DEVELOPMENT AND APPLICATION OF THE NITROGEN ISOTOPE TRACER IN EVALUATING HYDROGEOLOGIC CONTROLS ON ABATEMENT OF NONPOINT SOURCE POLLUTION FROM LIVESTOCK CONFINED FEEDING AREAS AND OTHER AGRICULTURAL OPERATIONS

Final Project Report

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submitted to

The Texas Institute for Applied Environmental Research

Tarleton State University

Stephenville, Texas 76402

by

R. Stephen Fisher

The Bureau of Economic Geology

W. L. Fisher, Director

The University of Texas at Austin

Austin, Texas 78713-7508

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INTRODUCTION

The U.S. Environmental Protection Agency (EPA) considers nonpoint source pollution to be the largest single category of contamination affecting the nation's waters. Agricultural nonpoint source pollution, which can be a significant component of this overall problem, requires further research to establish and implement best management practices. Improved land-management methods to control nonpoint source pollution must be based on an understanding of the geological and hydrological characteristics of soils, bedrock, and aquifers, as well as the social and economic impacts of recommended or regulated management practices. In particular, determining the source, mobility, and transport paths of contaminants to and within surface-water and ground-water systems is essential in developing prevention and remediation procedures and in formulating management policy for agriculture.

In areas of multiple land use practices and nonpoint sources of contaminants, identifying contaminant sources and flow paths, and therefore establishing best management practices, can be exceedingly difficult. One such area is the Western Cross Timbers physiographic province of North-Central Texas, where land uses include crop agriculture, dairy farming, and tree growing. The Western Cross Timbers physiographic province includes Erath County and the drainage basin of the Upper North Bosque River. In 1989, the Texas State Soil and Water Conservation Board designated the Upper North Bosque River watershed as the watershed most severely affected by agricultural nonpoint source pollution (Texas Institute for Applied Environmental Research, 1992).

In Erath County, Texas, one of the major dairy-producing areas in the United States, the number and size of dairy operations have increased dramatically during the past decade. In 1989 there were 187 dairy operations with a cumulative herd of 48,542 cows in Erath County (Texas Institute for Applied Environmental Research, 1992). Concentrated animal-feeding operations (CAFOs) are prevalent in the dairy industry and can significantly impact nonpoint source contamination of surface-water and ground-water systems. Analyses of shallow ground waters in

Erath County show that some nitrate concentrations are more than three times the EPA maximum contaminant level (Greene and Klemt, 1992). Other preliminary data suggest that nutrients, including nitrate and ammonium, are moving to the water table through the unsaturated zone beneath and near dairy waste lagoons (Nelson and others, 1992). Potential contributors include animal waste, fertilizers applied to lawns and cultivated fields, and natural accumulation of atmospheric nitrogen in soil and ground-water systems. The relative amount contributed from each source and the mobility and fate of each nitrogen-containing species in the various geologic and hydrologic environments must be evaluated before informed policy decisions regarding the dairy industry can be made.

This research is part of a national pilot research program, which is intended to develop a model of agricultural pollution associated with CAFOs and various dairy management practices. Such a model is needed to design strategies to abate nonpoint source pollution associated with livestock production agriculture and to evaluate the economic impact of various management practices. Research results should have national applications, including (1) site-specific methods for evaluating effects of CAFOs and management practices on ground-water quality and (2) design of institutional and technological approaches to minimize degradation of surface-water and ground-water quality.

Using deterministic process models to evaluate movement of pollutant nutrients and chemicals under various land-management scenarios requires understanding natural physical and chemical reactions and flow and transport rates in the unsaturated zone and underlying perched and regional ground waters, as well as quantifying the effects of land-management practices on the dairy and agriculture industry. The critical first step is identifying and quantifying sources, residence times, transport rates, and fates of nutrients and pollutant chemicals in the environment.

This initial project had a single objective: to investigate the utility of stable nitrogen isotope compositions ($\delta^{15}N$) of nitrate and ammonium as an indicator of the source of nitrogen in soils and shallow waters, as a tracer of contaminant movement, and as a record of the volumetrically significant sources of nitrate and ammonium in surface- and ground-water systems. After the utility

of the nitrogen isotope tracer is established, we will be able to proceed with associated geologic and hydrologic investigations that are necessary to accurately model nutrient transport in the Upper North Bosque watershed.

RESEARCH TASK: EVALUATE THE NITROGEN ISOTOPE TRACER

Previous studies in other areas have shown that various sources of nitrogen have distinctly different isotopic compositions (summarized by Letolle, 1980; Heaton, 1986). Relative to atmospheric nitrogen, animal waste is enriched in ^{15}N by 10 to 25 parts per thousand (‰), whereas nitrogen derived from organic matter in cultivated soils is enriched by only about 2 to 8‰, and nitrogen derived from artificial fertilizers is typically depleted by 1 to 2‰ (fig. 1). Precise values for each source of nitrogen vary with climate and soil conditions and therefore must be established for any given study area. Because compositional differences similar to those in previous studies are expected to exist in Erath County, analyses of nitrogen isotope compositions would be a powerful tool for assessing contaminant sources. Research tasks were directed toward establishing methods for collecting and isotopically analyzing nitrogen from nitrate and ammonium in Erath County soils and waters.

Task 1a. Develop procedures for sampling and nitrogen isotope analysis

The Bureau of Economic Geology (BEG) has a stable isotope ratio mass spectrometer suitable for nitrogen isotope analysis and a vacuum line for sample preparation of nitrate and ammonium extracted from soils and water. However, some specialized equipment required for preparation of samples for nitrogen analysis had to be obtained and installed before stable nitrogen isotope compositions could be routinely measured. Acquiring this equipment, establishing procedures for our laboratory, and creating formal Quality Assurance Specific Work Instructions for nitrogen isotope analysis was our first task.

Task 1b. Determine the range of nitrogen isotope compositions

After our analytical capabilities were established, we wished to demonstrate the utility of stable nitrogen isotope investigations in Erath County, Texas. Specifically, we needed to demonstrate that dairy waste in Erath County had approximately the same $\delta^{15}N$ composition as animal waste reported in other nonpoint source pollution investigations, and that our sample collection and analysis methods could detect and quantify the concentrations and isotope compositions of nitrogen species in soils and waters from Erath County. Collecting and analyzing soil and water samples, as well as comparing the results of our analyses with those of other published investigations, constituted the second task of this project.

RESULTS

We organized our work into eight subtasks. Those subtasks and our results are described below.

Subtask 1. Establish methods for chemical analysis of ammonium and nitrate in water and soil leachates. Prepare Specific Work Instructions (SWI's) for chemical analysis of dissolved nitrate and ammonium in waters and soil extracts.

Result: We have determined that standard methods at the BEG Mineral Studies Laboratory (MSL) described in SWI 1.15, "Determination of anions by ion chromatography," and SWI 1.17, "Determination of ammonium, nitrate, and nitrite forms of nitrogen in waters," are sufficient. Following these methods, we can routinely measure nitrate at levels as low as 0.5 ppm NO_3. Ammonium can be measured at levels as low as 0.2 ppm N. No changes are needed to our current Specific Work Instructions for nitrate, nitrite, and ammonium chemical analysis.

Subtask 2. Obtain standard reference materials for nitrogen isotope analysis.

Result: We have obtained five National Institute of Standards and Technology standard reference materials that cover a range of $\delta^{15}N$ compositions from –25‰ to +60‰. We have also established and tested methods to extract and purify atmospheric nitrogen for use as a laboratory standard. Because the range of $\delta^{15}N$ in soils, fertilizer, and animal waste is typically –5 to +30‰,

these reference materials allow us to fully monitor the performance of our stable isotope mass spectrometer for the nitrogen isotope study.

Subtask 3. Test the performance of our mass spectrometer for nitrogen isotope analysis using analytical grade nitrogen gas. Determine optimum operating conditions for isotope analysis and establish expected values for analytical precision.

Result: We have established optimum operating conditions for the instrument. Analytical precision, including sample preparation, is routinely better than 0.4‰, or approximately 1% of the total range of values anticipated.

Subtask 4. Establish procedures for extracting nitrogen species from waters and soils and for analyzing $\delta^{15}N$ in waters and soil extracts. Purchase necessary equipment, build and test extraction equipment, and write Specific Work Instructions for sample preparation and analysis.

Result: We reviewed various established methods and selected procedures that allow us to efficiently prepare samples and perform stable isotope analyses using existing facilities and equipment. Parameters such as minimum and maximum sample size and optimum extraction conditions have been established. We are able to analyze the nitrogen-15 composition of solutions that contain as little as 5 to 10 ppm NO_3-N or NH_4-N. The EPA maximum contaminant level for nitrate in drinking water is 10 mg/L NO_3-N. Therefore, we can perform nitrogen isotope analyses on all waters that exceed EPA maximum contaminant levels for nitrate. We have prepared SWI 1.24, "Preparation of ammonium chloride specimens from dissolved ammonium-, nitrate-, or Kjeldahl-nitrogen for stable nitrogen isotope determinations," and SWI 1.25, "Preparation of nitrogen gas from ammonium salts for nitrogen stable isotope analysis," which are enclosed as appendix 1a and 1b.

Subtask 5. Test nitrogen isotope analysis using solutions prepared from a variety of materials. Analyze duplicate samples and evaluate results.

Result: We tested the recovery and analytical precision for nitrogen in solutions that were prepared from a variety of nitrate and ammonium salts. The estimate of precision for sample preparation and analysis cited in the results of subtask 3 are based in part on the data obtained in

this subtask for single salt solutions. Because nitrate and ammonium are stable nitrogen species under drastically different oxidation conditions, encountering solutions that contain both nitrate and ammonium will be rare. Furthermore, results of previous studies indicate that the $\delta^{15}N$ composition of nitrate and ammonium will be similar if both are derived from the same source (Heaton, 1986). However, to reduce the possibility that trace amounts of ammonium could affect the measurement of $\delta^{15}N\text{-}NO_3$, we conducted preliminary tests to establish optimal conditions for separating NO_3-N and NH_4-N from solutions prepared by mixing known quantities of isotopically characterized nitrate and ammonium salts. Results were used to refine the chemical separation methods described in SWI 1.24. If future studies find samples with subequal amounts of NO_3-N and NH_4-N from different sources, we will further refine these methods to provide a means for isotopically characterizing the end-member nitrogen species.

Subtask 6. Prepare a Specific Work Instruction for field collection of water and soil samples for nitrogen isotope analysis.

Result: We reviewed methods cited in recent publications as well as those recommended by the U.S. Geological Survey and the Environmental Protection Agency. The resulting SWI 3.18, "Collecting and preserving soil, sediment, and water samples for measuring the concentration and stable nitrogen isotope composition of aqueous nitrate and ammonium," is enclosed as appendix 1c.

Subtask 7. Conduct field sampling in Erath County.

Result: Sampling was coordinated with Texas Institute for Applied Environmental Research staff and completed in October 1993. We collected both surface-water samples and soil samples from fields irrigated with dairy lagoon waste.

Subtask 8. Analyze soil and water samples from Erath County for nitrogen species and nitrogen isotope composition.

Result: Analyses are complete, and results are listed in table 1.

DISCUSSION

We collected three water samples from the North Bosque River: one at the river in Stephenville, Texas, a second at the river immediately below the outflow of the Stephenville municipal water treatment plant, and a third at the U.S. Highway 281 bridge south of Hico, Texas. An additional water sample was collected from a secondary-treatment lagoon at a dairy in Erath County. Four soil samples were obtained from fields irrigated with lagoon water at a large dairy. Sample identification information is listed in table 1.

The $\delta^{15}N$ of nitrogen recovered from the secondary-treatment lagoon is 23.6‰, in excellent agreement with reported values for the nitrogen isotope composition of nitrate derived from animal waste (fig. 1). However, filtered water from the secondary-treatment lagoon had no detectable nitrate or ammonium. All nitrogen species were incorporated in particles or colloids, which were filtered from the sample and then analyzed. Further research is needed to determine whether this is true for other waste lagoons and whether the incorporation of nutrients in particles and colloids rather than in solution impacts calculations of nutrient consumption by crops or grasses in fields irrigated with dairy waste.

Nitrate was extracted from each soil sample using both double deionized water and a 2N KCl solution. In three of the four cases, $\delta^{15}N$ of nitrate extracted in 2N KCl is isotopically heavier than that extracted in double deionized water (table 1). Nitrate extracted from two of the irrigated soils (soil 2s and soil 3, table 1) has a $\delta^{15}N$ value of about 19 to 24‰, suggesting that nutrients in lagoon water are mineralized to nitrate with no significant isotopic fractionation from the composition of lagoon nitrogen. Nitrate extracted from two other irrigated soils (soil 1 and soil 2d, table 1) has a $\delta^{15}N$ value of about 14 to 15‰. Several hypotheses may be offered to explain the difference in $\delta^{15}N$ between the two sets of soil samples: (1) it may be an artifact of the very limited number of samples measured, (2) it may reflect different transport rates of nitrate through the shallow unsaturated zone caused by differences in soil properties and thus represent irrigation applied under different temperature or precipitation conditions, and/or (3) it may reflect microbial processes in the soils. In

any event, the $\delta^{15}N$ of nitrate derived from all soils (14 to 24‰) is sufficiently like that of nitrate from lagoon nitrogen (23.6‰) that there would be no problem in distinguishing between an animal waste source of the nitrogen and a fertilizer source (0 to 5‰) (fig. 1).

The North Bosque River immediately downstream of the Stephenville water treatment plant has high nitrate concentrations and a $\delta^{15}N$ composition of 18.3‰. Because of the sample location and isotopic composition, the source of this nitrate is probably treated municipal waste. North Bosque River water sampled in Stephenville above the water treatment plant has moderate amounts of nitrate with a $\delta^{15}N$ value of 9.7‰. The simplest interpretation of this value is that the nitrate reflects a mixture of dairy or municipal waste ($\delta^{15}N$ value of about 20‰) and fertilizer applied to crop fields or lawns ($\delta^{15}N$ of about 0 to 5‰). Nitrate levels in the North Bosque River at Hico, approximately 20 mi downstream from Stephenville, are below detection limits, and $\delta^{15}N$ was not measured. Whether nitrate is removed by denitrification, converted to algae or other particulate organic matter between Stephenville and Hico, or simply diluted by inflow from tributaries cannot be determined with the available information. However, dairies in the Upper North Bosque River watershed between Stephenville and Hico do not appear to have contributed significant amounts of nitrate to the surface-water system immediately before sample collection.

CONCLUSIONS

Nonpoint source pollution of water supplies and resources is unquestionably one of the major environmental issues facing the United States. Agriculture and concentrated animal-feeding operations contribute not only to nonpoint source contamination of surface and ground water but also to the economy of many parts of Texas. Formulation of policy and regulations to best manage potential sources of nonpoint source pollution must have a firm basis in the physical sciences as well as in economics if the issues are to be resolved in the best interests of all concerned parties.

Stable nitrogen isotope analyses have proven useful in many other areas as a way to identify sources of nitrate in regions of multiple land uses and to quantify the contributions of various

sources to surface-water and ground-water systems. This study has demonstrated that a similar approach can identify and quantify nitrate sources in Erath County, Upper North Bosque River Basin, Texas. We have extended the capability of an established stable isotope ratio mass spectrometer laboratory to include facilities to (1) collect and preserve soil and water samples, (2) determine the chemical composition and concentration of various nitrogen species, (3) prepare nitrogen gas, and (4) measure the stable isotopic composition of nitrogen in soils and waters. We have also demonstrated that these methods produce realistic results for soil and water samples collected from Erath County dairy operations.

We are now ready to begin a thorough investigation of sources, transport rates, and fates of nitrogen nutrients produced by various nonpoint sources in the Cross Timbers province. We recommend that scientific hydrogeological experiments and monitoring of both the ground-water and vadose-zone systems be conducted to assess water quality under ambient conditions at livestock production centers and at controlled sites. Environmental geologic maps detailing land-use capability and recharge potential should be made of the North Bosque River watershed. These studies are needed to document the reactions controlling fate and transport of nutrients or pollutants in the unsaturated and saturated subsurface and to provide realistic estimates of nutrient transfer rates for use in nutrient-transport and economic-impact modeling.

ACKNOWLEDGMENTS

We appreciate the interest and assistance of the Texas Institute for Applied Environmental Research, particularly that of Ron Jones, Larry Hauck, and Tim Jones. Steve Tweedy and Bruce Turbeville of the BEG Mineral Studies Laboratory established and tested the chemical and isotopic analytical techniques and prepared SWI 1.24 and SWI 1.25. Alan Dutton, Steve Tweedy, and Bruce Turbeville reviewed an early draft of this report.

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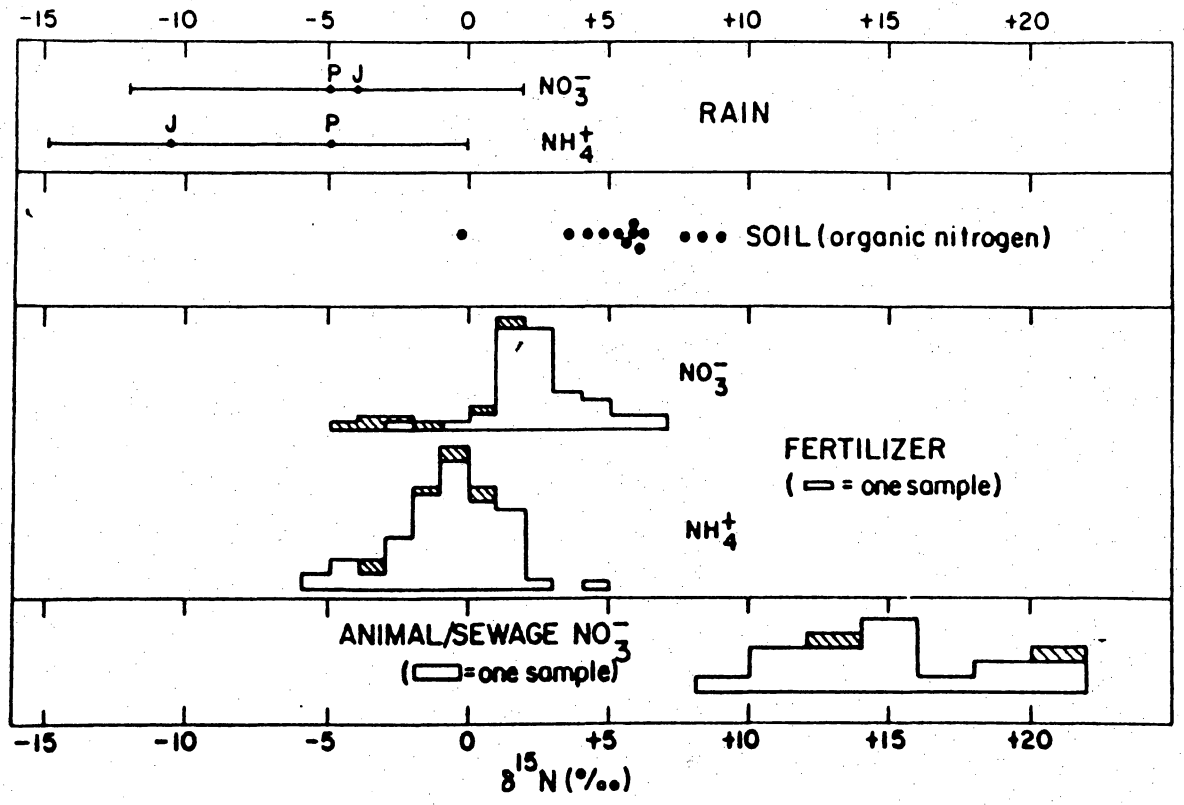


Figure 1. Summary of the range of $\delta^{15}N$ values for the various potential sources of nitrogen, ammonium, and nitrate in soils, surface water, and ground water (from Heaton, 1986).

Table 1. Results of analyses of water and soil extracts.

Water samples	NO_3 (mg/L)	$\delta^{15}N$ (‰)
North Bosque River at Stephenville	3.4	9.7
North Bosque River downstream from the Stephenville water treatment plant	24.0	18.3
North Bosque River at Hico	bd	na
Filtered water from secondary-treatment lagoon	bd	na
Particulate matter from secondary-treatment lagoon		23.6

Soil extracts	NO_3 (mg/kg)$_{ddw}$	$\delta^{15}N$ (‰)$_{ddw}$	$\delta^{15}N$ (‰)$_{KCl}$
Soil 1	330	14.0	14.6
Soil 2s	490	19.2	20.6
Soil 2d	430	14.9	14.4
Soil 3	530	21.4	23.7

Notes:

bd: below detection limit
na: not analyzed

Filtered water from secondary-treatment lagoon had no detectable nitrate or ammonium. All nitrogen species were incorporated in particulate, organic, and colloidal material, which was filtered from the sample and then analyzed.

Soil nitrate was obtained by extracting soluble material in double deionized water (ddw) and in 2N KCl solution (KCl) at a soil:solution ratio of 1:10 by weight.

Soil 1: Windthorst (?) soil from irrigated dairy field
Soil 2s: Surface soil sample from irrigated dairy field
Soil 2d: Red clayey soil approximately 6 inches beneath soil 2s
Soil 3: Soil from irrigated dairy field

APPENDIX 1a: SPECIFIC WORK INSTRUCTION SWI 1.24

Preparation of Ammonium Chloride Specimens from Dissolved Ammonium-, Nitrate-, or Kjeldahl-Nitrogen for Stable Nitrogen Isotope Det ions

SPECIFIC WORK INSTRUCTION

BUREAU OF ECONOMIC GEOLOGY
THE UNIVERSITY OF TEXAS AT AUSTIN

SWI 1.24

TITLE:

PREPARATION OF AMMONIUM CHLORIDE SPECIMENS FROM DISSOLVED AMMONIUM-, NITRATE-, OR KJELDAHL-NITROGEN FOR STABLE NITROGEN ISOTOPE DETERMINATIONS

APPROVAL:

DIRECTOR DATE

CONCURRENCE:

DEPUTY ASSOCIATE DIRECTOR DATE 01/04/94

QUALITY ASSURANCE MANAGER DATE 1.4.94

1.0 SCOPE

This procedure describes the laboratory activities required to produce ammonium chloride specimens from dissolved nitrate-nitrogen (NO_3-N) and ammonium-nitrogen (NH_4-N) of natural waters and sample digests (i.e., Kjeldahl) for the purpose of determining the nitrogen stable isotope signature ($\delta^{15}N$).

Because this procedure follows the quantification of forms of nitrogen (SWI 1.17), many aspects of that procedure have been retained in this document.

2.0 METHODOLOGY

2.1 Summary of Method

Isolation of ammonium (NH_4^+), nitrate (NO_3^-), and Kjeldahl (organic + NH_4^+) forms of nitrogen is accomplished through the steam distillation of ammonia (NH_3) under varied conditions. The ammonia is subsequently trapped, quantitatively measured by titration with standardized hydrochloric acid (when required), then recovered as an ammonium chloride solid specimen suitable for the determination of $\delta^{15}N$ by companion procedures (SWI's 1.22, 1.25).

In this procedure, (1) NH_4^+-N is steam distilled in the presence of a slurry of magnesium oxide and (2) (NO_3^- + NO_2^-)-N are subsequently distilled in the presence of magnesium oxide plus Devarda's alloy. The "magnesium oxide slurry" presents a buffered, pH 10.2 medium appropriate for steam distillation of ammonia. The finely divided Devarda's alloy acts as a reducing agent/catalyst.

Distillation flask of 1000-mL capacity, with an extra stoppered opening for introducing the Devarda's alloy

Metrohm Titroprocessor, Model 672

Metrohm Dosimat, Model 655

Titrant assembly; 10 mL burette, amber Metrohm type 6.1518.220, with anti diffusion tip

Combination glass electrode, Metrohm type 6.02, or equivalent

Glass beakers, 200-500 mL

Magnetic stirring plate with Teflon-coated stirring bars

Thermometer, capable of reading to within 0.1°C

Controlled temperature heating block, 40 x 1" holes, capable of maintaining 80±5°C

Purge system: compressed air, 16 port, exchangeable delivery tips, high capacity ammonia scrubber (conc. H_2SO_4, with indicator), adjustable delivery volume (1 to 10 L/min), see Figure 2.

2.5 Reagents/Materials

De ionized water (DI-H_2O), ASTM type III (Standard Specification D1193-77).

Boric acid (2 %), Dissolve 20 g of boric acid (H_3BO_3) in 1 L of water.

Devarda's alloy (50% Cu + 45% Al + 5% Zn), Grind to pass a 60-mesh sieve.

Magnesium oxide, Ignite reagent-grade MgO at 800°C for 3 hr. Cool to room temperature in a desiccator and store in a screw-cap jar.

Ammonium-N (1 mg NH_4^+-N/mL) standard solution, Dissolve 4.7170 g of $(NH_4)_2SO_4$ (dried at 105°C) in water; add 5 mL of concentrated (12N) HCl, and make up to 1 L by adding DI-H_2O.

Nitrate-N (1 mg NO_3^--N/mL) standard solution, Dissolve 7.2187g of KNO_3 (dried at 105°C), in water; add 5 mL of concentrated (12N) HCl, and make up to 1 L with DI-H_2O.

Hydrochloric acid (standardized titrant/around 0.018N), Pipette approximately 1.5 mL of concentrated (12N) HCl into a 1 L flask half filled with DI-H_2O, then dilute to 1 L and mix. Transfer this solution to the reservoir of the auto-titrator assembly. Standardize this solution with THAM prior to each use. (see Sections 2.6.4 and 2.6.5.)

THAM [Tris-(hydroxymethyl)aminomethane] (0.100 N), Dissolve 12.114 g dry (1 hr @105°C) THAM in 1 L of de-ionized CO_2-free DI-H_2O. Stopper in an airtight container. (This is the primary standard.)

Reference pH buffer solutions, pH 4.00 (±0.01), 7.00 (±0.01), 10.00 (±0.02); NBS traceable. Check the expiration date on each buffer solution, discard any solution whose expiration date has elapsed.

Sulfuric acid, concentrated, reagent grade

3. The printed calibration data include the relative slope and the measured isopotential point (IP). Theoretically, the value for the slope is 1 and the IP is 0 mV. The slope will normally vary by ±0.02 units and is an indication of the relative response of the electrode to the theoretical Nernstian response of 59.16 mV (@ 25°C). A deviation of greater than ±0.02 units indicates a problem with the glass electrode or with the buffer solutions. Investigate and correct any problem and repeat this procedure until electrode calibration is satisfactory.

4. Check the calibration with the pH 7 buffer solution by inserting the electrode in the buffer solution and pressing the **[enter]** key; when two consecutive printed pH measurements agree within ±0.01 units, then the last value is the measured pH of the buffer. The measured pH should be 7.00±0.05 pH units. If not, then the calibration procedure must be repeated.

5. Proceed with HCl standardization.

2.6.4 Preparation of Titration System for HCl Standardization

1. Load the standardization method into memory by pressing **[user methods]**. The display will show "recall". Key in **[2-1]** and **[enter]**. Display will show "GET pH 2-1". The standardization method is now recalled and in a ready state.

2. Check all parameters, formulas, constants, and preparation steps before beginning the analysis. Typical values are listed below with each parameter. Some of the titration control parameters such as the pulse range, drift, (t)delay, and dv/dt are sample-dependent variables and may be optimized by the analyst. The goal in optimization is to achieve a reasonably fast yet accurate titration. Whatever values are derived for these parameters, they should remain the same throughout the entire analysis.

NOTE: Each parameter will appear in turn by pressing **[enter]**. Refer to the Instruction Manual for a more detailed explanation.

a) Press **[prep. steps]**. The prep. steps contain any commands that precede the titration.

"add vol.	.00 mL"	Reagent dump
"pause	30 s"	Titration delay (seconds)
"electr. input	1"	Measuring input

b) Press **[parameters]**. The parameters control the various aspects of the titration:

"titration rate	0.50"	Delivery rate (mL/min)
"anticip.	20"	Slows delivery near endpoint
"stop V	10.00 mL"	Maximum titrant per sample
"stop pH	4.00"	Stop when this pH is reached
"stop EP#	OFF"	
"start V	5.00 mL"	Add volume of titrant to begin
"start pH	OFF"	
"start slope	OFF"	
"temp.	(xx.x) °C"	Key in temp. of solutions
"Stop V	OFF"	Stop volume - max. volume

c) The normality calculation will be done by the microprocessor. Verify the formula for calculating the normality of HCl by pressing **[2nd]** **[fmla]**. The display shows "F?"; enter **[1]**; the formula now appears:

F1 = C00 x C01/EP1;4;mole/L (1)

"start slope	OFF"	not used
"temp	xx.x°C"	enter temperature of solutions
"EP crit	1"	(1-10) controls EP sensitivity

c) Press **[2nd]**, **[fmla]**. "F?" appears; enter **[1]**; the formula is displayed:

F1 = (EP1-C01)*C02*C03*C04/C00;2;ppm (2)

where

F1 = RS1 = ammonium-nitrogen (mg/L NH_4^+-N)
EP1 = titrant volume (mL) required for sample
C01 = titrant volume (mL) required for sample blank
C02 = normality of HCl titrant
C03 = atomic weight for nitrogen
C04 = 1000 = conversion to desired units of "ppm"
C00 = sample volume (mL) prior to distillation
2 = decimal places in result
ppm = units of result (parts per million).

d) Press **[fmla const]**. Verify the formula constants by pressing **[fmla const]**, each constant will appear in turn by pressing **[enter]**. When "C02 = " appears, enter the new value calculated in standardization of the hydrochloric acid. Store this value by pressing **[user methods]** until "store 20" appears, then press **[enter]** twice.

2.6.7 Preparation of the Steam Distillation Apparatus

1. Assemble the steam distillation apparatus by referring to Figure 1.

2. Turn on the power to the variable transformer of the steam generator at a setting of 100%. Ensure that the 4-L boiling flask contains approximately 3 L DI-H_2O plus 50 mL sulfuric acid (this acid need only be added when filling an empty flask). Turn on the cooling water to the condenser.

3. Attach an empty distillation flask and close all valves leading to atmosphere. When constant boiling is achieved (approximately 20 minutes), adjust the setting of the variable transformer to obtain distillate from the condenser at a rate of 7 to 8 mL per minute.

2.7 Calibration/Standardization

Perform pH standardization and HCl-titrant standardization as described in Sections 2.6.3 and 2.6.5.

2.7.1 Standardization Test for Sample Analysis

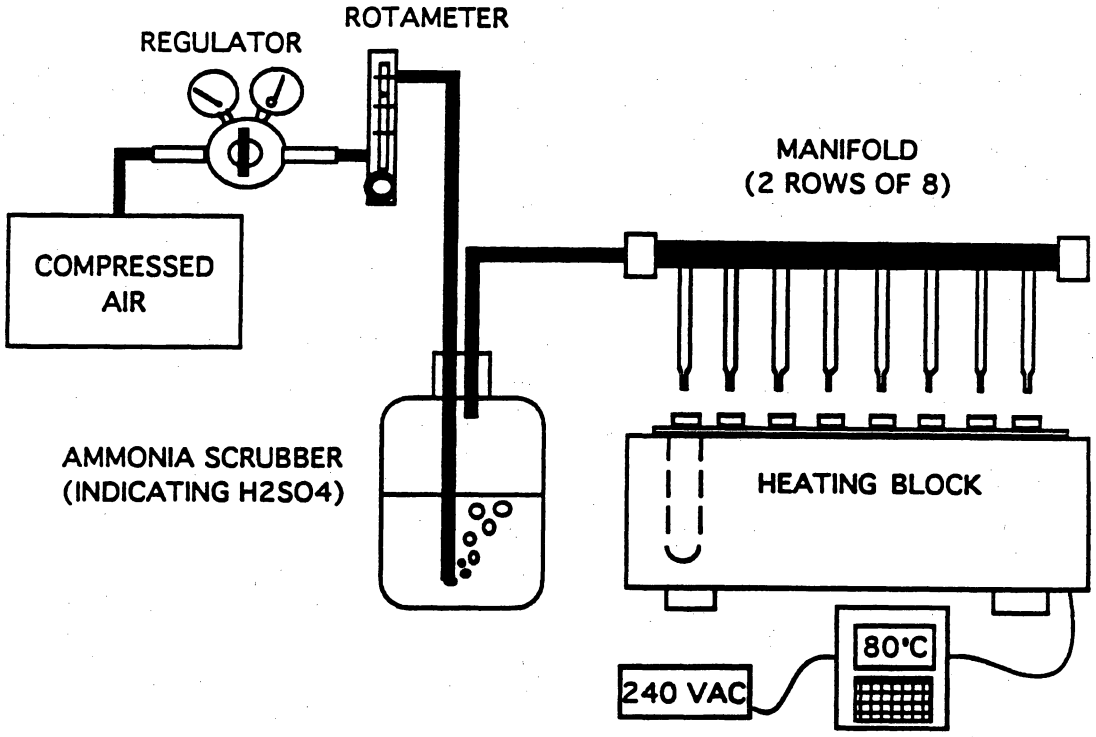
1. Set up the titration system for sample analysis as described in Section 2.6.6.

2. Place the electrode and the titrant delivery tip into the solution obtained from the steam distillation of a known quantity of nitrogen (boric acid plus the distilled ammonium-nitrogen for the species of nitrogen sought). Include a magnetic stir-bar. Enter the sample specific data, as detailed previously, then press [enter]. Start the titration by pressing [Go].

3. A printout of the report will automatically follow when the titration stops. The ammonium-nitrogen concentration is the RS1 value.

Note: The results obtained from the above steps will indicate the accuracy of the steam-distillation/titration procedure. If quantitative recovery is not achieved, investigate and correct any possible problems until satisfactory recovery is attained.

Figure 2.
Sample Drying Apparatus



14. Composite samples to one vial during or after evaporation. Resulting ammonium chloride residues are scraped from the bottom of the vials and used for the determination of $\delta^{15}N$ by SIRA-MS.

2.8.2 Steam Distillation of NH_4^+-N for Quantitative Determination

1. Blanks and standards: Two blanks and three standards should be run with each batch of samples to ascertain reliability of results.

   a) Steam-wash the distillation apparatus for 10 minutes by assembling the complete apparatus with a boiling flask containing only 0.05 grams of magnesium oxide. Follow with distillation of the blanks, consisting of all reagents minus any sample solution.

   b) Distill three NH_4^+-N standards (0.10 to 1.0 mg) using aliquots of the NH_4^+-N standard solution.

2. Place a 50 mL beaker containing 5 mL boric acid under the condenser tip to absorb the ammonia in the distillate. It is not necessary to submerge the tip in the boric acid, provided the distillate is cooled to a temperature below 25°C and condensed.

1. Ammonia: When reporting results from the determination of NH_4^+-N, multiply the result by a factor of 1.216X, round off to the nearest tenth, and report as mg/L NH_3.

2. Nitrate: After determining the component of the analytical results that represents the concentration of NO_3^--N in the sample, multiply this result by a factor of 4.427X, round off to the nearest tenth, and report as mg/L NO_3^-.

3.0 TRAINING

Individuals performing analyses specified in this SWI shall receive training in its requirements and shall demonstrate acceptable performance in a supervised trial run. Documentation of this qualification shall be maintained in the Records Center.

4.0 QUALITY ASSURANCE/CONTROL

Replicate analysis of standards and samples is required to provide an estimate of the accuracy and precision of the method. For each set of analyses, three aliquots of a suitable reference standard shall be analyzed along with the samples. In addition, 1 sample shall be selected from each group of 10 and 3 sub samples from it shall be analyzed.

Reference Standards

No water/brine standards exist with certified or recommended nitrate concentration values. The standards are prepared from pure compounds as described in Section 2.5.

The standard of choice for MSL nitrate analyses is purchased commercially from Fisher Scientific, 1 mg/L, as NO_3.

At the discretion of the Chief Chemist, other reference materials (if they can be obtained from a reputable source) may be substituted for the above.

The method of "spike recovery" is also appropriate to illustrate the applicability of this procedure. When working with unknown or questionable matrices, spike a representative sample with a known amount of nitrogen and verify quantitative recovery. If quantitative recovery is not achieved, this procedure is not valid for the associated sample matrix.

All analytical results, including accuracy and precision results, are reported in the MSL Analysis Report. Copies of the Analysis Report are distributed to the investigator, the QA Group, the Administrative Group, and the MSL report files.

5.0 RECORDS

Keep detailed records of the analyses and record the data from acceptable titrations on the "Analytical Data Log and Worksheet."

All pertinent information regarding the analyses can be recorded by instrument printout. The pH calibration data, the standardization results, and the sample analysis data will be recorded automatically. When the analyses are complete, record all other analysis information on the same printout by using the following keystrokes:

[report] [parameters] [enter]
[report] [prep steps] [enter]
[report] [2nd] [fmla] [enter]
[report] [fmla const] [enter]

Store the printout containing all data with the worksheet in the "Forms of Nitrogen" Procedure Log Book. These records will be filed and stored at the MSL facility of the Bureau.

APPENDIX 1b: SPECIFIC WORK INSTRUCTION SWI 1.25

Preparation of Nitrogen Gas from Ammonium Salts for Nitrogen Stable Isotope Analysis

SPECIFIC WORK INSTRUCTION

BUREAU OF ECONOMIC GEOLOGY
THE UNIVERSITY OF TEXAS AT AUSTIN

SWI 1.25

Date: December 20, 1993 **Revision:** 0
Supersedes: Not applicable

TITLE:

PREPARATION OF NITROGEN GAS FROM AMMONIUM SALTS FOR NITROGEN STABLE ISOTOPE ANALYSIS

APPROVAL:

DIRECTOR **DATE**

CONCURRENCE:

01/04/94
DEPUTY ASSOCIATE DIRECTOR **DATE**

1.4.94
QUALITY ASSURANCE MANAGER **DATE**

1.0 SCOPE

This method describes the preparation of high-purity N_2 gas from ammonium salts derived from water samples for $^{15}N/^{14}N$ isotopic determinations. The actual determination of the isotopic ratios of gas samples by mass spectrometry is covered in SWI 1.22.

Nitrogen isotopes are not fractionated using this method provided that the combustion of ammonium salts to produce N_2 gas is carried out to completion. Therefore, ammonium chloride or ammonium sulfate samples can be prepared individually or in batches with or without ammonium standards. Standards, however, should be included each time to test the method and to verify complete combustion with new reagents.

2.0 METHODOLOGY

2.1 Summary of Method

Ammonium chloride or ammonium sulfate samples are combusted with hot cupric oxide and calcium oxide (at 550°C) in an evacuated reaction tube to release N_2. Other gases produced during the combustion are effectively absorbed by the calcium oxide. The N_2 is then measured directly on a mass spectrometer to determine the $^{15}N/^{14}N$ ratio.

The relevant partial reaction is

$$NH_4Cl \text{ [or } (NH_4)_2SO_4\text{]} + CuO == {}^{(550°C)} ==> N_2$$

Figure 1
High Vacuum Sample Preparation Line

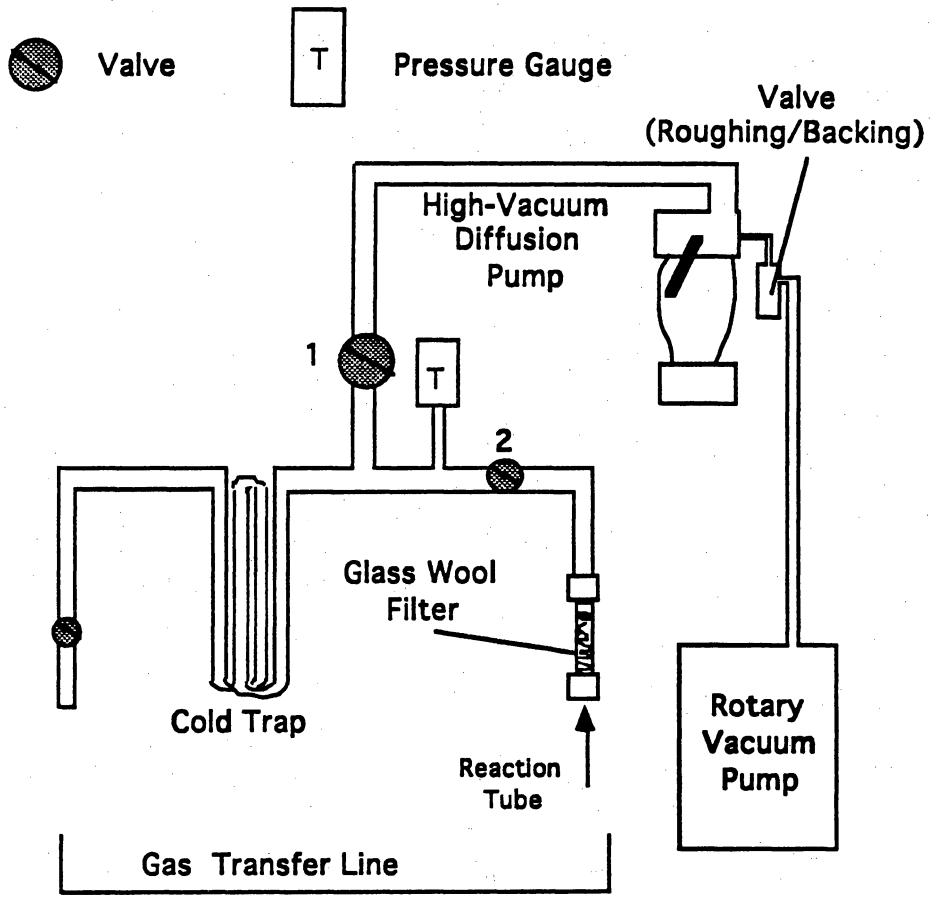
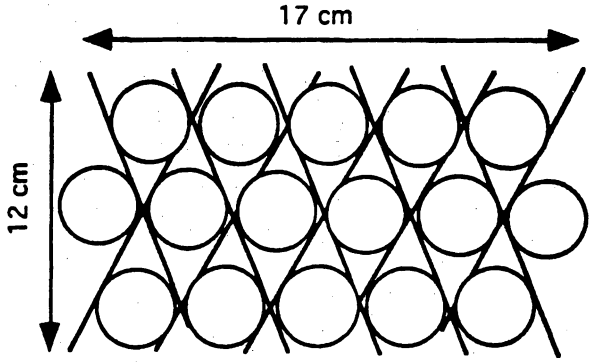


Figure 2
Reaction Tube Layout



Load reaction tube with approximately 5 cm of Pyrex glass wool, 3-4 grams of cupric oxide wire pieces, 10-20 mg of sample (as an ammonium salt), 75-100 mg calcium oxide, 3-4 grams of cupric oxide wire pieces, and 5 grams of copper shot.

Attach reaction tube to vacuum port below valve #2; an extra glass wool filter is placed between the reaction tube and this valve to prevent reagents from entering the vacuum system; tighten Ultra-Torr union.

Open valve #2 to evacuate tube.

Gently heat the base of the reaction tube and reagents to drive off any moisture.

Pressure will go up temporarily in the vacuum system; allow pressure to fall to 0.0004 torr or less.

Close valve #2.

Seal off reaction tube with a natural gas/oxygen torch at a point approximately 1-inch below the Ultra-Torr. If quartz or Vycor tubing is used instead of Pyrex, much higher temperatures (i.e., longer contact time with torch flame) are required before tubing will effectively seal.

Repeat above steps for up to 20 samples or standards.

Place the sealed reaction tube in one of the stainless steel tubes in the rack configuration of Figure 2.

Place the expanded metal rack inside the muffle furnace and heat to 550°C.

Heat reaction tubes for at least 3 hr, which should allow for 1 hr of gradual heating and 2 hr maintained at maximum temperature. Turn off muffle furnace and leave tubes in the oven for at least 12 hr to ensure gradual cooling of tubes. This cooling period is critical to the complete absorption of gas impurities by calcium oxide.

The nitrogen gas is now ready for isotope analysis without further processing. These gases, in the cooled reaction tubes, can be stored indefinitely.

Mercury-Ram Transfer System

In the event that very small quantities of gas are anticipated during the combustion, an additional gas transfer is accomplished with the mercury-ram transfer apparatus shown in Fig. 4. This allows further cryogenic purification, if necessary, and the transfer system is fitted with a mercury manometer for accurate gas pressure measurement.

Attach either a collection vessel with high-pressure valve (see SWI 1.20) or a Pyrex sample tube below valve #5, and insert sample reaction tube into cracker assembly (see SWI 1.22) below valve #1; tighten Ultra-Torr fittings of both connections.

Open valves #5, #4, #3,#2, and #1 to evacuate the transfer line, cracker assembly, and collection vessel/tube; allow pressure to fall to 0.0004 torr or less.

Close valves #1, #2, #3, #4, and #5; crack reaction tube at bend in cracker assembly.

Open valve #4, and pump the squeeze bulb to fill column with mercury. The two-way valve (Fig. 4) is used to regulate pressure in the mercury reservoir.

Immerse the LN_2 trap in a Dewar filled with liquid nitrogen.

Close valve #4, and open valve #3.

Pump the squeeze bulb to again fill column with mercury; this will force the sample gas out of the column and into the collection vessel/tube.

Repeat this process at least three times to ensure that as much gas as possible is transferred from the reaction tube to the collection vessel/tube (total recovery should be about 90-95%).

Close valve #5, and either close valve on reaction vessel, or seal collection tube with natural gas/oxygen torch.

This nitrogen gas in the collection vessel/tube can be stored indefinitely.

3.0 TRAINING

Individuals performing analyses specified in this SWI shall receive training in its requirements and shall demonstrate acceptable performance in a supervised trial run. Documentation of this qualification shall be maintained in the Records Center.

4.0 QUALITY ASSURANCE/CONTROL

Record in a lab book the proper sample identification, the date and temperature of sample reaction, and any unusual conditions that occurred during sample preparation. Ensure that proper labels are copied from original sample material to gas/reaction sample tubes.

5.0 RECORDS

Documents generated and completed by this SWI are Quality Assurance records and shall be submitted to the Records Center by the responsible initiator/authorizer. Prior to submittal, the sender shall assure that each document is complete, legible, and adequately identifiable. Control of these records shall be in accordance with QAP 17.1, "Quality Assurance Records Management Procedures."

The Quality Assurance records generated by this and associated SWI's include the following:

- Analysis Report
- Analysis Request Form
- Training documentation

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APPENDIX 1c: SPECIFIC WORK INSTRUCTION SWI 3.18

Collecting and Preserving Soil, Sediment, and Water Samples for Measuring the Concentration and Stable Nitrogen Isotope Composition of Aqueous Nitrate and Ammonium

SPECIFIC WORK INSTRUCTION

BUREAU OF ECONOMIC GEOLOGY
THE UNIVERSITY OF TEXAS AT AUSTIN

SWI 3.18

Date: December 31, 1993 **Revision:** 0
Supersedes: Not applicable

TITLE:
COLLECTING AND PRESERVING SOIL, SEDIMENT, AND WATER SAMPLES FOR MEASURING THE CONCENTRATION AND STABLE NITROGEN ISOTOPE COMPOSITION OF AQUEOUS NITRATE AND AMMONIUM

APPROVAL:

DIRECTOR DATE

CONCURRENCE:

DEPUTY ASSOCIATE DIRECTOR 01/10/94 DATE

QUALITY ASSURANCE MANAGER 1.10.94 DATE

1.0 SCOPE

This Specific Work Instruction (SWI) describes procedures for collecting and preserving soil, sediment, surface-water, and ground-water samples for measuring concentrations and the stable nitrogen isotope composition ($\delta^{15}N$) of nitrate-nitrogen (NO_3^--N) and ammonium-nitrogen (NH_4^+-N). These procedures are intended for use in agricultural non-point source pollution investigations where elevated (greater than 5 mg/L NO_3^--N) nitrate levels are anticipated, and where the samples are being collected specifically for nitrogen species analysis.

2.0 PROCEDURES

2.1 Soil and Sediment Samples

Approximately 1 to 2.5 kilograms of soil or sediment should be collected to provide material for extraction and analysis of NO_3^--N and NH_4^+-N. Additional material may be needed if textural analysis or mineral identification studies are also anticipated.

Sample containers should be washed with soap and water, rinsed with deionized water, and dried before use. Soil or sediment samples should be collected in clean, labeled, wide-mouth glass jars and tightly sealed at the time of collection. Borehole samples can be recovered using pre-cleaned Shelby tubes, split spoons, drive barrels, or as whole core. Sampling apparatus should be washed with soap and water and rinsed with deionized water before samples are collected. Samples should be sealed in appropriate clean containers and properly labeled. BEG water-sample labels (SWI 3.1, "Collection, Determination of Unstable Properties, and Field Treatment of Water Samples for Isotopic and Ionic Analyses") may be used for soil and sediment samples; only the top portion of the sample label need be completed. Labels on sample containers and field

of these records shall be in accordance with QAP 17.1, "Quality Assurance Records Management Procedures."

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