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ASSESSMENT OF THE ORIGIN AND FATE OF NITRATE FROM SOIL LYSIMETERS USING STABLE NITROGEN ISOTOPES

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ASSESSMENT OF THE ORIGIN AND FATE OF NITRATE FROM SOIL LYSIMETERS USING STABLE NITROGEN ISOTOPES

By

Keith Edwin Knoke

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ABSTRACT

ASSESSMENT OF THE ORIGIN AND FATE OF NITRATE FROM SOIL LYSIMETERS USING STABLE NITROGEN ISOTOPES

By

Keith Edwin Knoke

The concentration and $\delta^{15}N$ of nitrate in groundwater samples collected from two mid-Michigan lysimeters beneath farmland were determined on a weekly basis from March to November of 1993. The soil within one of the lysimeters was tilled and the other was not tilled. Nitrate concentrations in the samples from the tilled lysimeter were approximately twice those of the untilled lysimeter throughout the study. The nitrogen stable isotopic abundances varied similarly on a seasonal basis in the tilled and untilled lysimeters. The $\delta^{15}N$ values ranged between -3.9 $^{0}/_{00}$ in the spring to 9.4 $^{0}/_{00}$ in fall (September). An increasing trend in the $\delta^{15}N$ of nitrate from both lysimeters occurred from spring to late summer, that was likely the result of fractionation due to denitrification and mineralization of the less readily mineralizable organic fraction. A decrease in $\delta^{15}N$ was observed from the middle of September to early November that reflected a decrease in the influence of microbial denitrification on the isotopic composition of nitrate.

ACKNOWLEDGMENTS

I would like to thank Dr. Nathaniel Ostrom for all his help and patience during the time in which it took to complete this project. I would also like to thank my thesis committee members Dr. Grahame Larson, Dr. Lars Hedin and Dr. Steve Hamilton for valuable and thought provoking comments. Partial funding for this project was made available through the Long Term Ecological Research (LTER) program at KBS (NSF DEB 92-11771). The sample collection and analysis of NO_3^- concentrations was completed by Mike Brown, Jeff Hamelink, and Brian Kennedy of KBS. Without their help, this project would have been difficult to complete. In addition, I would like to thank fellow graduate students Jon Kolak, Michelle Thompson and Alan McCloud for providing the needed encouragement and just as needed distractions. Last and most importantly I would like to thank my wife Amy, who, in her caring way kept reminding me and encouraging me to complete this project.

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CHAPTER 1: INTRODUCTION

In the midwestern United States and numerous other locations around the world, nitrate contamination of ground water has become a major concern (Follett, 1989). High nitrate concentrations in groundwater have been linked to methemoglobinemia which is also know as "blue baby" syndrome (Schuphan, 1974). In response to this, the EPA has set recommended allowable limits of nitrate in drinking water of 10 mg NO_3-N/L (U.S. E.P.A., 1991). The health hazard associated with elevated concentration of nitrate in drinking water is, responsible for the scientific research dedicated to the better understanding of the causes and solutions to this problem. One area of scientific research used to assess this problem is the nitrogen stable isotope technique. The nitrogen stable isotope technique has primarily been used in determining the source of nitrate in groundwater (Kreitler, 1979, Gormly and Spaulding, 1979, Krietler and Browning, 1983, Heaton, 1984 and Flipse and Bonner, 1985). In addition to determining the source of the nitrate, the nitrogen stable isotope technique has been used to provide insight into microbial processes which may be altering the isotopic composition of inorganic nitrogen (Delwiche and Steyn, 1970, Vogel et. al., 1981, Heaton 1984, and Mariotti et al., 1988). The purpose of this thesis is to present a detailed consideration of how processes within the microbial nitrogen cycle in agricultural soils vary seasonally and as a function of tilling practices. This thesis accomplished this

objective by using the stable nitrogen isotopic technique to determine both the source of inorganic nitrogen and the microbial processes that alter its isotopic composition.

STABLE ISOTOPE TECHNIQUE

Stable isotope ratios are expressed in per mil ($^{0}/_{00}$) notation:

$$\delta^{15}N(^{0}/_{00}) = [(Rsample / Rstandard) - 1] * 1000$$

Where R is the abundance ratio of the heavy to light isotope. The internationally recognized standard for nitrogen is atmospheric nitrogen gas, which, by definition has a per mil value of 0.

In order to use stable isotope abundances as indicators of origins, pathways and fate of inorganic nitrogen in groundwater, two criteria must be satisfied. First, the primary sources of interest must be isotopically distinct from each other. Second, the isotopic signature of the source must not be altered during transport and transformation (Macko and Ostrom 1994). When isotopic alteration does occur, it must be altered in a predictable manner if the origin of the nitrate is to be determined. Nonetheless, the extent of isotopic alteration can be indicative of the fractionating process.

Considerable research has been conducted to improve the ability of the natural abundance stable isotope technique to quantify the extent of fractionation during equilibrium or kinetic reactions. Fractionation that occurs during an equilibrium reaction is a function of bond energies and may be calculated empirically (Urey, 1947). Fractionation that occurs during kinetic reactions is a function of the difference in mass of the isotopes. The fractionation

factor (α) was developed as an indicator of the extent of fractionation that may occur during kinetic and equilibrium reactions (O'Neal, 1981). In this study the reactions of interest are primarily unidirectional and kinetic. The fractionation factor for kinetic reactions:

A--->B

is a function of reaction rates for the light (k_1) and heavy (k_2) isotopes.

$$\alpha_{A\text{-}B} = k_1 / k_2$$

Isotopic fractionation may also be expressed in terms of the isotopic enrichment factor, ε:

$$\varepsilon = (\alpha - 1) * 1000$$

The fractionation factor for a specific reaction is constant (Macko and Ostrom 1994), however, the magnitude of isotopic fractionation between a source and a product is a function of the extent of reaction and substrate availability. By calculating the isotopic ratios at different times in a uni-directional reaction in a closed system, the (α) of the reaction can be determined.

THE MICROBIAL NITROGEN CYCLE

The microbial nitrogen cycle entails four primary processes: nitrogen fixation, mineralization, nitrification and denitrification. Nitrogen/fixation is the transformation of elemental dinitrogen into ammonia. In a terrestrial system there are two primary sources of fixed nitrogen. The first source is provided by the process of dinitrogen reduction into ammonia by a few select species of bacteria. The second primary source of fixed nitrogen to a terrestrial system is the application of nitrogen fertilizers. The majority of fixed nitrogen in the fertilizers is provided by the Haber process. The Haber Process reduces $N_{2(g)}$ to ammonia gas at a specific temperature and pressure.

$$N_{2(g)} + 3H_{2(g)} \Rightarrow 2NH_{3(g)}$$

As the fixed nitrogen enters a soil system, it may either volatilize to the atmosphere or be assimilated into plant or microbial matter. Much of organic matter is readily degradable by microbes (Figure 1, step 1). Decay of organic matter results in the mineralization of the organic nitrogen and the release of ammonia.

$$\text{Organic N} \Rightarrow NH_{3(g)}$$

Under aerobic conditions, ammonia may follow one or more of three available pathways: plant and microbial uptake, volatilization and microbial nitrification. Nitrification is the oxidation of ammonia to nitrite and nitrate (Figure 1, step 2).

$$2NH_3 \Rightarrow NO_2 \Rightarrow NO_3$$

Nitrate produced in this step may follow one or more of the three following pathways: it may be assimilated by plant growth, leached to ground water, or under anaerobic conditions, be reduced to $N_{2(g)}$ by microbial denitrification (Figure 1, step 3). The production of $N_{2(g)}$ by denitrificaiton completes the nitrogen cycle.

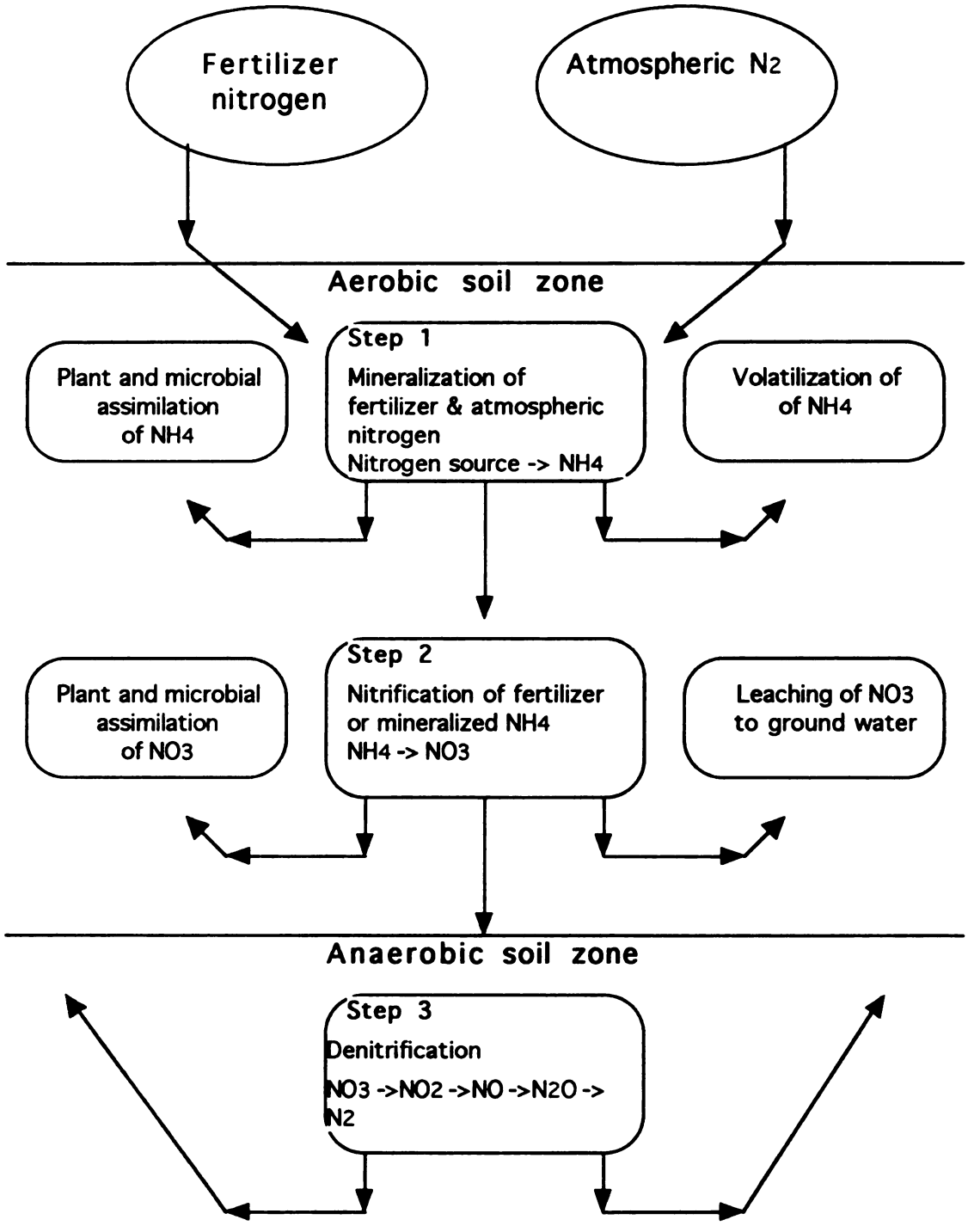

Figure 1. Microbial nitrogen cycle

SOURCES OF INORGANIC NITROGEN TO GROUNDWATER

The main sources of nitrate contamination of ground water have been found to be, nitrate derived from soil organic matter, human and animal waste nitrate and fertilizer nitrate (Heaton 1986). Over 90% of the nitrogen found in the surface layer of most soils is found is the form of organic nitrogen (Stevenson 1982). In many soils, the large reservoir of organic nitrogen can act as a source of nitrate through the mineralization process. Mineralization of organic nitrogen has been shown to increase with the initiation of cultivation. Mineralization of organic nitrogen is often responsible for the high nitrate concentration found in groundwater (Heaton, 1986). Elevated nitrate concentrations derived from geologic parent material are not generally considered a major source of nitrate contamination. Portions of the Fort Union geologic formation in North Dakota, Montana and Wyoming, however, are an example in which geologic sources are important. The elevated nitrate levels found in these shallow aquifers result from nitrification of high levels of ammonia derived from lignite coal (Fedkiw, 1981).

The concentration of naturally occurring nitrate in groundwater is often less than 3mg/L (Fedkiw 1981). However, the agricultural practices of fertilizer application and the operation of large feed lots has resulted in an increase in the concentration of nitrate found in many rural wells to well over 10 mg/L (Follett, 1989).

The U.S. Geological Survey has noted that approximately 20% of 124,000 wells tested nation wide, exceeded the 10 mg NO_3-N/L groundwater standard. These exceedances were generally attributed to anthropogenic activities (Follett, 1989). The sudden growth of urban population into rural areas not serviced by municipal sewers has resulted in an increased dependence on single household septic systems. Throughout the rural U.S and Canada, septic systems are often located on top of unconfined aquifers, thus creating a direct conduit for nitrate leaching to groundwater. In many environments, sufficient nitrate has leached to the groundwater to raise concentrations above the drinking water standard (Kreitler, 1979).

THE $\delta^{15}N$ OF NITRATE SOURCES TO GROUNDWATER

Stable isotopic analysis may provide information leading to the determination of the sources, fate and transformation of nitrate in groundwater. Three main sources of nitrate contamination can be distinguished on the basis of $\delta^{15}N$: soil derived nitrate (2 to 8 $^0/_{00}$), animal waste nitrate(10 to 20 $^0/_{00}$) and fertilizer nitrate (-3 to 2 $^0/_{00}$) (Kreitler, 1979).

Over the past few decades, considerable research has been directed towards the use of the nitrogen stable isotope technique to determine the source of groundwater nitrate contamination. Soil derived nitrate has been found to be one of the main sources of nitrate found in groundwater (Kreitler, 1975). Cultivation has been found to increase organic nitrogen mineralization (Heaton, 1985) and

may explain why some areas have high nitrate concentration in groundwater even though fertilizers have not been used. For example, in Runnels County in Texas, groundwater collected in the vicinity of cultivated fields in which no fertilizer had been applied had average nitrate concentrations of 250 mg/L and $\delta^{15}N$-values between 2 ‰ to 8 ‰. Dating measurements indicated that the water was less than 20 years old. Thus, $\delta^{15}N$ of the water and the lack of fertilizer application suggest that the nitrate found in the groundwater was soil derived (Kreitler, 1975).

Although cultivation enhances the mineralization of organic nitrogen it is not a prerequisite for soil-derived nitrate to be found in ground water. For example, water of a confined aquifer in the western Kalahari had $\delta^{15}N$-values between 4 and 8 ‰ . Water within this aquifer was dated at 3000 yrs B.P. and predated human cultivation practices. $\delta^{15}N$-values of the water were indicative of soil derived nitrate (Heaton, 1984).

Of the three primary sources of nitrate contamination, nitrate from animal and or human waste has the most distinctive isotopic signature. Nitrate from animal waste has been shown to have $\delta^{15}N$-values between +10 and +22 ‰ (Kreitler and Jones, 1975). Nitrate found in ground water of Grand Cayman island had $\delta^{15}N$-values between +14 to +24 ‰. The Cayman area is largely comprised of small communities and houses with individual septic systems. This observation in conjunction with high $\delta^{15}N$-values is indicative of nitrate contamination from animal and human waste (Kreitler and Browning, 1983). Nitrate contamination of the Ironshore Formation in Texas is another example where animal

waste was the main source of nitrate. The $\delta^{15}N$-values for ground water samples in this area ranged from +18 to +23.9 ‰ and thus, were strongly indicative of animal waste (Krietler and Browning, 1983).

The high $\delta^{15}N$-values characteristic of animal and human waste result from ammonia volatilization, which leaves the residual ammonia enriched in ^{15}N. Animal waste nitrate is excreted in the form of urea which is rapidly hydrolyzed to ammonia in the environment. Hydrolysis of urea causes an increase in the pH of the waste mixture that readily allows the ammonia to volatilize. Upon volatilization, the pH is lowered and the remaining ammonia is stabilized (Krietler and Jones, 1975). Preferential release of ^{14}N during volatilization leaves the residual ammonium enriched in ^{15}N. As a result, nitrification of the residual ammonia results in nitrate with ^{15}N values often greater than 10 ‰ (Kreitler, 1979).

Nitrogen-based fertilizers have isotope values between -8 to +6 ‰ (Krietler, 1979, Flipse and Bonner, 1985), although the majority of fertilizers range between -3 to +2 ‰ (Krietler, 1979). Groundwater samples taken from a housing development in Medford, New York, had $\delta^{15}N$-values that ranged from +1.1 to 7.1 ‰ and averaged 4.3 ‰. These $\delta^{15}N$-values are indicative of nitrate contamination from fertilizers and are probably the result of lawn fertilization. (Flipse et. al., 1984). A similar study in the central Platt region of Nebraska found that application of commercial fertilizers was the only reasonable source of nitrate contamination. This was confirmed by $\delta^{15}N$-values for nitrate in groundwater samples of 3.5 to 6.5 ‰ (Gormly and Spaulding, 1979).

ISOTOPIC FRACTIONATION IN THE MICROBIAL NITROGEN CYCLE

Mineralization, nitrification and denitrification can significantly affect the isotopic composition of groundwater nitrate and thereby compromise the determination of origin based on $\delta^{15}N$ (Heaton 1984 and Delwiche and Steyn, 1970). For example, it was found that a 20% removal of the nitrate pool via denitrification can an increase the $\delta^{15}N$ of residual nitrate by 8 o/oo (Heaton, 1984). Rapid denitrification within the Chalk Aquifer in northern France left the residual nitrate enriched in ^{15}N with $\delta^{15}N$ of 4.7 to 5.0 o/oo (Mariotti et. al., 1988), thus illustrating the influence of microbial processes on the isotopic signature of the nitrate.

The direction of fractionation may allow for the identification of the process causing isotope alteration. For example, when artesian ground water from the western Kalahari was sampled to determine the nitrogen isotopic composition, it was found that fractionation due to denitrification enriched the residual nitrate in ^{15}N by as much as 30 o/oo (Vogel et al., 1981). Although other processes within the nitrogen microbial cycle cause isotopic fractionation only denitrification can cause such large enrichments.

Mineralization and subsequent nitrification of the NH_3 in soils usually produces NO_3 similar in $\delta^{15}N$ to the organic matter from which it was derived (Feigin et al., 1974). Due to the slow rate of mineralization there is generally only a small pool of mineralized nitrogen available for nitrification. As result the the $\delta^{15}N$ of the

resulting nitrate is similar to the $\delta^{15}N$ of the original substrate (Vogel et al., 1981). In environments where large reservoirs of ammonium are present, the rate of nitrification limits the extent of fractionation. The nitrate produced in this scenario is often depleted in ^{15}N relative to the original substrate (Marriotti et al., 1988). The expression of fractionation during nitrification is often observed following the application of ammonium-based fertilizers (Kreitler, 1979).

ISOTOPIC COMPOSITION OF SOIL NITROGEN

There are four main reservoirs of soil nitrogen: (1) nitrate, (2) ammonium, (3) readily mineralizable organic nitrogen compounds and (4) organic compounds not usually available to plants or microbes (Feigin et. al., 1974a). The first three reservoirs provide the vast majority of the inorganic nitrogen needed for plant uptake and microbial reactions in soils. The wide range of $\delta^{15}N$ values of soil organic matter is indicative of the complexity of the soil biosphere system. A number of studies have found that there is high spatial variability in the $\delta^{15}N$ of soils (Rennie et. al., 1976, Broadbent et. al., 1980, Ledgard et. al., 1984). For example, in a small drainage basin the $\delta^{15}N$ of soil organic matter ranged from 5.0 to 7.9 o/oo (Ledgard et. al., 1984).

In a study of $\delta^{15}N$ content of two soil profiles from central Illinois, it was discovered that the $\delta^{15}N$ of the top 30cm of soil increased over the summer from 1.2 to +10 o/oo(Feigin et. al., 1974).

The enrichment of soils in ^{15}N is attributed to the conservation of the heavier isotope over time (Delwiche and Steyn, 1970) since the microbial nitrogen cycle favors the consumption of ^{14}N. Therefore, the remaining mineralizable pool of organic nitrogen and the subsequent nitrate is isotopically enriched in ^{15}N.

ISOTOPIC COMPOSITION OF PRECIPITATION

On a global scale, the amount of nitrogen available to soils from precipitation is on the same of order of magnitude as the amount of fertilizer nitrogen applied to cultivated fields (Freyer, 1978). Nitrogen deposition from precipitation at the study site is approximately 13 -15 kg/ha. Precipitation nitrogen is therefore, not a primary source of NO_3 in leachate. The $\delta^{15}N$ values of nitrate and ammonium in precipitation have been found to be highly variable (Macko and Ostrom, 1994). Studies that have weighted $\delta^{15}N$ values by their respective concentrations of nitrate and ammonium have shown that precipitation nitrate and ammonium are depleted in ^{15}N relative to soil organic matter. For example, rain samples collected in Pretoria, South Africa, over a period of one year had weighted average $\delta^{15}N$ values for nitrate and ammonium of -3.5 $^{0}/_{00}$ and -3.8 $^{0}/_{00}$, respectively (Heaton, 1987). In another study, precipitation samples collected over a two-year period in Julich, Germany had mean nitrate and ammonium $\delta^{15}N$ values of -3.1 $^{0}/_{00}$ and -9.1 $^{0}/_{00}$ respectively (Freyer, 1978).

FIELD INVESTIGATIONS

Kellogg Biological Station is located 15 miles northeast of the city of Kalamazoo in Kalamazoo County, Michigan. Two test plots at the Long-Term Ecological Research (LTER) Agricultural Ecology site were utilized in this study: (1) lysimeter No. 2, conventionally farmed and tilled; and (2) lysimeter No. 9, conventionally farmed and not tilled. Both conventionally farmed plots were on an annual cropping system of rye-corn rotation.

To gain a better understanding of the factors influencing the isotopic transformations and fate of inorganic nitrogen in a terrestrial farming system, leachates from soil lysimeters placed in the tilled and non-tilled plots were collected and analyzed for the $\delta^{15}N$ and concentration of NO_3^- and NH_4^+. Leachate from each of the two test plot lysimeters was sampled weekly from March to November 1993. Lysimeter samples were collected to determine the seasonal flux and isotopic composition of nitrate leaching from the upper soil column. The $\delta^{15}N$ of wet/dry atmospheric deposition and soil organic matter was determined to define the initial isotopic composition of these sources.

SYNOPSIS

The purpose of this research is to determine how processes within the microbial nitrogen cycle in agricultural soils vary seasonally and as a function of tilling practices. The stable nitrogen isotopic technique can provide insight into the dominant microbial

nitrogen cycle processes on a seasonal and spatial basis. An understanding of the influence of seasonal variation and tillage practices on the microbial nitrogen cycle and nitrogen speciation will provide insight into the the fate of inorganic nitrogen in agricultural systems.

CHAPTER 2: MATERIALS AND METHODS

LYSIMETER HISTORIES

Table 1 provides a chronological review of crop rotation and chemicals applied to lysimeters 2 and 9. Lysimeters 2 and 9 were installed in May of 1990 and have been on a rye/ corn rotation since that time. They were installed with a minimum of disturbance to the soil column. Lysimeters 2 and 9 were 4 feet by 5 feet and 10 feet deep and have been conventionally tilled and not tilled, respectively. The lysimeters were accidentally fertilized at a rate of 172 lbs N./acre with ammonium nitrate (34-0-0) fertilizer in July of 1990. The lysimeters were flushed with water for several days to remove the fertilizer. There has been no fertilizer added since. Round-up pre-plow herbicide, Dual pre-emergence herbicide, Banvel post-emergence herbicide and Difonate 20G insecticide have been applied as needed. However, Roundup pre-plow herbicide was accidently over applied in 1991.

Table 1. Chronological Review of Lysimeters 2 & 9 Crop Rotation and Chemical Applications

Year	crop	Insecticide	Herbicide	Fertilizer	Notes
1990	Corn Rye	none	Round-up pre plow Dual pre-emergence Banvel post- emergence	Ammonium nitrate (34-0-0)	Lysimeters were fertilized in error
1991	Corn	none	Round-up pre plow Dual pre-emergence	none	Round-up pre plow was misapplied at ten times the usual application rate
1992	Corn	Difonate 20G	Round-up pre plow Dual pre-emergence Accent	none	
1993	Corn	none	Round-up pre plow Dual pre-emergence	none	

SAMPLE COLLECTION AND PREPARATION

Lysimeter samples were collected once a week from March to November 1993. Clean 500 mL Nalgene sample bottles were placed in the lysimeters for one 24-hour period each week. To minimize degradation, samples were filtered with a 40-micron filter and frozen immediately upon removal from the lysimeters. Prior to

distillation, the samples were thawed and the volume of sample was determined. Nitrate concentrations were determined in the (KBS) lab of L.O. Hedin using a Dionex ion chromatograph equipped with a membrane supressor and a conductivity detector.

Soil samples from the top 5 cm of topsoil from lysimeter 2 and 9 were collected to determine the $\delta^{15}N$ of the soil nitrogen. The samples were dried in a vacuum oven and ground to a fine powder, after which they were acidified and dried again. Approximately 100 ug of dried soil was placed in a quartz tube with excess Cu and CuO. The quartz tube was then placed on a vacuum line. Upon evacuation, the tube was sealed with a torch and shaken vigorously to homogenize the metal and sample. The sealed quartz tube was then combusted at 850°C for 1 hour and allowed to cool gradually (Macko, 1981), after which the tube was cracked under vacuum to cryogenically remove all H_20 and CO_2. The N_2 created was then trapped on a molecular sieve. The $\delta^{15}N$ of the cryogenically purified N_2 was determined on a VG Prism stable isotope ratio mass spectrometer.

Wet and dry precipitation samples were collected throughout the study. Upon collection, the sample was immediately frozen and then thawed prior to distillation. Precipitation and temperature data were provided by Lolita Krievs of Kellogg Biological Station. The weekly precipitation and temperature data are averages over the 7 day periods ending on the sample collection date.

ISOTOPIC ANALYSIS

The success of the stable nitrogen isotope technique depends on the ability to convert nitrate and ammonium to N_2 without altering the $^{15}N/^{14}N$ ratio. For this to occur, the procedure must provide complete and separate recovery of ammonium and nitrate without isotopically fractionating the sample (Bremner and Keeney, 1966; Cline, 1975; Heaton and Collett, 1985; Velinsky et, al., 1989; Ostrom, 1992). In this procedure, ammonia is separated by steam distillation using a Labconco Rapid Kjeldahl system (Rapid Still II). The Rapid Still II consists of a 100 or 500 mL sample flask, a water cooled condenser and a heating element. To assure complete recovery, cooling water within the condenser was kept at 5.0°C using a Neslab recirculating chiller.

The distillation of ammonium was completed by first raising the pH of the sample to greater than 10 with the addition of 1mL 5N NaOH to the sample. In an earlier analysis of this method, it was found that the NaOH would cause the breakdown of organic nitrogen to ammonia, thus contaminating the sample (Bremner and Keeney, 1966). The use of MgO instead of NaOH and short distillation times was found to eliminate contamination from organic nitrogen compounds (Bremner and Keeney, 1966). Velinsky, however, found that there was little difference in the $\delta^{15}N$ of the samples when using NaOH or MgO (Velinsky et, al., 1989). It was reasoned that dissolved organic nitrogen (DON) was present in concentrations too small to affect the isotope ratio or that significant decomposition of the organic nitrogen did not occur (Velinsky et, al., 1989). In a

similar test, the $\delta^{15}N$ of fertilizer and rainwater where determined and were in close agreement when using either MgO or NaOH (Heaton and Collett, 1985). Therefore, based on these results, the breakdown of organic matter by NaOH should not cause contamination of samples.

After raising the pH of the sample with the addition of NaOH, boiling was initiated and adjusted to achieve a collection rate of 9 mL min^{-1}. Faster or slower distillation rates led to incomplete recovery. Condensate was collected through tygon tubing, passed through an attached glass pipet which was immersed in 25 mL of 0.084N HCL acid. Quantitative recovery of ammonium from 100 and 500 mL samples was obtained within 6 and 15 minutes, respectively (Figures 2 & 3)

After distillation, the condensate flask was set aside for binding of the ammonium. A second distillation for nitrate was commenced. The reduction of nitrate to ammonium was accomplished with the addition of 0.3 g of Devarda's alloy (50% Cu, 45% Al, 5% Zn) by the following reaction:

$$3NO_3^- + 8Al + 5OH^- + 2H_2O \rightarrow 8AlO_2^- + 3NH_3$$

The Devarda's Alloy was quickly added to the ammonia-free sample, which was then distilled at a rate of 9 mL min^{-1} into an 25 mL 0.084N HCL acid trap. Quantitative recovery of nitrate from 100 and 500 mL samples was obtained within 6 and 15 minutes, respectively (Figure 3). To assure that complete recovery was achieved, standards were run with a known concentration of ammonium. Once

the time required for quantitative recovery was established, sample distillations were conducted.

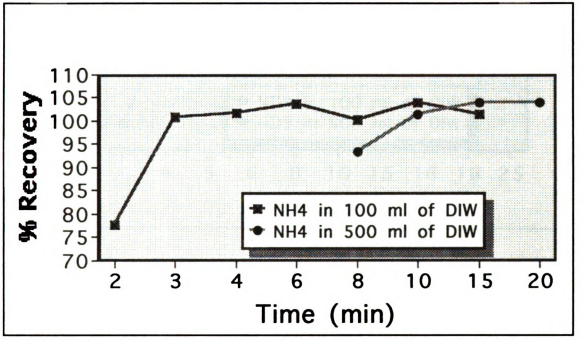

Figure 2. Average Recovery During Distillation vs. Time: 20 μmols of NH_4^+ in 100ml & 500ml of Deionized Water

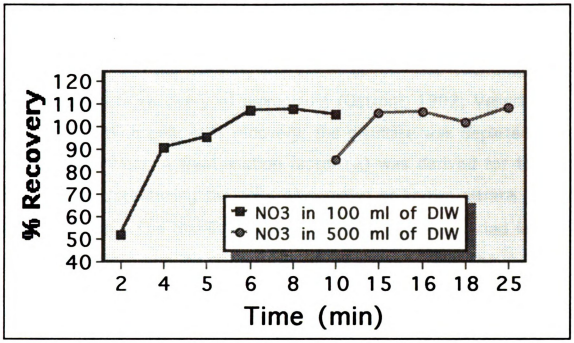

Figure 3. Average Recovery During Distillation vs. Time: 20 μmols of NO_3^- in 100ml & 500ml of Deionized Water

Incomplete recovery of ammonium or nitrate during distillation may result in a loss of isotopic integrity (Cline, 1975; Heaton and Collett, 1985; Ostrom, 1992; Velinsky et, al., 1989). Incomplete recovery during both ammonium and nitrate distillation results in appreciable fractionation in which the distillate is isotopically depleted. Other studies have shown that upon 90% recovery during distillation of ammonium, the distillate was depleted in ^{15}N by ≈ 0.5 ‰. The fractionation was even greater during nitrate distillation, in which 90% recovery resulted in the distillate being depleted by ≈ 2.5 ‰ (Heaton and Collett, 1985). In a similar study, an isotopic fractionation factor(α) of 1.0058 ± 0.0004 was determined for incomplete recovery of ammonia during

nitrate distillation (Cline, 1975) and agrees well with the (α) calculated in this study.

As in the other studies, there was substantial fractionation with incomplete recovery of ammonium (Ostrom, 1992; Velinsky et, al., 1989). With only 40% recovery, the distillate was depleted in ^{15}N by $\approx$ 3.5 $^{0}/_{00}$. A fractionation factor (α) was derived for this reaction by determining the $\delta^{15}N$ of samples of known nitrate concentration. The distillation time of these samples varied in the length causing a varying degree of fractionation. Using the information derived from this study and a modified Rayleigh equation (Macko et al. 1986). A fractionation factor of 1.0063 was determined (Figure 4).

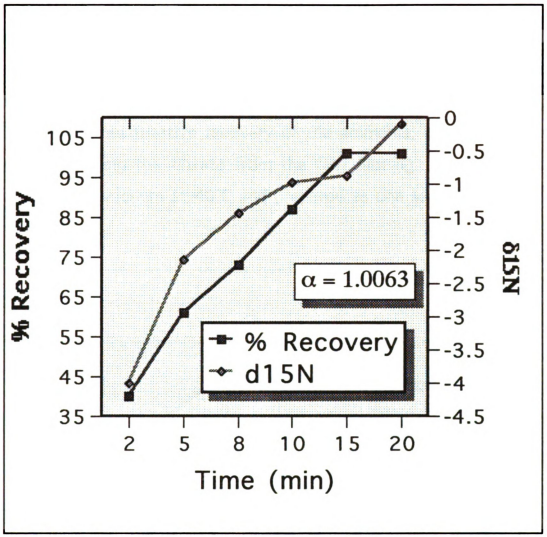

Figure 4. Changes in the $\delta^{15}N$ of the Distilled $NH4^+$ over the Course of the Distillation (Note: The $\delta^{15}N$ of the standard used was -0.910/00)

After the distillation of the ammonium or nitrate was complete, 0.1g of dried zeolite molecular sieve and a teflon coated stir bar were added to the distillate. The distillate was covered and stirred for 1/2 hour while maintaining a pH of 4.5 to 5.0. Ph was maintained by adding 1 normal HCL and Na_2HCO_3 to the distillate. Quantitative recovery was best accomplished within this pH range

(Figure 5). At the end of the 1/2 hour, the sieve was vacuum filtered onto a precombusted Whatman GF/C glass fiber filter. After 1/2 hour of zeolite binding, 96% of the ammonia was bound (Figure 6). To achieve quantitative recovery of the ammonia, the procedure was repeated using the filtrate from the first binding. The filters were dried under vacuum at 40°C and prepared as one sample for combustion.

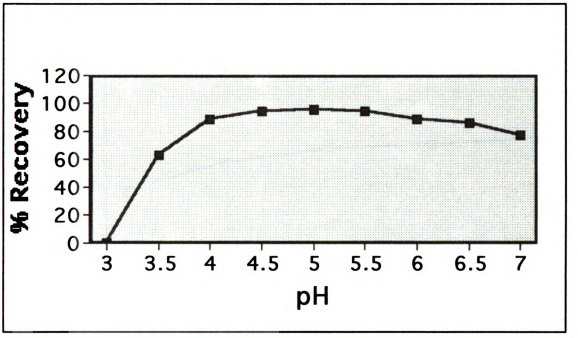

Figure 5. Recovery of NH_4^+ Using Zeolite with Varying pH: 20 μmols NH_4^+ in 300ml Deionized Water and 60 mg Zeolite

In preparation for isotopic analysis, the dried zeolite was removed from the filter and placed in an precombusted quartz tube. In addition to the zeolite, 2 grams of Cu and 2 grams of precombusted CuO (500°C for 1 hour) were added to the tube. The quartz tube was then placed on a vacuum line. Upon evacuation, the tube was sealed

with a torch and shaken vigorously to homogenize the metal and sample. The sealed quartz tube was then combusted at 850°C for 1 hour and allowed to cool gradually. Prior to analysis the tube was cracked in a vacuum line to cryogenically remove H_2O and CO_2. The liberated N_2 was then trapped in molecular sieve and the $\delta^{15}N$ was determined on a VG Prism stable isotope ratio mass spectrometer. The precision of this technique is 1 ‰ or better (Table 2).

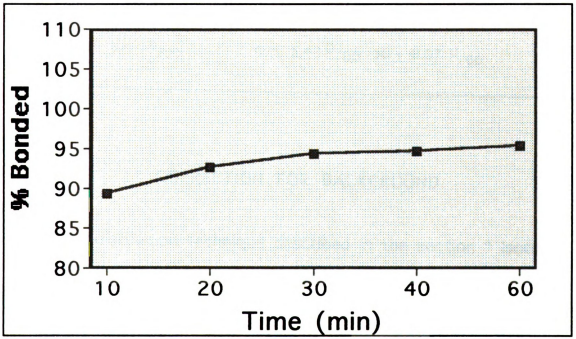

Figure 6. Percent NH_4^+ Bounded Using Zeolite with Varying Time: 20 μmols NH_4^+ in 300ml Deionized Water and 60 mg Zeolite

Table 2. $\delta^{15}N$ Values for IAEA N3 and KNO3 Standards Determined Using the Sealed -Tube Combustion Technique

IAEA N3		
1.)	4.11 $^{0}/_{00}$	
2.)	4.11 $^{0}/_{00}$	
3.)	4.25 $^{0}/_{00}$	Avg. 4.16 $^{0}/_{00}$ SD ± 0.08 $^{0}/_{00}$
Baker		
1.)	5.35 $^{0}/_{00}$	
2.)	5.45 $^{0}/_{00}$	
3.)	5.48 $^{0}/_{00}$	Avg. 5.43 $^{0}/_{00}$ SD ± 0.07 $^{0}/_{00}$

CORRECTION FOR BACKGROUND

The distillation technique described in the section " Isotopic Analysis " has been shown to provide quantitative recovery of nitrate and ammonium from standards in the range of 3 to 40 μmols. Nitrate and ammonium yields averaged 107.9 +/- 3.1% and 102.1 +/- 1.3%, respectively (Table 3). These values are similar to those obtained by Velinsky et al., 1989a and Ostrom 1992. Like other studies, (Velinsky et al., 1989a and Ostrom 1992), a substantial background of ammonium was discovered during the steam distillation technique. Background ammonium and nitrate derived from the steam distillation technique averaged 0.07 μmoles (Table 4) and 1.41 μmols/L, respectively. (Table 5)

Table 3. Percent Recovery of 20 µmol of IAEA KNO_3 and NH_4 Standards in 100 ml Deionized Water

Standard	Initial Abundance	Final Abundance	% Recovery
IAEA NH_4	20 υmoles	20.12 υmoles	101
IAEA NH_4	20 υmoles	20.16 υmoles	101
IAEA NH_4	20 υmoles	20.33 υmoles	102
IAEA NH_4	20 υmoles	20.80 υmoles	104
IAEA NH_4	20 υmoles	20.40 υmoles	102
IAEA NH_4	20 υmoles	20.14 υmoles	101
IAEA NH_4	20 υmoles	20.61 υmoles	103
IAEA NH_4	20 υmoles	20.21 υmoles	101
IAEA NH_4	20 υmoles	20.16 υmoles	101
IAEA NH_4	20 υmoles	20.20 υmoles	101
IAEA NH_4	20 υmoles	20.34 υmoles	102
IAEA NH_4	20 υmoles	20.74 υmoles	104
IAEA NH_4	20 υmoles	20.95 υmoles	105
IAEA NH_4	20 υmoles	20.45 υmoles	102
IAEA NO_3	20 υmoles	21.56 υmoles	108
IAEA NO_3	20 υmoles	22.30 υmoles	112
IAEA NO_3	20 υmoles	23.45 υmoles	117
IAEA NO_3	20 υmoles	21.03 υmoles	105
IAEA NO_3	20 υmoles	21.61 υmoles	108
IAEA NO_3	20 υmoles	21.60 υmoles	103
IAEA NO_3	20 υmoles	21.31 υmoles	107
IAEA NO_3	20 υmoles	21.81 υmoles	109
IAEA NO_3	20 υmoles	21.25 υmoles	106
IAEA NO_3	20 υmoles	21.08 υmoles	105
IAEA NO_3	20 υmoles	21.38 υmoles	107
IAEA NO_3	20 υmoles	21.32 υmoles	106
IAEA NO_3	20 υmoles	21.64 υmoles	108
IAEA NO_3	20 υmoles	21.96 υmoles	110
IAEA NO_3	20 υmoles	21.44 υmoles	107
IAEA NO_3	20 υmoles	21.68 υmoles	108
	Average	105% +/- 3.8	

Table 4. Abundance of Background NH_4 Associated with the Distillation Technique

Blank #	umols recovered
Blk #1	0.1
Blk #2	0.1
Blk #3	0.0
Blk #4	0.1
Blk #5	0.1
Blk #6	0.1
Blk #7	0.1
Blk #8	0.1
Blk #9	0.1
Blk #10	0.1
Blk #11	0.1
Average	0.1

Table 5. Abundance of Background Nitrate with the Distillation Technique

Blank #	umols recovered
Blk #1	1.8
Blk #2	2.2
Blk #3	0.9
Blk #4	1.2
Blk #5	0.9
Average	1.4 +/- 0.56

Ammonium background during the distillation processes was most likely derived from air, base (NaOH) or Devarda's alloy. In order to reduce background NH_4 from the NaOH, the base was distilled and diluted back to its original volume (Ostrom, 1992).

Additionally, the steam distillation apparatus was cleaned after every sample run by distilling deionized water.

In order to obtain the actual isotopic signature of a sample, the background nitrogen must be accounted for in both ammonium and nitrate distillations (Cline, 1975; Ostrom, 1992). However, in this study background contamination during the distillation of ammonium was found to negligible. The average ammonium background concentration was found to be 0.07 μmoles/L (Table 4) and was too low for $\delta^{15}N$ to be determined. The release of ammonia from Devarda's Alloy during the reduction of nitrate was a concern. In blanks in which 15g of alloy was used, 7 to 10 umoles of NH_3 was produced (Cline, 1975). In another study, 0.97 umoles of NH_3 was produced when 0.3 g of the alloy was used (Ostrom, 1992). In this study the average background concentration was 1.41 umols of NH_3 produced upon using 0.3 g of the alloy (Table 5). The isotopic composition of the background was previously found to be depleted in ^{15}N relative to atmospheric nitrogen (Cline, 1975; Ostrom, 1992). In this study, however, the nitrate background was found to be isotopically enriched in ^{15}N relative to atmospheric nitrogen. The $\delta^{15}N$ of the nitrate background ranged from 7.0 to 15.6‰ and averaged 12. 3 ‰ (Table 6).

Table 6. $\delta^{15}N$ of Nitrate Blanks

Blank #	$\delta^{15}N$
Blk # 1	10.3
Blk # 2	8.8
Blk #3	7.0
Blk #4	15.4
Blk #5	16.6
Blk #6	15.5
Average	12.3 +/- 3.7

In order to obtain the isotopic composition of the sample, background must be accounted for. In this study, background correction was accomplished by using the following mass balance equation (Ostrom, 1992):

$$\delta^{15}N_{sam} = \frac{\delta^{15}N_{meas}(A_{sam} + A_{back}) - (\delta^{15}N_{back} * A_{back})}{A_{sam}} \quad \text{(Eq. 1)}$$

where: $\delta^{15}N_{sam}$ = the $\delta^{15}N$ of the sample.

$\delta^{15}N_{meas}$ = the $\delta^{15}N$ measured.

$\delta^{15}N_{back}$ = the $\delta^{15}N$ of the background.

A_{sam} = the abundance of nitrate or ammonium placed in the distillation apparatus (umoles of N).

A_{back} = the abundance of ammonium associated with background nitrate or ammonium

The isotopic composition of the sample was obtained by subtracting the background portion of ^{15}N from the uncorrected sample using equation 1. The background concentration and its isotopic composition were determined by analyzing deionized water with an equal amount of Devarda's Alloy as was used in the distillation of the sample (Tables 5 & 6). Prior to the use of equation 1, KNO_3 standards of known isotopic composition and concentration were distilled to determine the accuracy of the procedure. After subtracting out the background by mass balance, the $\delta^{15}N$ of nitrate standards matched the projected $\delta^{15}N$ of the standard (Table 7).

Table 7. $\delta^{15}N$ Correction for Background U.S.G.S. KNO_3 Standard (Standard $\delta^{15}N = 180\ ^0/_{00}$)

Uncorrected		Corrected
1.) 168.8 $^0/_{00}$	→	179.6 $^0/_{00}$
2.) 169.6 $^0/_{00}$	→	180.4 $^0/_{00}$
3.) 170.6 $^0/_{00}$	→	181.4 $^0/_{00}$
	Avg. 180.5 $^0/_{00}$ + 0.9 $^0/_{00}$	

CHAPTER 3: RESULTS AND DISCUSSION

RESULTS

Groundwater samples from two mid-Michigan lysimeters were collected on a weekly basis from March of 1993 to November of 1993. Lysimeters 9 was untilled and lysimeter 2 was tilled. Nitrate concentrations were determined as well as the nitrogen isotopic abundances. Statistical analysis of the analytical results were completed to determine significant relationship between variables. Finally, the analytical and statistical results were assessed to determine if possible, the feasibility of using the stable isotope technique in studying the dynamics of nitrogen cycling in a terrestrial system.

Lysimeter flow rates were monitored during the same period as the sample collection. Flow rates in both the tilled and untilled lysimeters were similiar with both reaching maximum and minimum flows in the spring and late summer, respectively (Figure 7 and Appendix A). Nitrate concentrations in the tilled lysimeter were approximately twice that of the untilled lysimeter and varied in a similar manner (Figure 8 and Appendix B). The variation in nitrate fluxes was largely driven by variation in the lysimeters flow rates. (Figure 9 and Appendix C).

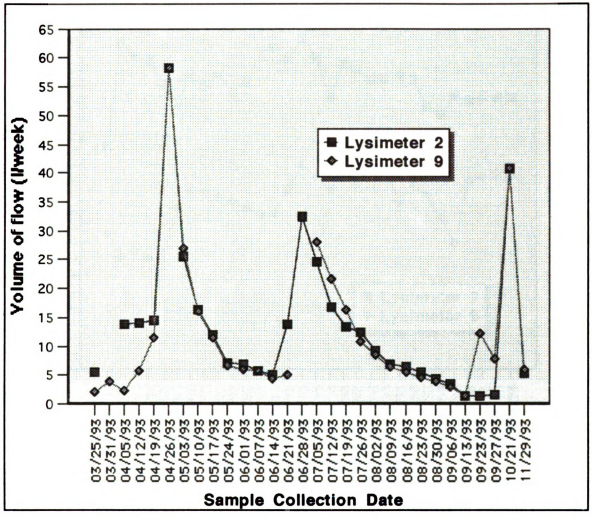

Figure 7 Seasonal Variations in the Flow Rates of Lysimeters 2 and 9

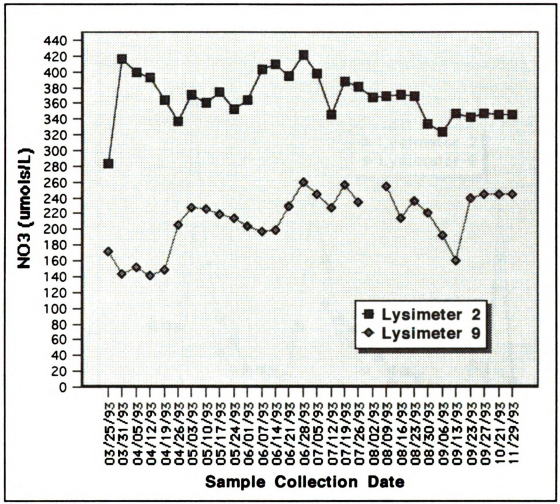

Figure 8 Lysimeters 2 and 9 Dissolved Nitrate Concentrations

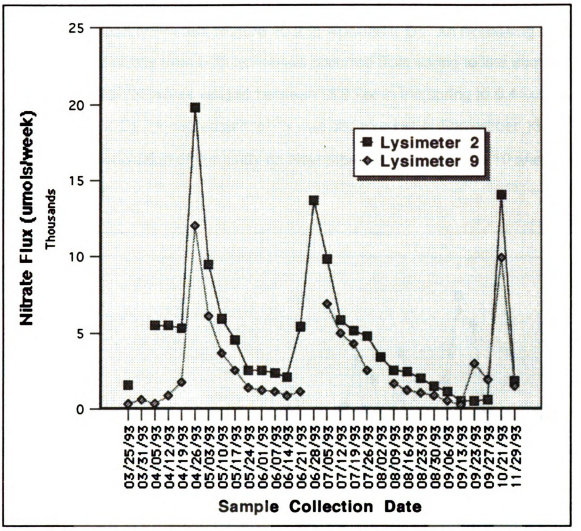

Figure 9 Seasonal Variation in the Nitrate Flux of Lysimeters 2 and 9

Variations in nitrogen isotopic abundances were similar between the untilled and tilled lysimeters (Figure 10 and Appendix D). An increasing trend in the $\delta^{15}N$ of nitrate from both lysimeters occurred from spring to late summer of 1993. The $\delta^{15}N$ values ranged between -3.9 ‰ in the spring to 9.4 ‰ in fall (Appendix D). A decrease in $\delta^{15}N$ was observed after September 13, 1993 to the conclusion of the study on November 29, 1993 (Figure 10 and Appendix D).

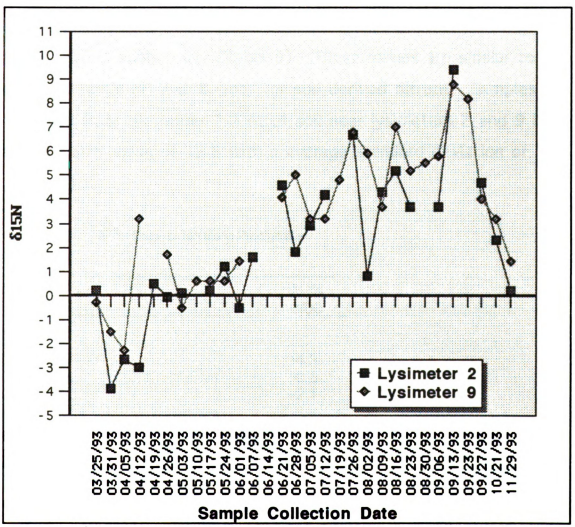

Figure 10 Seasonal Variations in the $\delta^{15}N$ of Dissolved Nitrate

$\delta^{15}N$ OF PRECIPITATION AND SOIL ORGANIC NITROGEN

Precipitation and soil organic matter from the study site were analyzed for $\delta^{15}N$. The $\delta^{15}N$ of the combined wet and dry precipitation nitrate samples (3 total) had an average $\delta^{15}N$ of -2.3 $^{0}/_{00}$ with a standard deviation of 1.5 $^{0}/_{00}$ (Table 8). Soil samples were collected from the top 2 cm of topsoil and from a homogenized mixture of the upper 2 feet of topsoil. Samples collected from the top 2 cm of top soil from lysimeters 2 and 9 had $\delta^{15}N$ values of 6.6 and 8.3 $^{0}/_{00}$, respectively (Table 9). These values are similar to the expected range of 2 to 8 $^{0}/_{00}$ for soil derived nitrate. Samples collected from the upper 2 feet of soil near lysimeters 2 and 9 had average $\delta^{15}N$ value of 13.6 with a average standard deviation of 1.95 (Table 9).

Table 8 $\delta^{15}N$ Data for Nitrate in Precipitation

Date	$\delta^{15}N$ 0/00
03/31/93	-4.3
08/15/93	-0.6
09/15/93	-2.1
	Average -2.3

Table 9. $\delta^{15}N$ Data for Soil Organic Matter.

Lysimeter	Sample 1 $\delta^{15}N$ 0/00	Sample 2 $\delta^{15}N$ 0/00	Average $\delta^{15}N$ 0/00
Ly 2 top 2 cm	6.6		
Ly 9 top 2 cm	8.3		
Ly 2 top 2 ft	15.9	11.5	13.7*
Ly 9 top 2 ft	10.9	16.1	13.5*

* Note: The $\delta^{15}N$ determinaitons were performed on a homogenius mixture of the upper 2 feet of the soil column. The samples were taken from two randomly selected sampling points near each lysimeter. Direct sampling of the lysimeter was considered too destructive. Samples from the top 2 cm were taken from the surface soil of each lysimeter.

LINEAR REGRESSION ANALYSIS OF FACTORS CONTROLLING THE $\delta^{15}N$ OF GROUNDWATER NITRATE

In an attempt to better understand factors controlling variation in the $\delta^{15}N$ of the dissolved nitrate, linear regression analysis and F-tests were performed. The $\delta^{15}N$ of dissolved nitrate from lysimeters 2 and 9 (dependent,X variable) were compared to flow rates, nitrate flux, temperature, soil moisture B horizon, soil moisture C horizon, precipitation and nitrate concentration (independent, Y variable). The variables of nitrate flux, precipitation, nitrate concentration, soil moisture B and C horizons and flow rates were not correlated with the $\delta^{15}N$ of dissolved nitrate from lysimeters 2 and 9 (R^2 values for these independent variables ranged from 0.018 to 0.199, Table 10). The Null hypothesis was accepted for the relationships between lysimeter 2 and these

variables and lysimeter 9 and these variables with F- test values in the range of 0.01 to 4.46, α=0.05 (Table 10).

Temperature had a significant correlation with $\delta^{15}N$ values of dissolved nitrate from lysimeters 2 (R^2 = 0.50, n= 19) and 9 (R^2 = 0.45, n= 19) (Table 10). The Null hypothesis was rejected for the relationship between lysimeter 2 and temperature and lysimeter 9 and temperature with F- test values of 19.55 and 15.65 respectively, α=0.05 (Table 10).

Table 10 Linear Regression Analysis (R^2) and F-test (F) Data

Variable	Lysimeter 2	Lysimeter 9
Soil Moisture B-hor (R^2)	0.0209	0.0008
Soil Moisture B-hor (F)	0.34 p = 0.95	0.01 p = 0.95
Soil Moisture B-hor (R^2)	0.0934	0.0811
Soil Moisture B-hor (F)	1.86 p = 0.95	4.46 p = 0.95
Flow Rates (R^2)	0.0811	0.0175
Flow Rates (F)	2.03 p =0.95	0.42 p = 0.95
Nitrate Flux (R^2)	0.0836	0.0093
Nitrate Flux (F)	2.10 p = 0.95	0.24 p = 0.95
Temperature (R^2)	0.5037	0.4516
Temperature (F)	19.55 p = 0.95	15.65 p = 0.95
Precipitation (R^2)	0.0641	0.0486
Precipitation (F)	1.30 p = 0.95	0.96 p = 0.95
Nitrate Concentration (R^2)	0.04524	0.09873
Nitrate Concentration (F)	1.09 p = 0.95	2.63 p = 0.95

Slopes of the weighted- averaged lines for lysimeters 2 and 9 were nearly identical, with a slope value of 0.0369 and 0.0396 respectively (Appendix E and Figure 11). Similar slopes in both lysimeters indicate that the processes altering the $\delta^{15}N$ of the nitrate are the same in both lysimeters and are independent of the tillage practices.

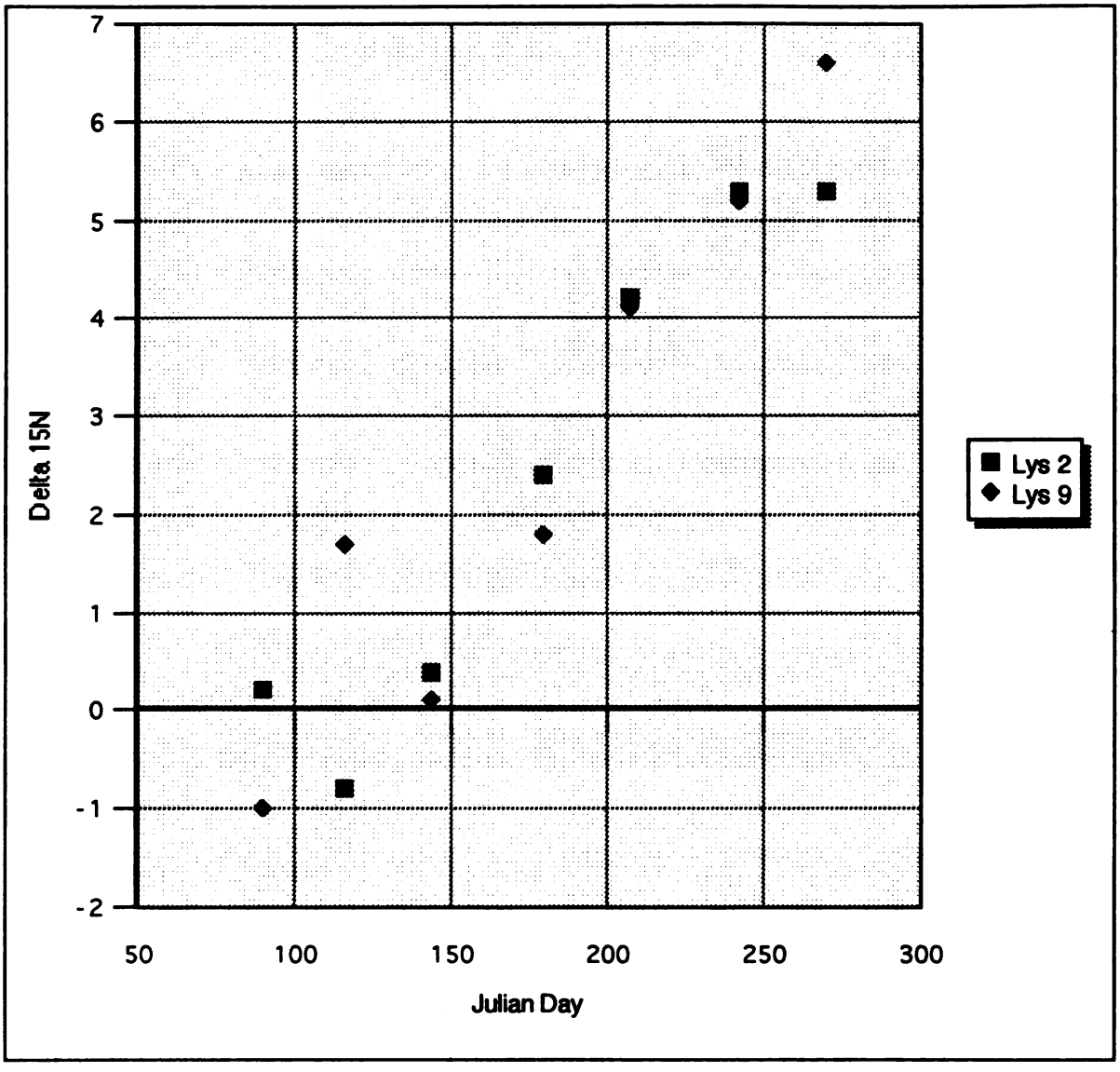

Figure 11 Monthly running weighted average of $\delta^{15}N$ of lysimeters 2 and 9

DISCUSSION

The $\delta^{15}N$ values of leachate collected in the early spring from both lysimeters 2 and 9 were depleted in ^{15}N relative to other times of the year (a low of -3.9 ‰ was observed in March 31, 1993). These values may have resulted from (1) isotopic fractionation

during nitrification (Marriotti et. al., 1988), (2) mineralization of readily mineralizable organic matter (Feigin et. al., 1974, Tiessen et. al., 1984 and Ledgard et al., 1984) or (3)^{15}N-depleted nitrate in precipitation (Moore, 1977; Freyer et. al.; 1978 and T.H.E. Heaton, 1987).

When a large reservoir of ammonium is present, fractionation during nitrification is fully expressed and the nitrate produced is depleted in ^{15}N relative to the ammonium (Marriotti et. al., 1988). In contrast, when a low concentration of ammonium is present, nitrification of the ammonium pool is complete and as a result, fraction during nitrification is not expressed (Feigin et. al., 1974b). The concentrations of ammonium in the leachate collected from lysimeters 2 and 9 were near or below detection limits throughout the study. As a result, isotopic fractionation during nitrification is unlikely to be the cause of the low δ^{15}N values.

The readily mineralizable portion of soil organic matter has been shown to be depleted in ^{15}N relative to the less mineralizable fraction of soil (Ledgard et. al., 1984 and Tiessen et. al., 1984). As a result, the initial mineralization of the readily mineralizable organic matter will result in the release of ^{15}N depleted nitrate and the accumulation of ^{15}N enriched residual organic matter (Ledgard et. al., 1984). The depleted ^{15}N values observed in the early spring may be the result of the initial mineralization of a readily mineralizable fraction of soil organic matter.

Precipitation samples collected from KBS averaged -2.3 $^{0}/_{00}$ +/- 1.5 $^{0}/_{00}$ in δ^{15}N (Table 8). The average monthly concentration of inorganic nitrogen at the Kellogg Biological Station in 1993 was

53.8 μM (G.P. Robertson, unpublished data on Web site http://KBS.MSU.EDU/lter/data/000_toc.htmlx). This concentration is less than 1/3 of that found in leachate from the non-tilled lysimeter and less than 1/6 of that found in leachate from the tilled lysimeter (Figure 8). This suggests that precipation nitrogen is not a primary source of NO_3. Due to the fact that neither of the lysimeters had recent applications of nitrogen fertilizers, and that precipitation nitrogen is not a primary source of NO_3, it appears likely that soil nitrogen is the primary of source of NO_3.

The leachate samples collected in the early spring to late summer from both lysimeters 2 and 9 show an increasing trend in $\delta^{15}N$ (a maximum of 9.4 $^{0}/_{00}$ was observed on September 13, 1993). This trend may have resulted from isotopic fractionation during denitrification (Marriotti et. al., 1988), or mineralization of an increasingly refractory pool of organic matter that is enriched in ^{15}N (Feigin et. al., 1974, Tiessen et. al., 1984 and Ledgard et al., 1984). As a result of warmer soil temperatures as spring progresses, microbial activity increases. Nitrate produced can either be assimilated by plants and/or the microbial population or undergo denitrification. The process of denitrification favors the lighter isotope of nitrogen and leaves the residual nitrate enriched in ^{15}N. Enrichment of the residual nitrate will continue until the nitrate pool has been expended or soil temperatures drop and thus decrease the rate of denitrification. The continued enrichment of the nitrate pool by denitrification, therefore, may be responsible for the increase in $\delta^{15}N$ values of leachate collected in the early spring to late summer from both lysimeters 2 and 9.

Another possible explanation for the observed increase in the $\delta^{15}N$ values from early spring to late summer may be the mineralization of an increasingly refractory and ^{15}N enriched pool of organic matter. Four separate nitrogen pools have been defined in soils. They consist of (1)nitrate, (2) readily available ammonium and other nitrogenous materials, (3) less readily available organic nitrogen and (4)unavailable or unmineralizable nitrogen (Feigin et. al., 1974). During incubation studies, Feigin et. al., (1974) found that pool 1 leached out almost immediately and pool 2 produced an initial nitrate spike. As a result, the less readily available organic nitrogen, pool 3, provided substrate for the longer term production of nitrate.

The readily mineralizable organic fraction has been shown to be depleted in ^{15}N relative to the other pools of soil nitrogen (Delewiche and Steyn, 1970). As a result, the mineralization of the readily available organic matter fraction of the soil may have led to the production of ^{15}N depleted nitrate in the early spring. The readily mineralizable organic fraction of soils is a finite reservoir. As mineralization of the readily mineralizable organic fraction of soils reaches completion, microbial mineralization proceeds to mineralize the less mineralizable and ^{15}N-enriched organic nitrogen. Subsequent mineralization and nitrification of this soil fraction and the resulting fractionation will result in the release of ^{15}N-enriched nitrate. Incubation studies found that the process of mineralization and subsequent enrichment of the less readily available organic fraction of soils continues until the $\delta^{15}N$ of the less readily available organic fraction of soils is approximately

equal to the $\delta^{15}N$ of total soil nitrogen (Feigin et. al., 1974). Therefore, the increasing trend of $\delta^{15}N$ values of leachate observed from early spring to late summer may be the result of the fractionation associated with mineralization and nitrification of the less readily available organic fraction of soils.

The increasing trend of $\delta^{15}N$ values of leachate observed from early spring to late summer may therefore be the result of either isotopic fraction during denitrification or the mineralization and subsequent nitrification of less readily mineralizable organic matter. However, it is unlikely that either is solely responsible for the observed trend. Rather, it is more likely that the observed trend is result of a combination of these two processes.

The $\delta^{15}N$ values of leachate decreased from late summer to the conclusion of the study on 11/29 from both lysimeters 2 and 9. This trend appears to reflect a change in the predominant microbial process controlling the $\delta^{15}N$ values observed in leachate.

With the onset of fall, both air and soil temperatures decreased. As a result, much of the microbial activity probably decreased. Denitrification rates have been shown to decrease significantly with cooler temperatures and are not appreciable below 4-6 degrees celsius. (Ryden, 1986; Jordan, 1989; Ruz-Jerez et al., 1994). The rates of mineralization and nitrification also likely decreased. However, site specific data suggests that these processes were still active despite low soil temperatures (G.P. Robertson, unpublished data on Web site http://KBS.MSU.EDU/lter/data/000_toc.htmlx). In addition, 15N-depleted plant material (harvest residuales) may have also provided

an additional source of mineralizable organic nitrogen. As a result, mineralization and nitrification of the ^{15}N-depleted soil fraction, may explain the decreasing trend of $\delta^{15}N$ values of leachate observed from late summer to the end of the study.

Based on this discussion, it is evident that no single source or microbial process is responsible for trends observed in the $\delta^{15}N$ values of leachate from lysimeters 2 and 9. In fact, these trends could be the result of a combination of nitrogen source and fractionation due to microbial processes. The findings of this study, suggest that soil organic matter was the primary source of nitrate to lysimeter leachate. Whereas, precipitation and fertilizer nitrogen did not appear to be appreciable sources of nitrate to lysimeter leachate. In addition, it appears likely that fractionation due to mineralization and nitrification was at least partially responsible for the trends observed in the $\delta^{15}N$ values of leachate from lysimeters 2 and 9 during the spring and early summer. Whereas, denitrification and/or mineralization of a less mineralizable soil fraction were in part responsible for the trends observed in the summer and fall.

As result, of the dynamic nature of the terrestrial system and the multitude of variables influencing the $\delta^{15}N$ values of leachate, the feasibility of using the stable isotope technique to assess origins in such an environment can be limited. It is evident from this study that influence of microbial processes on the $\delta^{15}N$ values of leachate is appreciable and it is this line of research that may be most informative. Therefore, any use of the stable isotope technique to determine the source of nitrogen without considering

the effect of fractionation may lead to questionable results. However, it is in studies such as this one where there is at least a limited understanding of the sources and processes affecting the final product (in this case the $\delta^{15}N$ values of leachate) that the feasibility of using the stable isotope technique in investigating the dynamics of nitrogen cycling in a terrestrial system is evident.

APPENDIX

APPENDIX A

FLOW RATE DATA FOR LYSIMETERS 2 AND 9

Date	Lysimeter 2 Volume of flow (l/week)	Lysimeter 9 Volume of flow (l/week)
03/25/93	5.4236	2.086
03/31/93		3.9634
04/05/93	13.7676	2.2946
04/12/93	13.9762	5.8408
04/19/93	14.602	11.473
04/26/93	58.408	58.408
05/03/93	25.6578	26.9094
05/10/93	16.4794	16.0622
05/17/93	12.0988	11.473
05/24/93	7.0924	6.6752
06/01/93	6.8838	6.0494
06/07/93	5.8408	5.8408
06/14/93	5.0064	4.3806
06/21/93	13.7676	5.0064
06/28/93	32.5416	
07/05/93	24.6148	28.161
07/12/93	16.8966	21.6944
07/19/93	13.3504	16.4794
07/26/93	12.516	10.8472
08/02/93	9.1784	8.5526
08/09/93	6.8838	6.4666
08/16/93	6.4666	5.6322
08/23/93	5.4236	4.5892
08/30/93	4.3806	3.9634
09/06/93	3.5462	2.9204
09/13/93	1.4602	1.4602
09/23/93	1.4602	12.3074
09/27/93	1.6688	7.9268
10/21/93	40.677	40.677
11/29/93	5.4236	6.0494

APPENDIX B

NITRATE CONCENTRATION FOUND IN LEACHATE COLLECTED FROM LYSIMETERS 2 AND 9

Date	Lysimeter 2 Nitrate Concentration (µmols/L)	Lysimeter 9 Nitrate Concentration (µmols/L)
03/25/93	282.9	172.1
03/31/93	416.8	143.7
04/05/93	398.6	150.9
04/12/93	393.1	142.3
04/19/93	363.6	148.1
04/26/93	337.8	206.4
05/03/93	370.0	226.8
05/10/93	360.7	226.3
05/17/93	373.9	219.3
05/24/93	352.6	214.7
06/01/93	363.3	204.4
06/07/93	402.3	198.0
06/14/93	409.5	199.2
06/21/93	394.5	228.7
06/28/93	421.1	259.2
07/05/93	398.4	244.1
07/12/93	346.3	226.9
07/19/93	387.5	256.5
07/26/93	380.7	235.0
08/02/93	366.8	
08/09/93	369.2	255.3
08/16/93	370.8	213.8
08/23/93	369.5	235.8
08/30/93	333.0	220.4
09/06/93	323.7	191.9
09/13/93	346.4	160.5
09/23/93	342.1	238.6
09/27/93	347.3	244.0
10/21/93	345.0	245.0
11/29/93	345.0	245.0

APPENDIX C

NITRATE FLUX DATA FOR LYSIMETERS 2 AND 9

Date	Lysimeter 2 Nitrate Flux (umols/week)	Lysimeter 9 Nitrate Flux (umols/week)
03/25/93	1534.3	359
03/31/93	569.5	
04/05/93	5487.8	346.3
04/12/93	5494	831.
04/19/93	5309.3	1699.2
04/26/93	19730.2	12055.4
05/03/93	9493.4	6103.1
05/10/93	5944.1	3634.9
05/17/93	4523.7	2516
05/24/93	2500.8	1433.2
06/01/93	2500.9	1236.5
06/07/93	2349.8	1156.5
06/14/93	2050.1	872.6
06/21/93	5431.3	1145
06/28/93	13703.3	
07/05/93	9806.5	6874.1
07/12/93	5851.3	4922.5
07/19/93	5173.3	4227
07/26/93	4764.8	2549.1
08/02/93	3366.6	
08/09/93	2541.5	1650.9
08/16/93	2397.8	1204.2
08/23/93	2004	1082.1
08/30/93	1458.7	873.5
09/06/93	1147.9	560.4
09/13/93	505.8	234.4
09/23/93	499.5	2936.5
09/27/93	579.6	1934.1
10/21/93	14033.6	9965.9
11/29/93	1871.1	1482.1

APPENDIX D

$\delta^{15}N$ OF DISSOLVED NITRATE

Date	Lysimeter 2 Corrected $\delta^{15}N$ ($^0/_{00}$)	Lysimeter 9 Corrected $\delta^{15}N$ ($^0/_{00}$)
03/25/93	0.2	-0.3
03/31/93	-3.9	-1.5
04/05/93	-2.7	-2.3
04/12/93	-3	3.2
04/19/93	0.5	
04/26/93	-0.1	1.7
05/03/93	0.1	-0.5
05/10/93	0.8	0.6
05/17/93	0.2	0.6
05/24/93	1.2	0.6
06/01/93	-0.5	1.4
06/07/93	1.6	
06/14/93		
06/21/93	4.6	4.1
06/28/93	1.8	5
07/05/93	2.9	3.2
07/12/93	4.2	3.2
07/19/93	4.8	
07/26/93	6.7	6.8
08/02/93	7	5.9
08/09/93	4.3	3.7
08/16/93	5.2	7
08/23/93	3.7	5.2
08/30/93	5.5	
09/06/93	3.7	5.8
09/13/93	9.4	8.8
09/23/93	8.2	
09/27/93	4.7	4
10/21/93	2.3	3.2
11/29/93	0.2	1.4

Note: All data corrected for background

APPENDIX E

MONTHLY RUNNING WEIGHTED AVERAGE OF $\delta^{15}N$

Julian Date	Ly 2 Running Monthly Weighted Avg. $\delta^{15}N$-NO3	Ly 9 Running Monthly Weighted Avg. $\delta^{15}N$-NO3
90	0.2	-1
116	-0.8	1.7
144	0.4	0.1
179	2.4	1.8
207	4.2	4.1
242	5.3	5.2
270	5.3	6.6

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