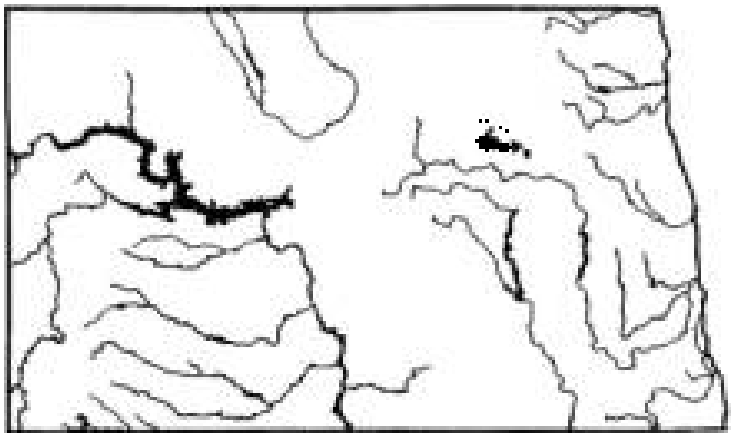

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February 2008

North Dakota Water Resources Research Institute
North Dakota State University, Fargo, North Dakota

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by

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February 2008

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ABSTRACT

The Karlsruhe Aquifer of north-central North Dakota has experienced considerable nitrate contamination sparking an investigation by the North Dakota State Water Commission. Certain portions of the aquifer have experienced nitrate-N concentrations of up to seven times the drinking water standard. Although contamination is evident it is possible the NO_3^- is naturally being eliminated by denitrification, whereby bacteria reduce NO_3^- to nitrogen gas. Evidence of denitrification may be illustrated by accelerated reduction of NO_3^- relative to the conservative tracer Cl^- coupled with an increase in the heavy isotopes of ^{15}N and ^{18}O within the NO_3^- ion. Such an increase is not evident in other nitrate attenuation processes. This dual isotope method may even be extended to identify the source of the contamination based upon isotopic signatures of specific nitrate sources.

Periodic sampling was conducted from the fall of 2003 to the spring of 2004 to monitor the relationship between NO_3^- concentrations and isotopic fractionation. All samples were tested for NO_3^- and Cl^- while select samples were tested for nitrate-$\delta^{15}N$ and nitrate-$\delta^{18}O$. An inverse relationship between nitrate concentrations and isotopic fractionation and a direct linear trend between ^{15}N and ^{18}O enrichment shows that denitrification did occur at

some locations. Additionally the fractionation trends of ^{15}N and ^{18}O indicate that NO_3^- in the aquifer is predominantly derived from the oxidation of ammonia fertilizer.

DESCRIPTION OF THE WATER PROBLEM ADDRESSED

Contamination of groundwater is of increasing concern due to the world's expanding population and the resulting depletion of water resources. Nitrate (NO_3^-) is the most common form of contaminant due to growing anthropogenic sources (Freeze and Cherry, 1979). The US EPA drinking water standard is 10.0 mg/L nitrate-N. At high concentrations NO_3^- may interfere with the O_2-carrying capacity of hemoglobin in infants, a disorder known as methemoglobinemia.

Denitrification is a natural process in which bacteria reduce NO_3^- to N_2 through oxidation of organic or inorganic compounds that act as electron donors. The end product, nitrogen gas (N_2), is no longer a contaminant and has a triple bond that resists conversion back to nitrate (Korom, 1992). This process may result in NO_3^- elimination to levels that are below detection. The four general requirements for denitrification are (Korom, 1992; Firestone, 1982):

1) N oxides (NO_3^-, NO_2^-, NO, and N_2O) as electron acceptors.

2) The presence of bacteria possessing the metabolic capacity.

3) Suitable electron donors.

4) Anaerobic conditions or restricted O_2 availability.

Inherent to denitrification is the resulting increase in heavy isotopes of oxygen (^{18}O) and nitrogen (^{15}N). As denitrification proceeds the undenitrified nitrate becomes enriched in these stable isotopes. Such an increase is direct evidence of denitrification.

The purpose of this study is to use the relationship between decreasing NO_3^- concentrations and isotopic enrichment as evidence of denitrification within the Karlsruhe Aquifer of north-central North Dakota (Figure 1). Additionally this study used the heavy isotopes of NO_3^- as an indicator of the source of nitrate contamination. The methodology consisted of periodic sampling of groundwater from five multi-port sampling wells. All samples were analyzed for NO_3^--N and Cl^- contents. Chloride served as a conservative tracer for nitrate to measure dilution. Some samples were analyzed for ^{18}O and ^{15}N in NO_3^- .

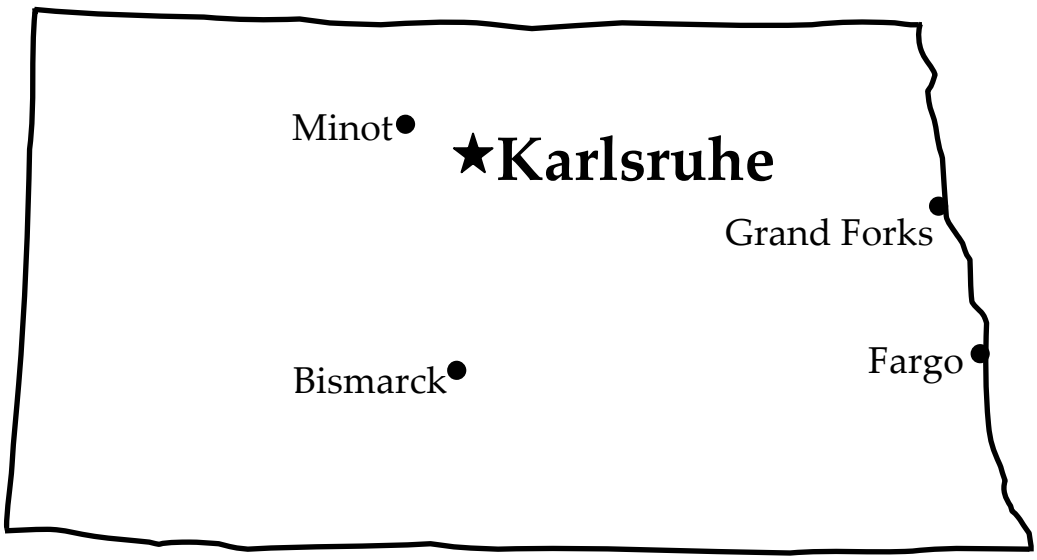


Figure 1. Location of Karlsruhe, North Dakota.

BACKGROUND

Kinetic Isotopic Fractionation Of Nitrate

Kinetic isotope effects on oxygen and nitrogen in NO_3^- have become important indicators of the processes that affect NO_3^- in groundwater. Stable isotopes may impart measurable fractionation compared to lighter isotopes during physical and chemical reactions (Clark and Fritz, 1997). During denitrification bacteria prefer to attack bonds of the lighter isotopes because they are easier to break. As denitrification proceeds, corresponding increases in the ratios of ^{15}N to ^{14}N and ^{18}O to ^{16}O in the remaining nitrate result. Other processes that decrease nitrate concentrations, such as dilution, do not cause such fractionation. Even in the case of assimilation of the nitrate ion where the N-O bond is broken, isotopic enrichment is not demonstrated (Mariotti et al., 1982). Thus, noting an increase in these ratios provides evidence of denitrification.

Isotopic ratios are measured relative to a known standard. Standards for nitrate isotopes are atmospheric nitrogen (^{15}N) and Standard Mean Ocean Water (^{18}O). Isotopic ratios are reported in delta (δ) notation as parts per thousand (‰), also referred to as permil. Fractionation is calculated as:

$$\delta^{15}N = 1000 \times (R_{sample} - R_{standard}) / (R_{standard}) \quad (1)$$

where $\delta^{15}N$ is the isotopic variation between the sample and standard, R_{sample} is the isotopic ratio ($^{15}N/^{14}N$) of the sample, and $R_{standard}$ is the isotopic ratio of the standard. Thus a sample with $\delta^{15}N$ of 10‰ has 1% more ^{15}N than the standard. A similar relationship exists for ^{18}O.

Under idealized conditions, such as in in-situ mesocosms (ISMs), denitrification may result in a linear relationship between $\delta^{15}N$ and natural log of NO_3^--N concentration, as shown in Figure 2.

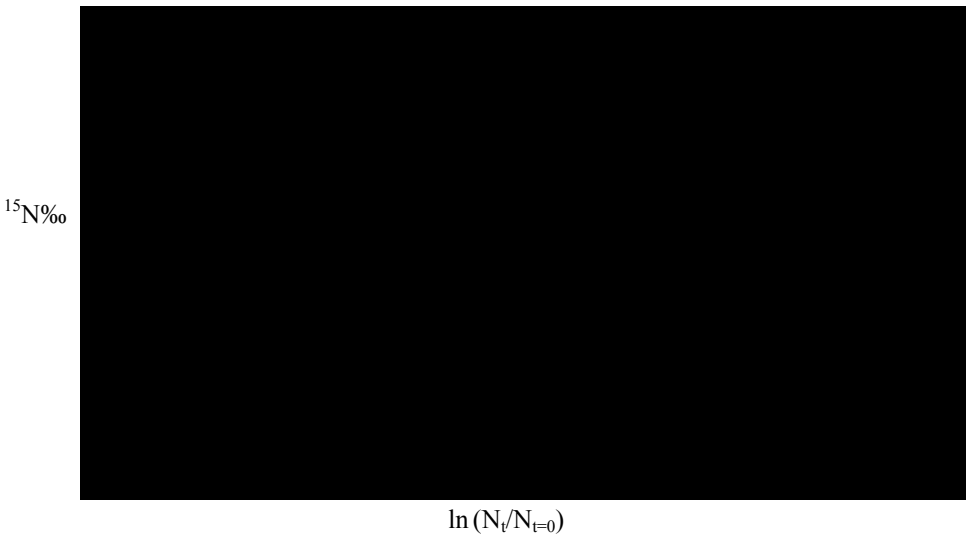


Figure 2. $\delta^{15}N$ versus the natural logarithm of the nitrate concentrations remaining in the in situ mesocosms during the first tracer test at the Larimore site (Adapted from Schlag, 1999; Korom et al. 2005).

In this experiment a known amount of nitrate with a specific initial $\delta^{15}N$ was placed into the ISM at the beginning of the test and observed in isolation from other nitrate sources. Contrarily however, nitrate entering an aquifer may vary in its source, concentration, and time of introduction into the aquifer. Without the confinement of an ISM, groundwater may have multiple NO_3^- sources with various isotopic signatures, as shown in Figure 3. Thus for field conditions, plots of $\delta^{15}N$ versus NO_3^--N concentrations may not produce good correlations. Such is shown in Figure 4 from a site beneath a field fertilized with hog manure in the Assiniboine Delta Aquifer of Manitoba. It is difficult to see a relationship between NO_3^--N and $\delta^{15}N$. Additionally it is difficult to determine from what material the NO_3^- originated.

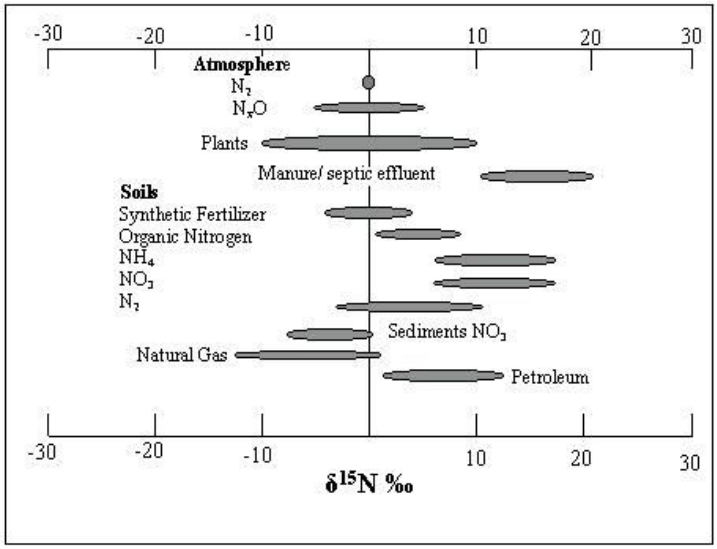


Figure 3. Isotopic signatures for ^{15}N of select nitrogen bearing materials (Adapted from Clark and Fritz, 1997).

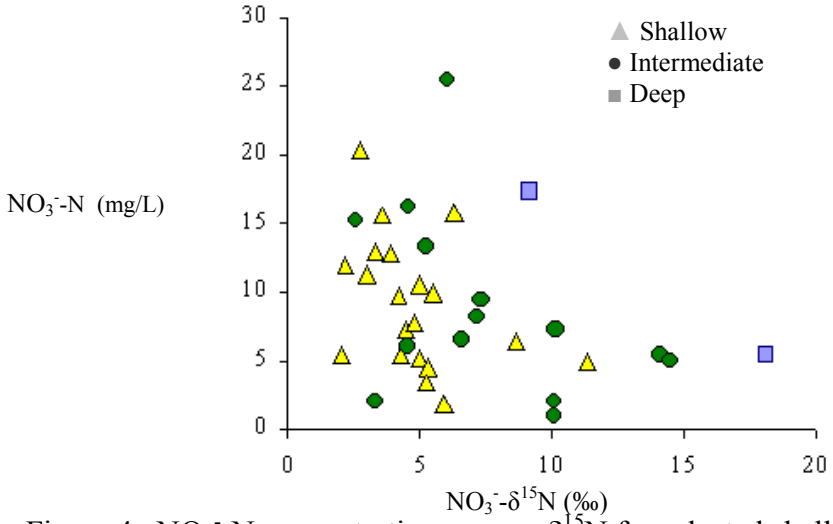


Figure 4. NO_3^--N concentrations versus $\delta^{15}N$ for selected shallow, intermediate, and deep groundwater samples (Adapted from Phipps and Betcher, 2003).

Analyzing both $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- may enhance the ability to identify denitrification and the NO_3^- source. Laboratory experiments have demonstrated that microbial nitrification derives two-thirds of its oxygen from local groundwater and one-third

from the atmosphere (Andersson and Hooper, 1983; Hollocher, 1984). Therefore the initial $\delta^{18}O$ of NO_3^- derived from the oxidation of NH_4^+ from all sources can be estimated with the following equation.

$$\text{Initial } \delta^{18}O = \tfrac{2}{3}(\text{local groundwater}^{18}O) + \tfrac{1}{3}(\text{local atmospheric}^{18}O) \qquad (2)$$

This constrains the initial ^{18}O and allows identification of the NO_3^- source based on the initial ^{15}N. Consider Figure 5.

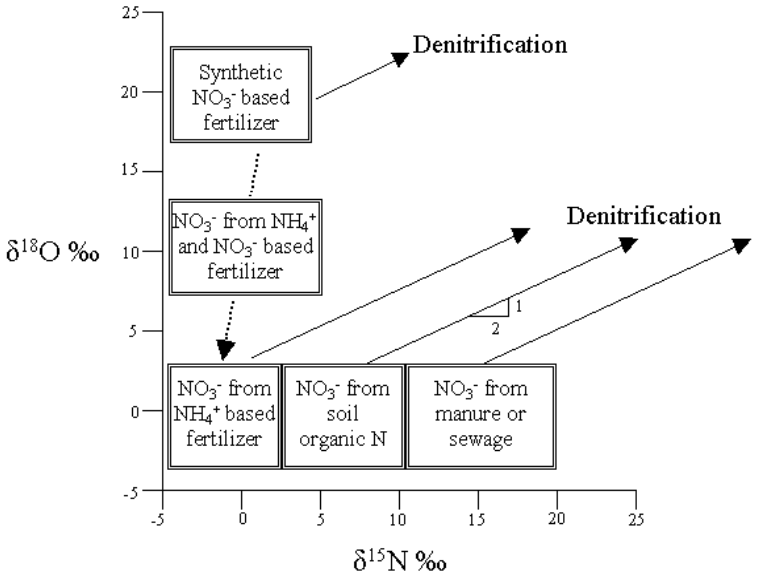


Figure 5. Denitrification trends based upon NO_3^- source (Adapted from Mengis et. al. 2001).

Nitrification of ammonia fertilizer by Equation 2 (dashed line of Figure 5) will produce NO_3^- depleted in both ^{15}N and ^{18}O. If this NO_3^- is denitrified both isotopes will follow a fractionation trend similar to that as shown in Figure 5. Specifically the remaining nitrate will become enriched in both isotopes with ^{15}N enriching twice as much as ^{18}O. NO_3^- derived

from manure or soil organic nitrogen will have the same range of ^{18}O and will follow a similar fractionation trend, although the initial ^{15}N would be more enriched.

Now consider the same data shown in Figure 4 with the addition of ^{18}O data, as shown on Figure 6.

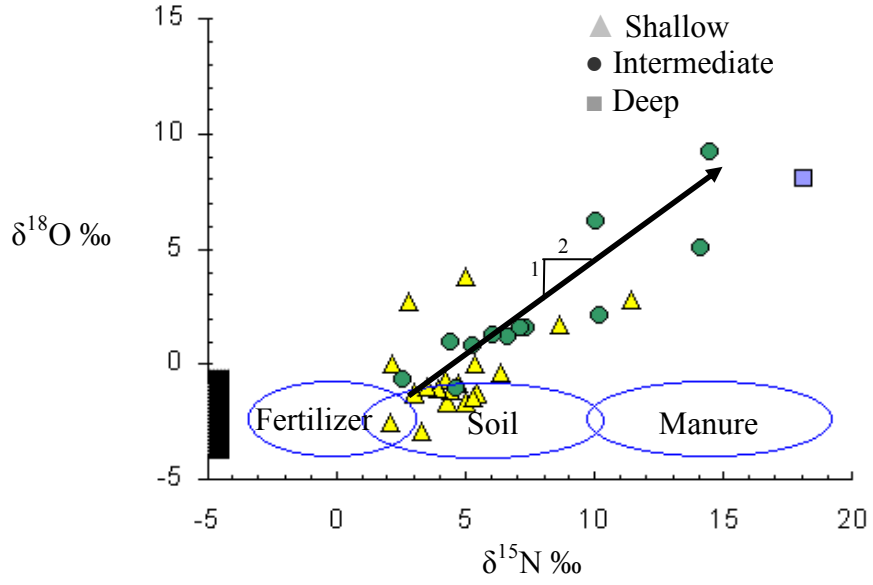


Figure 6. Plot of nitrate-$\delta^{18}O$ versus nitrate-$\delta^{15}N$ showing enrichment attributed to denitrification (Adapted from Phipps and Betcher, 2003).

The resulting trend was attributed to denitrification because of the direct linear relationship and the 1:2 slope between the increased enrichment of both isotopes. By Equation 2 the ^{18}O of local groundwater (-14.1 ± 1.5‰) and atmosphere (+23.5‰) produced an expected range of nitrate-^{18}O of -3.4 to 0.1‰ (black shaded box of Figure 6). This combined with ^{15}N data indicated that NO_3^- originated primarily from organic soil nitrogen (Phipps and Betcher, 2003).

Previous Work On The Karlsruhe Aquifer

The Karlsruhe Aquifer of north-central North Dakota occupies a surficial outwash plain in south-central McHenry County (Randich, 1981). The aquifer has a surface area of about 9300 hectares (23,000 acres) (Figure 7) and is underlain by the New Rockford Aquifer. Two distinct periods of glacial activity resulted in deposition of glacial till and sand and gravel outwash to form both aquifers. The aquifers are mostly separated although they are hydraulically connected in some discrete sections (Wanek, 2002).

Relatively high NO_3^--N concentrations within the aquifer sparked an investigation by the North Dakota State Water Commission (NDSWC). The NDSWC began an extensive program in the fall of 2001 by monitoring nitrate levels within thirty-eight nests of wells. Total nitrate-N load, nitrate-N load density, and the potential mixed concentration index (PMCI) are three indices used to evaluate the extent of contamination. Total nitrate load and nitrate-N load density quantify the total mass of nitrate in the aquifer and the total mass per unit area. PMCI is the total nitrate-N load mixed throughout the entire saturated thickness of the aquifers. These variables were used to assess the economic loss of nitrate to the aquifer as well as the potential nitrate contamination if the groundwater were fully mixed throughout its saturated thickness (Schuh et al., 2002).

Initial results during the fall of 2001 indicated that about 1.8 million kilograms (4 million lb) of nitrate-N were present within the aquifer while roughly 1300 hectares (3200 acres) had a PMCI above the EPA drinking water standard of 10.0 mg/L Testing was expanded in the following sampling sessions to include sixty-five well nests and multi-port samplers. Total nitrate-N load decreased by 0.18 million kilograms (0.4 million lb) in the spring of 2002, but then increased to 1.9 million kilograms (4.2 million lb) over the growing

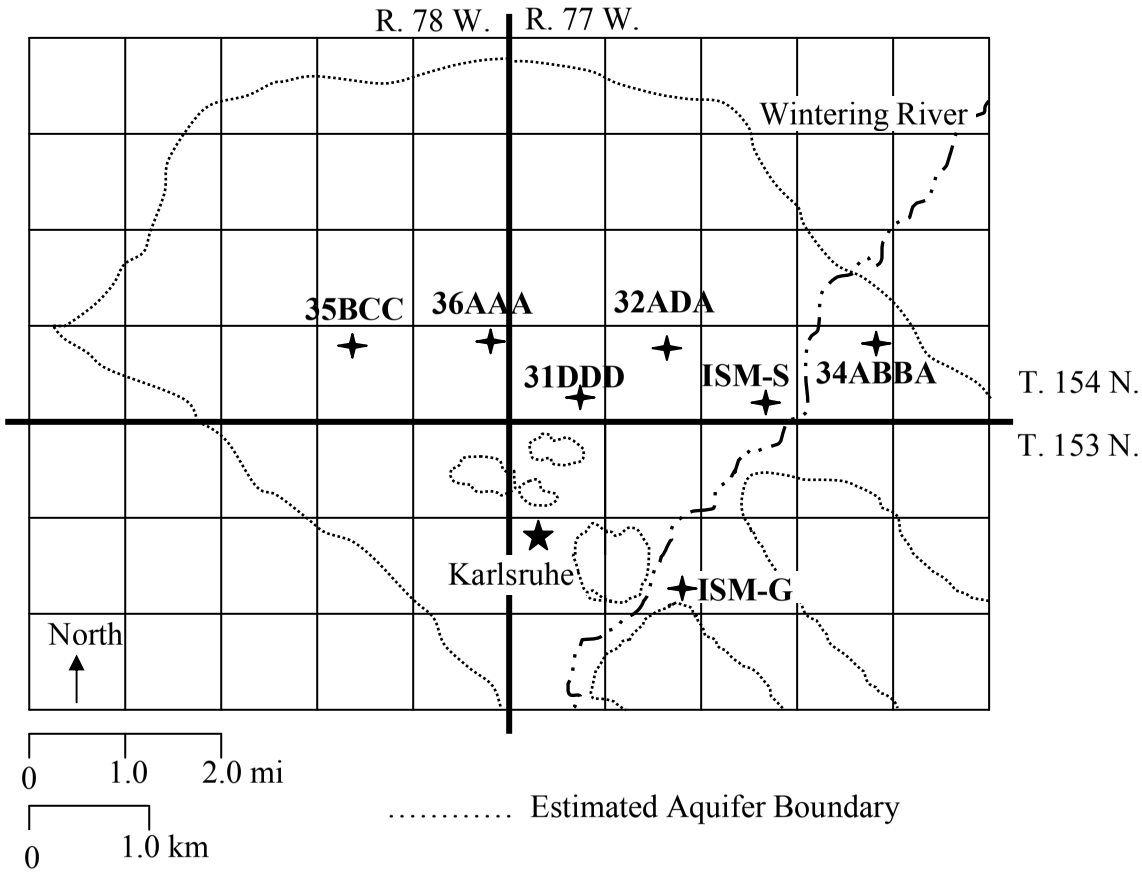


Figure 7. Arial extent of the Karlsruhe Aquifer showing locations of multilevel sampling wells and ISMs (Modified from Wanek, 2002).

season into the fall of 2002. Additionally over 2400 hectares (6000 acres) had a PMCI above 10.0 mg/L, an increase of almost 80% from the previous year. By the fall of 2003 total nitrate load decreased to about 1.5 million kilograms (3.2 million lb) while area with a PMCI > 10.0 mg/L decreased to about 2000 total hectares (5000 acres). Overall an improved nitrate status was exhibited in 78.5% of the aquifer. This provides optimism that better management is being applied in the use of nitrogen-based fertilizers. It is also possible that lower nitrate concentrations in the Karlsruhe Aquifer are a result of denitrification (Schuh et al., 2004).

The NDSWC found a casual relationship between nitrate-N concentrations and irrigation. Generally the highest nitrate-N loads are on or near irrigated sections and tend to decrease with distance from the source. Quarter sections with irrigation permits had the highest nitrate load density with an average of 400 kg/ha (362 lb/acre). Quarter sections 2.4 km (1.5 mi) away or more from a source had the lowest density with an average of 45 kg/ha (41 lb/acre). A positive relationship between nitrate and TDS ($R^2 = 0.71$) is evident in groundwater samples with nitrate-N greater than 4.0 mg/L. Increased leaching as a result of accelerated percolation of water through the vadose zone may be the cause. Additionally, 75% of wells correlated by nitrate and TDS were within irrigated quarter sections. Therefore it is apparent that fertilizer application coupled with irrigation is contributing to high nitrate concentrations within the Karlsruhe Aquifer (Schuh 2002).

Warne (2004) tested denitrification rates with in-situ mesocosms (ISMs) at two locations within the Karlsruhe Aquifer (Figure 7). NO_3^-, bromide (Br^-), and ^{15}N fractionation were monitored over 273 days. In one ISM (ISM-S) nitrate-N decreased by 21.8 mg/L beyond what could be explained dilution while $\delta^{15}N$ increased from 0.63 to 10.05‰. This

indicated denitrification occurred within this ISM. The other ISM (ISM-G) showed little evidence of denitrification (Warne 2004).

METHODS

On October 21, 2003, thirty-one groundwater samples were withdrawn with a peristaltic pump from five multilevel sampling wells adapted from a design by Pickens et al. (1978). Individual wells were located between T. 154 N., R. 77 W. Section 34 and T., 154 N., R. 78 W. Section 35 (Figure 7). Wells were chosen based upon previous water quality data provided by the NDSWC. These wells exhibited high nitrate loading in the past and appeared to have the potential for denitrification. Additionally wells were selected over a large part of the aquifer. Sampling depths ranged from 1.5 to 10.4 meters (5.1 to 34.1 ft.) below the ground surface. All wells were initially purged to ensure removal of stagnant groundwater. Groundwater samples were passed through 0.45-micron filters and stored in plastic 0.50- and 1.0-L bottles. Additional samples were collected on December 22, 2003 and March 22, 2004. Fewer samples were taken in the later sessions based on initial analytical results. Samples are named based upon the well from which it was taken and the specific sampling port. For example sample 31DDD4 was taken from Well 15407731DDD, port 4.

All samples were transported to the University of North Dakota Environmental Analytical Research Laboratory (EARL) for analysis. Each was tested for NO_3^--N and Cl^- concentrations with a DIONEX® AS50 Autosampler and DX 120 Ion Chromatograph. Samples were then sterilized with a saturated HgCl solution (1 drop of solution per 100 mL of sample) to stop biological activity and refrigerated until isotopic analysis. On

April 7, 2004, forty-six samples were sent to the Environmental Isotope Laboratory at the University of Waterloo for analysis by mass spectrometry. Of the forty-six samples all were analyzed for nitrate-$\delta^{15}N$, twenty for nitrate-$\delta^{18}O$, and four for $\delta^{2}H$ and $\delta^{18}O$ in water.

Identification of the nitrate source was based upon $\delta^{15}N$ of possible sources as presented in Figure 3 and the initial $\delta^{18}O$ as computed by Equation 2. Initial fractionation of each isotope is considered to be indicative of nitrate derived from the nitrification of ammonia from a specific source as shown in Figure 5.

Ratios of Cl^-/NO_3^- were assumed to be relatively constant for groundwater in the Karlsruhe Aquifer. This allowed relative concentration profiles to serve as an initial indicator of denitrification. All relative concentrations were compared to NO_3^- and Cl^- concentrations from the October 21st sampling session. Sampling ports 32ADA3 and 34ABBA7 are not included in the relative profiles because concentrations at these sites fell below detection limits or were not accessible during sampling. Depth profiles show NO_3^--N concentrations relative to depth beneath the ground surface. The number of points included in each plot depended on the accessibility of ports. Depth categories were based upon the following: shallow, 0 to 1.8 meters (0-6.0 ft); intermediate, 1.8 to 4.6 meters (6.0-15.0 ft); deep, greater than 4.6 meters (15.0 ft).

The Rayleigh Equation was applied to ^{15}N, ^{18}O, and ln (NO_3^--N) plots through linear regression to show evidence of denitrification. This is calculated as:

$$\delta_s = \delta_{s_o} + \varepsilon \ln(C/C_0) \tag{3}$$

where δ_s is enrichment at time t, δ_{s_o} is initial enrichment prior to fractionation, and C/C_0 is normalized NO_3^- concentration at time t. The isotopic enrichment factor (ε) is the slope of a plot of ln (C/C_0) versus δ_s and allows for comparison of denitrifying environments. The initial

NO_3^--N (C_0) cannot be determined for this study; thus the natural log (ln) of NO_3^--N (C) was used. This will produce the same enrichment factor (ε) as Equation 3.

Evidence of denitrification was based upon an accelerated reduction of NO_3^--N relative to Cl^- and a reduction of NO_3^--N with depth plus one or more of the following:

(1) An inverse relationship between ^{15}N fractionation and natural log (ln) of NO_3^--N concentrations.

(2) A direct linear relationship between increased fractionation of ^{15}N and ^{18}O.

(3) Samples enriched in ^{15}N and ^{18}O beyond what could be explained from possible NO_3^--N sources.

RESULTS AND DISCUSSION

Results are reported individually for each well including relative concentration profiles, nitrate and isotopic variation with depth, ^{15}N versus ln (NO_3^--N), and ^{15}N versus ^{18}O. Three of the five wells showed strong evidence of denitrification, one showed moderate evidence of denitrification, and one showed no evidence of denitrification. Data for all wells were then combined as a cumulative interpretation of results. This includes ^{15}N versus ln (NO_3^--N), ^{18}O versus ln (NO_3^--N), and ^{15}N versus ^{18}O. Cumulative plots are indicative of denitrification. Table 1 of Appendix A in Spencer (2005) provides all raw data including sample name, sample depth, nitrate-N concentration, chloride concentration, and isotopic ratios.

Well 31DDD

Relative concentration profiles for all ports show only slight differences between chloride and nitrate (Figures 8, 9, and 10); any change in Cl^- is mirrored by NO_3^-. Depth

profiles show reduction of NO_3^--N with depth (Figure 11), although this is accompanied by minimal fractionation of ^{15}N with a peak enrichment of only 2.3‰ (Figure 12). Additionally there is poor correlation between ^{15}N and ln (NO_3^--N) (Figure 13) and ^{15}N versus ^{18}O (Figure 14). The cumulative data make it unlikely that denitrification took place within this section of the aquifer.

Well 32ADA

Relative profiles for all ports show considerable reduction of NO_3^--N over Cl^- (Figures 15, 16, and 17). Reduction of NO_3^--N with depth is evident for each sampling date (Figure 18). Shallow samples show the highest NO_3^--N values, near 30 mg/L, at 1.9 m and fall below 2.0 mg/L at 3.7 m. Additionally ^{15}N consistently increases with depth and shows a peak enrichment of 38.1‰ (Figure 19). Such enrichment is well beyond the range of any possible NO_3^--N source (Figure 3) and is likely the result of denitrification. A reasonable correlation is evident between ^{15}N and ln (NO_3^--N) (Figure 20) and an exceptionally strong linear relationship is evident between enrichment of ^{15}N and ^{18}O (Figure 21). Together these trends provide compelling evidence of denitrification.

Well 34ABBA

Relative concentrations of NO_3^--N and Cl^- in ports 3 and 5 show little fluctuation during winter followed by a distinct separation in spring (Figures 22 and 23). In port 7 the NO_3^--N concentrations decreased below detection (< 0.1 mg/L) by spring and were therefore too low for comparison with Cl^- and were too low for isotopic analysis. Denitrification may have caused these observations. Evidence of denitrification with depth is observed below 3.0 m of depth (Figures 24 and 25). This is most evident during the fall with NO_3^--N falling to 6.9 mg/L and $\delta^{15}N$ reaching 34.8‰. A relatively weak positive correlation is evident

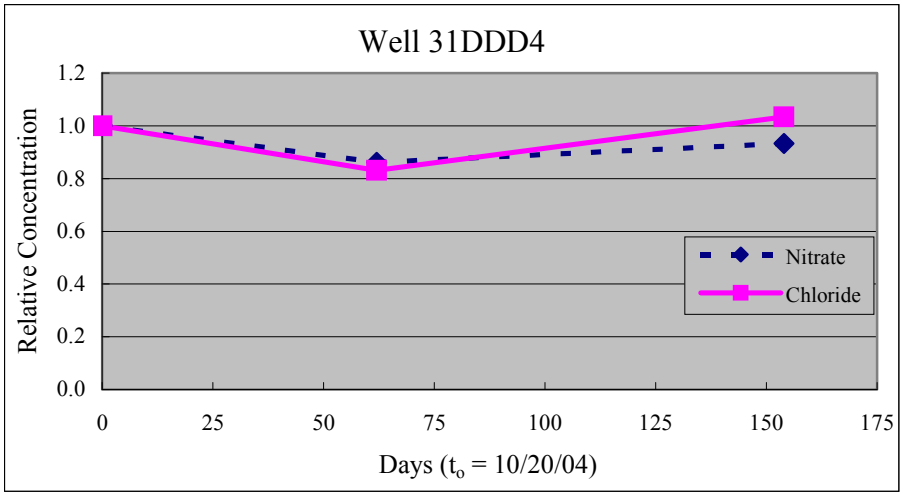


Figure 8. Relative concentration profile for Well 15407731DDD, Port 4.

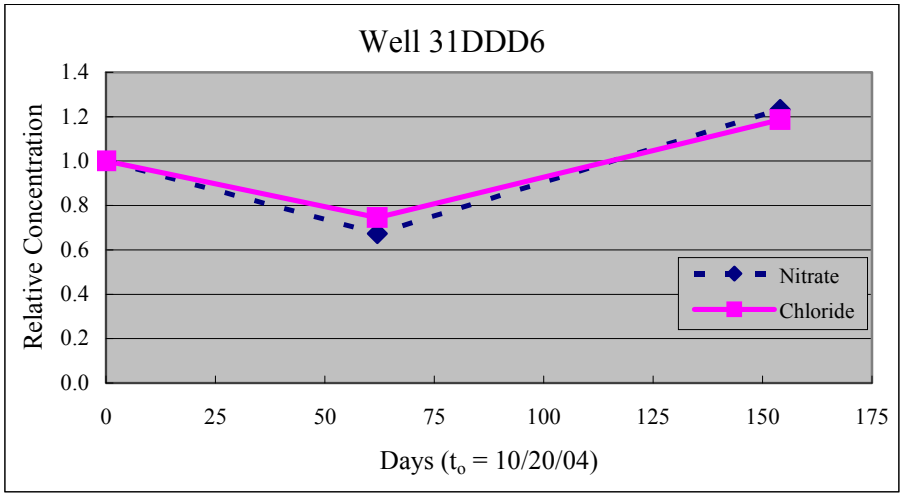


Figure 9. Relative concentration profile for Well 15407731DDD, Port 6.

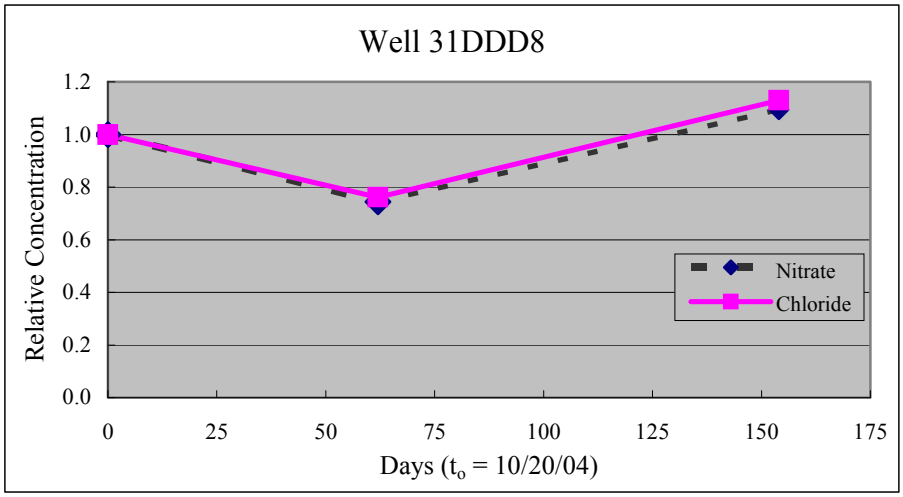


Figure 10. Relative concentration profile for Well 15407731DDD, Port 8.

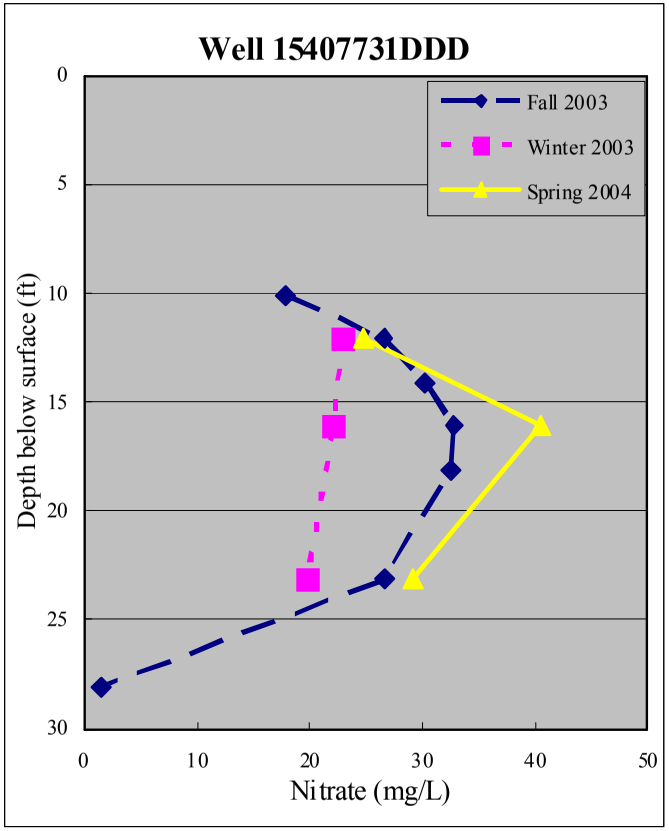


Figure 11. Depth profiles of nitrate-N concentration for Well 15407731DDD.

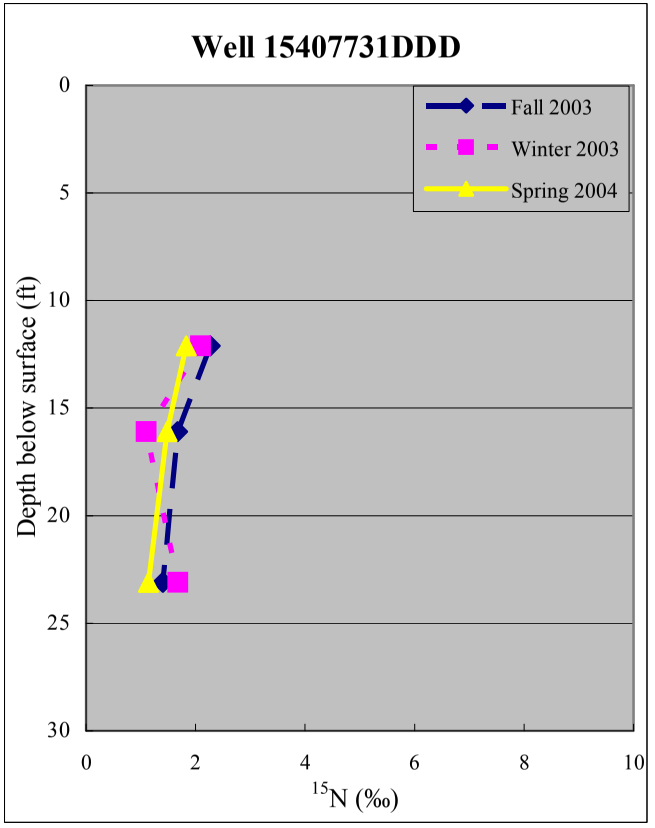


Figure 12. Depth profile of ^{15}N fractionation for Well 15407731DDD.

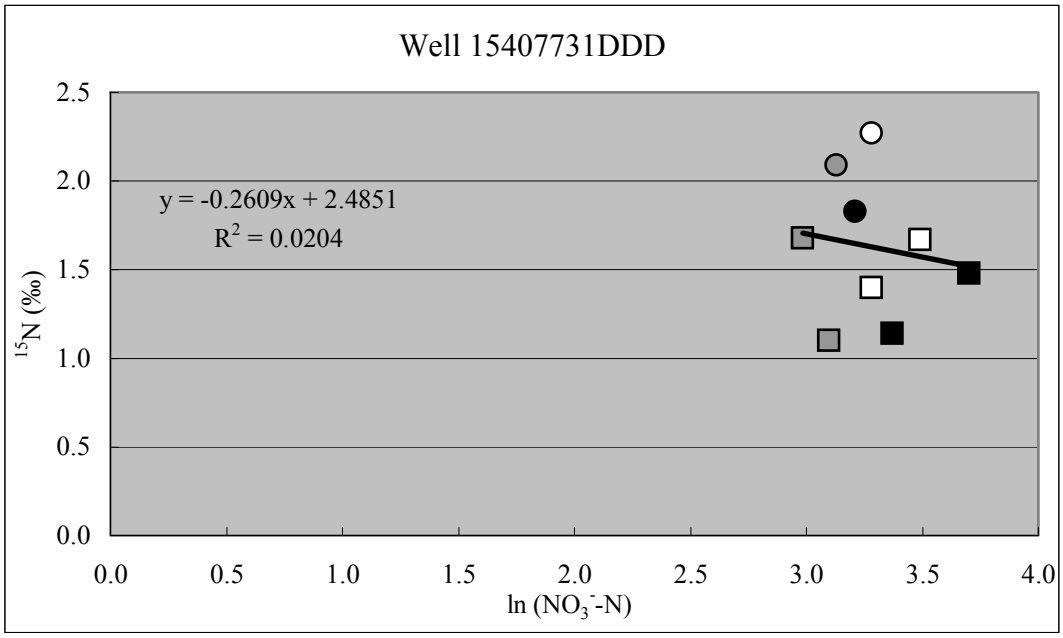
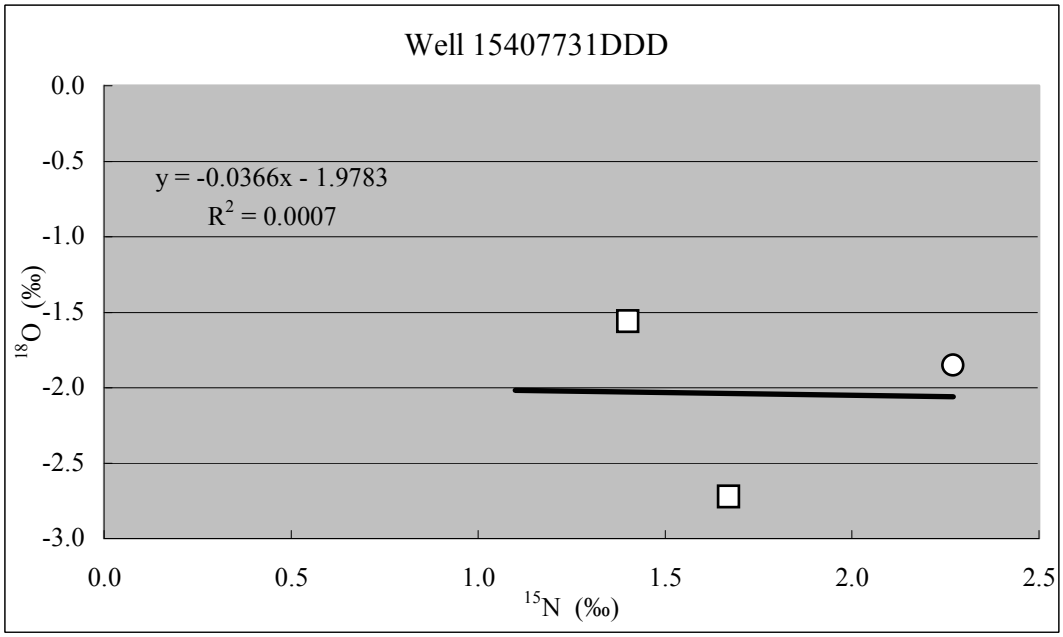


Figure 13. ^{15}N fractionation versus ln (NO_3^--N) for Well 15407731DDD.



▲ Shallow White fill = Fall
● Intermediate Grey fill = Winter
■ Deep Black fill = Spring

Figure 14. ^{15}N versus ^{18}O fractionation for Well 15407731DDD.

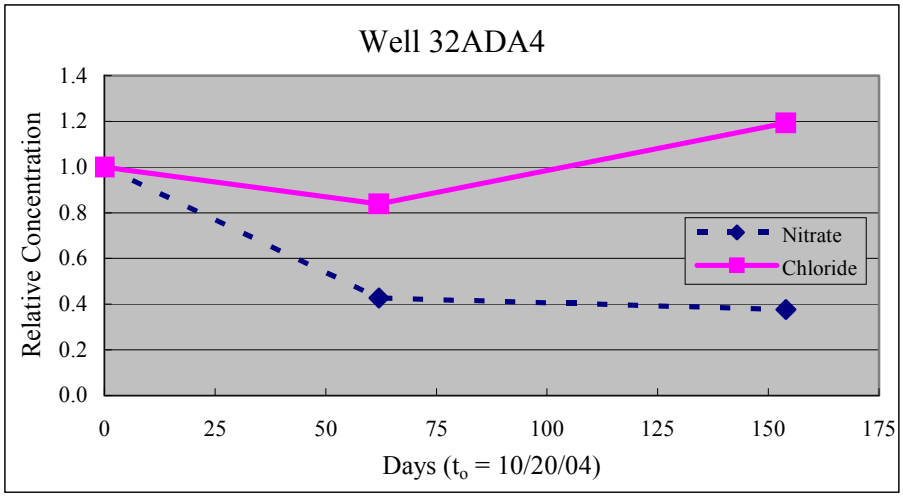


Figure 15. Relative concentration profile for Well 15407732ADA, Port 4.

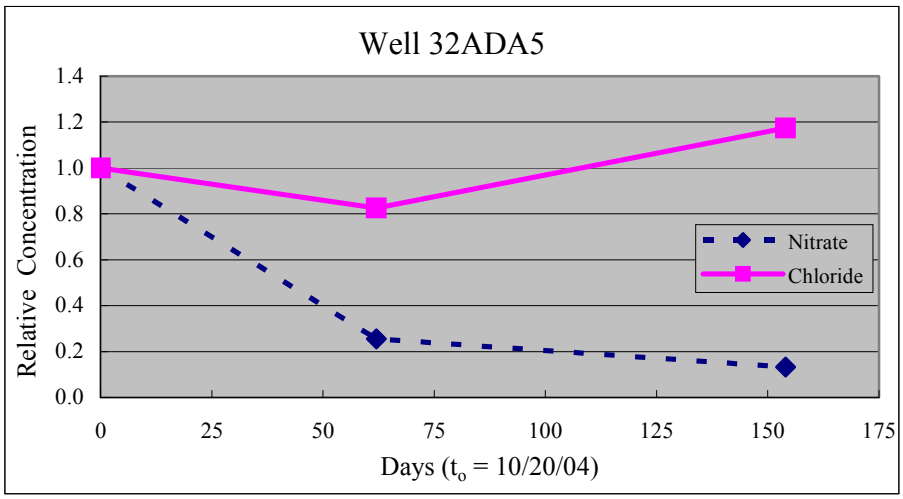


Figure 16. Relative concentration profile for Well 15407732ADA, Port 5.

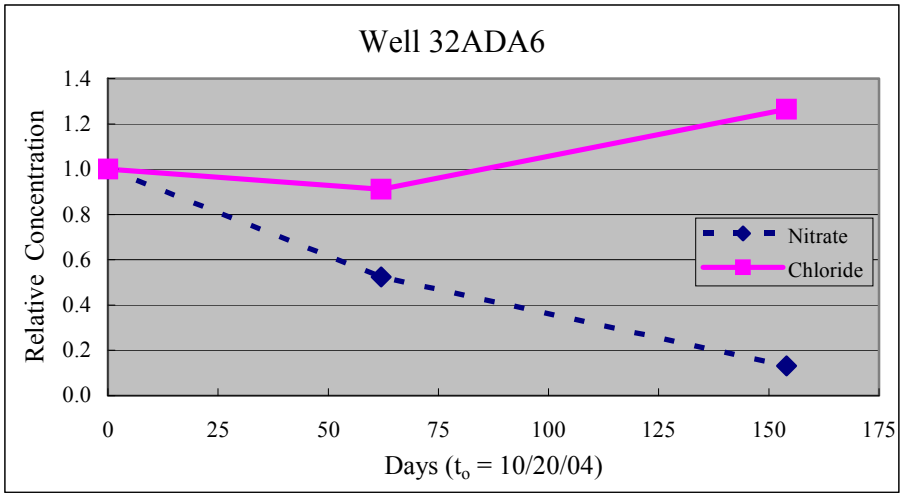


Figure 17. Relative concentration profile for Well 15407732ADA, Port 6.

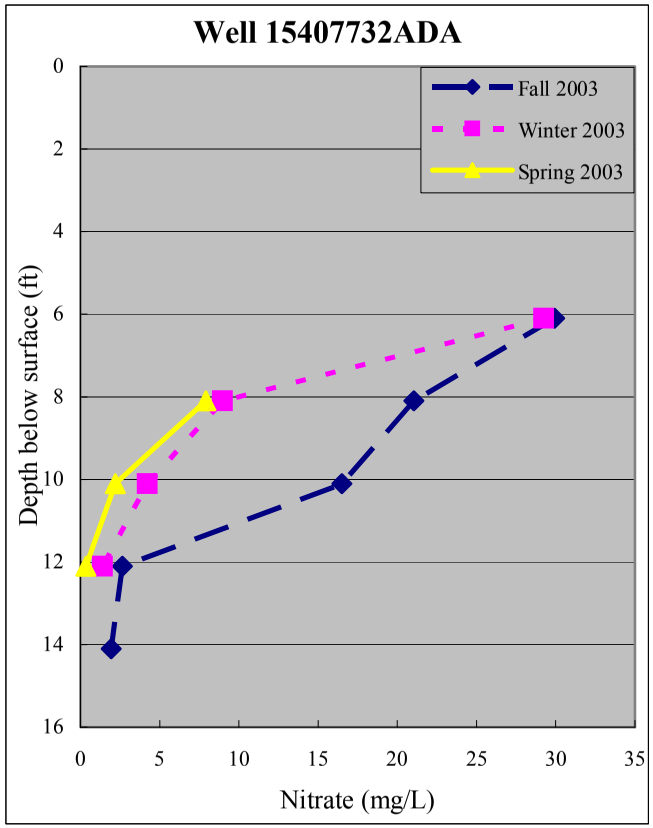


Figure 18. Depth profiles of nitrate-N concentration for Well 15407732ADA.

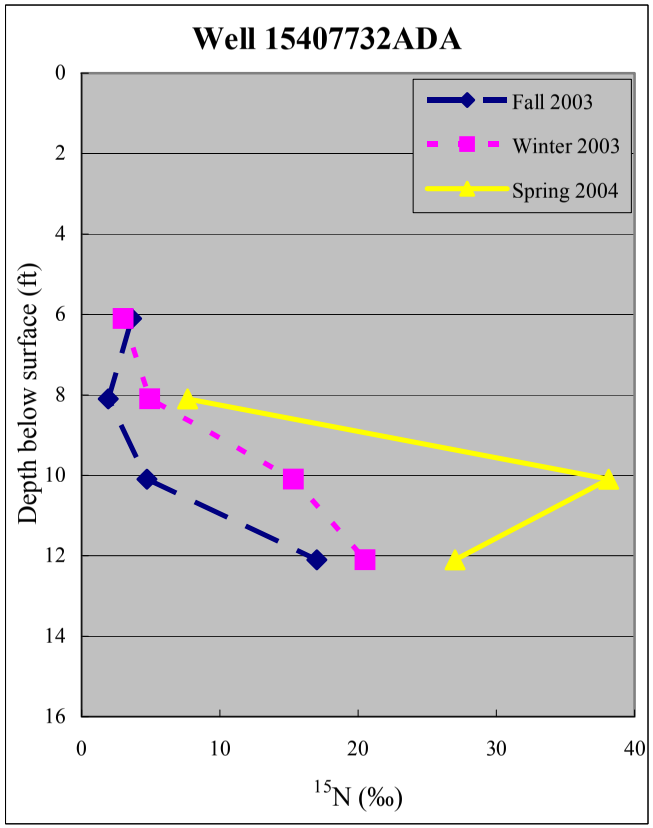


Figure 19. Depth profile of ^{15}N fractionation for Well 15407732ADA.

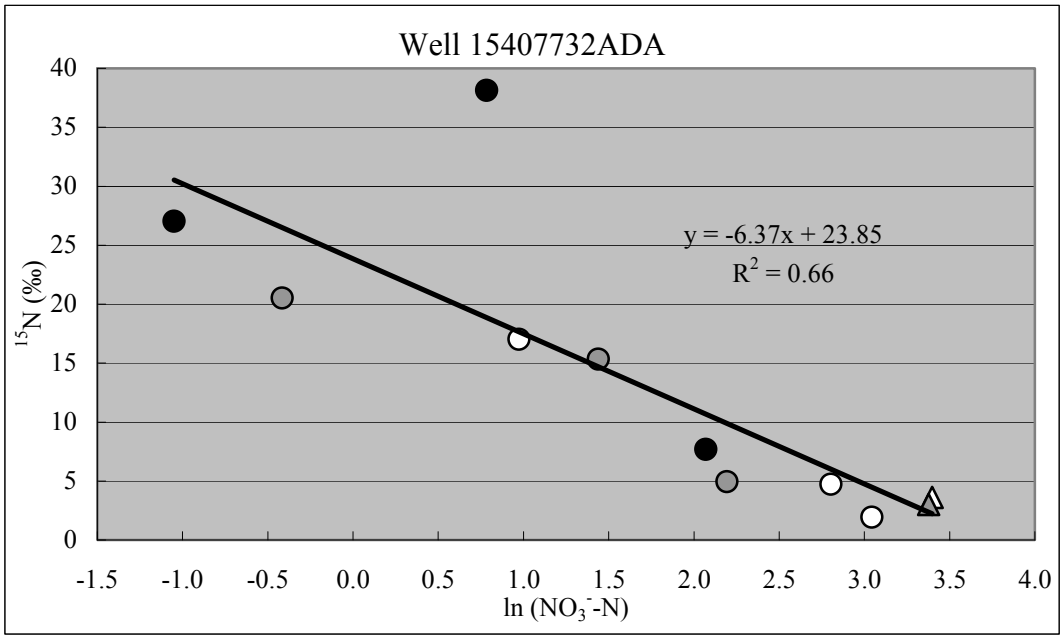
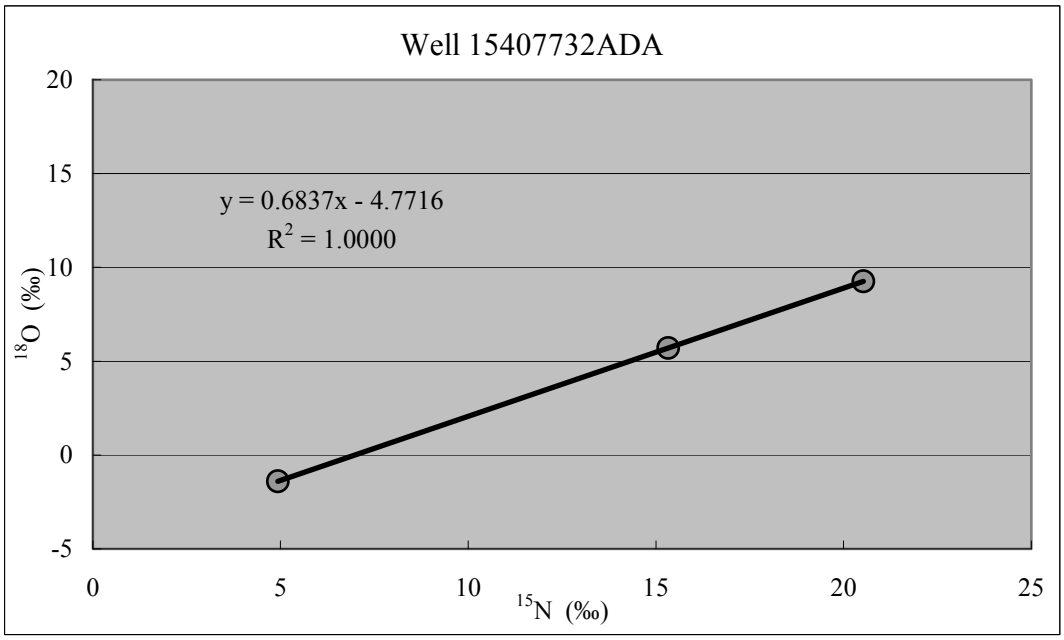


Figure 20. ^{15}N fractionation versus ln (NO_3^--N) for Well 15407732ADA.



▲ Shallow White fill = Fall
● Intermediate Grey fill = Winter
■ Deep Black fill = Spring

Figure 21. ^{15}N versus ^{18}O fractionation for Well 15407732ADA.

between ^{15}N and ln (NO_3^--N) (Figure 26), which is contrary to denitrification. However, the strong correlation with a slope near 0.5 for the increasing fractionation of ^{18}O and ^{15}N (Figure 27) is indicative of denitrification. More research is necessary to clear up the apparent discrepancy among these data. Nevertheless, the preponderance of data support the conclusion that denitrification occurred at this site.

Well 35BCC

All ports show little change in relative concentrations of NO_3^--N and Cl^- from fall to winter (Figures 28, 29, and 30). During the spring there is a distinct separation between NO_3^--N and Cl^- for ports 6 and 12, which is consistent with denitrification. Decreasing NO_3^--N concentrations with depth (Figure 31) are also indicative of denitrification. This is accompanied by a moderate increase in ^{15}N with depth during fall and winter and a significant increase during spring with peak enrichment of 29.9‰ (Figure 32). A strong negative correlation is apparent between ^{15}N and ln (NO_3^--N) (Figure 33) and an even stronger positive correlation with a slope near 0.5 is exhibited for the fractionation of ^{15}N and ^{18}O (Figure 34). Together these results provide compelling evidence of denitrification.

Well 36AAA

Relative concentrations for NO_3^--N and Cl^- are generally consistent with each other, with only moderate separations in ports 9 and 12 for the spring (Figures 35, 36, and 37). Reduction of NO_3^--N takes place with depth for all ports for all three sampling dates (Figure 38) and the reduction is accompanied by increases in ^{15}N (Figure 39). ^{15}N and ln (NO_3^--N) exhibit a linear negative relationship and are consistent with denitrification (Figure 40). ^{18}O and ^{15}N also show a positive relationship with a slope near 0.5 (Figure 41). The peak value of 13.6‰ for ^{15}N and 6.1‰ for ^{18}O on Figure 41, because it was collected in the fall, likely

represents enrichment caused by denitrification that occurred before the study began. Overall, the preponderance of data indicate that denitrification occurred at this locale.

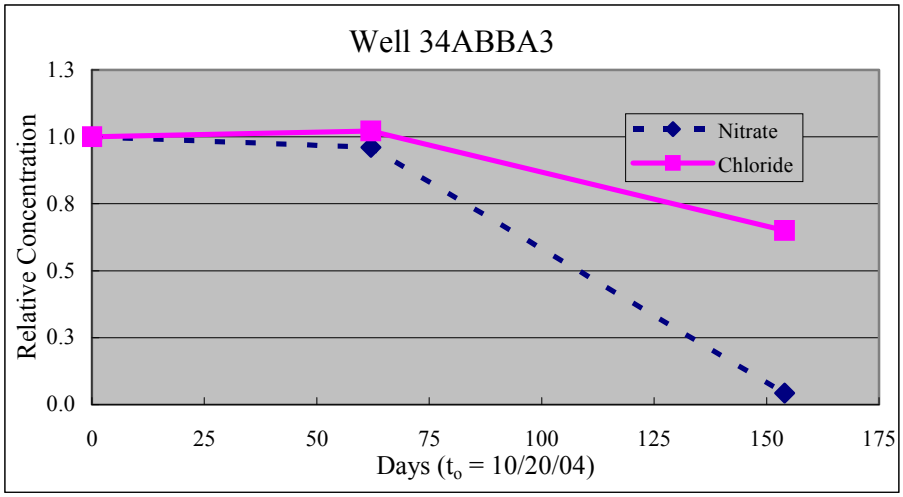


Figure 22. Relative concentration profile for Well 15407734ABBA, Port 3.

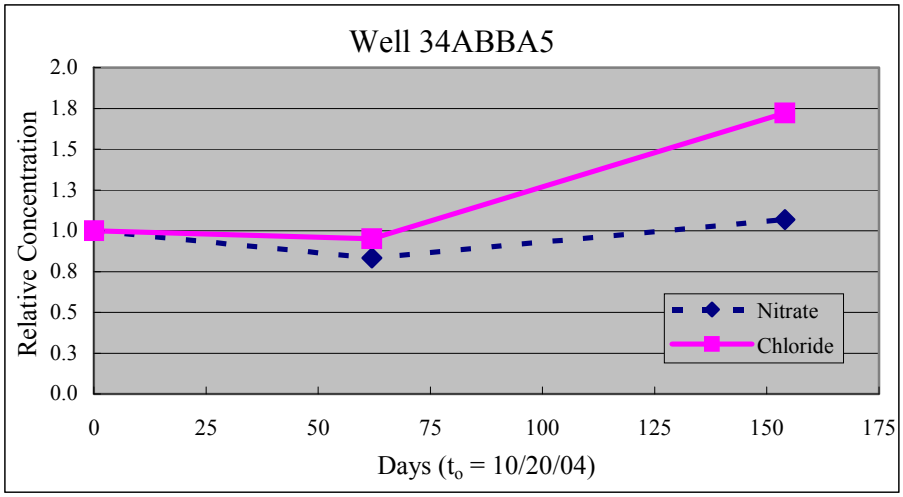


Figure 23. Relative concentration profile for Well 15407734ABBA, Port 5.

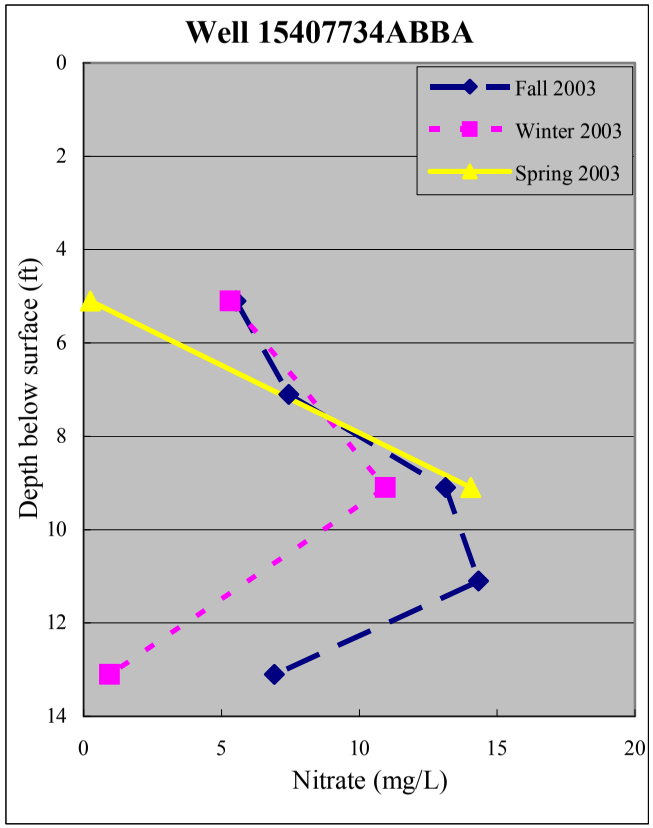


Figure 24. Depth profiles of nitrate-N concentration for Well 15407734ABBA.

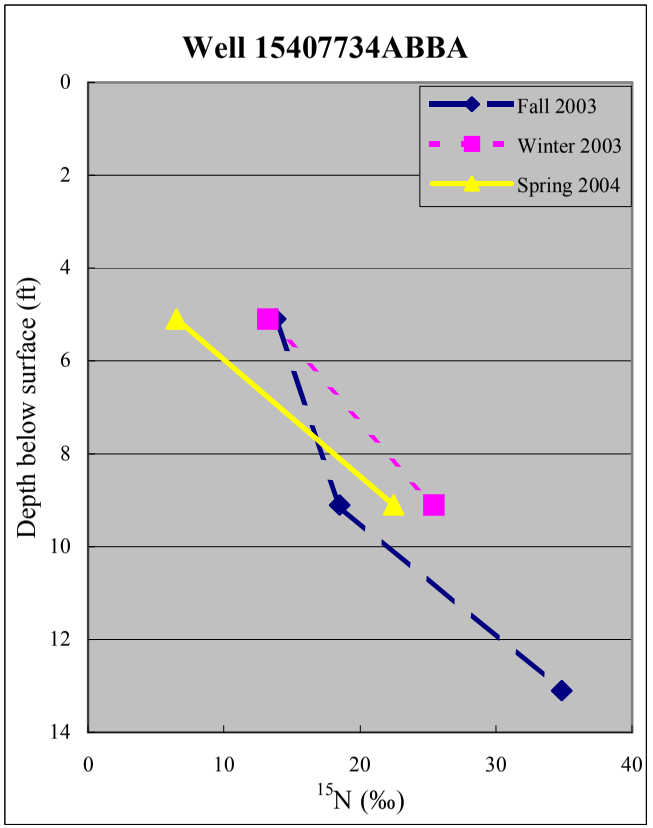


Figure 25. Depth profile of ^{15}N fractionation for Well 15407734ABBA.

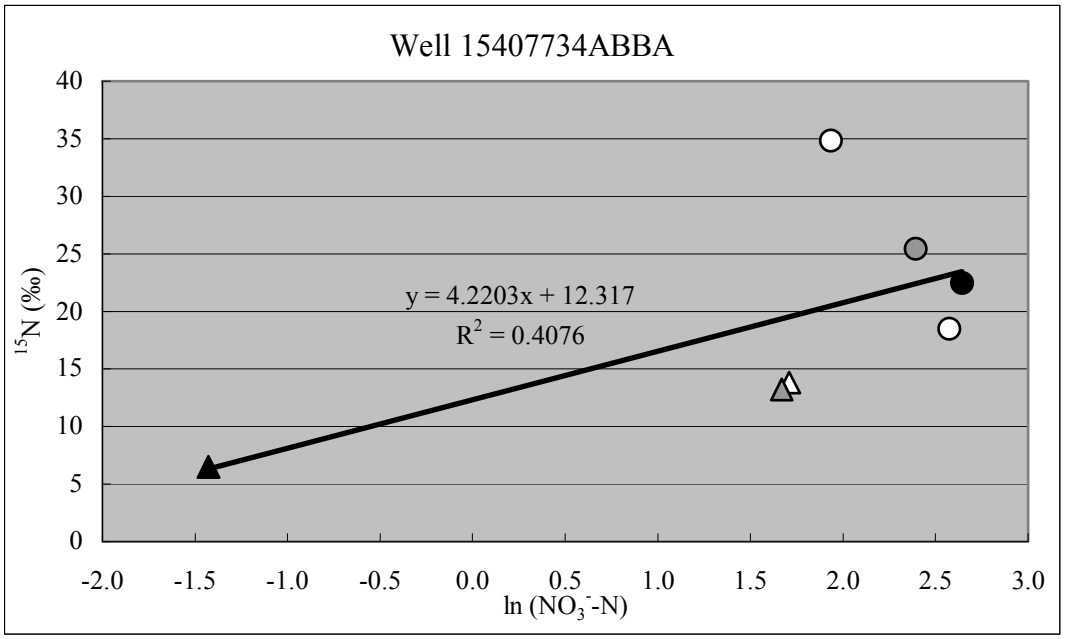
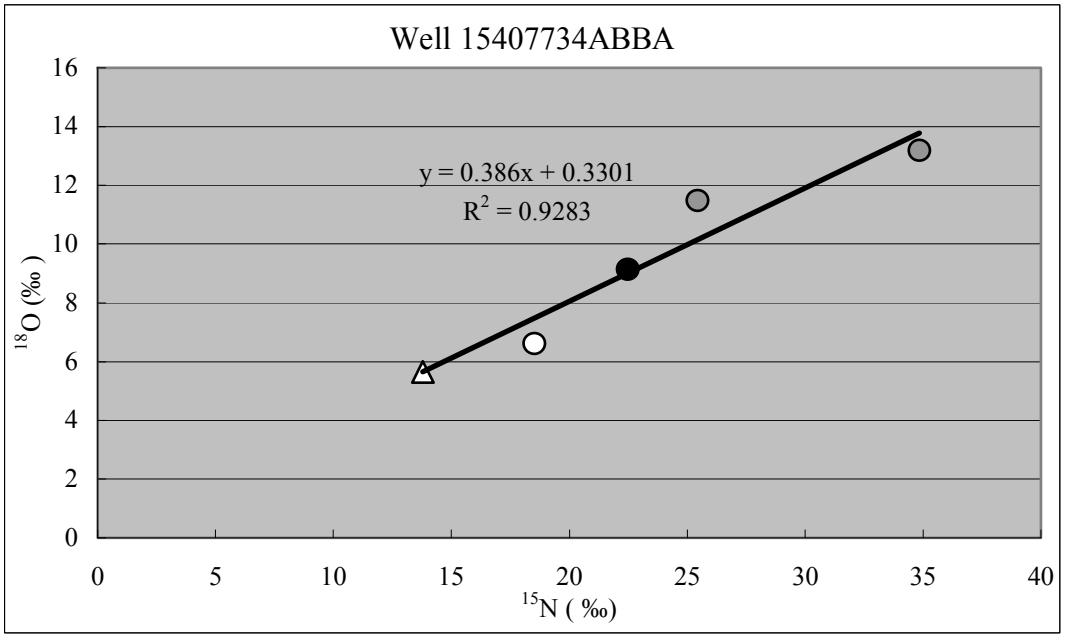


Figure 26. ^{15}N fractionation versus ln (NO_3^--N) for Well 15407734ABBA.



▲ Shallow White fill = Fall
● Intermediate Grey fill = Winter
■ Deep Black fill = Spring

Figure 27. ^{15}N versus ^{18}O fractionation for Well 15407734ABBA.

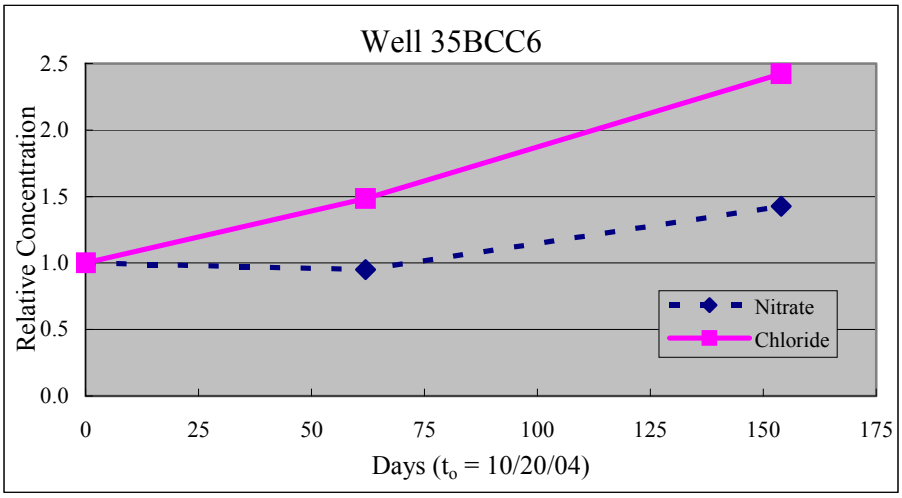


Figure 28. Relative concentration profile for Well 15407835BCC, Port 6.

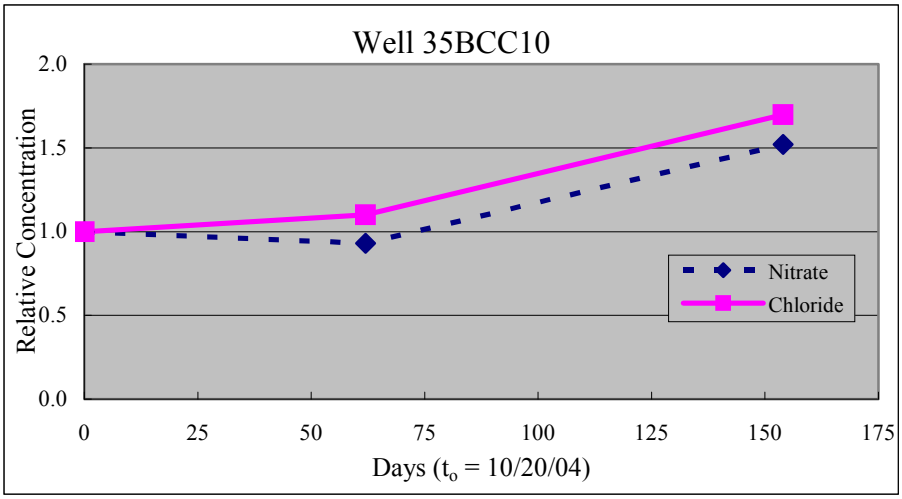


Figure 29. Relative concentration profile for Well 15407835BCC, Port 10.

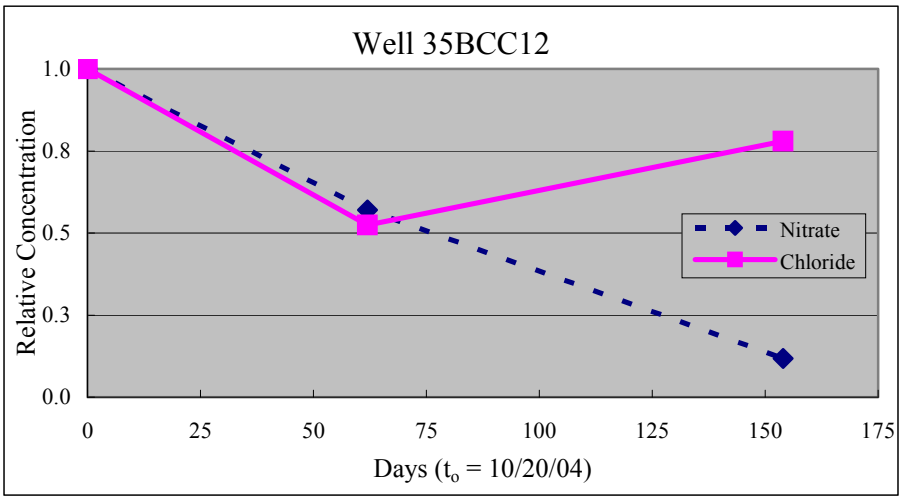


Figure 30. Relative concentration profile for Well 15407835BCC, Port 12.

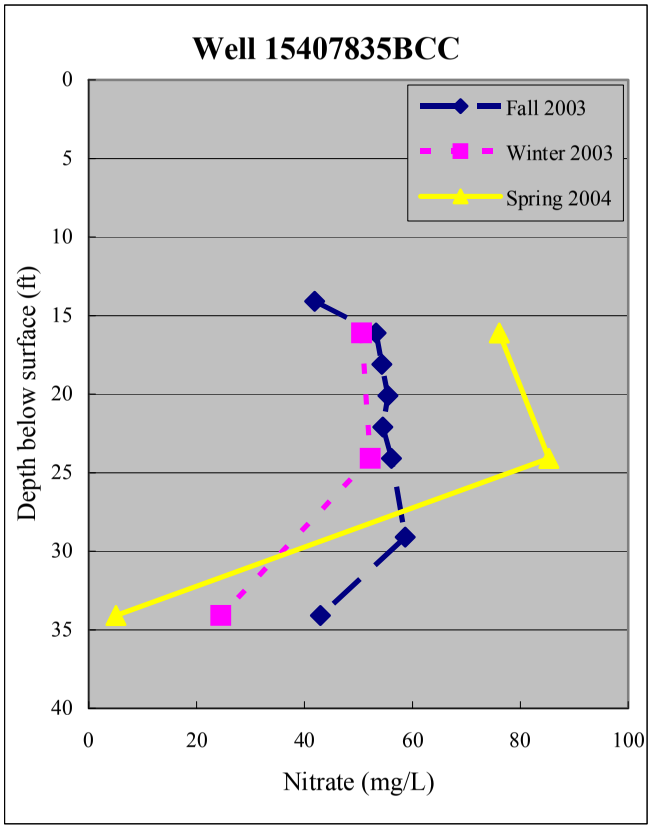


Figure 31. Depth profiles of nitrate-N concentration for Well 15407735BCC.

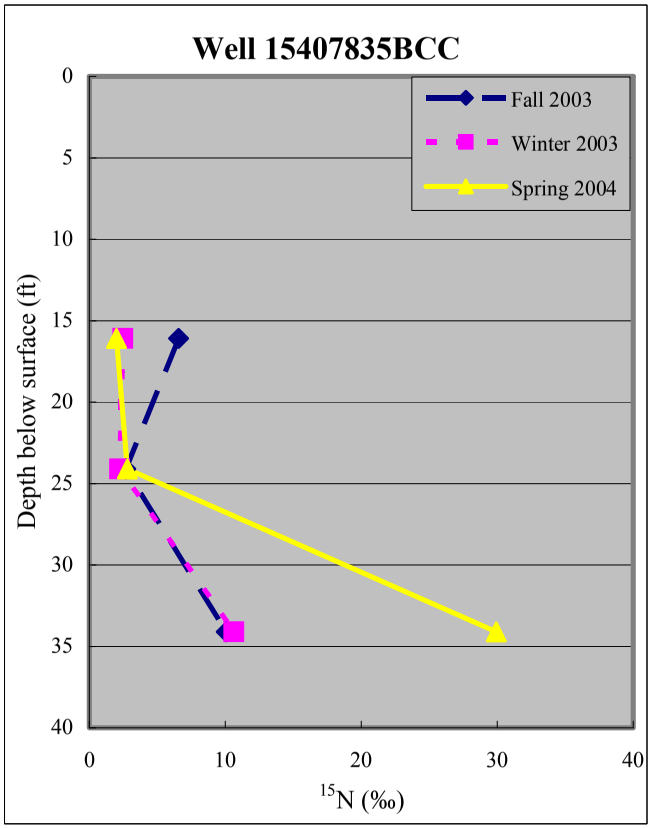


Figure 32. Depth profile of ^{15}N fractionation for Well 15407734BCC.

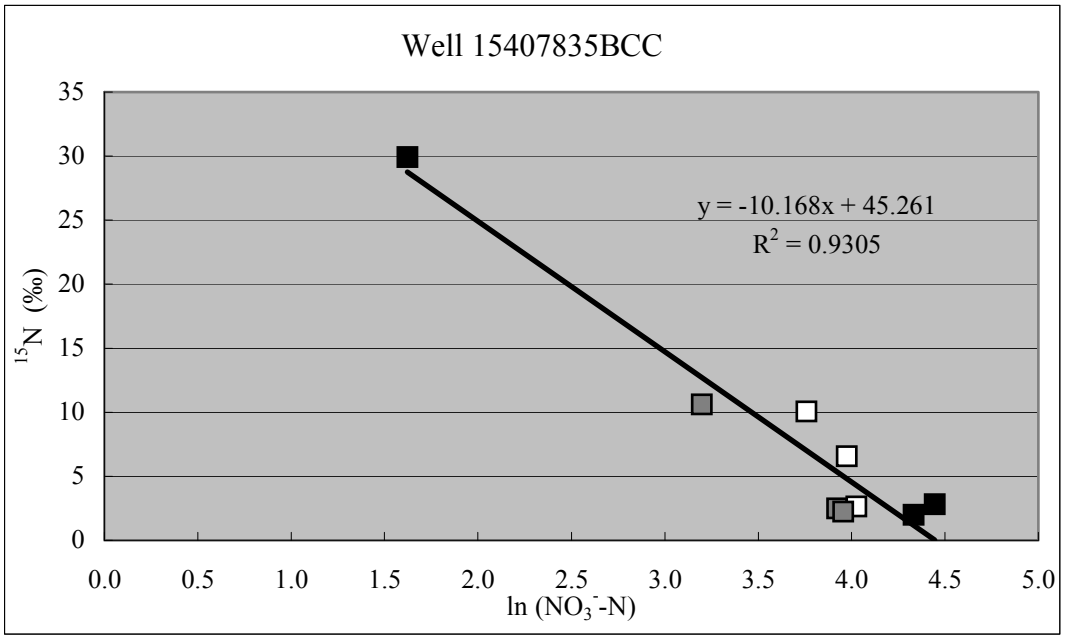
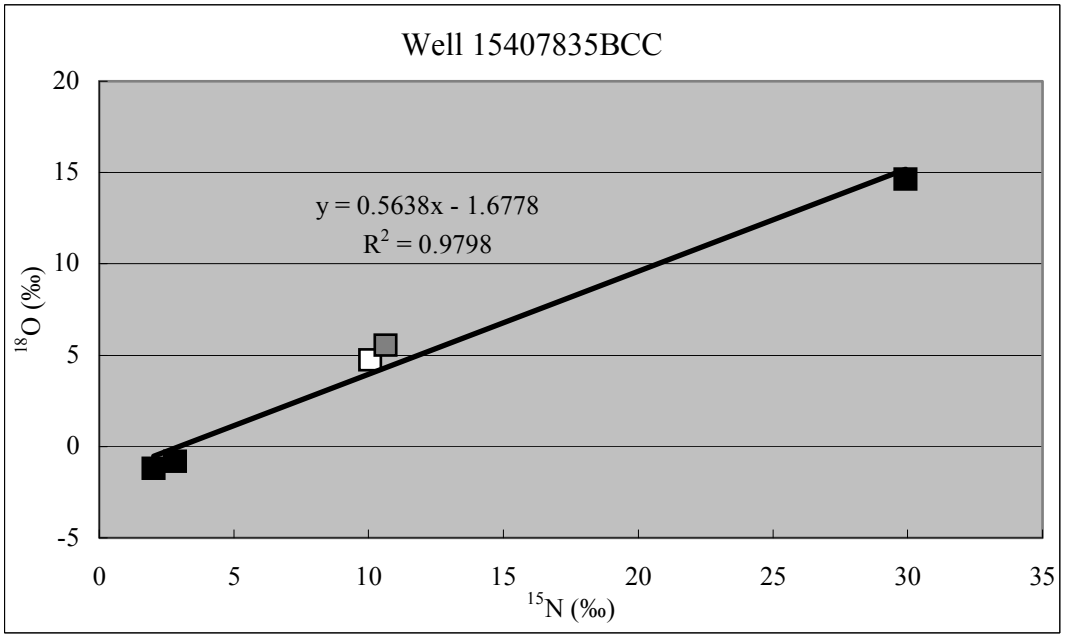


Figure 33. ^{15}N fractionation versus ln (NO_3^--N) for Well 15407835BCC.



▲ Shallow White fill = Fall
● Intermediate Grey fill = Winter
■ Deep Black fill = Spring

Figure 34. ^{15}N versus ^{18}O fractionation for Well 15407835BCC.

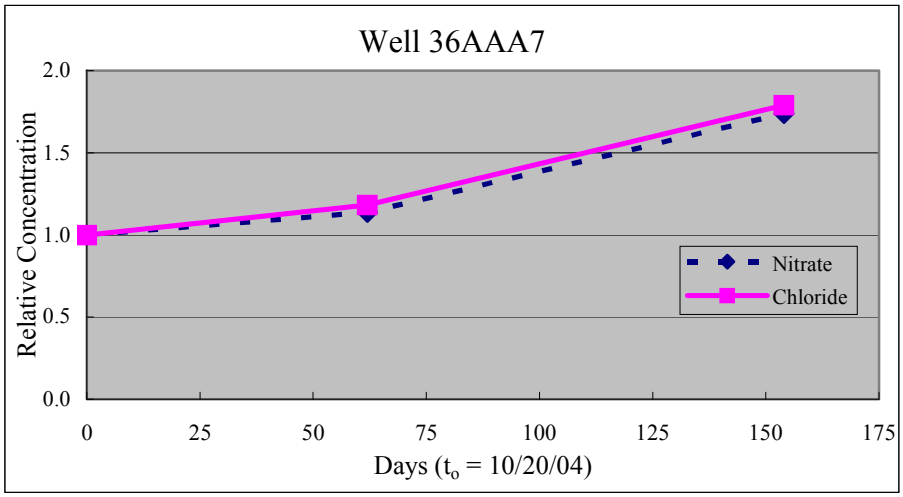


Figure 35. Relative concentration profile for Well 15407836AAA, Port 7.

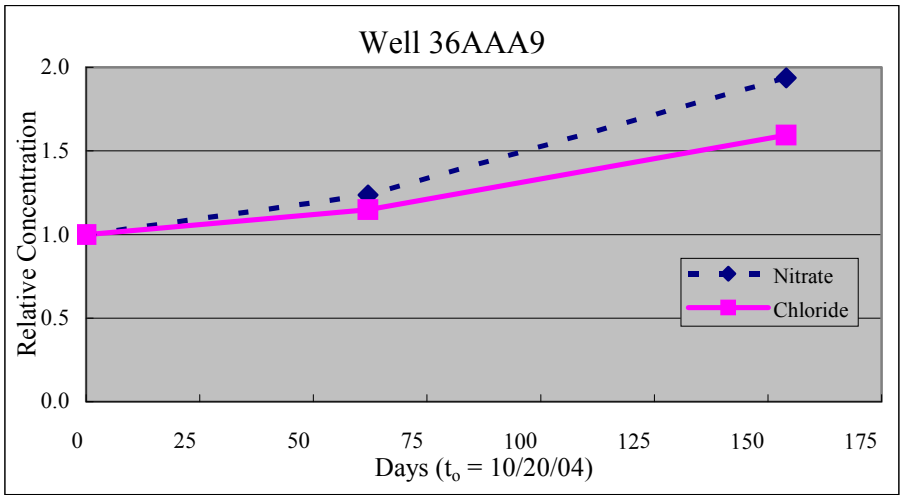


Figure 36. Relative concentration profile for Well 15407836AAA, Port 9.

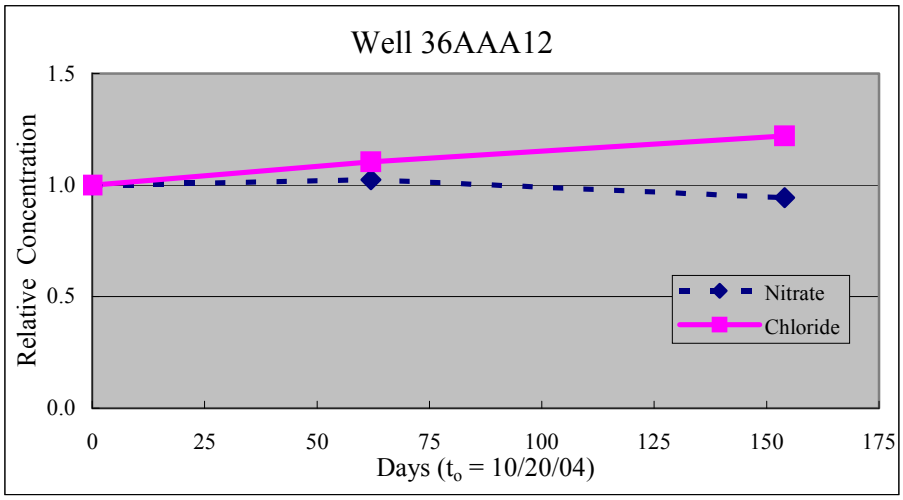


Figure 37. Relative concentration profile for Well 15407836AAA, Port 12.

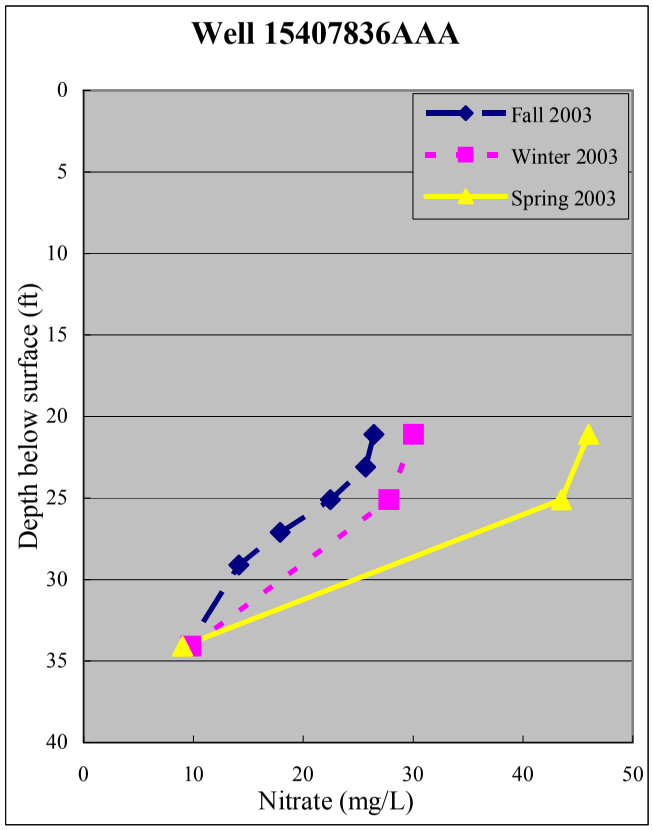


Figure 38. Depth profiles of nitrate-N concentration for Well 15407836AAA.

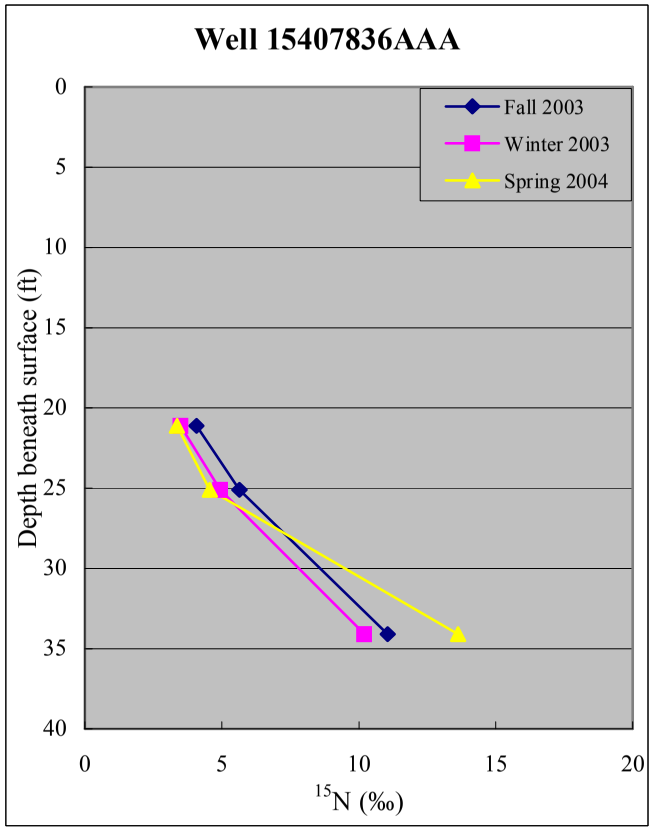


Figure 39. Depth profile of ^{15}N fractionation for Well 15407836AAA.

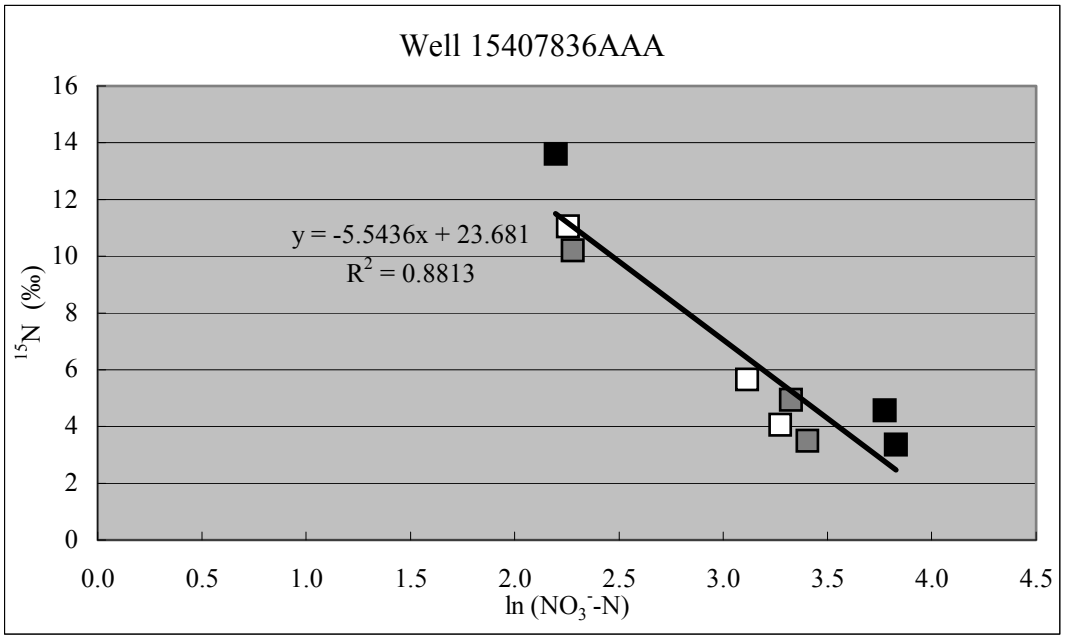
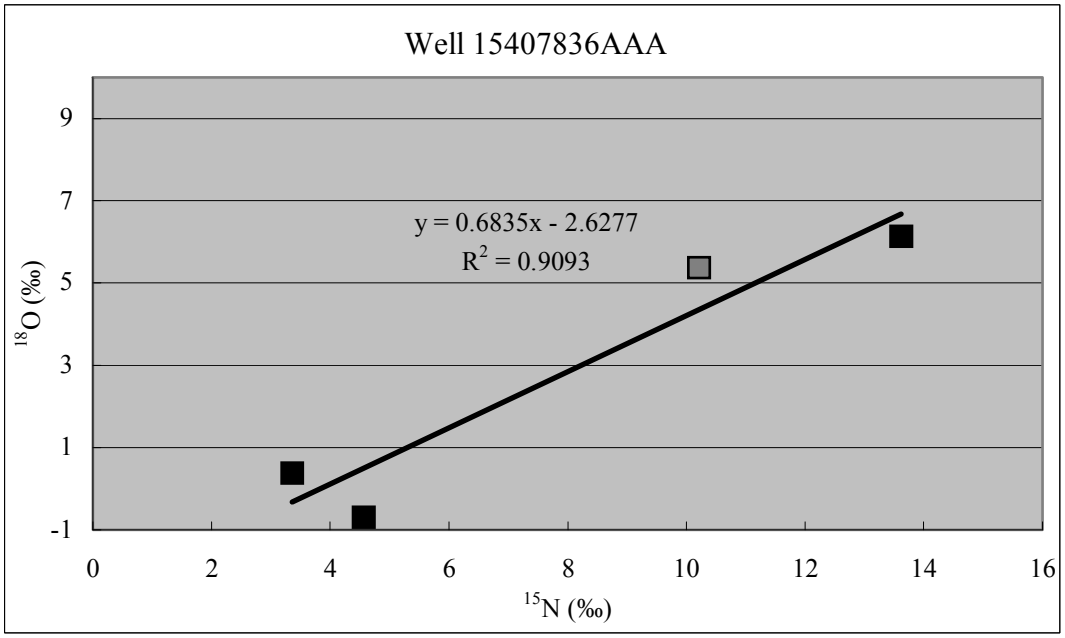


Figure 40. ^{15}N fractionation versus ln (NO_3^--N) for Well 15407836AAA.



▲ Shallow White fill = Fall
● Intermediate Grey fill = Winter
■ Deep Black fill = Spring

Figure 41. ^{15}N versus ^{18}O fractionation for Well 15407836AAA.

Cumulative Evidence Of Denitrification

Relatively weak relationships exist between isotopic enrichment and ln (NO_3^--N) (Figures 42 and 43) for all samples. However these figures show that fractionation is inversely proportional to nitrate concentration and that those samples that are enriched are likely the result of denitrification.

Figure 44 may be the single most important line of evidence supporting denitrification in the Karlsruhe Aquifer. As predicted the ^{18}O and ^{15}N show a direct linear trend of increasing fractionation. Additionally ^{15}N enrichment is nearly twice that of ^{18}O. Other than for denitrification, it is highly unlikely that any other type of nitrate attenuation or consumption process could produce these results.

The four isotope samples tested for ^{18}O of H_2O gave an average value of -12.98±0.70‰ (Spencer, 2005). Atmospheric ^{18}O was assumed as +23.5‰ (Kroopnick and Craig, 1972.) Thus nitrate derived from the oxidation of ammonia (Equation 2) would have an expected initial $\delta^{18}O$ of -0.80±0.70‰ (solid rectangle on y-axis of Figure 44). This indicates that fractionation lies within the range of NO_3^- derived from the oxidation of ammonia fertilizer (dashed ellipse of Figure 44). If the NO_3^- was derived from another source of NH_4^+ the cluster of initial fractionation would be more enriched in ^{15}N.

These results are consistent with the work of Phipps and Betcher (2003) where a moderate relation between ^{15}N and NO_3^--N was significantly enhanced by the addition of ^{18}O (Figures 4 and 6). Additionally, their estimate of initial $\delta^{18}O$ (-3.4 to -0.1‰) was similar to that reported herein. Their ratio of ^{18}O to ^{15}N enrichment also produced a slope of about 0.5.

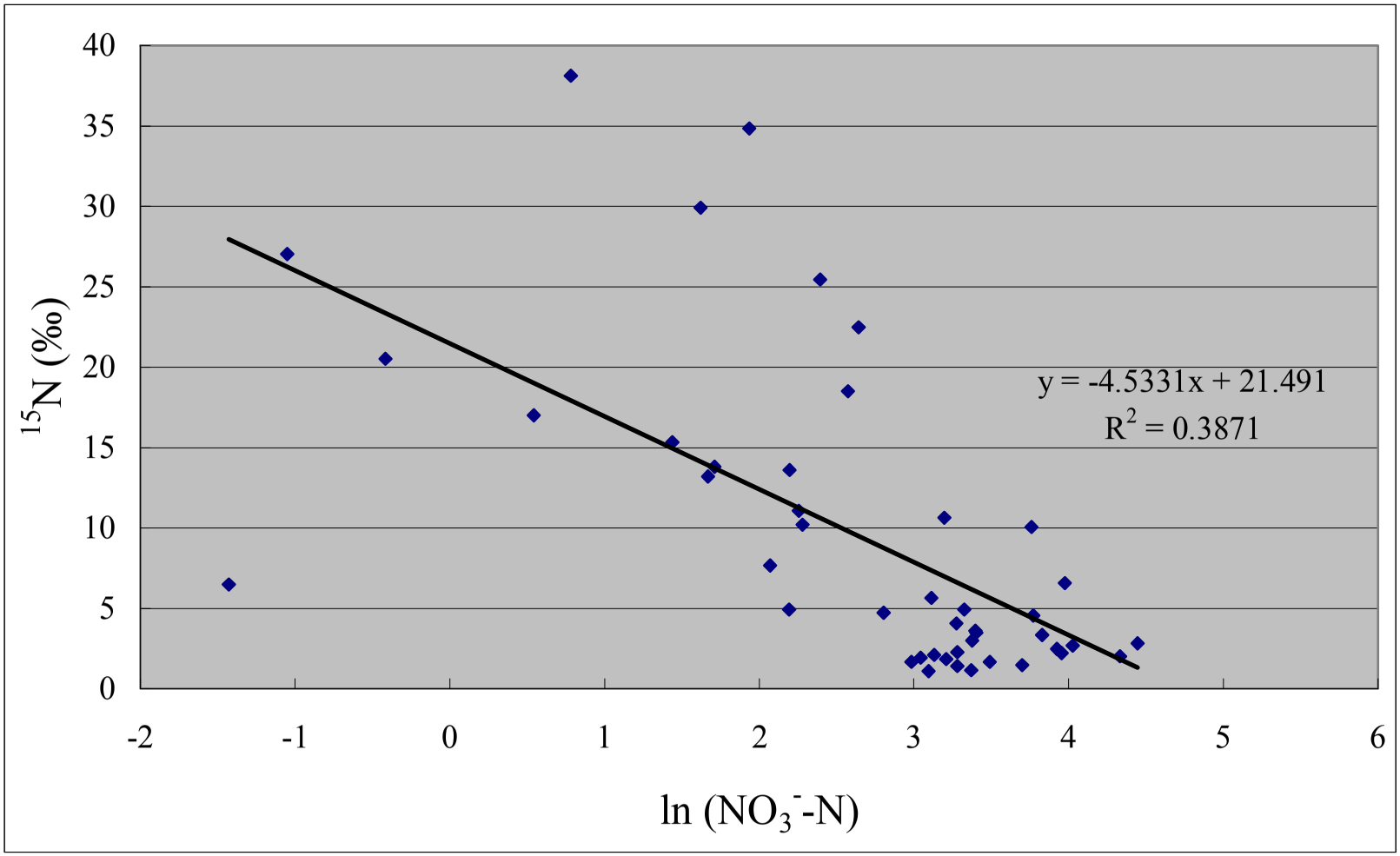


Figure 42. ^{15}N versus ln (NO_3^--N) for all samples with ^{15}N data.

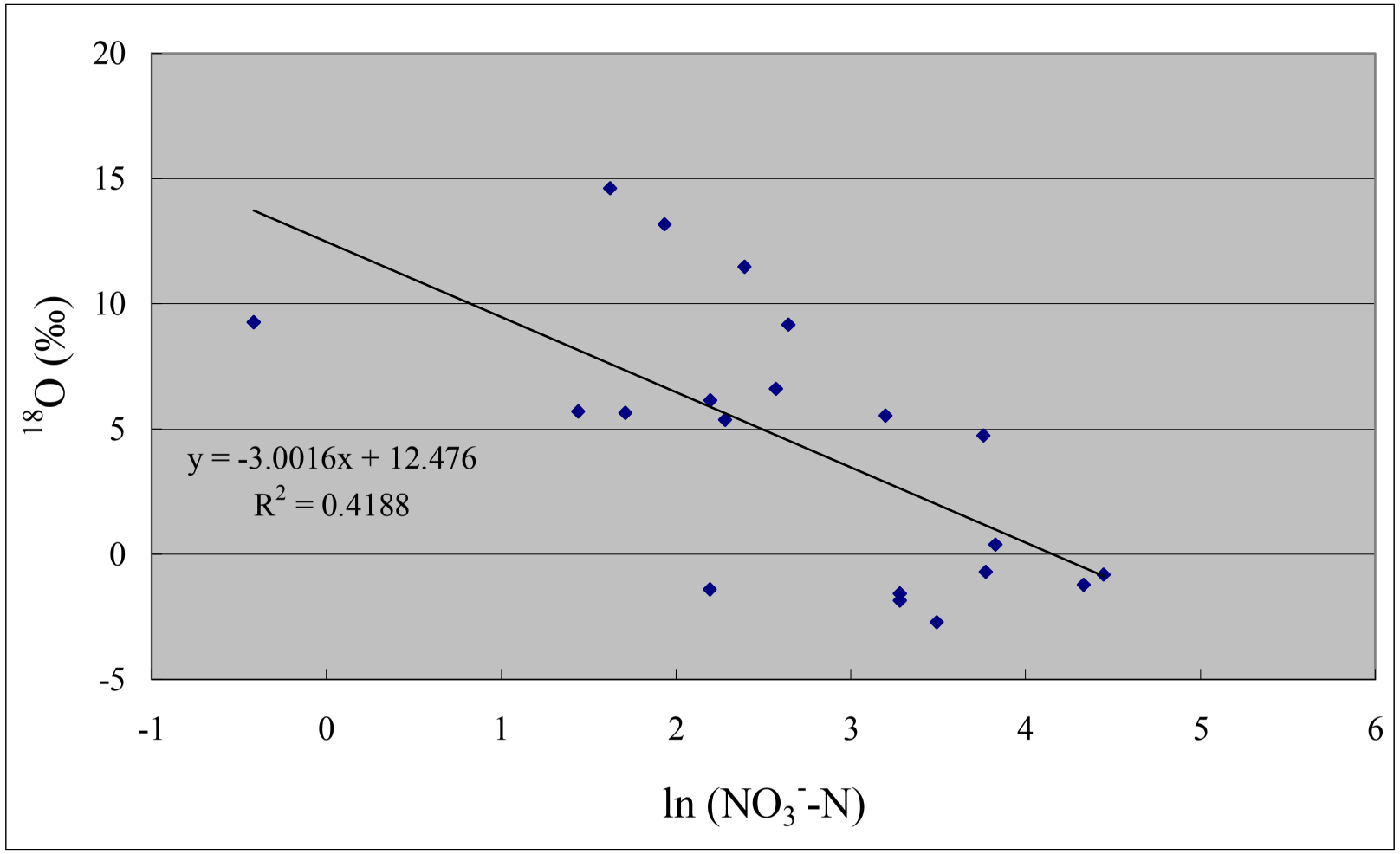


Figure 43. ^{18}O versus ln (NO_3^--N) for all samples with ^{18}O data.

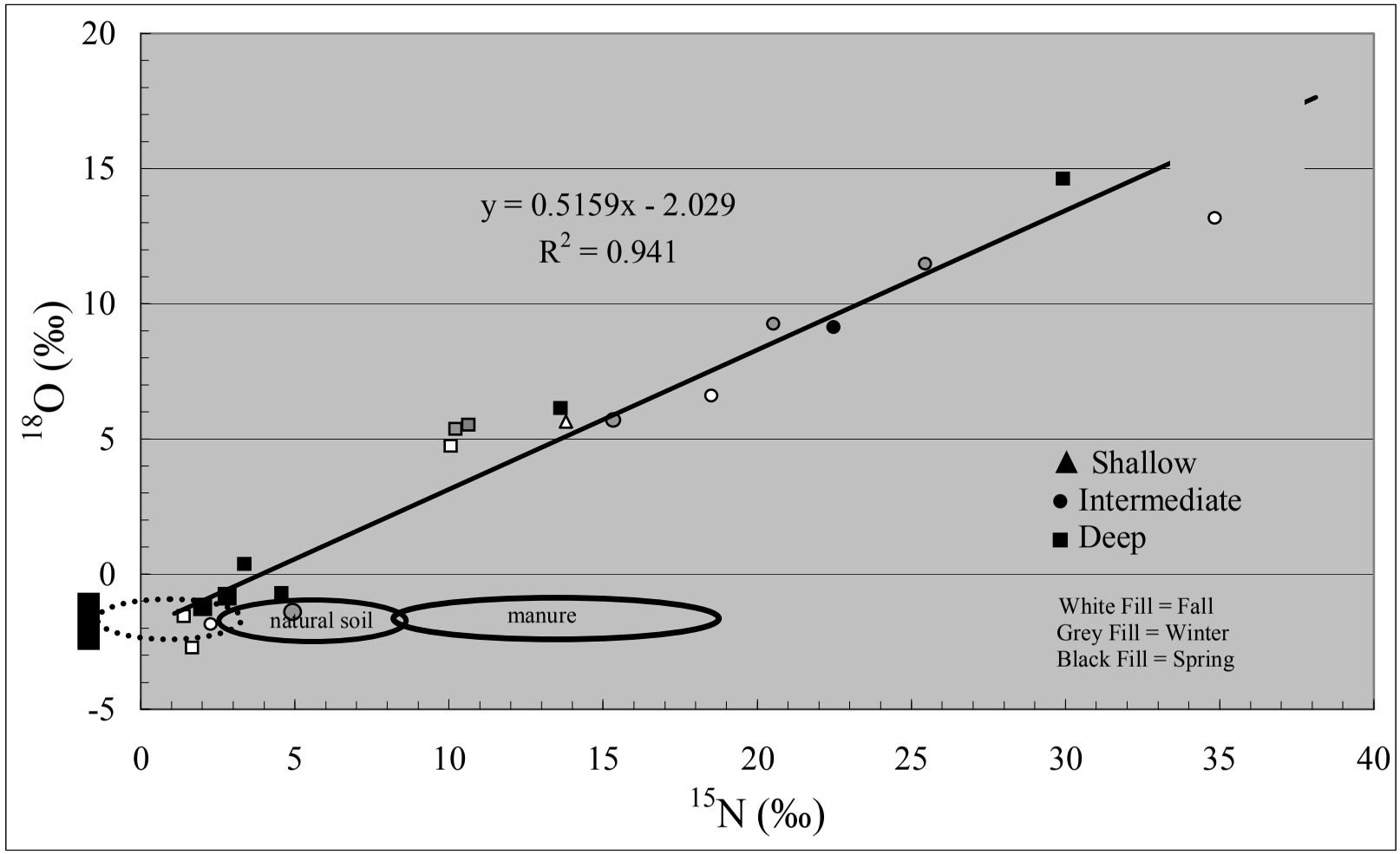


Figure 44. ^{15}N versus ^{18}O fractionation as an indicator of nitrate source.

Enrichment factors (ε) based on all samples are -4.53‰ for ^{15}N and -3.01‰ for ^{18}O (Figures 42 and 43). These are low compared to previous experiments as conducted by Botcher et al. (1990) (ε -^{15}N = -15.9‰ and ε -^{18}O = -8.0‰) and Mengis et al. (1999) (ε -^{15}N = -27.6‰ and ε -^{18}O = -18.3‰). Warne (2004) also received a higher enrichment of ^{15}N (-9.3‰) in the ISM experiment. Low enrichment factors for this study may simply be the result of specific site conditions such as temperatures, denitrification rates, or the availability of electron donors.

CONCLUSIONS

Denitrification occurs in certain portions of the Karlsruhe Aquifer. The relationship between decreased NO_3^--N and increased fractionation of ^{15}N and ^{18}O is direct evidence of this. Figure 44 is in itself conclusive evidence of denitrification. It is unlikely that this relationship between increasing fractionation coupled with the peak enrichment values could have resulted from any other process. High nitrate loading in the Karlsruhe Aquifer is likely the result of the application of ammonia-based fertilizer. Initial ^{15}N and ^{18}O fractionation supports this conclusion. It is possible that other nitrogen sources are present but ammonia-based fertilizer is the most significant contributor of the nitrate in this study.

Although denitrification is evident it did not take place in all of the wells. Only Wells 32ADA, 34ABBA, and 35BCC show satisfactory evidence of denitrification. Moderate fractionation within Well 36AAA supports the hypothesis of denitrification, but to a lesser extent. Well 31DDD is not influenced by denitrification. Denitrification rates in Wells 32ADA and 34ABBA correlate well with the ISM-S installed by Warne (2004). All three locations are near each other. Furthermore, denitrification occurs with depth. This is

supported by a decrease of NO_3^--N with depth coupled with an increase in ^{15}N. This is most apparent in Wells 32ADA and 36AAA and to a lesser extent in Wells 34ABBA and 35BCC.

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