Application of Nitrogen and Oxygen Isotopes to Identify Sources of Nitrate

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ABSTRACT

The goal of this project was to establish the effectiveness and applicability of using nitrogen and oxygen isotope analyses on dissolved nitrate in groundwater as a useful technique to help identify sources and fate of nitrate found in the aquifers of Illinois. The initial focus of the project was to characterize the isotopic shift that occurs during denitrification. Three sites were investigated. The first and primary site was located in rural Mason County, Illinois at a research tree nursery facility, where the surrounding land use is completely agricultural. The second site is located near the banks of the Illinois River in Marshall County, Illinois, where there is the possibility of multiple sources of nitrate. The third site was in Shelby County, Illinois near the Kaskaskia River where a municipal well field contains elevated nitrate levels and there has not been any previous investigation as to the source. Groundwater samples from all three sites had isotopic values that followed a linear relationship typical of denitrification processes. The results for the Mason and Shelby County sites showed isotopic values for the nitrate that were characteristic of primarily originating from nitrogen-fertilizer applications and the oxidation of soil organic matter. At the Mason site there is a redoxcline at a depth of 40 feet which suggests there may be significant amount of denitrification occurring. However, the isotopic composition and chloride concentrations indicate that very little denitrification is actually occurring across the redoxcline. The Marshall County site had some isotope values that suggested there may be some contribution of nitrate from septic systems, however, much of the nitrate appeared to be from up gradient agricultural sources.

INTRODUCTION

Nitrate contamination can be a severe problem in some Illinois aquifers where the drinking water standard of 10 milligrams per liter as nitrogen (10 mg/L NO_3^--N) in shallow public and private wells is often exceeded. The sources and fate of nitrate can be complicated and are not well understood due to the mixing of nitrate from different sources and the variation in the occurrence and amount of denitrification within an aquifer. The use of nitrogen isotopes is a well established method of identifying nitrate sources, however, this method has some severe limitations due to overlapping signals and changes in the isotopic signal with denitrification within an aquifer. During microbial denitrification of NO_3^- to N_2 there is an isotopic fractionation which preferentially utilizes ^{14}N in the conversion to N_2. This process leaves the remaining NO_3^- isotopically more enriched in ^{15}N. This fractionation effect can make nitrate from partially denitrified fertilizer have the same ^{15}N isotopic value as nitrate derived from animal waste. The newly developed technique of determining the oxygen isotopic ratio of nitrate has the potential to overcome these limitations. Like ^{15}N, the O isotopes of the NO_3^- compound also becomes more enriched in ^{18}O in the remaining NO_3^- during denitrification (Böttcher et al.,1990). Therefore, analysis of the oxygen isotopes and nitrogen isotopes of dissolved NO_3^- can help factor out the effects of denitrification and clarify the identification of the primary nitrate sources.

In this study, groundwater samples were collected from monitoring well networks at three sites; two research sites in Mason and Marshall Counties where there is nitrate contamination and the sources and fate of the nitrate have been previously studied, and a third site in Shelby County where a municipal well field has nitrate concentrations close to the drinking water standard. At the Mason County site, earlier studies identified a nitrate plume coming from fertilized corn fields that showed complete denitrification with depth. At the Marshall County site there is potential mixing of nitrate from fertilizer and septic wastes along the flow path from the surrounding agricultural uplands through a small town whose residents used to have individual septic systems. At the Shelby County site the source of nitrate in the municipal well field is probably from surrounding agricultural fields but has not been investigated previously. Studying the characteristics of the two nitrate isotopes plus the isotopic composition of certain other major constituents and the general water chemistry in water samples from these sites, the isotopic behavior of nitrate from different sources typically found in Illinois can be better understood. This information could be applied to many of the other contaminated supply wells in communities, such as South Jacksonville and Lawarenceville, where the source of nitrate is uncertain or needs to be verified. The results of this study will allow for more effective land use practices designed to reduce the amount of nitrate contamination to groundwater resources

LITERATURE REVIEW

Nitrogen and Oxygen Isotope Fractionation Processes Associated with Nitrate

Nitrogen occurs in several forms in natural waters, including the aqueous species nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) and the dissolved gases nitrogen (N_2) and ammonia (NH_3). Nitrogen also occurs in organic molecules (Org-N) that can be in dissolved or solid form. Because of the wide variety of sources, nitrogen can be loaded into the soil in any of the forms.

Aside from the many nitrogen reactions occurring within the overlying plants, there are three major reactions in the soil and groundwater. Microorganisms in the groundwater play an important role in all of these reactions as described by Ehrlich (1996). Because organic nitrogen occurs in many forms, there are numerous pathways by which ammonium or ammonia can be formed. In the denitrification reaction, nitrite and nitrous oxide are formed as intermediary steps and the electron donor for this reaction is commonly organic carbon or a sulfide mineral such as pyrite. The following are examples of typical nitrogen transformations, including 2 pathways for denitrification:

1) Ammonification: $\text{Org-N} \rightarrow NH_4^+ \leftrightharpoons NH_{3(g)} + H^+$
2) Nitrification: $NH_4^+ + \frac{1}{2}O_2 + 2H_2O \rightarrow NO_3^- + 8H^+$
3) Denitrification:
   Pyrite oxidation: $14NO_3^- + 5FeS_2 + 4H^+ \rightarrow 7N_{2(g)} + 10SO_4 + 5Fe^{2+} + 2H_2O$
   Organic matter oxidation: $4NO_3^- + 5CH_2O \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O$

Because oxygen is usually very plentiful in the soil, most of the nitrogen that reaches the groundwater has undergone nitrification and is in the oxidized form nitrate. Ammonium concentrations in groundwater tend to be more than an order of magnitude less than the nitrate concentrations except at some feedlots where the groundwater is reduced (Komor and Anderson, 1993). Denitrification occurs when groundwater migrates into a reducing zone.

Measurement of the nitrogen and oxygen isotope ratios, $^{15}N/^{14}N$ and $^{18}O/^{16}O$, of NO_3^- in groundwater can be used to help distinguish sources of nitrate and evaluate whether nitrate concentrations are changing due to mixing of different sources or to denitrification processes. The nitrogen and oxygen isotope ratios are expressed with the delta notation, $\delta^{15}N$ and $\delta^{18}O$ respectively. The delta values are reported as a parts per thousand deviation from specific standards, such as air for nitrogen isotopes and standard mean ocean water (SMOW) for oxygen isotopes. Often, during certain reactions the isotopes will fractionate, usually resulting in the products becoming more enriched in the lighter isotopes. Understanding the effects of these reactions on the isotopic composition related to nitrate can help us determine what types of geochemical reactions are occurring that are affecting the nitrate concentration within the hydrologic system.

Fractionation mechanisms for nitrogen isotopes include volatilization of $NH_{3(g)}$ after ammonification, conversion of ammonium to nitrate during nitrification, and conversion of nitrate to $N_{2(g)}$ during denitrification. The greatest amount of fractionation in the $^{15}N/^{14}N$ ratio, up to +45‰ (AIR), is found at animal feed lots where there is a significant amount of $NH_{3(g)}$ evaporation from wastes on the land surface. There can be significant fractionation of the nitrogen isotopes during nitrification due to the selectivity of the lighter isotopes by the microbes involved. However, due to a number of factors, such as nearly complete consumption of NH_4^+ to NO_3^-, decreases in nitrification rates with the progression of the reaction, and increased $\delta^{15}N$ values of NH_4^+ due to volatilization, the overall $\delta^{15}N$ of NO_3^- in the soil usually ends up similar or slightly greater than that of the original NH_4^+ (Feigen et al., 1974; Heaton, 1986; Kendall, 1998). Both the oxygen and nitrogen isotopes in nitrate are fractionated during denitrification. Again, this is a microbial reaction and the lighter isotopes are selectively enriched in the products. Böttcher et al. (1990) computed denitrification enrichment factors (ϵ) of -15.9‰ for nitrogen and -8.0‰ for oxygen isotopes in a sand aquifer in Germany. Böttcher et al. (1990) also points out that the degree of fractionation may depend on environmental conditions and that greater enrichment factors have been observed in the laboratory experiments. Additionally, some field studies have shown lower enrichments factors while others have observed comparable results for field enrichment values in a riparian environment next to a corn field in Ontario (Mengis et al., 1999).

Assuming two thirds of the oxygen in the nitrate comes from the local groundwater and one third for the atmosphere (Böttcher et al., 1990; Kendall, 1998), the oxygen isotopic composition of the nitrate will reflect that of the local water. Aravena et al. (1993) computed a $\delta^{18}O_{NO3-}$ of +1‰ for a site in Ontario using a measured $\delta^{18}O$ of the groundwater of -10‰ and assuming an atmospheric $\delta^{18}O$ of +23‰. All of the nitrate derived from the various sources of reduced nitrogen, such as organic nitrogen and ammonia, at a particular site should have similar $\delta^{18}O$ values. Because synthetic inorganic nitrate fertilizer is made by fixing atmospheric gases, the $\delta^{18}O$ of nitrate fertilizer has distinctively higher $\delta^{18}O$ values usually around +20‰ to +25‰.

Characteristic $\delta^{15}N_{NO3-}$ Values for Different Settings

One of the best sources of information concerning nitrogen isotopes is a review paper by Heaton (1986) which cites approximately 60 papers on the subject. Komor and Anderson (1993) determined isotopic ratios in groundwater downgradient from variety source areas in an alluvial aquifer along the Mississippi River in Minnesota, a setting similar to the two study sites examined for this project. From the data presented in these two studies, general $\delta^{15}N_{NO3-}$ ranges can be assigned to the different contaminant sources discussed below. These ranges are shown along the x axis of figure 1. The $\delta^{18}O$ ranges of the different nitrate sources are plotted on the y axis.

Precipitation. There are a variety of nitrogen sources to the atmosphere and a wide variety of reactions that involve nitrate and ammonium. Levels of these two species are relatively very low in precipitation and do not represent a major source for groundwater contamination. The $\delta^{15}N_{NO3-}$ of precipitation can have an important effect if a high-nitrate plume is being diluted by very large amounts of low-nitrate water that is isotopically different (Mariotti et al., 1988). Heaton (1986) reports a weighted annual mean $\delta^{15}N_{NO3-}$ of -3‰ for precipitation in Pretoria, South Africa and expects similar values elsewhere.

Streams. Establishing the $\delta^{15}N_{NO3-}$ value of streams is critical where stream-bank infiltration and flood waters impact the groundwater system. From samples of the Colorado River in Texas, Kreitler and Land (1978) established a typical range of $\delta^{15}N_{NO3-}$ values between +1‰ and +10‰ for oxidizing nutrient-sufficient surface-water systems. Values higher than +10‰ were found in anoxic waters which the authors attributed to denitrification. Along rivers that recharge the Edwards Aquifer in Texas, Kreitler and Browning (1983) found that flood waters were isotopically lighter (+1‰ to +6‰) than base flow waters (+6‰ to +8‰). The authors did not explain this shift, but a possible explanation may be an increased proportion of isotopically depleted nitrate from precipitation or runoff containing fertilizer. In the Hennops River in South Africa which drains unfertilized land, Heaton (1986) found low $\delta^{15}N_{NO3-}$ values consistent with the local precipitation.

Soil. In the soil, the nitrogen bound in organic material can mineralize and then undergo ammonification followed by nitrification. If the ammonium was created at a sufficiently slow rate with a short residence time, there will be minimal volatilization and the nitrification reaction will go to completion. Under such conditions, the net fractionation is low and the resulting $\delta^{15}N_{NO3-}$ value is generally similar to that of the soil organic matter, which is typically in the range of +4‰ to +9‰ (Heaton, 1986, Mariotti et al., 1988). In incubation studies of seven different soils, Bremner and Tabatabai (1973) found a lower $\delta^{15}N_{NO3-}$ range of -9.6‰ to +6.3‰ and that the enrichment increased with incubation time. The large range was attributed to variations in the $\delta^{15}N$ value of the different forms of nitrogen in the soils and to variations in the percentage contribution of each of these forms to the nitrate produced during the incubations.

Groundwater in Natural Settings. Establishment of a background $\delta^{15}N_{NO3-}$ value for larger-scale studies has been more difficult. This is primarily because of natural variations in nitrate concentrations and $\delta^{15}N_{NO3-}$ values, especially in lower permeability soils (Vanden Heuval, 1992). Komor and Anderson (1993) attempted to establish background groundwater $\delta^{15}N_{NO3-}$ values for their study in Minnesota by collecting samples from wells in areas they considered to be natural. With one exception, they found nitrate levels below 1.5 mg/L with the $\delta^{15}N_{NO3-}$ values averaging +3.9‰, close to Heaton's (1986) expected range for soil.

Groundwater Contaminated by Inorganic Fertilizer. Nitrogen in fertilizer, commonly in the ammonium nitrate or anhydrous ammonia form, is derived from the industrial fixation of atmospheric nitrogen by a quantitative process that, depending on variations in the manufacturing processes, yields fertilizers with a $\delta^{15}N_{NO3-}$ value between -4‰ and +4‰. Summarized data in Heaton (1986) shows that the median values for the ammonium and nitrate portions of ammonium nitrate are -1‰ and +2.5‰, respectively. Groundwater contaminated by applied nitrate fertilizer should have a $\delta^{15}N_{NO3-}$ value in the same range. However, studies by Kohl et al. (1973) and Meints et. al (1975) show that the fertilizer nitrogen can lose its isotopic signature due to exchange with the large mass of organic nitrogen in the soil (Heaton, 1986). Dealy (1995) suggests a range of +3.5‰ to +7‰ as being the range of $\delta^{15}N_{NO3-}$ values for contamination with a combined fertilizer and an organic soil nitrogen source.

Groundwater Contaminated by Animal Waste and Septic Systems. Nitrate derived from animal waste typically has $\delta^{15}N_{NO3-}$ values greater than +10‰. The enrichment is caused by volatilization of ammonia gas as the organic nitrogen in the waste, mostly urea, is converted to ammonium. The degree of $\delta^{15}N_{NO3-}$ enrichment is highly dependant on the physical conditions affecting volatilization, such as the length of time the waste is exposed directly to the atmosphere. The summary data from Heaton (1986) show $\delta^{15}N_{NO3-}$ values for manure and contaminated soils ranging between +8‰ and +22‰. Human waste is a potential source of nitrate contamination in a small community or rural subdivision where there are a large number of septic systems. The isotopic enrichment from human waste tends to be less than animal waste. Mariotti (1988) found $\delta^{15}N_{NO3-}$ values between +10‰ and +13.5‰ in sewer and septic tank discharges while groundwater samples in an urban area of France average +9.9‰. Aravena et al. (1993) were able to clearly distinguish nitrate from a septic field from other nitrate sources in an aquifer using isotope analyses.

Denitrification. When groundwater flows into a reducing zone, nitrate reduction will greatly increase due to bacteria which use it as an electron acceptor. Reported enrichment factors range widely from 4.7‰ in a chalk aquifer in France where sorption is occurring (Mariotti et al., 1988) to 30‰ in a sandy aquifer in the Kalahari desert where the denitrification rate is extremely slow (Vogel et al. 1981). Mariotti et al. (1988) observed decreased nitrate levels in groundwater flowing towards two different wellfields in northern France. At the Emmerin wellfield nitrate levels rapidly dropped and the $\delta^{15}N_{NO3-}$ values rapidly increased as flow in the aquifer changed from unconfined to confined conditions. This was attributed to the onset rapid denitrification with the separation from atmospheric oxygen supply and an ample organic carbon electron source. At the Les Ansereuilles wellfields, nitrate levels slowly dropped with flow towards the wells, however, the $\delta^{15}N_{NO3-}$ value remained fairly constant. This was attributed to mixing caused by the wellfield inducing a large amount of river water that had a low concentration of NO_3^- which was isotopically similar to the aquifer. Because the aquifer was unconfined, it remained open to atmospheric oxygen which inhibits denitrification.

Available $\delta^{18}O_{NO3-}$ Values for Groundwater

The available $\delta^{18}O_{NO3-}$ data for groundwater in the literature is increasing as the ability to measure this isotope becomes more widespread. The Böttcher et al. (1990) and Mengis et al. (1999) studies show $^{18}O_{NO3-}$ fractionation occurring with denitrification in groundwater. Böttcher et al. (1990) had data from 10 samples at 4 multi-level wells along a 3 kilometer flow path at a mixed agricultural and forested site in Germany that showed a good correlation between nitrate concentration and increases in $\delta^{15}N_{NO3-}$ and $\delta^{18}O_{NO3-}$. In one well, they found a decrease in nitrate from 21 mg/L (as N) to 4.5 mg/L from 4.0 to 7.0 meters in depth while the $\delta^{15}N_{NO3-}$ increased from +9.69‰ to +37.09‰ and the $\delta^{18}O_{NO3-}$ increased from +6.01‰ to +25.67‰. Mengis et al. (1999) used $\delta^{15}N_{NO3-}$ and $\delta^{18}O_{NO3-}$ to help study denitrification processes in a riparian zone next to an agricultural crop field in Ontario. At this site, identification of nitrate reduction by continuous decreasing nitrate concentration was complicated by varying loads of nitrate added to the system and by local hydrologic complexities. However, the isotopic results were characteristic of denitrification showing a linear relationship between the nitrogen and oxygen isotopes, with $\delta^{15}N$ values ranging from about +5‰ to +30‰ and $\delta^{18}O$ values ranging from about +0.5 to +17‰. The trend in isotopic enrichment during denitrification is shown on a crossplot of $\delta^{15}N_{NO3-}$ and $\delta^{18}O_{NO3-}$ values (figure 1).

In an area where fertilizer was one of the suspected nitrate sources, Aravena et al. (1993) found that the $\delta^{18}O_{NO3-}$ had a mean value of +3.5‰. This was 10‰ below their expected of +13‰ if all of the nitrate came from NH_4NO_3 fertilizer, assuming a $\delta^{18}O$ of +23‰ for the nitrate portion and a $\delta^{18}O$ of +1‰ for the nitrified ammonium. They suggest that the nitrate in the groundwater is not from the nitrate in the fertilizer, rather it is from nitrification of the ammonium portion along with ammonium from manure and mineralized organic nitrogen from the soil. This hypothesis is further evidenced by studies of nitrogen uptake by crops using isotopically labeled ^{15}N that showed the nitrate portion is used up more readily than the ammonium portion (Recous et al., 1988).

Nitrogen and oxygen isotopes were also used by Wassenaar (1995) to try and distinguish if the observed nitrate contamination in an aquifer in British Columbia was from poultry manure used as fertilizer or from inorganic nitrogen fertilizer. Because elevated $\delta^{18}O_{NO3-}$ values in samples from 117 irrigation, domestic and municipal wells were not found, he concluded that nitrate was largely from the poultry manure and ammonium fertilizers and that little denitrification is occurring in the aquifer.

Study Sites

Agricultural/Denitrification Site - Mason State Tree Nursery. In a detailed chemical study Kelly (1997) found denitrification to be occurring with depth in the groundwater. The nursery is in the heavily irrigation region of western Mason County, Illinois where the thick Sankoty-Mahomet sand aquifer is at the surface. Using water samples from 16 multi-level sampling wells and 10 regular monitoring wells, Kelly (1997) was able to define a nitrate plume from an irrigated field (figure 2) and the chemical changes in the aquifer as the plume was denitrified. Irrigated fields typically have a higher permeability and a lower organic content which, coupled with the higher volume of infiltration, allow for more nitrate to reach the water table either without reacting or cycling through the plant material. Because of the previous investigation and the existence of the multi-level sampling wells, this site was an excellent site for our isotope study.

We initially assumed conditions were still similar to those found in 1993 and 1994 when the original sampling was done, and were hopeful that analyzing the isotopes from a selected number of wells would provide some insight to characterizing and quantifying the fractionation that occurs during denitrification in a sandy aquifer typical of many aquifers in Illinois. Although geologically and hydrologically the site was the same, the fertilization practices had changed. After sampling and examining the isotopic and chemical results, it became evident that the tree nursery facility had applied N-fertilizers to their plots, whereas, during the previous study, no N-fertilizers had been applied to these plots. Although the deeper groundwater samples still exhibited reducing conditions and some denitrification still persists, the results are complicated due to variable N-fertilizer applications. However, the changes in N-fertilizer application practices allowed us to examine the chemistry and isotopic characteristics beneath sandy soils receiving abnormally high rates of N-fertilizer application.

Mixed Source Site - Henry. A good location to test the effectiveness using both the nitrogen and oxygen isotopes to differentiate the major source of nitrate is at Henry, in Marshall County, Illinois. The monitoring wells constructed at the Henry study site (Soong et al., 1998) were designed to examine stream bank infiltration during floods. Henry is located adjacent to the Illinois River, sixty miles north of the Mason County site in the same alluvial aquifer system. The nitrate concentration from the Henry supply wells is generally at or above the drinking water standard. The potential sources of nitrate, which vary across town, include the spring flood waters, fertilizer from upgradient irrigated and nonirrigated fields at the edge of town, a nearby fertilizer production plant, and the many septic systems that were active until a sewer system was installed in 1996. In four monitoring wells along a 2 kilometer groundwater flow path, the nitrate concentration drops from 14 mg/L near the irrigated fields to 10 mg/L near the supply wells next to the river and the corresponding $\delta^{15}N_{NO3-}$ values increased from +2.4‰ to +5.0‰ (unpublished data). These data suggest that either some denitrification is occurring or that there is mixing by isotopically heavier nitrate from the septic systems. For this study, groundwater samples were collected from five of the wells plus the Illinois River and analyzed for $\delta^{15}N_{NO3-}$ and $\delta^{18}O_{NO3-}$ as well as for chemical parameters including major anions and cations.

Shelbyville Site. The City of Shelbyville operates nine wells in an alluvial aquifer along the Kaskaskia River in Shelby County, Illinois. Wells 4, 5, and 6 are located in a line perpendicular to the river in the bottomland that is farmed with row crops. A four foot thick soil layer covers the aquifer. Wells 5 and 6 are 680 feet and 1,330 feet from the river, respectively, and had NO_3-N concentrations that frequently exceeded 10 mg/L in past samples (Farnsworth and Wiley, 1996). Well 4 is only 300 feet from the river and had a NO_3-N concentration less than 4 mg/L, which may be caused by infiltration of lower nitrate river water (Farnsworth and Wiley, 1996). Wells 1, 2, 3, 8, and 9 are grouped together in an upland area where there is approximately 15 ft of silty material overlying the aquifer. Wells 1, 2, and 3 had NO_3-N concentration less than 8 mg/L (Farnsworth and Wiley, 1996). Wells 8 and 9 are quite new and have no published chemical data.

Analytical Parameters and Methods

Water samples were collected and analyzed for their chemical and isotopic composition. Isotopic analyses primarily included the oxygen and nitrogen isotopes of the dissolved nitrate ($\delta^{18}O_{NO3-}$ and $\delta^{15}N_{NO3-}$). In addition to the nitrogen and oxygen isotopes, selected samples also were analyzed for the carbon isotopic composition ($\delta^{13}C$) of bicarbonate, the sulfur isotopic composition ($\delta^{34}S$) of sulfate sulfur, and the hydrogen and oxygen isotopes of the water (δD_{H2O} and $\delta^{18}O_{H2O}$). Analysis of the $\delta^{13}C$ and $\delta^{34}S$ of these other constituents help to determine what geochemical processes are controlling the denitrification process. For example, if denitrification occurs due to pyrite oxidation then the $\delta^{34}S$ values will become more negative, or if the denitrification is due primarily to oxidation of organic matter then the $\delta^{13}C$ values may become more negative. The samples also were analyzed for the different dissolved nitrogen species including nitrate, nitrite, ammonium, and organic nitrogen. To understand or confirm the geochemistry of the sites, additional analyses were performed for the metals, anions non-volatile organic carbon (NVOC), alkalinity, and total dissolved solids. The parameters pH, Eh, dissolved oxygen (DO), specific conductivity and temperature were measured in the field using the appropriate meters and electrodes. The water samples were filtered, preserved, and/or kept at 4 degrees Celsius as called for in the analytical protocols. Duplicate samples and sample blanks were also collected for quality assurance. Solid samples of the aquifer material were analyzed for their organic and inorganic carbon contents and their mineralogical composition. The chemical analyses were performed at the Illinois State Water Survey and the isotopic and mineralogical analyses were performed at the Illinois State Geological Survey.

The stable isotope analyses of nitrate, dissolved inorganic carbon, sulfate-sulfur and water were performed by converting the specific element of interest (such as N, O, C, S or H) to a purified gas phase (N2, CO_2, SO_2, or H_2) and measuring the isotopic composition on an isotope ratio mass spectrometer. The analytical techniques for measuring the nitrate-oxygen isotope are the most recently developed and are not as routine as the analyses for the other isotopes. For $\delta^{18}O$ determination of the nitrate, it was necessary to remove the dissolved organic matter and sulfate ions from the water samples prior to collecting the nitrate to minimize contamination from the oxygen in sulfate ions and in dissolved organic matter. The sulfate ions were removed by precipitation as $BaSO_4$ (Amberger and Schmidt, 1988) and the dissolved organic matter were removed using a silicalite molecular sieve. After removal of sulfate and dissolved organics, the nitrate in the water samples were extracted using an anion-exchange column packed with BioRad AG 2-X8 resin, similar to that described by Silva et al. (1998). Once the nitrate is collected on the ion-column, it was eluted with HBr solution (Hwang et al., 1999) and converted to silver nitrate by adding silver oxide and freeze drying in a vacuum system. The silver nitrate is converted to CO_2 and N_2 for $\delta^{18}O$ and $\delta^{15}N$ analysis respectively using techniques similar to those described by Silva et al. (1998). Both gases were analyzed either on a Finnigan Mat Delta E Isotope Ratio Mass Spectrometer (IRMS) or a Finnigan Mat 252 GC-MS IRMS at the Illinois State Geological Survey.

The δD and $\delta^{18}O$ of H_2O were determined by a modified Zn-reduction method and a modified H_2O-CO_2 equilibration method respectively as described by Hackley et al., 1999. The $\delta^{13}C$ of the dissolved inorganic carbon (DIC) were determined by acidifying the sample in an evacuated container and collecting the CO_2 gas released similar to that described by Sackett and Moore (1966) The $\delta^{34}S$ of the sulfate sulfur were determined by precipitating the sulfate as $BaSO_4$ and converting the precipitate to SO_2 for isotopic analysis using techniques similar to that described by Veda and Krouse (1986).

Collection of groundwater samples

A reconnaissance trip was made on September 27, 2000 to the field site at the Mason State Tree Nursery in Mason County to test the integrity of the existing multi-level sampling (MLS) wells, collect water samples to confirm the disappearance of the nitrate plume with depth, and identify which wells should be sampled for complete chemical analyses. The sampling tubes for three of the multi-level wells (3,7,18) were pumped and found to be in good working condition. Because groundwater levels were lower than when the wells were installed in 1993, the shallowest ports were dry. Well MLS-18 is in the upgradient corner of the tree nursery and is surrounded on three sides by irrigated fields that were planted with corn in 2000 and 2001. MLS-3 and MLS-7 are clustered together with a series of wells 600 feet downgradient of MLS-18 and 100 feet upgradient of the Nursery's irrigation well.

Full sets of samples were collected at the Mason site on October 11, 2000, February 27, 2001, and June 25, 2001 from MLS-7 and MLS-18. To help resolve the geochemical trends found in the bottom two ports of MLS-7, an addition well (#19) was installed 3 feet downgradient MLS-7 on April 16, 2002. The borehole was drilled with hollow-stem augers using the ISWS drill rig. Solid samples were collected throughout the hole with a split spoon. The well was completed to 42.5 feet with a 2-foot screen from 40.5 to 42.5 feet. Additional water samples were collected form MLS-7 on April 16, 2002 and from well 19 and selected ports of MLS-7 on June 5, 2002. Samples of the fertilizer used on the surrounding fields were also obtained from the local fertilizer dealer.

Samples were collected at the Henry site on September 19, 2001 from MLS-4, Henry municipal well #3, observation well SP3, and additional upgradient observation wells at Child Park and the water tower. The Henry sampling sites are described in Soong et al. (1998). The samples from the multi-level wells were collected with a multi-channel peristaltic pump. Samples from the observation wells were collected with a positive displacement submersible pump.

Samples were collected from Kaskaskia River and Shelbyville Wells 1, 3, 5, 6, and 8 on April 15, 2002. Unfortunately, the pump in Well 4 had been removed for repair at the time. Therefore, Well 4 was sampled on June 6, 2002 along with Wells 1,5, and 8 and the Kaskaskia River.

Results - Mason Site

Nitrate Distribution: The nitrite concentrations and isotopic results for the Mason County site are shown in Table 1. Unfortunately, in the first and second sampling events, insufficient volume was collected for those samples with small nitrate concentrations (below 2 mg/L) to obtain accurate values for $\delta^{15}N$ and $\delta^{18}O$ analysis on the mass spectrometer. In subsequent sampling larger volumes of sample was collected. Nitrite, ammonium, and organic nitrogen were not detected in any of the groundwater samples from the site.

The drop in nitrate concentration at a depth between 38 and 44.5 feet at MLS-7 and between 29.5 and 39.5 feet at MLS-18 strongly suggest the occurrence of denitrification at those depths. Along with the drop in nitrate is a large drop in dissolved oxygen concentration (Table 2), suggesting that a geochemical redox zone, or redoxcline, exists at these depths. However, other evidence discussed below suggests that the drop in nitrate may not be dependent on the redoxcline.

In the 2/27/01 samples, the nitrate levels decreased significantly in the uppermost ports of both wells and then increased again in the 6/25/01 samples. The winter decrease is likely due to the infiltration of low-nitrate rain water and snow melt. Nitrate levels increased dramatically in the 4/15/02 samples throughout the plume at MLS-7. This rise may be due to the spring application of fertilizer, an explanation which would require a significant amount mixing to occur in the upper 17 feet of the aquifer. Significant rains in May 2002 caused the water table to rise enough to saturate port 7-7 (6/5/02 sampling event). This loading of the systems also appears to have pushed nitrate in the bottom zone of MLS-7. In 1994 the nitrate concentrations in MLS-18 (Kelly and Ray,1999) were similar to 2001 values but the 1994 values at MLS-7 were only half those found in 2001. The 1994 data also showed lower concentrations of sulfate and chloride at both wells and much higher NVOC concentrations at MLS-18.

Table 1. Nitrate and isotopic analyses from the Mason State Tree Nursery

		10/11/2000					2/27/2001				
Well-Port	Depth (ft)	NO_3^--N (mg/L)	$\delta^{18}O_{NO3-}$ (SMOW)	$\delta^{15}N_{NO3-}$ (air)	$\delta^{34}S_{SO4--}$ (CDT)	$\delta^{13}C_{DIC}$ (PDB)	NO_3^--N (mg/L)	$\delta^{18}O_{NO3-}$ (SMOW)	$\delta^{15}N_{NO3-}$ (air)	$\delta^{34}S_{SO4--}$ (CDT)	$\delta^{13}C_{DIC}$ (PDB)
7-7	13										
7-6	17	14.9	5.5	6.8	-0.6	-8.04	6.66	-	7.5	-1.2	-9.64
7-5	23	18.3	6.0	6.6	3.9	-8.45	13.7	5.4	8.2	2.0	-7.81
7-4	27	19.5					19.9	6.0	9.1	1.2	-8.26
7-3	33	17.1	6.6	10.5	-5.9	-7.66	18.3	8.0	9.3	2.0	-8.44
7-2	38	18.1	6.2	10.1	-4.9	-7.93	14.0	6.7	8.1	0.6	-8.46
7-1	44.5	<0.06			-11.3	-7.86	0.63	-	-	-9.8	-7.90
19	41.5										
18-8	14.5	11.2	1.9	2.7	0.1	-9.12	8.42	1.9	3.9	0.5	-8.78
18-7	19.5						15.4	2.4	2.5	-0.1	-7.20
18-6	24.5	7.23	2.0	1.9	-0.4	-6.93	7.13	2.8	3.7	-2.6	-6.72
18-5	29.5						5.35	4.5	4.5	-5.8	-8.11
18-4	34.5	1.97	-	-	-8.1	-8.32	1.90	-	8.7	-8.0	-8.24
18-3	39.5						0.48	-	-	-6.9	-8.77
18.2	49.5						<0.06			-11.8	-8.63
18.1	59.5						<0.06			-9.7	-8.72

	6/25/2001					4/26/2002			6/5/2002		
Well-Port	NO_3^--N (mg/L)	$\delta^{18}O_{NO3-}$ (SMOW)	$\delta^{15}N_{NO3-}$ (air)	$\delta^{34}S_{SO4--}$ (CDT)	$\delta^{13}C_{DIC}$ (PDB)	NO_3^--N (mg/L)	$\delta^{18}O_{NO3-}$ (SMOW)	$\delta^{15}N_{NO3-}$ (air)	NO_3^--N (mg/L)	$\delta^{18}O_{NO3-}$ (SMOW)	$\delta^{15}N_{NO3-}$ (air)
7-7									5.80	6.9	6.7
7-6	11.8	5.8	5.9	2.2	-9.26	14.1	6.4	6.8			
7-5	17.1	4.6	7.5	1.8	-8.61	22.9	7.5	8.5			
7-4	21.6	5.9	7.6	1.9	-8.27	31.5	7.9	6.9			
7-3	18.6	6.3	9.1	-4.1	-8.19	33.0	7.7	6.5			
7-2	18.6	5.9	9.0	-3.5	-8.35	26.4	7.9	6.9	32.3	9.1	5.4
7-1	1.71	6.8	8.3	-8.9	-7.55	1.55	6.9	5.7	7.59	9.4	5.8
19									8.53	12.6	17.7
18-8	10.4	3.2	1.5	0.2	-7.78						
18-7	11.6	2.3	2.4	0.2	-7.03						
18-6	9.00	2.7	3.2		-6.41						
18-5	0.95	-	-		-7.47						
18-4	1.16	7.6	1.7		-7.80						
18-3	0.96	8.7	9.2	-8.6	-7.80						
18.2	<0.06			-12.3	-8.42						
18.1	<0.06			-8.8	-8.62						

Table 2. Chemical analyses from MLS-7 and MLS-18 from June 25, 2001.

Well-Port	Depth (ft)	pH (units)	Dis. O_2 (mg/L)	Cl^- (mg/L)	NO_3^--N (mg/L)	SO_4^{--} (mg/L)	TDS (mg/L	alkalinity (mg/L)	Fe (mg/L)	Mn (mg/L)
7-6	17	7.35	6.8	13.3	11.8	48.8	471	326	<0.009	<0.002
7-5	23	7.32	6.2	16.2	17.1	32.1	444	303	0.021	<0.002
7-4	27	7.36	5.5	17.4	21.3	31.4	447	272	0.012	<0.002
7-3	33	7.41	3.2	13.4	18.5	20.5	343	203	<0.009	<0.002
7-2	38	7.36	2.3	15.3	18.4	38.9	401	245	<0.009	0.076
7-1	44.5	7.29	0.8	4.80	1.71	62.9	325	192	<0.009	0.325
18-8	14.5	7.28	6.7	10.3	10.4	22.6	260	155	<0.009	<0.002
18-7	19.5	7.30	8.8	6.88	11.6	20.2	190	80	<0.009	<0.002
18-6	24.5	7.23	4.4	5.17	9.00	14.8	168	87	<0.009	<0.002
18-5	29.5	7.47	-	4.28	0.95	57.7	251	126	0.019	<0.002
18-4	34.5	7.19	0.8	5.93	1.16	56.0	269	126	<0.009	0.016
18-3	39.5	7.38	0.6	4.55	0.96	54.6	234	128	0.132	0.156
18-2	49.5	7.57	0.6	12.3	<0.06	70.9	298	156	0.689	0.206
18-1	59.5	7.50	0.6	15.3	<0.06	53.9	284	161	0.575	0.213

Initial Isotopic Values: The initial value for the δ^{15}N and δ^{18}O in the aquifer will depend on the type of fertilizer used, the type and degree of nitrogen transformations in the soil, and uptake by the plants. Table 3 shows lists the fertilizers believed to have been used on the irrigated fields and the nursery plots. The δ^{15}N and δ^{18}O values listed for the Liquid-28 and DAP fertilizers were from samples collected from the local fertilizer dealer and analyzed. The anhydrous ammonia is mixed from the same stock as the Liquid-28 and so the NH_4 in each type of fertilizer is expected to have similar δ^{15}N values. Representative ranges for the δ^{15}N of synthetic nitrate and ammonium from ammonium sulfate was compiled by Hubner (1986). The nitrate sampled from MLS-18, nearest the corn fields, have isotopic values which plot in the overlapping regions typically observed for nitrate originating from nitrogen fertilizers and organic nitrogen in the soil. If all of the nitrate in MLS-18 is from the fertilizer applied to the irrigated fields, then the initial δ^{15}N should be around 0‰. Because nitrogen cycling in the soil, the synthetic nitrate will likely be transformed to organic matter or ammonia prior to reaching the water table. During these transformations the oxygen atoms are lost to the system and any subsequently formed nitrate derives its oxygen in a mix of one-third atmospheric O_2 and two-thirds groundwater (Böttcher et al., 1990; Kendall, 1998). Using a value of 23‰ for the air and -7.8‰ for the groundwater from 18-8, the initial δ^{18}O would be around 2.5‰, very similar to what is observed in the shallowest groundwater samples from MLS-18.

On the isotopic crossplot (figure 3) the initial nitrate from MLS-18 falls near the overlapping areas of the characteristic signatures from fertilizer and soil nitrogen. This raises the possibility that a significant portion of the nitrate is coming from the decay of organic matter, such as the plant debris and root systems of the yearly crops. However, because of the strong correlation between nitrate and chloride (figure 4), most of the nitrate is probably directly associated with fertilizer applications. The strong correlation suggest that the chloride is probably coming from the KCl applied with the diammonium phosphate and chloride is therefore a tracer for the fertilizer. The nitrate-chloride correlation is maintained through the large seasonal fluctuations in nitrate concentrations, suggesting that whatever processes are affecting the nitrate concentrations, the chloride concentrations also seem to be equally affected. The bottom ports of MLS-18 showed

Table 3. Fertilizer applications and representative δ^{15}N values.

Irrigated Fields	**Nursery Plots**
February-March - Diammonium phosphate - DAP $(NH_4)_2HPO_4$ 18-46-0 100 lb/acre δ^{15}N = 1.3‰[*] - Potassium chloride KCl 0-0-60 200 lb/acre	Fall 1999 - 2001 - Diammonium phos. - DAP $(NH_4)_2HPO_4$ 18-46-0 300-400 lb/acre (1999, 2000) 200 lb/acre (2001) δ^{15}N = 1.3 ‰[*]
March-April and June - Anhydrous ammonia NH_4OH Variable application rate δ^{15}N = -0.8 ‰[^]	Spring-Summer 2000-2001 - Ammonium Sulfate $(NH_4)_2SO_4$ 300 lb/acre δ^{15}N = -2.7 to 2.7 ‰[**]
May-early July - if needed - Liquid 28 - UAN, Uran NH_4NO_3 and Urea 28-0-0 δ^{15}N NO_3 = 1.4‰[*] δ^{18}O NO_3 = 22.3‰[*] δ^{15}N NH_4 = -0.8‰[*] δ^{15}N Urea = -1.1 to 1.5‰[**]	2002 - No application between MLS-18 and MLS-7
Other - Potash - potassium chloride KCl 0-0-60 200 lb/acre - Limestone 1-2 tons/acre	Other - Potash - potassium chloride KCl 0-0-60 200 lb/acre

[*] locally measured values

[**] range from Hubner, 1986.

[^] Expected to be similar to NH_4 in Soln-28

elevated chloride levels but low nitrate which would suggest that denitrification is occurring upstream in the flow system. If the nitrate was primarily coming from the oxidation of organic matter in the soil, there should be no correlation with chloride because the chloride content of organic matter is very low. _

Mixing and Partial Denitrification: The δ^{15}N and δ^{18}O data of the dissolved nitrate in the samples collected from the Mason tree farm indicate that some denitrification and some mixing are occurring in the shallow portion of the aquifer. A crossplot of the oxygen and nitrogen isotopic values follow a relationship of increasing δ^{18}O values along with increasing δ^{15}N values (figure 3). The values follow along the denitrification trend as water moves along the flow path from the upper ports of MLS-18 to both the deeper port 18-3 and to well MLS-7. The higher nitrate concentrations at MLS-7 compared to the upgradient MLS-18 would suggest that there must be some additional input of nitrate from within the nursery.

A plot of the $\delta^{15}N$ versus NO_3^- concentration for the data collected from MLS-18 generally shows an inverse correlation and supports denitrification with depth (figure 5). Some of the other chemical parameters, such as DO, dissolved iron, and manganese, also supports a theory of a reducing zone with depth at MLS-18 (Table 2). Although, contrary to what would be normally expected in a groundwater flow system where denitrification is occurring, there is a lack of a correlation between the concentration and the isotopic composition of the nitrate in the upper zone of the aquifer when considering the data collected from the down gradient well MLS-7 (figure 6). However, this is a dynamic system where nitrate inputs have varied spatially and with time. For example, within the Nursery itself, ammonium application during June and July of 2000 was 200 lbs/acre while during August of 1999 the application was 300 to 400 lbs/acre. In the agricultural fields surrounding the Nursery, nitrogen is added in March and April and again in June primarily as anhydrous ammonium and in May, June and beginning of July nitrogen was added in the liquid form as "liquid-28" (28% nitrogen as ammonium and urea). Furthermore, nitrogen is only added in the fields for corn and not soybeans. Thus, the inputs of nitrate during different times of the year and during different years could be quite diverse considering variables such as crop rotation, seasonal fertilizer application, variation in the quantity of precipitation, fluctuation of the water table and mixing between deeper and shallow groundwater

As the groundwater migrates from MLS-18 to MLS-7 the isotopic composition of both nitrogen and oxygen does shift toward higher values with a slope of around 0.5, indicating a $\delta^{18}O:\delta^{15}N$ enrichment of 1:2 which is very similar to the trend observed by Böttcher et al. (1990) for denitrification. Obviously, denitrification of nitrate migrating from MLS-18 cannot completely explain the positive isotopic shift observed at MLS-7 because the nitrate concentration is greater at MLS-7 (nearly double that of MLS-18). The high nitrate concentration plus the positive isotopic shift observed at MLS-7 could be explained by a combination of nitrate from MLS-18 plus nitrate inputs from the high fertilizer application rates at the nursery (table 3). The nitrate input from the nursery plots would probably have also undergone partial denitrification during infiltration through the soil zone. Thus, a combination of highly concentrated, partially denitrified, NO_3 from fertilizer-N applied to the nursery would explain the greater NO_3^- concentration plus the general positive shift of both $\delta^{18}O$ and $\delta^{15}N$ values. The larger range of $\delta^{18}O_{NO3}$ values observed at MLS-7 could be due to small percentages of atmospheric NO_3 intermittently added to the system. Precipitation soon after fertilizer application would tend to increase the NO_3^- concentration because of flushing NO_3^- out of the upper soil zone and the $\delta^{18}O$ may increase due to the high $\delta^{18}O$ values of atmospheric NO_3^-.

An alternative possibility for a greater range of $\delta^{18}O_{NO3}$ values at MLS-7 would be the occurrence of greater evaporation in the upper soil zone on the less vegetated Nursery plots creating more positive $\delta^{18}O$ values in the soil water during dry periods. The isotopically more positive $\delta^{18}O$ values of the upper soil zone water would result in greater $\delta^{18}O$ values of NO_3^- during the nitrification processes (assuming there is two thirds oxygen contribution from surrounding water and one third from atmospheric oxygen). Thus, periodically, the NO_3^- infiltrating the groundwater may have greater $\delta^{18}O$ values due to evaporational processes at the surface. In either case, atmospheric inputs of NO_3^- or evaporational processes effecting the $\delta^{18}O$ of the upper soil zone water, the NO_3^- concentration and the $\delta^{18}O$ values of the overall nitrate in the sampled groundwater at MLS-7 would both increase. If evaporational processes were affecting the upper soil zone close to the time of fertilizer application, then it is possible that some of the applied N-fertilizer may have undergone volatilization and could have been released as NH_3. Isotopic fractionation occurs during this process resulting in the volatilized NH_3 becoming enriched in the lighter nitrogen isotope (^{14}N) and the remaining ammonium becoming isotopically enriched in ^{15}N. Because the overall trend in $\delta^{18}O$ and $\delta^{15}N$ values for the site fits a 1:2 ratio on a delta-delta plot, denitrification appears to be occurring. However, the spread of isotopic values, especially for MLS-7 suggests that other inputs or processes such as those described above may also be occurring.

Mixing models were developed to test whether inputs of atmospheric nitrate or evaporational effects plus denitrification of nitrate from MLS-18 and the tree nursery plot could actually cause the observed isotopic compositions at MLS-7. While the true mixing ratios are unknown, the 10 inches of annual aquifer recharge (Kelly, 1997) would suggest the infiltration at the nursery could account for up to 30% of the water in the upper portion of the aquifer at MLS-7.

The first mixing model includes NO_3^- originating from MLS-18 that follows a denitrification trend plus partially denitrified NO_3^- from the nursery plot originating primarily from the nitrification of fertilizer-N, plus small inputs of atmospheric NO_3^-. The model reveals that samples collected from MLS-7 could be composed of about 59 to 67 % inputs from MLS-18, 30 to 35 % inputs from fertilizer applications at the Nursery, and 3 to 6 % inputs from the precipitation. Because the fertilizer applications at the Nursery were 3 to 4 times that of the surrounding cropped fields relatively high NO_3^- concentrations were used in the models. Concentrations on the order of 35 to 80 mg/L of NO_3-N were used for inputs from the Nursery plot. These ranges are within reason considering measurements of NO_3^- at the water table beneath an agricultural field in Iowa that received normal rates of N-fertilization had ranges of 18 to 55 mg/L in the early 1990's (Littke and Hallberg, 1991).

The second mixing isotope model considers the possibility of evaporation of water in the upper soil zone that could increase the $\delta^{18}O$ of that water. This approach was also able to predict the observed variability at MLS-7. Using this model results in less atmospheric input of nitrate and slightly greater nitrate from fertilizer-N and SOM inputs from the Nursery plots.

<u>Redox zone upgradient, Mixing down gradient:</u> The change in chemistry across the redoxcline below port 18-6 and port 7-2 is very apparent from the chemical data, with the dissolved oxygen levels dropping below 1.0 mg/L (Table 2). The dissolved oxygen values below 1 mg/L are approximate because they are below the accuracy range of the probe. An increase in sulfate, along with a decrease in $\delta^{34}S$ values suggest sulfide minerals are probably responsible for the reducing conditions at depth. These results support the theory that the dissolved oxygen and probably nitrate, are oxidizing sulfide minerals such as pyrite in the aquifer, first suggested by Kelly (1997) . As oxygenated water, or water containing NO_3^-, encounters pyrite the oxygen will be reduced followed by the nitrate according to the following reactions:

$$3.5O_2 + FeS_2 + H_2O \rightarrow 2SO_4^{2-} + Fe^{2+} + 2H^+$$
$$14NO_3^- + 5FeS_2 + 4H^+ \rightarrow 7N_{2(g)} + 10SO_4^{2-} + 5Fe^{2+} + 2H_2O$$

As might be expected from the above reactions, other elements such as iron may increase after dissolved oxygen and NO_3^- is consumed. Iron was detected in water sampled from the bottom ports of MLS-18 where there is no nitrate remaining. However, the relatively low iron concentrations suggest that most of the iron released from the pyrite appears to be immediately reprecipitated. The $\delta^{34}S$ analyses help confirm that there has been significant pyrite oxidation. Pyritic sulfur usually has low $\delta^{34}S$ values and the sulfur isotope analyses show an inverse relationship with sulfate concentration. The greater the sulfate concentration the more negative the $\delta^{34}S$ values, indicating input of isotopically light sulfur. Examination of the unconsolidated sand core samples, collected from the borehole of well 19, identified pyrite grains between 40 to 42 feet. The mineralogical analysis (XRD of random packed samples) showed the presence of pyrite in the sand between a depth of 38 and 39 feet. However, a second XRD analysis, using a micronized sample found no pyrite at this level (Hughes, 2002). Pyrite was not observed in the nine sand samples from intervals above 38 feet from well 19.

If denitrification were occurring in the lower ports at MLS-7 (7-2 and 7-1), where the NO_3^- concentration drops by an order of magnitude, the $\delta^{15}N$ and $\delta^{18}O$ of the dissolved NO_3^- should increase significantly due to the fractionation that occurs with the microbial denitrification process. The lack of a $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ shift in samples collected from the lower ports at MLS-7 would suggest that perhaps instead of denitrification causing the decrease in NO_3^- concentration at this down gradient well, physical mixing of water with low or zero NO_3^- concentration is the main cause of the drop in NO_3^- concentration. The Cl- concentration data also suggests that there is physical mixing of water infiltrating from the Nursery plots with deeper water from the upgradient area. The profile of Cl- and NO_3^- with depth at MLS-7 have identical patterns (figure 7). If the water from the Nursery were infiltrating past the depths of the lower ports at MLS-7, and the nitrate were being reduced at 35 to 40 ft, then the Cl^- concentrations should remain elevated in the deeper ports. However, the Cl^- concentrations in the deeper ports drop to levels observed in the middle depths of upgradient well, MLS-18. In a tracer test Kelly and Ray (1999) observed downward movement of tracer when the irrigation well is turned on. It may be possible that the downward pull of the nitrate plume by the irrigation may be coincidently limited to about the depth of the redoxcline by the strong regional gradient and the hydrodynamics around the well. A layer of much finer sand also occurred at this depth which may help to hamper downward migration and dispersion. Thus, the isotopic and chemical data suggest that the high-nitrate water infiltrating from the Nursery and the adjacent fields forms a wedge-like plume at the top of the water column flowing (figure 2). The low nitrate and high Cl- concentrations at the bottom of MLS-18 would suggest that any nitrate from fields further upgradient than the adjacent fields has been denitrified before reaching the Nursery.

Only one sample, Well 19, collected in the down gradient area showed a positive shift in the $\delta^{15}N$ and $\delta^{18}O$ values, evidence of denitrification at depth. This well is constructed differently and instead of screened at a single point, as with the MLS ports, well-19 is screened over a two-foot span, across the zone in which pyrite was observed. It appears that the water sampled across this zone was subject to denitrification. Samples from the two lower ports of MLS-7, 7-2 and 7-1, were sampled at the same time as Well 19 but did not show any isotopic evidence of denitrification. It is possible that the pyrite abundance is less at MLS-7, even though it is only a few feet from well 19. The distribution of pyrite throughout the site is unknown.

Carbon: The carbon isotopic composition for the samples collected ranged from approximately -7 to -9 ‰. While there was significant variation in the $\delta^{13}C$ values of dissolved inorganic carbon (DIC) at MLS-18 (2 ‰), the $\delta^{13}C$ values at MLS-7 did not change very much (<1 ‰). The most negative $\delta^{13}C$ value at MLS-18 was located near the top of the water table and was probably controlled by the isotopic composition of soil CO_2, which is usually quite negative, -15 to -22 ‰ (Zlotnick, 1992). With depth at MLS18, the $\delta^{13}C$ shifts to more positive values and then back to more negative values. A similar trend was also observed at MLS-7 except the changes were much smaller than that in MLS-18. The shift to more positive $\delta^{13}C$ values would imply the groundwater DIC was equilibrating with solid carbonates in the system. Usually the $\delta^{13}C$ of carbonate minerals is around -2 to +2 ‰. It should also be noted that at both these wells the pH and HCO_3^- fluctuated with depth. The pH increased and then decreased while the HCO_3^- decreased and then increased. These chemical fluctuations agree with what would be expected if the system was basically controlled by carbonate equilibrium.

The shift in $\delta^{13}C$ composition of DIC toward more negative values near the base of the nitrate plume at MLS-18 is indicative of a contribution of an isotopically light carbon into the DIC phase. The isotopically light carbon source may be the oxidation of organic carbon to carbon dioxide in the system. The organic matter may be present either as sedimentary organic carbon or dissolved organic carbon. The dissolved non-volatile organic carbon (NVOC) concentration was low, however, the measured NVOC at MLS-18 has been quite high in the past, ranging from 0.6 to 19.6 mg/L (Kelly and Ray, 1999).

RESULTS - HENRY SITE

The nitrate and chloride concentrations and isotopic values from the Henry site are shown in Table 4. A preliminary look at the data show that the isotopic values from around the Henry Well #3 located next to the Illinois River (MP4 and SP-3) plot in a region between the MLS-18 and MLS-7 samples from the Mason Site (figure 8). One major exception is MP4-10, which shows a significant amount of denitrification. The nitrate concentration at MP4-10 is approximately half the concentration of the deeper ports from this multi-level sampling well. One explanation could be that there is some contribution of highly denitrified water coming along a flow path of induced river water that passes through silty river bottom sediments. The river itself has a much lower nitrate concentration and does show some denitrification, although its nitrate source may be very different than that of the groundwater. The upgradient wells at the water tower and Child Park locations have greater nitrate concentrations and show isotopic values that trend toward nitrate originating from nitrification of fertilizer-N. A mixing model shows that a small concentration of nitrate (3 mg/L) with a $\delta^{15}N$ typical of septic waste (10-15‰) could explain the observed nitrate concentration and isotopic composition in the city well. This is supported by a large increase in chlorides that would be typical of septic wastes. Alternatively, the increase in chlorides could be due to road salt that would have a more concentrated use in the town compared to the rural areas.

Table 4. Chemical and isotopic analyses from the Henry Site

Well-Port	Depth (ft)	Cl^- (mg/L) 9/19/01	NO_3^--N (mg/L) 9/19/01	$\delta^{18}O_{NO3-}$ (SMOW) 9/19/01	$\delta^{15}N_{NO3-}$ (air) 9/19/01
Water Tower ob well	95	15.6	12.2	4.3	3.1
Child Park ob well	65	22.1	14.5	5.7	4.6
SP-3	60	31.7	10.2	3.9	5.8
Henry #3	50	61.0	8.1	5.0	5.9
MP4-10	10	153	5.0	11.8	17.7
MP4-15	15	75.2	9.1	3.6	6.2
MP4-20	20	54.5	9.6	3.9	5.9
MP4-30	30	58.8	9.2	3.6	5.9
MP4-35	35	73.0	8.4	3.3	6.4
MP4-40	40	77.5	8.3	3.7	6.2
MP4-45	45	73.0	9.5	3.9	5.7
MP4-50	50	82.6	9.1	3.6	6.8
Illinois River	1	101	2.1	6.8	9.4

RESULTS - SHELBYVILLE SITE

The nitrate and chloride concentrations and isotopic values from the Shelbyville site are shown in Table 5. The $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- for the Shelbyville samples follow a denitrification trend that appears to originate from an area that would be characteristic of mineralized fertilizer nitrogen (figure 9). Those samples located closest to the river tend to exhibit the greatest degree of denitrification. The $\delta^{18}O$ values of the nitrate from this site are generally greater than most of the values from the other two sites. The greater $\delta^{18}O_{NO3}$ values may indicate that there is a greater amount of synthetic NO_3^- fertilizer used on the surrounding fields at Shelbyville compared to the other sites. Alternatively, it is possible that there is greater evaporation of soil water in the upper soil zone at the Shelbyville site which would lead to greater $\delta^{18}O$ values for the NO_3^- because, as discussed earlier, approximately two thirds of the oxygen in NO_3^- is derived from surrounding water during the nitrification process.

Table 5. Chemical and isotopic analyses from the Shelbyville Site

Well / Location sampled	Date Sampled	Depth (ft)	Cl^- (mg/L) 9/19/01	NO_3^--N (mg/L) 9/19/01	$\delta^{18}O_{NO3-}$ (SMOW) 9/19/01	$\delta^{15}N_{NO3-}$ (air) 9/19/01
Well 6	4/15/02	63	18.7	15.5	6.5	1.6
Well 5	4/15/02	61	21.7	3.72	9	7.2
Well 1	4/15/02	60	28.1	2.44	--	--
Well 3	4/15/02	54	22.9	4.35	8.4	5.8
Well 8	4/15/02	60	27.1	0.72	--	--
Kaskaskia R	4/15/02	-	29.9	10.1	9.4	4.8
Well 4	6/06/02	59	25.1	2.99	9.2	5.8
Well 5	6/06/02	61	20.7	11.4	8.4	2
Well 1	6/06/02	60	27.3	2.77	7.2	2.8
Well 8	6/06/02	60	27.3	1.96	8.8	6.2
Kaskaskia R	6/06/02	-	12.2	5.86	9.6	5.3

CONCLUSIONS

The isotopic results for all three sites sampled are typical of nitrate that primarily originates from agricultural row-crop land use practices. A correlation between the $\delta^{18}O$ and $\delta^{15}N$ values, showing a relationship of approximately 1:2, typical of microbial denitrification was observed at all three sites. Several of the samples collected near the water table had $\delta^{18}O_{NO3}$ values that suggest the initial NO_3^- formed by the microbial nitrification process obtains approximately two thirds of the NO_3^--oxygen from surrounding water and one third from atmospheric oxygen. However, there was significant variation of the $\delta^{18}O_{NO3}$ values from site to site and with depth. Besides the obvious effects of denitrification which increases both $\delta^{15}N$ and $\delta^{18}O$ values, some of the variation of $\delta^{18}O_{NO3}$ could be a result of several factors including variable applications of reduced-N fertilizer versus NO_3^-N fertilizer, variable cultivation practices, variable NO_3^- inputs from precipitation, differences in soil characteristics and plant density (which could affect infiltration of NO_3^-) and variable degrees of evaporation occurring in the upper portions of the soil zone from one site to another.

Considering the sites on an individual basis, the isotopic results helped determine whether changes in NO_3^- concentration were due to physical mixing or denitrification processes. For example, at the Mason County Tree Nursery site, the rapid drop in NO_3^- concentration at the down gradient MLS-7 well appears to be due more to a lack of physical mixing than geochemical denitrification processes. Differences in isotopic characteristics between upgradient wells and those from monitoring wells at Henry, Illinois suggested that there may be a contribution of nitrate from the septic systems that were once prevalent within the town. However, additional work should be completed at the Henry site to verify this possibility. The results from the Shelbyville municipal well field exhibit consistently greater $\delta^{18}O_{NO3}$ values than typically observed at the other two sites. The higher $\delta^{18}O$ values for the Shelbyville site implies there was either more synthetic NO_3-N fertilizer applied to the surrounding fields or perhaps there is greater evaporation of soil water from the fields compared to the other two sites.

Using both $\delta^{15}N$ and $\delta^{18}O$ analyses definitely help to differentiate between geochemical processes such as denitrification and mixing of isotopically distinct sources. Although additional questions have risen due to this research, the isotopic characteristics observed at the three sites investigated for this study will help us to better evaluate other sites in Illinois where nitrate concentration is a problem.

REFERENCES

Amberger, A. and H.-L. Schmidt, 1987. Natürliche Isotopengehalte von Nitrat als Indikatoren für dessen Herkunft, Geochimica et Cosmochimica Acta, Vol. 51, pp. 2699-2705.

Aravena, R., M.L. Evans, and J.A. Cherry, 1993. Stable Isotopes of Oxygen and Nitrogen in Source Identification of Nitrate from Septic Systems. Ground Water, vol. 31, pp. 180-186.

Aravena, R and W. D. Robertson, 1998, Use of multiple isotope tracers to evaluate denitrification in ground water: study of nitrate from a large-flux septic system plume. Ground Water, v. 36, pg 975-982.

Böttcher, J, O. Strebel, S. Voerkelius, and H.L Schmidt, 1990. Using Isostope Fractionation of Nitrate-Nitrogen and Nitrate-Oxygen for Evaluation of Microbial Denitrification in a Sandy Aquifer, Journal of Hydrology, vol. 114, pp. 413-424.

Bremner, J.M. and M.A. Tabatabai, 1973. Nitrogen-15 Enrichment of Soils and Soil-Derived Nitrate. J. Environmental Quality, vol. 2, pp. 363-365.

Clark, I. and P. Fritz, 1997. Environmental Isotopes in Hydrogeology, Lewis Publishers, New York, NY, 328 p.

Dealy, M.T., 1995. Investigation of Nitrate-Nitrogen Concentrations in the Equus Beds Aquifer, Southeast Reno County, Kansas. U.S. Geological Survey, Water Quality Invest. 95-01, 56 pp.

Erlich, H.L., 1996. Geomicrobiology, Marcel Dekker, Inc., New York, 719 pp.

Farnsworth and Wiley, 1996. Wellhead Protection Program. Report submitted to the City of Shelbyville, 59 pp.

Feigin, A., G. Shearer, D. H. Kohl and B. Commoner, 1974, The amount and nirogen-15 content of nitrate in soil profiles from two centroal Illinois fields in a corn-soybean rotation. Soil Sci. Coc. Amer. Proc., 38, pp. 465-471.

Gormly, J.R. and R.F. Spalding, 1979. Sources and Concentrations of Nitrate-Nitrogen in Ground Water of the Central Platte Region, Nebraska. Ground Water, vol 17, pp. 291-301.

Hackley, K. C., C. L. Liu and D. Trainor, 1999. Isotopic identification of the source of methane in subsurface sediments of an area surrounded by waste disposal facilities. Applied Geochemistry, v. 14, p. 119-131.

Heaton, T.H.E., 1986. Isotopic Studies of Nitrogen Pollution in the Hydrosphere and Atmos-phere: A Review. Chemical Geology, vol. 59, pp. 87-102.

Hubner, H., 1986. Isotope effects of nitrogen in the soil and biosphere, *in* Fritz, P. and Fontes, J. eds., Handbook of Environmental Isotope Geochemistry, v. 2: Amsterdam, Elsevier, p. 361-425.

Hwang, Hue-Hwa; Liu, C.-L.; and Hackley, K.C., 1999. Method improvement for oxygen isotope analysis in nitrates, 1999 North-Central GSA meeting, April 22-23, Champaign, Illinois.

Kelly, W.R., 1997. Heterogeneities in Ground-Water Geochemistry in a Sand Aquifer beneath and Irrigated Field. Journal of Hydrology vol. 198, pp. 154-176.

Kelly, W.R., and C. Ray, 1999. Impact of Irrigation on the Dynamics of Nitrate Movement in a Shallow Sand Aquifer. Illinois State Water Survey Research Report 128, 58 pp.

Kendall, C. 1998. Tracing nitrogen sources and cycling in catchments. In: Kendall, C., and J.J. McDonnell (Eds.), Isotope Tracers in Catchment Hydrology. Elsevier Science B.V., Amsterdam, pp. 517-576, Chap. 16.

Kohl, D.H., G.B. Shearer, and B. Commoner, 1971. Fertilizer Nitrogen: Contribution to nitrate in Surface Waters in a Corn Belt Watershed. Science, vol. 174, pp. 1331-1334.

Kohl, D.H., G.B. Shearer, and B. Commoner, 1973. Variation of ^{15}N in Corn and Soil Fol-lowing Application of Fertilizer Nitrogen. Soil Science Society of America Procedings, vol. 37, pp. 888-892.

Komor, S.C. and H.W. Anderson Jr., 1993. Nitrogen Isotopes as Indicators of Nitrate Sources in Minnesota Sand-Plains Aquifers. Ground Water, vol. 31, pp. 260-270.

Kreitler, C.W. and L.A. Browning, 1983. Nitrogen-Isotope Analysis of Groundwater Nitrate in Carbonate Aquifers: Natural Sources Versus Human Pollution. J. Hydrology, vol. 61, pp. 285-301.

Mariotti, A., 1984. Utilisation des Variations Naturelles d'Abondance Isotopique en ^{15}N pour tracer l'Origine des pollutions des Aquifers par les Nitrates. In: Isotope Hydrology 1983, I.A. E.A., Vienna, pp. 605-633.

Mariotti, A., A. Landreau, and B. Simon, 1988. ^{15}N Isotope Biogeochemistry and Natural Denitrification Process in Groundwater: Application to the Chalk aquifer of Northern France. Geochimica et Cosmochimica Acta, vol. 52, pp. 1869-1878.

McMahon, P.B., and J.K. Böhlke, 1996. Denitrification and mixing in a stream-aquifer system: effects on nitrate loading to surface water, Journal of Hydrology, 186, no. 1-4: 105-128.

Meints, V.W., L.V. Boone, and L.T. Kurtz, 1975. Natural ^{15}N Abundance in Soil, Leaves, and Grain as Influenced by Long Term Additions of Fertilizer N at Several Rates. J. Environmental Quality, vol. 4, pp. 486-490.

Mengis, M., S. L. Schiff, M. Harris, m. C. English, R. Aravena, R. J. Elgood, and A. MacLean, 1999. Multiple geochemical and isotopic approaches for assessing ground water NO_3^- elimination in a riparian zone. Ground Water, vo. 37, No. 3, pg. 448-457.

Soong, D.T., C. Ray, G.S Roadcap, and D. Borah, 1998. Flood Induced Loading of Agricultural Chemicals to Public Water Supply Wells in Selected Reaches of the Illinois River, Proceedings: Illinois Groundwater Consortium, Southern Illinois University, Carbondale, IL.

Sackett, W. M. and W. S. Moore, 1966. Isotopic variations of dissolved inorganic carbon. *Chemical Geology*, v. 1, p. 323-328.

Silva, S.R., Kendall, C., Wilkison, D.H., Chang, C.C.Y., Zeigler, A.C., and Avanzino, R.. 1998. Collection and analysis of nitrate from fresh water for nitrogen and oxygen isotopes. (J. of Hydrology, accepted).

Veda, A. and H. R. Krouse, 1986. Direct conversion of sulphide and sulphate minerals to SO2 for isotope analysis. Geochemical Journal, v. 20, p. 209-212.

Vogel, J.C., A.S. Talma, and T.H.E. Heaton, 1981. Gaseous Nitrogen as Evidence for Denitrification in Groundwater. J. Hydrology, vol. 50, pp. 191-200.

Wassenaar, L.I., 1995. Evaluation of the Origin and Fate of Nitrate in the Abbotsford Aquifer using the Isotopes ^{15}N and ^{18}O in NO_3^-, Applied Geochemistry, vol. 10, pp. 391-405.

Zlotnick, A. R., 1992. The geochemistry and carbon isotope composition of soil gases at the Sycamore Farm Experimental Agricultural Research Station, Madison Township, Montgomery County, Ohio. Thesis (M.S.), Wright State University. Dayton OH.

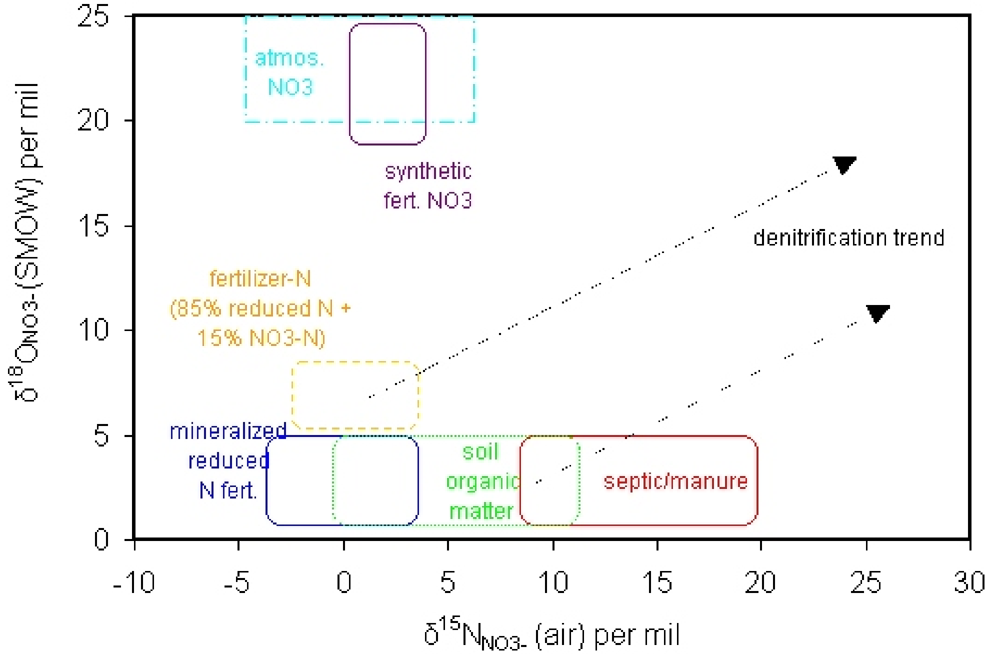

Figure 1. Crossplot of typical $\delta^{18}O_{NO3-}$ and $\delta^{15}N_{NO3-}$ values for different nitrate sources and the trend of isotopic compositions obtained due to denitrification processes (modified from Clark and Fritz, 1997).

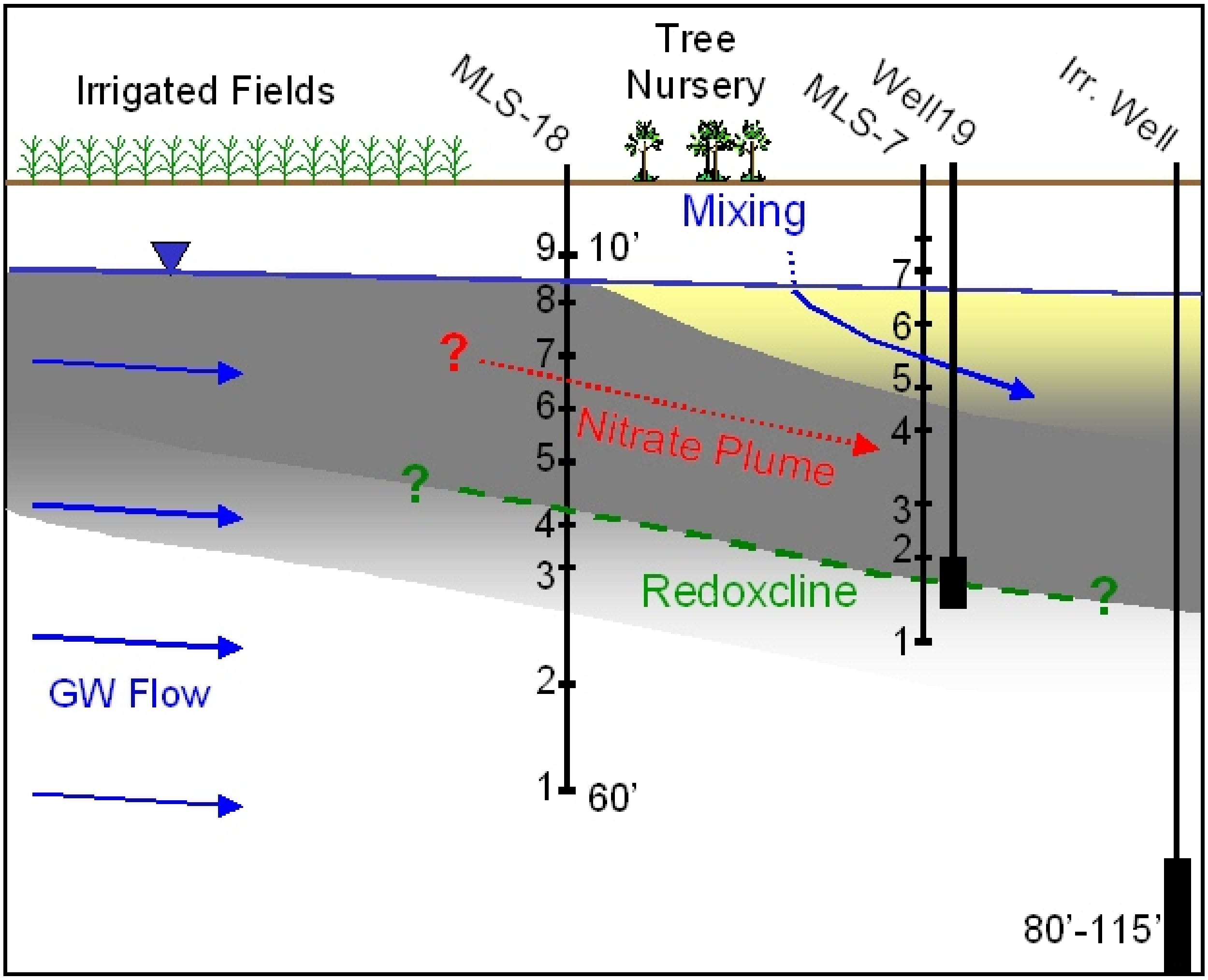

Figure 2. Cross-sectional view of Mason Co. site showing the conceptual model of the nitrate plume originating from fertilized fields (modified from Kelly, 1997) . The multi-level port numbers are indicated next to the well.

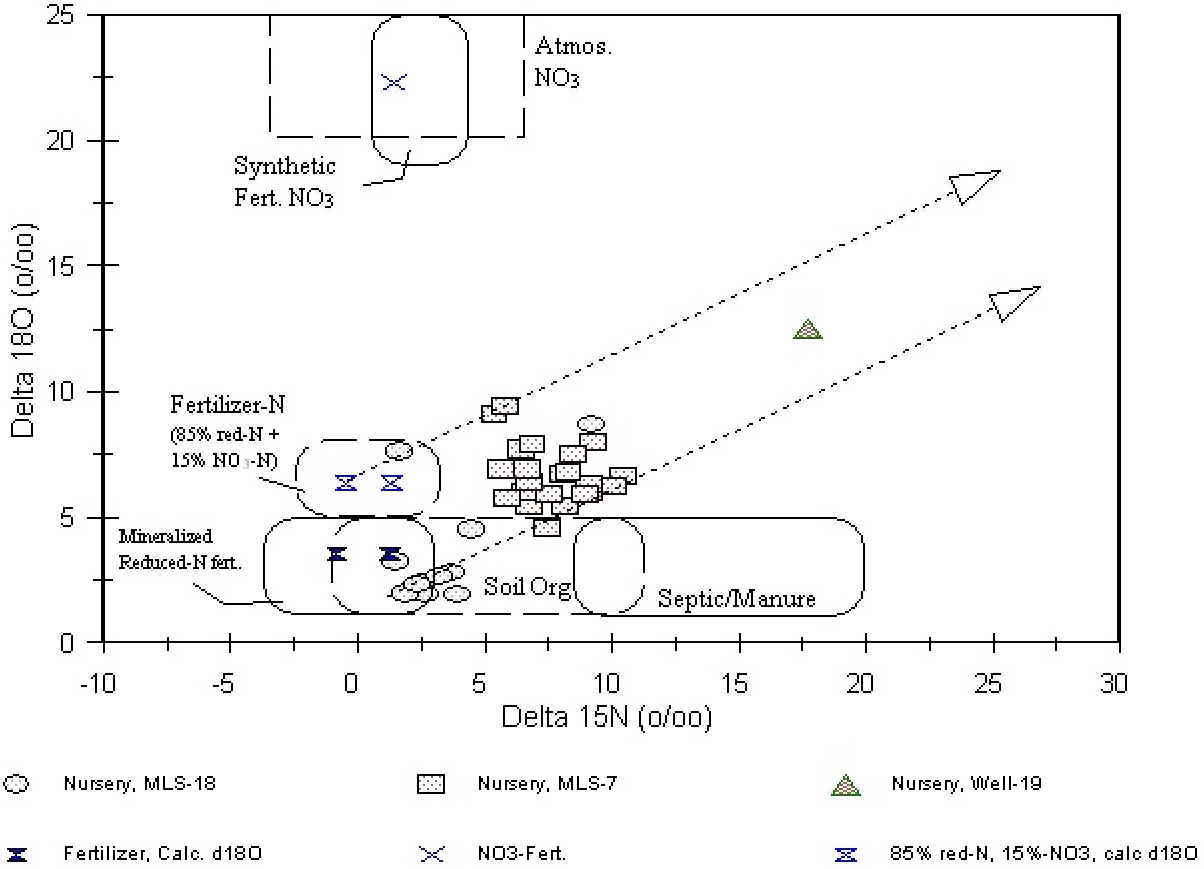

Figure 3. Plot of δ^{15}N and δ^{18}O for nitrate in water samples from the Mason County Tree Nursery Site. The dashed arrows show the direction of isotopic values for NO$_3^-$ remaining in the groundwater due to typical microbial denitrification processes.

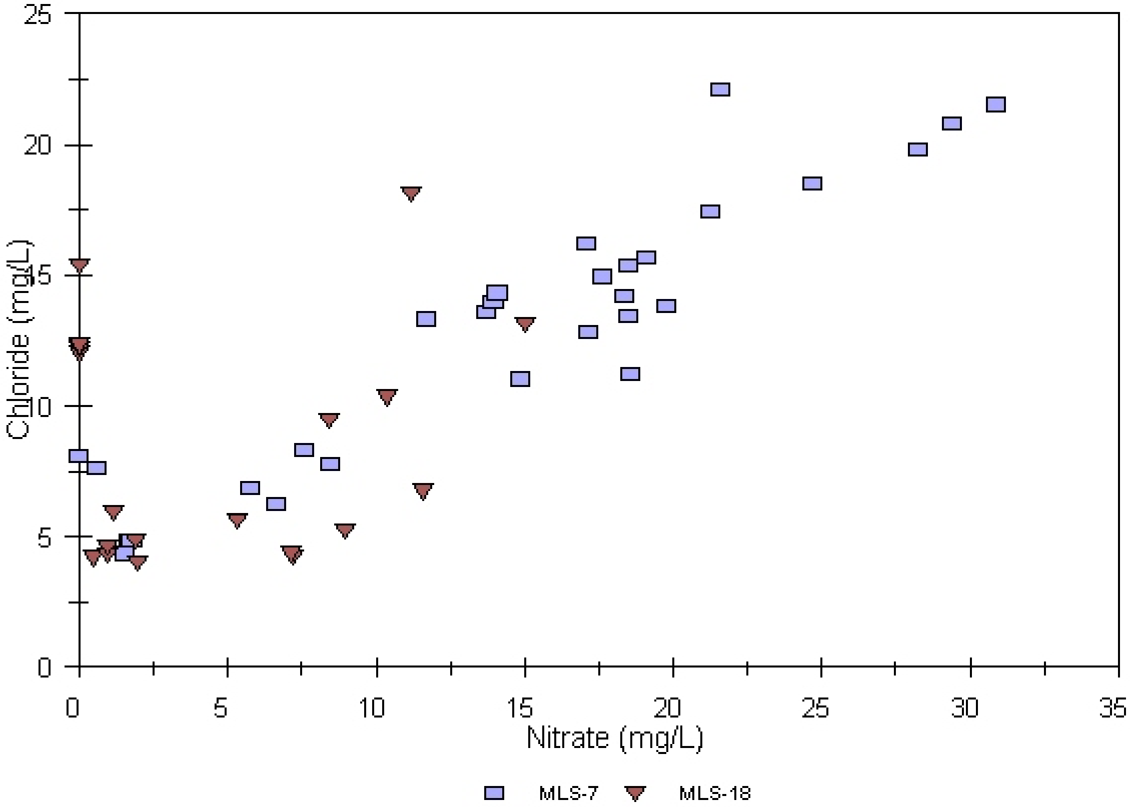

Figure 4. Chloride versus nitrate concentration at the Mason County Tree Nursery site.

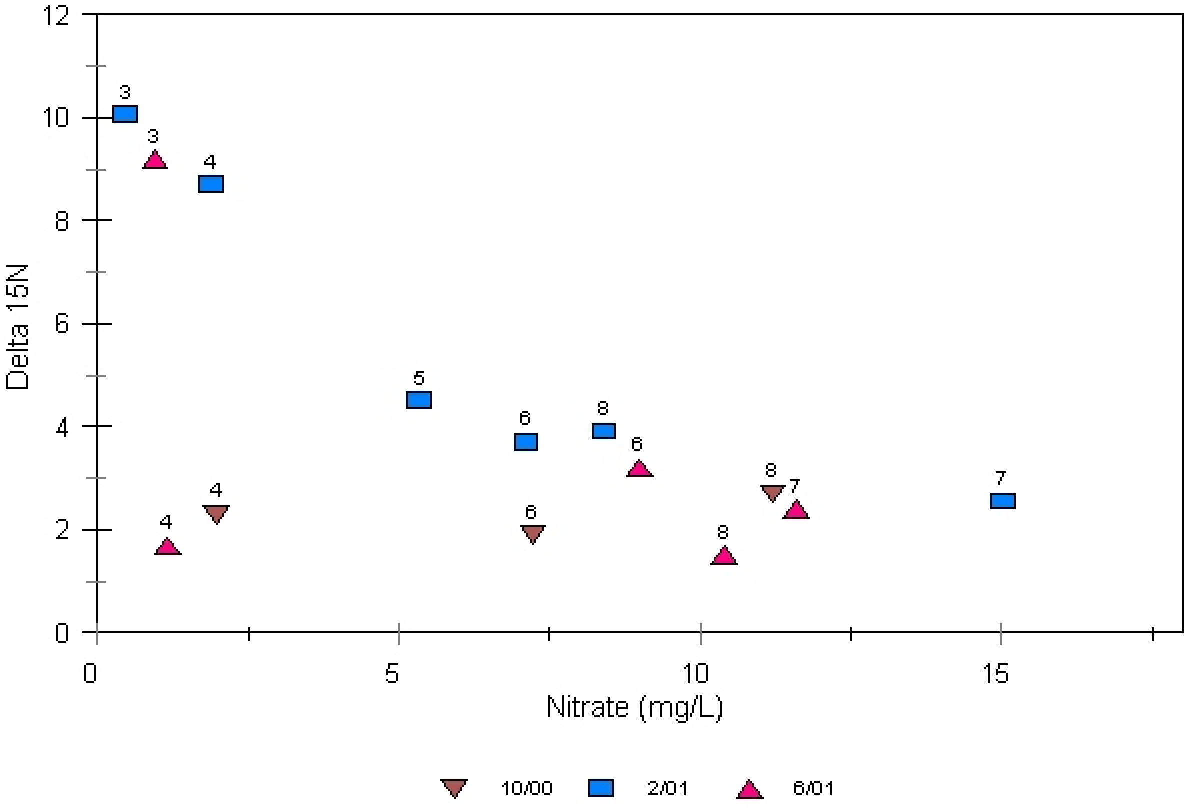

Figure 5. Plot of $\delta^{15}N$ versus NO_3^- concentration for MLS-18 at the Nursery Site.

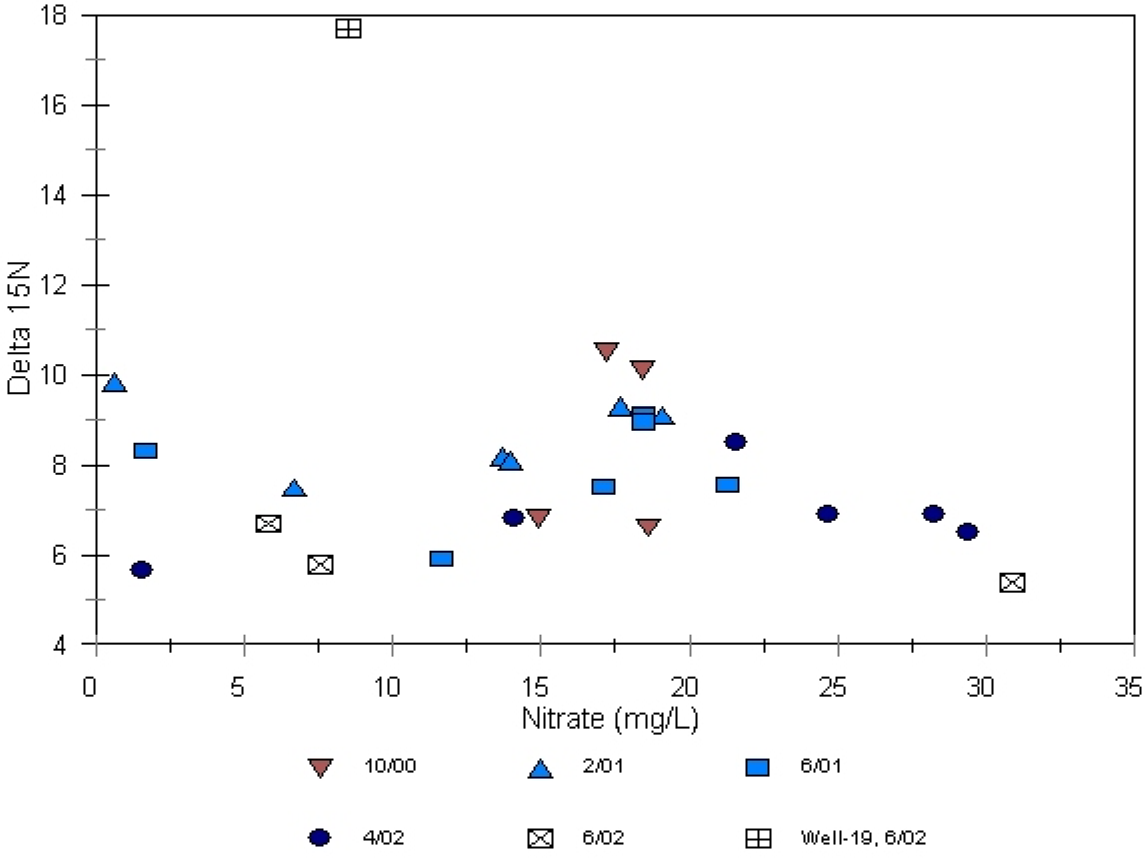

Figure 6. Plot of $\delta^{15}N$ versus NO_3^- concentration for MLS-7 and well-19 at the Nursery Site.

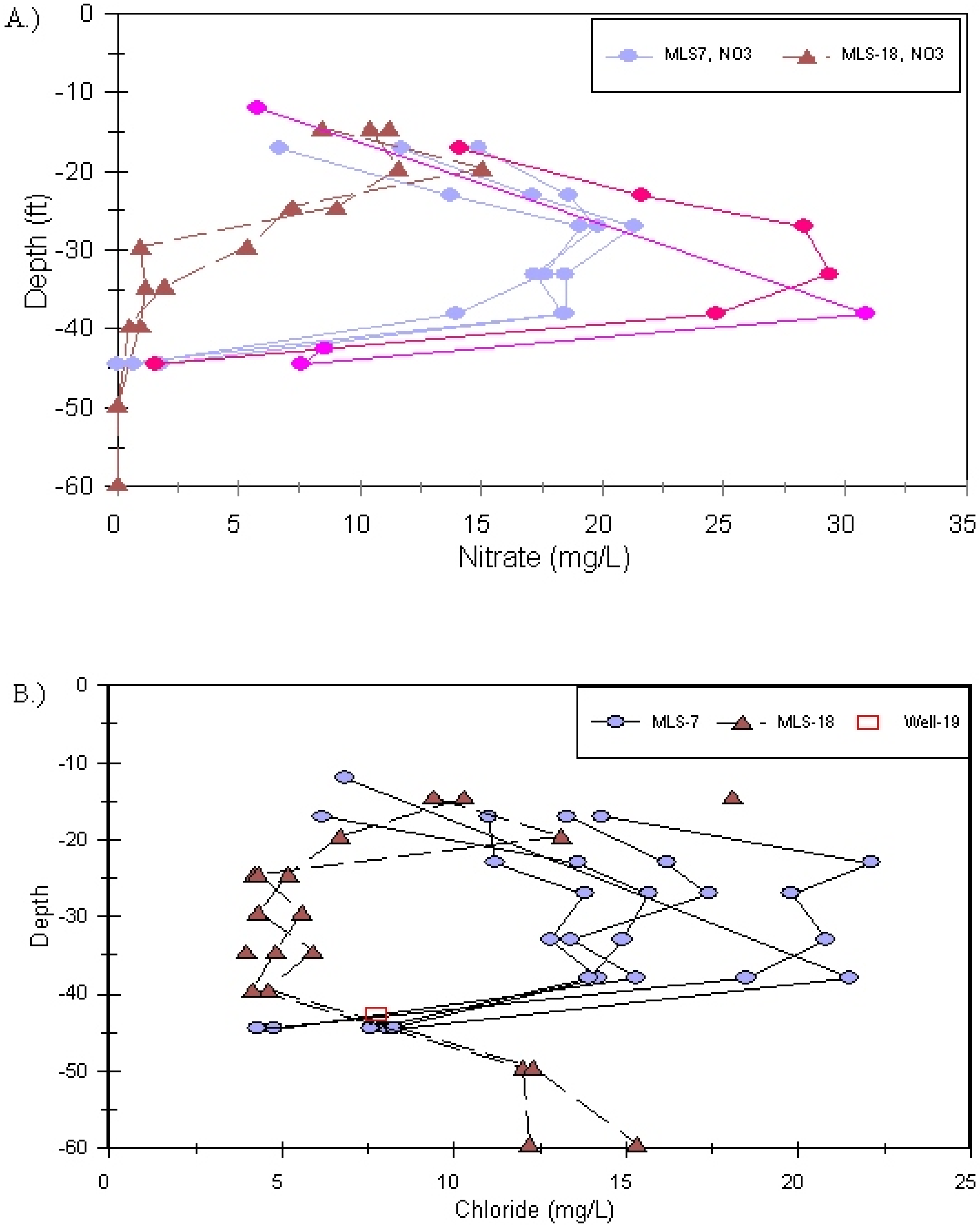

Figure 7. Nitrate (A) and Chloride (B) plotted versus depth for Nursery Site.

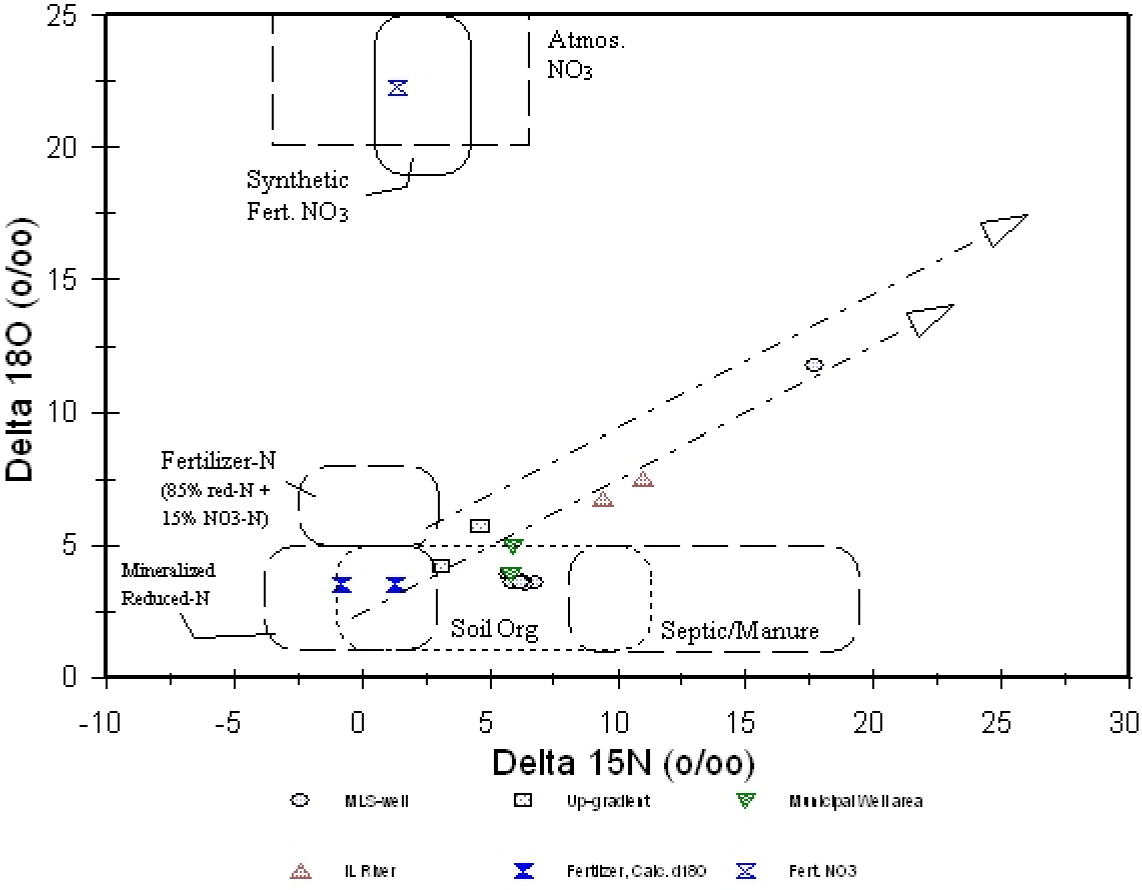

Figure 8. Plot of δ^{15}N and δ^{18}O for nitrate in water samples from the Marshall County site in Henry, Illinois.

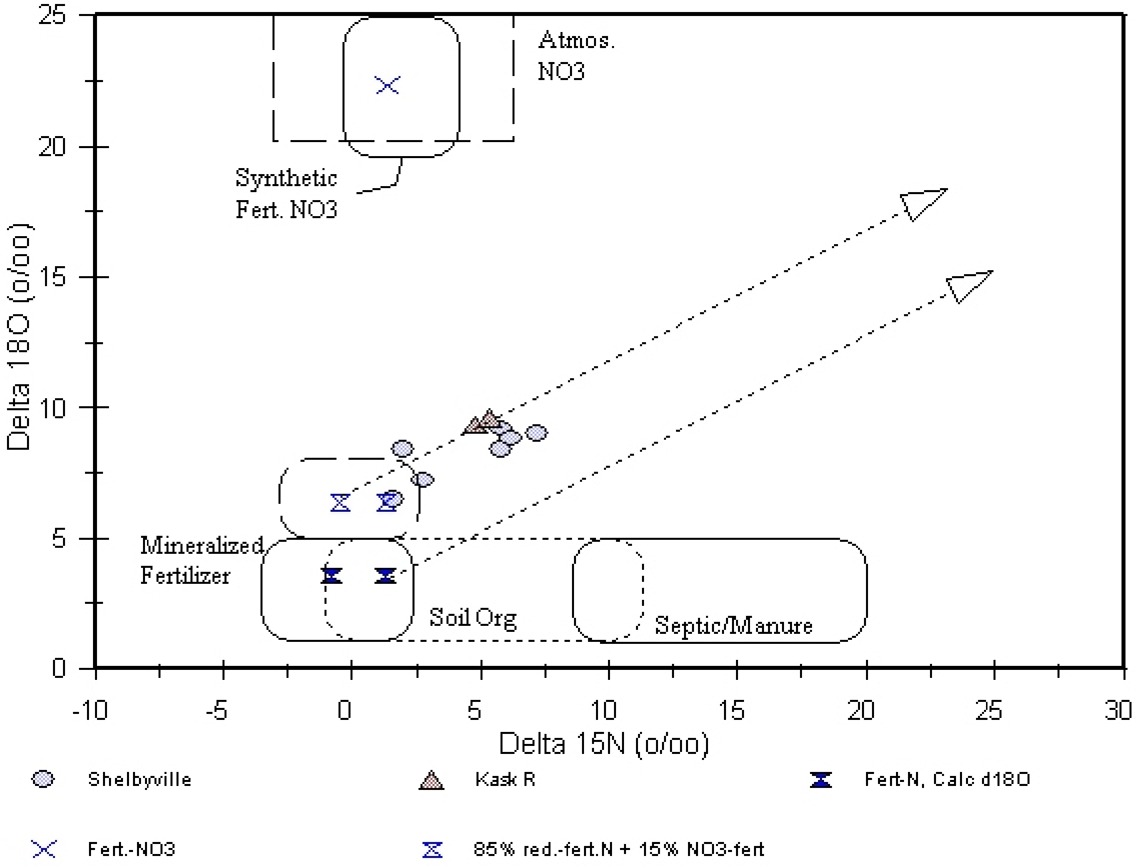

Figure 9. Plot of δ^{15}N and δ^{18}O for nitrate in water samples from the Shelbyville site.