth Erlenmeyer flask, immerse the flask in crushed ice, and add 60 ml of Br_2 over a period of 30 minutes. Stir the solution vigorously during addition of the Br_2, and regulate the rate of addition so that the temperature of the solution does not exceed 5°C. When the addition of Br_2 has been completed, add the remainder of the NaOH solution; and, after stirring the mixture for a few minutes, stopper the flask, and place it in a refrigerator for 4 to 6 days. Remove the copious precipitate of NaBr which forms during this period of cold storage by filtration with suction through a glass-fibre filter and dilute the filtrate with an equal volume of a solution prepared by dissolving 2 g of KI in 1 liter of water. Store the hypobromite-iodide solution in a tightly stoppered bottle in a refrigerator. Take care during preparation and storage of the reagent to protect it from atmospheric CO_2. One milliliter of this solution will oxidize 5 to 6 mg of NH_4 to N_2, and the reagent will retain its activity for at least 6 months if stored in a refrigerator.

APPENDIX 2. ANALYSES OF WATER SAMPLES FROM SOUTHERN RUNNELS COUNTY, TEXAS.

Water Well Number[1] and Sample Number	Owner	Location	del N15 (ppt)	NO_3 (mg/l)		
			1972	1972	1971	1969
15	Steinback	In barnyard	+ 3.3	200	340	280
16	Steinback	In field	+ 2.0	243	250	220
18	A. Halfman	In barnyard	+ 6.4	288	791	540
67	C. Robinson	In pasture	+ 7.5	154	288	330
105	Connelley	Near house	+ 7.4	21	> 0.4	56
211	R. Hohensee	Near house	+ 7.5	211	220	320
165	Nitsche	In field	- 1.1	78	326	490
201	H. Book	In field	+ 2.2	96	220	130
233	W. Beimer	Near house	+ 7.0	416	572	600
234	W. Beimer	Near house	+ 3.3	290	378	390
309	L. Harter	In field	+ 4.5	390	294	233
366	Kvapil	In field	+ 2.5	32	56	62
369a	W. Ransbarger	In pasture	+ 7.0	257	326	380
386	E. O. Eggemeyer	In field	+ 5.8	366	315	310
388	E. O. Eggemeyer	In barnyard	+ 10.3	978	1428	
419	Busenlehner	Near house	+ 5.3	186	240	250
421	Busenlehner	In pasture	+ 8.6	105		
506	B. Wilde	In field	+ 5.2	174	299	300
551	Pieper	Near house	+ 6.6	184	294	200
551a	Pieper	Near house (seep)	+ 8.3	470		
552	Pieper	In field	+ 1.2	94		
728	Teplicek	In field	+ 2.7	61	130	
865a	O. Halfman	Near house	+ 14.1	1360	1360	
865b, c	O. Halfman	Near house	+ 13.0	1260		
867a	O. Halfman	In barnyard	+ 10.4	579	1898	
868	O. Halfman	In field	+ 13.1	21		920
1002	R. Schwertner	In field	+ 5.0	223	280	
1003	E. Holubec	In field	+ 6.3	255		
1034a	F. Bachous	In barnyard	+ 12.0	1238	2162	2240
1004	W. Lange	Near house	+ 10.0	250		
1005	Carl Wilde	Near house	+ 6.8	75		

[1]Water wells are located on figure 7.

APPENDIX 3. ANALYSES OF SOILS FROM SOUTHERN RUNNELS COUNTY, TEXAS.

Sample Location[1]	Depth (ft)	Owner	Land Use	NO_3 (mg/kg)	Cl (mg/kg)	del N15 (ppt)[2]	Soil Association[3]
a	2	Frank Gully	turnrow	348		4.4	Rowena-Tobosa
	3			458		3.8	
	4			381		4.5	
	5			237		3.6	
	7			105		2.7	
	8			104		2.0	
	9			44		2.5	
b	1	Paul Pieper	cornfield with cattle	42.5		15.3	Rowena-Tobosa
	3			25		17.9	
	4			19		11.8	
	6			43		11.6	
	7 + 8			32		7.0	
c	1	Paul Pieper	cottonfield	43		3.3	Rowena-Tobosa
	3			65		3.9	
	5			75		5.0	
	7			104		3.8	
d	1	Paul Pieper	barnyard	180		13.6	Rowena-Tobosa
	3			1682		16.5	
	4			1300		15.2	
	5			429		13.7	
	6			706		14.1	
	10			700		14.0	
e	2	Santa Fe Railroad	right-of-way	30	225	24.6	Portales-Potter-Mereta
	4			126	200	31.0	
	7			36	45	19.2	
g	2	H. H. Gully	deserted barnyard	135		21.9	Portales-Potter-Mereta
h	2 + 3	H. H. Gully	cornfield	10		12.4	Rowena-Tobosa
	4 + 5			25		7.8	
j	0	Homer Eggemeyer	barnyard soaked with pig urine			46	
	3		barnyard	805		9.7	Portales-Potter-Mereta
k	1 + 2	Homer Eggemeyer	cottonfield	13		5.8	Portales-Potter-Mereta
	3 + 4			36		6.5	
	6			58		5.6	
	7			49		4.6	
	8			45		4.9	
l	1	Emil Kvapil	cottonfield	78		4.4	Rowena-Tobosa
	3			36		5.7	
	5			32		6.2	

m	3	Emil Kvapil	barnyard	1032	14.0	Rowena-Tobosa
n	1	Walter Beimer	cottonfield	37	3.3	Rowena-Tobosa
	3			32	8.6	
	5			19	6.2	
o	2	Walter Beimer	turnrow	225	6.7	Rowena-Tobosa
	3			450	5.7	
	7			65	6.4	
	9			70	6.2	
p	2	James Jones	septic tank drain field	33	17.8	Spur-Colorado-Miles
	3			33	13.0	
q	5	James Teplicek	barnyard	17	15.9	Rowena-Tobosa
r	6 + 7	Paul Busenlehner	pasture	10	10.4	Portales-Potter-Mereta
s	0	Omar Halfman	soil seep	631	9.8	Rowena-Tobosa
t	3	Omar Halfman	barnyard	306	14.6	Rowena-Tobosa
u	2	Omar Halfman	septic tank drain field	711	10.3	Rowena-Tobosa
	3			523	12.4	

[1]Samples are located on figures 6 and 7.

[2]All del N15 values are positive.

[3]Wiedenfeld and others, 1970.

APPENDIX 4. SOIL ASSOCIATIONS OF RUNNELS COUNTY, TEXAS.[1]

(Condensed from Wiedenfeld and others, 1970)

1. Portales-Potter-Mereta association: Nearly level to undulating, loamy soils, moderately deep to very shallow over caliche.

2. Rowena-Tobosa association: Nearly level to gently sloping, deep, loamy and clayey soils.

3. Spur-Colorado-Miles association: Nearly level to gently sloping, deep, loamy soils mainly on floodplains and old stream terraces, but also on Permian limestone and shale.

4. Olton-Vernon-Rowena association: Nearly level to gently sloping, deep, loamy soils on Permian limestone and shale, and gently sloping to steep, shallow, clayey soils on uplands.

5. Cobb-Winters association: Gently sloping to moderately sloping, moderately deep to deep, loamy soils on uplands and Permian limestone and shale.

6. Tarrant-Rough stony land association: Undulating to steep, very shallow, clayey soils and steep stony areas.

7. Talpa-Kavett association: Undulating to steep, loamy and clayey soils, very shallow and shallow over limestones on uplands.

[1]Soil associations located on figure 6.

APPENDIX 5. ANALYSES OF GROUND-WATER SAMPLES, MACON COUNTY, MISSOURI.

Well Number	Owner	Well Type	Well Age (yrs)	Pump Type	Depth (ft)	Location	NO_3 (mg/l) 1973	del N15 (ppt)
1	Les Ayers	Dug		Hand		In barnyard	75	+ 15.9
	(Comment: Old barnyard, 75 to 100 years old; Smith (1969) measured high nitrate profile for this barnyard.)							
2	Wendell Baker	Dug	30	Electric		Next to barnyard	62	+ 10.8
3	Caryle Carter	Drilled		Electric		Next to barnyard	105	+ 18.8
4	Caryle Carter	Buried cistern (rock-walled)				Next to septic tank	260	+ 14.5
5	Norman Damron			Electric	30-40	Next to barnyard	330	+ 13.7
6	Junior Hartung	Drilled		Electric	60	Next to cattle pen	60	+ 16.6
7	Junior Hartung	Dug		Electric	30	In barnyard	95	+ 13.5
8	Junior Hartung	Dug		Electric	20	Next to house	145	+ 16.2
9	Herschel Lane	Dug		Electric		Next to barnyard	215	+ 14.8
10	Paul Roan	Dug		Hand		15 feet south of house	180	+ 12.2
11	Grisham White	Dug	55	Hand		20 feet northeast of house (near deserted turkey pen)	145	+ 15.2

APPENDIX 6. GROUND-WATER DATES OF WATERS IN RUNNELS COUNTY, TEXAS, BY TRITIUM ANALYSIS.

If agricultural terracing and cultivation were the prime causes of contamination of the ground waters of southern Runnels County, Texas, then the ground waters must be as young or younger than the terracing. To confirm this, three water samples from the deepest wells in the limestone aquifers were analyzed for tritium content by the Teledyne Isotope Corporation (table 22).

Table 22. Tritium ages of ground water, Runnels County, Texas.

Well Number	Owner	Depth (ft)	Measured T.U.[a]	Corrected T.U.[a]	Approximate Age of Water (years)
1003	E. R. Holubec	70	57 ± 12	47	13 - 17
552	P. Pieper	75	241 ± 11	205	8 - 13
	City of Miles	130	371 ± 13	316	9 - 12

[a]1 T.U. (Tritium Unit) equals 1 tritium atom (H^3) per 10^{18} hydrogen atoms.

Tritium is a radioactive isotope of hydrogen with a half-life of 12.5 years, that is, half of the original tritium will decay to hydrogen in 12.5 years. In 1954, the New Castle atmospheric nuclear explosions injected large amounts of tritium into the atmosphere. Since then, tritium concentrations in rainwater have been much higher than natural tritium levels (2 to 10 Tritium Units) (Stewart and Hoffman, 1966). With the use of liquid or gas scintillation counters, tritium concentrations can be determined and post-nuclear bomb waters theoretically can be distinguished from pre-bomb waters. However, the mixing of young and old waters causes interpretation problems. Tritium concentrations slightly above the background levels indicate at least some recent addition of rainwater, whereas high concentrations of tritium confirm the young age of the water.

A more precise date may be calculated by estimating the yearly tritium input in rain for a given area, the tritium output at the well, the decay coefficient, the dispersion coefficient, and the tritium fractionation caused by evaporation.

Table 23 lists weighted average annual tritium concentrations based on T. U. contour maps of the United States (T. Wyerman, personal communication, March 1972).

Table 23. Approximate tritium content of precipitation for 31° to 33° N. latitude and 100° to 105° W. longitude.

Year	T. U.[a]
1952	8
1954	100
1955	16
1956	45
1957	40
1958	160
1959	170
1960	50
1961	70
1962	470
1963	1300
1964	600
1965	270
1966	180
1967	110
1968	90

[a]1 T.U. (Tritium Unit) equals 1 tritium atom (H^3) per 10^{18} hydrogen atoms.

Ground-water ages can be calculated from two mathematical models developed by Dincer and Davis (1968). The first model is a simple piston flow equation with a radioactive decay coefficient:

$$C = Co\ e^{-\frac{0.693\ t}{12.5}}$$

where:

C = output tritium concentration

Co = input tritium concentration

t = transit time of ground water

This model assumes no mixing of ground waters of different years.

The second model, which more likely represents the carbonate aquifer conditions of southern Runnels County, Texas, is a binomial dispersive flow equation with a radioactive decay coefficient:

$$f_o(t) = \sum_{o}^{+} f_i(t - T)e^{-\frac{0.693\ t}{12.5}} \left(\frac{n}{x}\right)_T (0.50)^n$$

where:

$f_o(t)$ = output function

$f_i(t - T)$ = input function

T = transit time of ground water

$\left(\frac{n}{x}\right)$ = binomial coefficient which corresponds to the transit time T

n = function of binomial dispersion

This model assumes a mixing of ground waters of a particular year with ground waters a year younger and a year older.

Before the percolating water reaches the water table, clay mineral adsorption, plant absorption, and evaporation may alter the tritium concentration by isotopic fractionation. Zimmerman and others (1967) found that plant transpiration had no isotopic effect on H^2, thus, no effect on tritium. Stewart (1972) showed that adsorbed meteoric water on clay had a small fractionation.

There is a significant isotopic fractionation of H^2 with evaporation. With approximately 50 percent evaporation of the original water, there will be an isotopic fractionation of 110 ppt between hydrogen and deuterium (fig. 24) (Craig and others, 1963). Zimmerman and others (1967) observed a difference of approximately 80 ppt between rain and water vapor in the same system. Since the isotopic fractionation factor is directly proportional to the mass of the involved molecules, the fractionation of tritium should be 3/2 the fractionation of deuterium. The fractionation of tritium in the residual water in comparison to the original water should be 165 ppt. This means that the measured tritium concentrations may be 165 ppt more enriched in tritium than the original precipitation. Table 22 shows the corrected tritium values based on the assumption that there has been 50 percent evaporation.

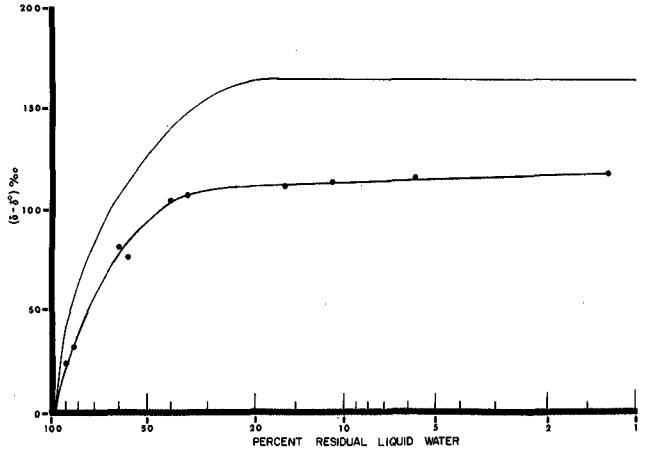

Figure 24. Enrichment of deuterium and tritium in liquid water during evaporation. Deuterium curve determined experimentally by Craig and others (1963). Hypothetical tritium curve calculated from deuterium curve.

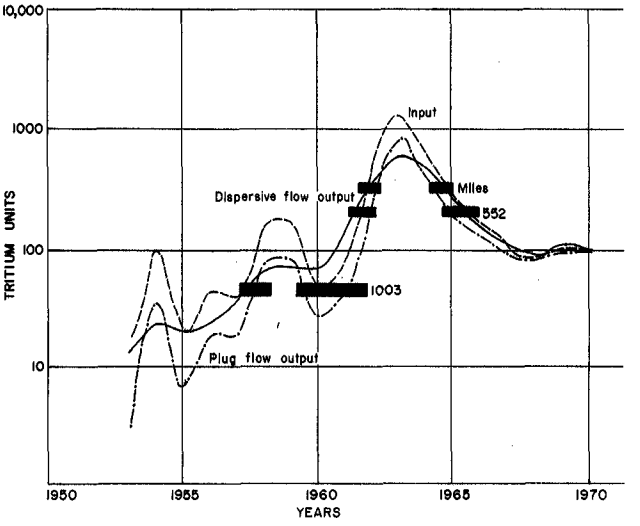

Figure 25. Tritium in ground water and precipitation.

The tritium input curve, the tritium plug flow output curve, the tritium dispersive flow output curve, and the corrected tritium concentrations of the three samples are plotted on a semilogarithmic graph (fig. 25). The intersection of the tritium concentrations of the samples with either the dispersive flow output curve or the plug flow curve indicates the approximate age of the ground water. The approximate ages of ground waters from wells 1003, 552, and the City of Miles are 13 to 17 years old, 8 to 13 years old, and 9 to 12 years old, respectively (table 22). These waters are younger than the mid-1950's, thus they are younger than terracing.