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Tracing Nitrogen in the Pedosphere and Hydrosphere of Agricultural Plots near Lethbridge, Alberta using Isotopic Techniques

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Tracing Nitrogen in the Pedosphere and Hydrosphere of Agricultural Plots near Lethbridge, Alberta using Isotopic Techniques

by

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Abstract

The source(s) and fate of nitrogen in soils and in shallow groundwater beneath agricultural plots with different fertilizer amendments were determined using isotopic techniques for an experimental field located near Lethbridge, Alberta. Isotopic compositions of nitrogen and of oxygen were determined for soil total nitrogen, soil nitrate, and groundwater nitrate. Treatment differences and temporal trends in groundwater nitrate concentrations indicated that synthetic fertilizer and fixed nitrogen had an impact on groundwater nitrate. Isotopic analyses identified mineralization of soil organic matter as the main source of soil and groundwater nitrogen, whereas fertilizer, fixed nitrogen, and manure did not appear to have had a large direct contribution. This may have been due to alteration of the original isotopic source signals of fertilizer, fixed nitrogen, and manure by nitrogen transformation processes (nitrification and denitrification) during nitrogen cycling in the soil and groundwater. Denitrification occurred in the groundwater within the three-year study period.

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List of Symbols, Abbreviations, and Nomenclature

AAFC	Agriculture and Agri-Food Canada
AGS	Alberta Geological Survey
Alf	Alfalfa treatment
ANOVA	Analysis of variance
CF200	Corn faba-bean treatment
CLTN	Corn with long-term nitrogen treatment
CRN	Corn with recent nitrogen treatment
CRN+m	Corn with recent nitrogen and manure treatment
HDPE	High density polyethylene
LNID	Lethbridge northern irrigation district
m ASL	Meters above sea level
PRS	Plant root simulator
SD	Standard deviation
SOM	Soil organic matter
VSMOW	Vienna standard mean ocean water
WHO	World Health Organization
WT	Water table

CHAPTER 1: INTRODUCTION

1.1. Nitrogen in the environment

Nitrogen exists naturally in the soil, plants, atmosphere, and hydrosphere, with the atmosphere containing approximately $4x10^9$ Tg of N (Söderlund and Svensson, 1976; Winteringham, 1980; Schlesinger, 1997). Over the last few decades, anthropogenic activities, such as agriculture and burning of fossil fuels, have drastically increased the amount of nitrogen in groundwater and surface water (Vitousek *et al*., 1997).

Synthetic fertilizers and manure are often used in agricultural settings to increase crop quality and yield, but inappropriate or excessive use can lead to pollution of the soil and groundwater (Addiscott, 2005; Olson *et al*., 2005). In a review of nitrogen budgets in Canadian agricultural environments using 1996 data, Janzen *et al*. (2003) estimated the total nitrogen inputs by biological N_2 fixation, atmospheric deposition, and fertilizer application to have been approximately 2.35TgN/yr. Given that less than 50-60% of the total nitrogen input is removed through crop harvesting and animal products (Smil, 1999; Janzen *et al*., 2003), roughly half of the nitrogen added annually is available for potential losses through leaching, ammonia volatilization, and denitrification, or for accumulation in the soil zone. Losses of fertilizer nitrogen through leaching are estimated to be roughly 10% for fertilizer application rates of less than 150kgN/ha/yr and 20% for rates greater than 150kgN/ha/yr (Frissel and Kolenbrander, 1978).

Inappropriate disposal of sewage and its treated effluents can also lead to nitrogen contamination of groundwater (Wakida and Lerner, 2005). Nitrate is a particularly pervasive contaminant, due to its widespread use and disposal, low chemical reactivity, and high solubility (Korom, 1992; Spalding and Exner, 1993; Rivett *et al*., 2007). Many cases of surface water and groundwater nitrate contamination have already been documented in North America (e.g., the Gulf of Mexico, Rabalais *et al*., 2002; the United States, Spalding and Exner, 1993) and in Europe (European Environment Agency, 2000).

According to the World Health Organization's *Guidelines for Drinking-water Quality* (2007), nitrite, the reduced form of nitrate, can lead to methaemoglobinaemia and asphyxiation, particularly in infants, when ingested at high concentrations. This is commonly known as the "blue baby syndrome". Nitrite can also form carcinogenic N-nitroso compounds when taken into

the human body (WHO, 2007). Because of these adverse health effects, nitrate levels should not exceed 10mg/L of nitrate-N (45mg/L as nitrate) in drinking water (WHO, 2008). Excessive nitrogen in the environment may also lead to eutrophication of lakes and rivers (Vitousek *et al*., 1997).

As a result of the negative health and environmental effects caused by excessive nitrate, a thorough understanding of nitrogen transformation processes in soils and the extent of nitrate export to the groundwater is vital. Many studies of groundwater nitrate contamination have been conducted in the past (e.g., Wassenaar, 1995; Aravena and Robertson, 1998; Rodvang *et al*., 2004), but few studies have utilized ion exchange membrane, soil, and groundwater data in conjunction to assess the long-term effects of crop cultivation and fertilizer usage on soil and groundwater quality. In this study, ion exchange membranes in the form of plant root simulator probes (PRS, Western Ag Innovations), soil cores, and an extensive set of groundwater samples taken over three years (July 2006-October 2009) were analyzed and studied through the use of isotope ratio analyses to better understand the processes affecting nitrate concentrations in the soil and shallow groundwater beneath an agricultural plot receiving multiple nitrogen amendments.

1.2. Nitrogen Transformation Processes in Soils and in Groundwater

Nitrogen occurs in the atmosphere primarily as N_2 gas, and less commonly as ammonium and nitrate (Schlesinger, 1997). In soils and in plants, nitrogen occurs mainly in the form of organic compounds (e.g., amino-acids), and inorganic compounds, such as ammonium and nitrate (Schlesinger, 1997; Brady and Weil, 2002). The dominant form of nitrogen found in oceans, groundwater, and surface water is nitrate (Schlesinger, 1997).

Major nitrogen transformation processes in the nitrogen cycle include biological fixation, mineralization (ammonification and nitrification), ammonia volatilization, and denitrification (Hiscock *et al*., 1991; Kendall, 1998; Galloway *et al*., 2004). Other processes affecting the nitrogen cycle include atmospheric deposition, fertilizer input, leaching of inorganic nitrogen, and assimilation of inorganic nitrogen by plants and micro-organisms (Keeney and Olson, 1986; Kendall, 1998). A simplified nitrogen cycle is shown in Figure 1.1.

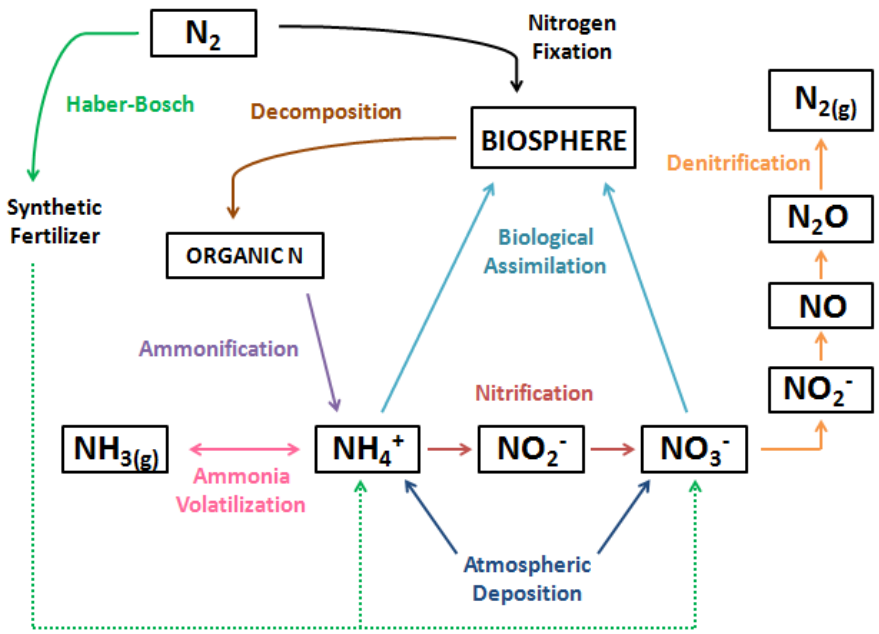

Figure 1.1: Schematic diagram of the nitrogen cycle showing major nitrogen transformation processes. Modified after Kendall (1998).

Nitrogen fixation is the process in which atmospheric N_2 gas is converted into NH_3 and subsequently organic forms of nitrogen by symbiotic (for example, by *Rhizobia* in leguminous plants, such as alfalfa) and non-symbiotic nitrogen-fixing bacteria (Brady and Weil, 2002). Ammonification is the process in which ammonium is produced from organic nitrogen, and ammonia volatilization is the process by which ammonium is converted into ammonia gas, which is subsequently lost into the atmosphere (Keeney and Olson, 1986; Kendall, 1998; Brady and Weil, 2002). The reversible reaction is represented by the equation (Brady and Weil, 2002):

$$NH_4^+ + OH^- \leftrightarrow NH_{3(g)} + H_2O_{(l)} \quad [1.1]$$

Nitrification is the oxidation of ammonium to nitrate and occurs in two separate steps (Kendall, 1998). The first step involves the oxidation of ammonium into nitrite, which is facilitated by the bacteria *Nitrosomonas*, while the second step is the oxidation of nitrite into nitrate, which is facilitated by *Nitrobacter* (Keeney and Olson, 1986; Kendall, 1998). The two reactions are as follows (Brady and Weil, 2002):

$$NH_4^+ + 1\tfrac{1}{2}O_2 \rightarrow NO_2^- + 2H^+ + H_2O_{(l)} + 275\ kJ \quad [1.2]$$

$$NO_2^- + \tfrac{1}{2}O_2 \rightarrow NO_3^- + 76\ kJ \quad [1.3]$$

Denitrification is the reduction of nitrate into N_2 gas, facilitated by autotrophic and heterotrophic denitrifying bacteria, such as *Thiobacillus denitrificans* and *Pseudomonas* (Knowles, 1982; Clark and Fritz, 1997; Rivett *et al*., 2008). Intermediate products of denitrification include NO_2^-, NO, and N_2O gas (Knowles, 1982). The denitrification process requires electron donors, which can include organic carbon, reduced sulphur, reduced iron, and reduced manganese (Korom, 1992; Appelo and Postma, 2005). Denitrification can occur at temperatures between 2 and 50°C, with the optimum temperature being between 25 and 35°C (Brady and Weil, 2002). Denitrification is inhibited at pH levels < 5 and > 8.3 (Rust *et al*., 2000; Brady and Weil, 2002). Autotrophic and heterotrophic denitrification can be represented by the equations (McMahon *et al*., 1999; Hiscock *et al*., 1991):

$$2FeS_2 + 6NO_3^- + 2H_2O \rightarrow 3N_2 + 4SO_4^{2-} + 2FeOOH + 2H^+ \quad [1.4]$$

$$5CH_2O + 4NO_3^- + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O \quad [1.5]$$

Soil processes, such as nitrification, leaching, and denitrification, can heavily influence the amount of nitrate exported to the groundwater; therefore, it is important to evaluate the extent of these soil nitrogen transformation processes in addition to groundwater studies.

1.3. Previous Related Studies

Several groundwater quality studies have been conducted in the past in southern Alberta, especially in the vicinity of the Lethbridge northern irrigation district (LNID). Rock (2005) analyzed groundwater samples obtained in July and August of 2003 from thirty-seven wells for δ^2H-H_2O, $\delta^{18}O$-H_2O, $\delta^{13}C$-HCO_3, $\delta^{34}S$-SO_4, $\delta^{18}O$-SO_4, and $\delta^{15}N$-NO_3 values in the eastern Lethbridge area to determine the sources of these compounds. It was found that carbonate dissolution and, to a lesser extent, decay of organic matter were the sources of dissolved inorganic carbon in groundwater; that oxidation of reduced sulphur species and manure (in certain locations) were the sources of groundwater sulphate; and that manure was the source of groundwater nitrate in eastern parts of the Oldman River Basin.

McCallum *et al.* (2008) studied denitrification in groundwater below a manured field in the LNID using the $\delta^{15}N$ and $\delta^{18}O$ values of nitrate. It was determined that the source of groundwater nitrate was manure and that denitrification had occurred when younger, manure-influenced groundwater had mixed with older groundwater of low redox potential.

Hendry *et al*. (1984) used geochemical analyses and stable isotopes to determine the

source of nitrates in an oxidized till of southern Alberta. The high concentrations (> 100mg/L of NO_3-N) of nitrate in isolated groundwater enclaves were found to be from nitrification of till ammonium.

A groundwater quality assessment was conducted by Rodvang *et al*. (2004) under irrigated fields in the LNID. Groundwater nitrate concentrations ranging from 0.1 to 74mg/L of nitrate-N were detected in the fine-textured glacio-lacustrine sediments. The authors stated that the risk of nitrate contamination of shallow groundwater beneath the fields was high.

Beke *et al*. (1993a) evaluated the effect of irrigation on shallow groundwater quality in the Bow River irrigation district and the St. Mary irrigation district. Two of the sites were located in the Lethbridge area and the respective groundwater sodium concentrations and groundwater calcium + magnesium concentrations were measured to be from 4.5 to 22mmol/L and from 17.4 to 63.7mmol/L.

In a past study at the Lethbridge Research Center, the influence of a cattle feedlot on soil and groundwater quality was investigated between May 1996 and November 2000. Olson *et al*. (2005) found that there was less NO_3-N in the overall soil profile (0-1.5m depth) in 1999 than in 1996, when the cattle feedlot was first constructed. The authors explained that denitrification may have occurred, which led to a decrease, rather than an increase, in the soil nitrate levels. Taking sixteen groundwater wells into consideration, Olson *et al*. (2005) found that average groundwater nitrate levels increased by roughly 20 to 30mg/L after October 1998; however, the average groundwater nitrate concentration was found to have been the highest during the baseline period, before the feedlot was established. Olson *et al*. (2005) argued that previous land-use activities, such as agricultural fertilizer application, may have caused the high baseline nitrate concentrations.

Previous literature and research in the southern Alberta region have shown that there are several different sources that can contribute to groundwater nitrates, and that source determination is not always straightforward. In addition to geochemical analyses, the use of stable isotopes can potentially aid in the determination of the source(s) of nitrate.

1.4. Use of Stable Isotopes

Nitrogen has two stable isotopes, ^{14}N and ^{15}N, with natural abundances of 99.636% and 0.364%, respectively (Berglund and Wieser, 2011). Oxygen has three stable isotopes, ^{16}O, ^{17}O,

and ^{18}O, with respective natural abundances of 99.757%, 0.038%, and 0.205% (Berglund and Wieser, 2011). The standard delta-notation for isotope variations is as follows:

$$\delta_{sample}\ (‰) = [(R_{sample} / R_{reference}) - 1] \times 1000 \qquad [1.6]$$

where R represents a ratio of the heavy isotope to the light isotope (e.g., $^{15}N/^{14}N$). The reference materials for nitrogen and oxygen isotope analyses are air and Vienna Standard Mean Ocean Water (VSMOW), respectively.

During denitrification, the lighter isotope, ^{14}N, in NO_3^- is preferentially converted into N_2 gas, thereby causing the remaining NO_3^- to be enriched in the heavier isotope, ^{15}N, and the produced N_2 gas to be enriched in the lighter isotope, ^{14}N (Kendall, 1998). Likewise, nitrates containing the lighter oxygen isotope, ^{16}O, are preferentially converted into NO_2^-, NO, and N_2O first, so that the remaining nitrates are consequently enriched in the heavier oxygen isotope, ^{18}O (Kendall, 1998). Hence, increasing $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values, with corresponding decreasing nitrate concentrations, are indicative of denitrification (Gormly and Spalding, 1979; Böttcher *et al*., 1990; Aravena and Robertson, 1998).

For nitrification, the lighter nitrogen isotope, ^{14}N, in NH_4^+ is preferentially converted into NO_2^- and subsequently into NO_3^-, causing the produced NO_3^- to be relatively enriched in ^{14}N (Kendall, 1998). As a result, the residual NH_4^+ ions are increasingly enriched in the heavier ^{15}N as the reaction proceeds. As the reaction progresses, the heavier ^{15}N will also eventually be converted and the produced NO_3^- will have increasing $\delta^{15}N\text{-}NO_3$ values over time (Kendall, 1998). Therefore, increasing nitrate concentrations and low initial $\delta^{15}N\text{-}NO_3$ values, which increase gradually over time, are suggestive of nitrification (Kendall, 1998). When the nitrification reaction approaches completion (i.e., when the source ammonium is exhausted), however, the $\delta^{15}N$ value of the produced nitrate will be similar to the $\delta^{15}N$ value of the original ammonium source (Kendall, 1998).

Two oxygen atoms are obtained from H_2O (typically soil pore-water) and one oxygen atom is obtained from O_2 during the nitrification process (Hollocher *et al*., 1984; Durka *et al*., 1994; Mayer *et al*., 2001). Therefore, the theoretical $\delta^{18}O\text{-}NO_3$ value of the produced nitrate can be calculated if the $\delta^{18}O\text{-}H_2O$ and $\delta^{18}O\text{-}O_2$ values are known, using the equation:

$$\delta^{18}O\text{-}NO_3 = \tfrac{2}{3}\,\delta^{18}O\text{-}H_2O + \tfrac{1}{3}\,\delta^{18}O\text{-}O_2 \qquad [1.7]$$

The degree of isotopic fractionation caused by a particular transformation process is typically described with the isotopic enrichment factor, defined as:

$$\varepsilon = \delta_{product} - \delta_{reactant} \quad [1.8]$$

Denitrification, ammonium volatilization, and nitrification produce the largest nitrogen isotope fractionation effects (for example, ε = −12 to −29‰ for nitrification), whereas plant uptake, biological fixation, and ammonification have minor isotopic fractionation effects of only a few permil or less (Kendall, 1998). Nitrogen isotope enrichment factors for denitrification can range from −5‰ to −40‰ (Mariotti *et al*., 1982; Hübner, 1986; Kendall, 1998), and oxygen isotope enrichment factors for denitrification can range between −8 and −15‰ (Olleros, 1983; Böttcher *et al*., 1990). The extent of isotopic fractionation varies depending on the processes involved, the amount of substrate available, and environmental conditions such as temperature (Mariotti *et al*. 1982; Mariotti et al., 1988).

The $\delta^{15}N$ values of nitrate from atmospheric deposition typically range from −15 to +15‰ (Kendall *et al*., 2007), and vary between +8 to over +20‰ for manure- and sewage-derived nitrates (Kreitler, 1975; Aravena *et al*., 1993; Wassenaar, 1995). Synthetic fertilizers are produced from atmospheric nitrogen through the Haber-Bosch process. Consequently, these fertilizers have $\delta^{15}N$ values near 0‰. Nitrates derived from synthetic fertilizers have $\delta^{15}N$ values between approximately −4 to +4‰ (Kendall, 1998). Soil nitrification produces nitrates with $\delta^{15}N$ values less than about +8‰ (Heaton, 1986; Kendall, 1998). The $\delta^{18}O$ values of nitrates sourced from atmospheric deposition, synthetic fertilizers, and soil nitrification are typically greater than +50‰ (Voerkelius, 1990; Durka *et al*. 1994; Kendall *et al*., 2007), from +18 to +22‰ (Amberger and Schmidt, 1987), and from −10 to +10‰ (Kendall, 1998), respectively.

Geologic, till-derived nitrate is another potential source of groundwater nitrates in southern Alberta (Hendry *et al*., 1984; Mayer *et al*., 2004). Hendry *et al*. (1984) determined the $\delta^{15}N$ values of the till-derived nitrate to be from +8‰ to +26‰, while Mayer *et al*. (2004) measured $\delta^{15}N\text{-}NO_3$ values from +9 to +17‰ and $\delta^{18}O\text{-}NO_3$ values from +5 to +9‰.

Nitrates sourced from nitrification, synthetic fertilizers, and atmospheric deposition have fairly distinct ranges of $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values, as shown in Figure 1.2. Hence, nitrogen and oxygen isotopic ratios can serve as a method to assist in nitrate source determination and in the evaluation of nitrogen conversion processes.

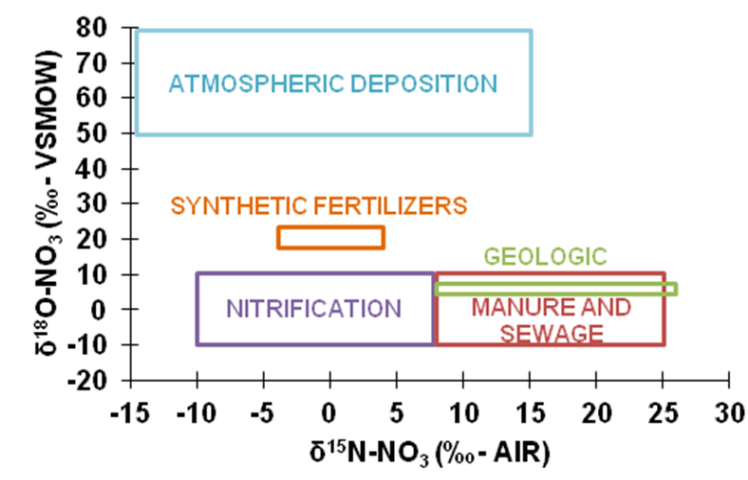

Figure 1.2: Ranges of $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values for various sources of nitrate, modified after Kendall *et al*. (2007), with geologic nitrate source range from Hendry *et al*. (1984).

1.5. Study Objectives

It is essential to have a thorough understanding of nitrogen transformation processes and the cycling of nitrogen through the soil zone and groundwater, due to the adverse health and environmental effects of nitrate loading. As indicated by previous research, nitrogen conversion processes in soils and in groundwater are highly complex. The effect of manure and synthetic fertilizers on soil and groundwater quality is dependent on many factors, such as location, crop or vegetation type, and land-use history. As a result of these factors, it is difficult to trace the path of fertilizer nitrogen in agricultural soils with chemical analyses alone. In this thesis, isotopic ratios were used in conjunction with geochemical analyses to determine the fate of nitrogen in an agricultural environment. The objectives of this study were to:

i. evaluate the extent of nitrate contamination in shallow groundwater;
ii. better understand the fate of manure- and fertilizer-derived nitrogen in agricultural soils and the associated groundwater using stable isotopes;
iii. determine the source of nitrate in the soil and shallow groundwater using isotopic techniques;
iv. identify the occurrence of any nitrogen transformation processes, such as nitrification and denitrification; and
v. distinguish differences in groundwater nitrate sources, nitrate concentrations, and nitrogen transformation processes caused by varying agricultural treatments.

CHAPTER 2: STUDY AREA

2.1. General Area

The site investigated in this thesis is located in the Oldman River Basin of southern Alberta, which spans from the Rocky Mountains to the Plains, and covers an area of approximately 28,200km^2 (Rock, 2005). There are nine irrigation districts in the Oldman River Basin, including the Lethbridge northern irrigation district, Bow River irrigation district, and St. Mary irrigation district just east of Lethbridge (Rock, 2005). The site is located at the Lethbridge Research Center managed by Agriculture and Agri-Food Canada (AAFC). Over the past century, the Lethbridge Research Center has been conducting research to increase the efficiency and profitability of beef and crop production, while maintaining a stable balance with the environment (AAFC, 2013). The study site is an experimental cropping system known as "Rotation U".

2.2. Regional Geology

The Upper Cretaceous Foremost Formation, Oldman Formation, Bearpaw Formation, Blood Reserve Formation, and St. Mary River Formation constitute the bedrock in the southern Alberta region near the city of Lethbridge (Tokarsky, 1974; AGS, 1999; see Figure 2.1). The Foremost Formation consists of light grey sandstones, siltstones, mudstones, and dark grey shales (Tokarsky, 1974; AGS, 1999), while the overlying Oldman Formation consists of light grey sandstones, grey siltstones, and dark grey shales (Nielsen, 1970; Tokarsky, 1974; AGS, 1999). The Bearpaw Formation overlies the Oldman Formation, and contains mostly marine shales and clayey sandstones (Irish, 1967; Tokarsky, 1974). The Blood Reserve Formation is composed of thickly-bedded, grey sandstones, and the overlying St. Mary River Formation consists of pale grey sandstones, siltstones, mudstones, and thin coal beds (AGS, 1999). The study site just east of Lethbridge is underlain by bedrock from the Oldman Formation (Tokarsky, 1974; AGS, 1999).

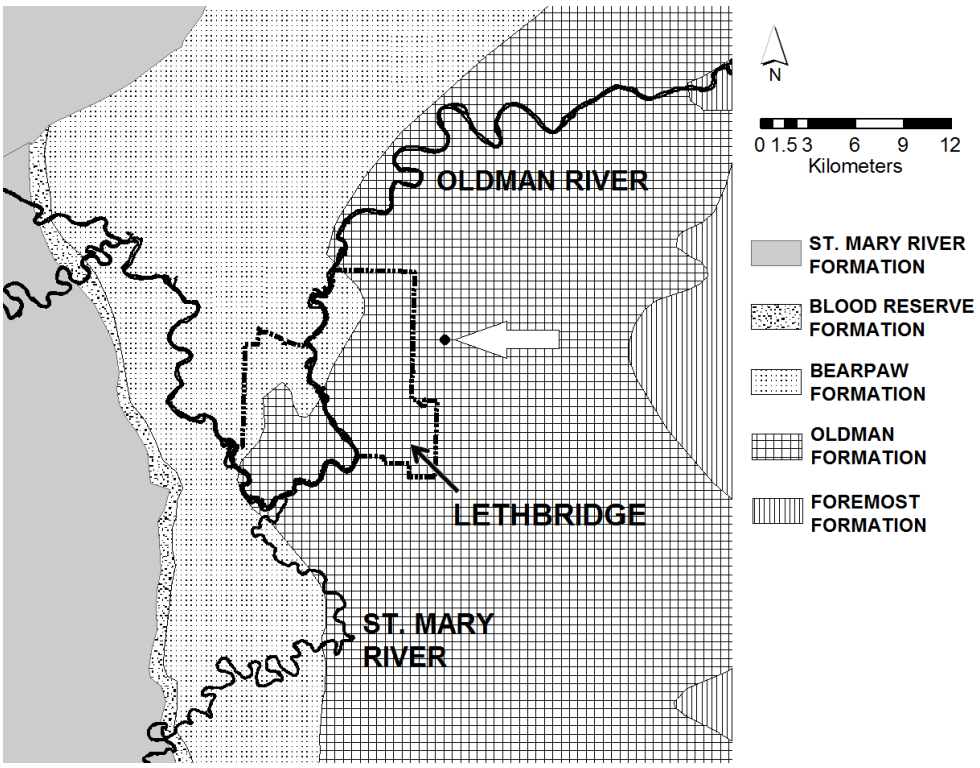

Figure 2.1: Simplified map of Lethbridge area showing bedrock geology created with data from the Alberta Geological Survey (AGS, 2004) and GeoBase Secretariat (2011). Lethbridge municipal boundaries were plotted using data from DMTI Spatial Inc. (2011). Study site is identified with white arrow.

Quaternary till covers the Upper Cretaceous bedrock in much of southern Alberta, and glacio-lacustrine deposits with clayey-silt to silty-sand textures overlie the Quaternary till at certain sites (Hendry *et al*., 1986; Hendry, 1988; Rodvang *et al*. 2004). The till is associated with the Wisconsin ice sheet and is rich in organic sulphur (Hendry *et al*., 1986; Hendry *et al*., 1989; Rodvang *et al*., 1998). The till contains discontinuous sand layers and is divided into an upper weathered till of roughly 9 to 18m in thickness and a lower non-weathered till of roughly 10 to 30m in thickness (Hendry, 1988).

2.3. Regional Hydrogeology

The upper weathered till at a site near Vauxhall, Alberta (~68km NE of Lethbridge) contains fractures of both small scale and large scale, and has a vertical groundwater flow velocity of roughly 0.1m/yr (Hendry, 1988). The small-scale fractured till and large-scale

fractured till have respective disturbed hydraulic conductivities of $5x10^{-9}$m/s and $2x10^{-7}$m/s, according to constant head permeameter experiments conducted by Hendry (1982) on disturbed cores of the upper weathered till from the Bow River irrigation district.

Rodvang *et al*. (2004) performed slug tests at a site approximately 25km north of Lethbridge, and the calculated average hydraulic conductivities were $1.3x10^{-7}$m/s and $6.7x10^{-9}$m/s for the fine-grained glacio-lacustrine deposits and the underlying till, respectively. Groundwater flow is primarily lateral in the glacio-lacustrine deposits and predominantly vertical in the underlying till (Rodvang *et al*., 2004). The fine-grained and coarse-grained glacio-lacustrine deposits have horizontal Darcy velocities of 0.05-0.24m/yr and 30-55m/yr, respectively, given horizontal hydraulic gradients of 0.01-0.02m/m and 0.0075-0.009m/m (Rodvang *et al*., 2004).

The age of the groundwater in the upper weathered till near Vauxhall ranges from 13,000 years before present to present, based on tritium and ^{14}C analyses conducted by Hendry (1986). Groundwater within the oxidized till deposits contain high concentrations of Na^+, SO_4^{2-}, and HCO_3^- (Hendry *et al*., 1986).

At the Lethbridge Research Center, the glacio-lacustrine deposits overlying the oxidized till are roughly 1m thick and, on average, the water table was detected within 1.23 to 2.50m of the soil surface, according to a study by Olson *et al*. (2005). Depths to the Quaternary till deposit range from 0.15m at a site just north of Rotation U, to 1.6m at a site less than 1km southeast of Rotation U (Beke *et al*., 1993b). Beke *et al*. (1993b) calculated the average disturbed hydraulic conductivities of the till and of the overlying glacio-lacustrine deposits and soils (averaged over all nine sites in southern Alberta) to be $2.14x10^{-6}$m/s and $1.25x10^{-5}$m/s, respectively.

2.4. Climate

Southern Alberta is dominated by a semi-arid climate, with high potential evapo-transpiration rates and low annual precipitation in many areas. Lethbridge had an average annual precipitation of approximately 400mm and an average annual temperature of 6.2°C from 1991 to 2005 (Ellert and Janzen, 2008). From 2006 to 2009, average annual precipitation was 404mm, and the average mean, average maximum, and average minimum temperatures were 6.3°C, 13.2°C, and −0.7°C, respectively (calculated from data provided by Environment Canada's National Climate Data and Information Archive; see Table 2.1 for climate data statistics). Mean

open pan evaporation was 1490mm from April to October (Ellert and Janzen, 2008).

Table 2.1: Annual precipitation and annual average mean, average maximum, and average minimum temperatures for years 2005 through 2009. Annual averages were calculated using daily mean, maximum, and minimum temperatures.

Year	**Average Temperatures (°C)**			**Annual Precipitation (mm)**
	Mean	**Max**	**Min**	
2005	5.9	13.1	−1.3	645
2006	6.8	14.1	−0.6	331
2007	6.5	13.9	−0.9	343
2008	6.2	12.7	−0.4	525
2009	5.7	12.2	−0.9	417

2.5. Site Description – Rotation U

Rotation U, located at the Lethbridge Research Center, is an experimental cropping system that was first initiated in 1911. The experimental field is roughly 946′ long by 445′ wide (~288m x ~137m) and covers about 4 hectares. The soils beneath Rotation U have loam to clay loam textures and are classified as Orthic Dark Brown Chernozems (Kocaoglu and Pettapiece, 1980; Ellert and Janzen, 2008). The calcareous soils are derived from glacio-lacustrine material, and have an average bulk density of approximately 1.4g/cm^3 (unpublished data, AAFC).

Rotation U originally had one 10-year crop rotation on ten plots of land, but in 1989, nine of the original plots, each 0.405 ha in size, were turned into three replicates of three different crop rotations, where corn (*Zea mays* L.), alfalfa (*Medicago sativa* L.), barley (*Hordeum vulgare* L.), and soft white spring wheat (*Triticum aestivum* L.) were planted. The first rotation was the alfalfa rotation (alfalfa-alfalfa-alfalfa-wheat-barley), the second rotation was the corn rotation (corn-wheat-corn-wheat-barley), and the third rotation was a hybrid of the alfalfa and corn rotations (corn-wheat-corn-wheat-barley-alfalfa-alfalfa-alfalfa-wheat-barley).

Each of the nine original plots were subdivided into twenty 73′ x 24.5′ (roughly 22m x 7.5m) subplots, which received various amounts of synthetic nitrogen fertilizer and organic fertilizer (manure). Synthetic nitrogen fertilizer was applied and incorporated into the soil annually at a rate of 100-200kgN/ha as ammonium-nitrate in late April to early May before seeding, and solid beef cattle (*Bos taurus)* manure from an unpaved feedlot was applied circa

once every five years (most recently in the fall seasons of 2001 and 2007) at a rate of 33.5MgN/ha (wet weight) to specific plots in the fall. In years up to 1989, none of the plots received synthetic fertilizer, but all plots were amended with manure once every five years. Irrigation water was applied with impact sprinklers over a period of three to four days, two to six times during the summer (~25-50mm each time).

The five main agricultural treatments to be investigated in this thesis are: i) alfalfa, ii) corn with recent nitrogen, iii) corn with recent nitrogen and manure, iv) corn with long-term nitrogen, and v) corn faba-bean. The alfalfa treatment plots were from the alfalfa crop rotation and did not receive any synthetic fertilizer or manure. Plots under the corn with long-term nitrogen (CLTN) treatment were from the corn rotation and received synthetic fertilizers at a rate of 200kgN/ha/yr in years up to and including 2003. Starting in 2004, the CLTN treatment plots received synthetic fertilizers at a rate of 100kgN/ha/yr. The corn recent nitrogen (CRN) and corn recent nitrogen & manure (CRN+m) treatment plots were from the corn rotation and did not receive any synthetic fertilizers before 2004, but were fertilized at a rate of 100kgN/ha/yr since 2004. The CRN+m treatment also received manure approximately once every five years. Plots under the corn faba-bean (CF200) treatment were from the alfalfa-corn hybrid rotation. The CF200 treatment plots received 200kgN/ha/yr of inorganic fertilizers in years up to and including 2003 (except in 1991, 1994, 1996, and 2001, when faba-bean was planted), and none since 2004. Table 2.2 summarizes the synthetic fertilization and manure application rates for each of the five treatments before and after 2004. Table 2.3 lists the crops grown on the plots of each treatment from 2006 to 2011. Figure 2.2 shows the locations of each of the treatment plots and wells.

Table 2.2: Synthetic fertilizer and manure application rates for each treatment before and after 2004.

Treatment	**Fertilization Rate (kgN/ha/yr)**		**Manure Application Rate since 1989 (Mg/ha, wet weight)**
	Before 2004	**Since 2004**	
Alfalfa	0	0	0
CRN	0	100	0
CRN+m	0	100	33.5 (approx. once every five yrs)
CLTN	200	100	0
CF200	200	0	0

Table 2.3: Crops planted from years 2006 to 2011 for each treatment.

Treatment	2006	2007	2008	2009	2010	2011
Alfalfa	Alfalfa	Wheat	Barley	Alfalfa	Alfalfa	Alfalfa
CRN	Corn	Wheat	Barley	Corn	Wheat	Corn
CRN+m	Corn	Wheat	Barley	Corn	Wheat	Corn
CLTN	Corn	Wheat	Barley	Corn	Wheat	Corn
CF200	Corn	Wheat	Barley	Alfalfa	Alfalfa	Alfalfa

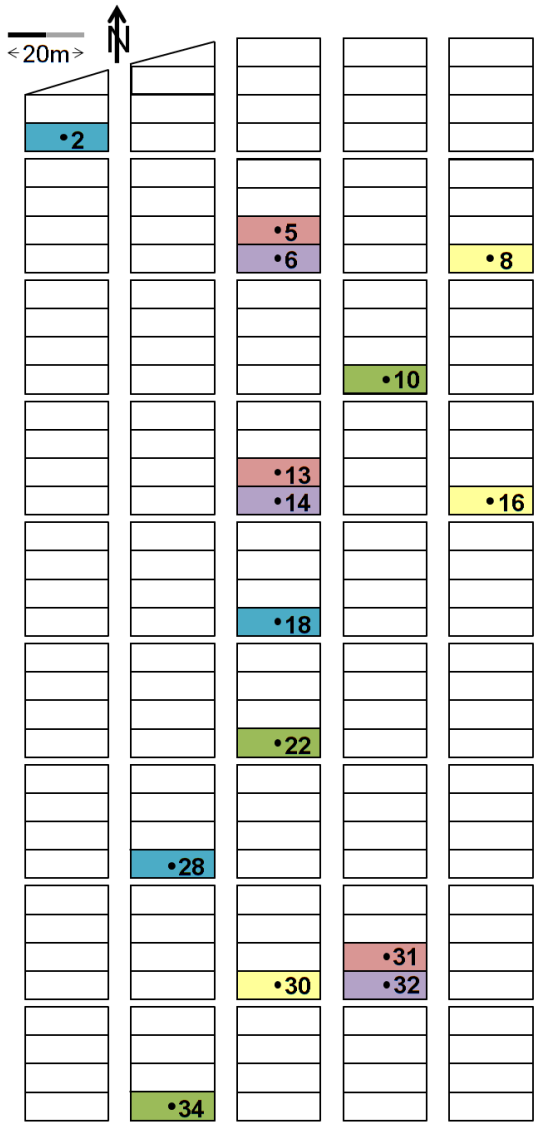

Figure 2.2: Plot plan of Rotation U. Green = Alfalfa treatment, pink = CRN+m treatment, purple = CRN treatment, yellow = CLTN treatment, blue = CF200 treatment. Well numbers and locations are identified with black circles.

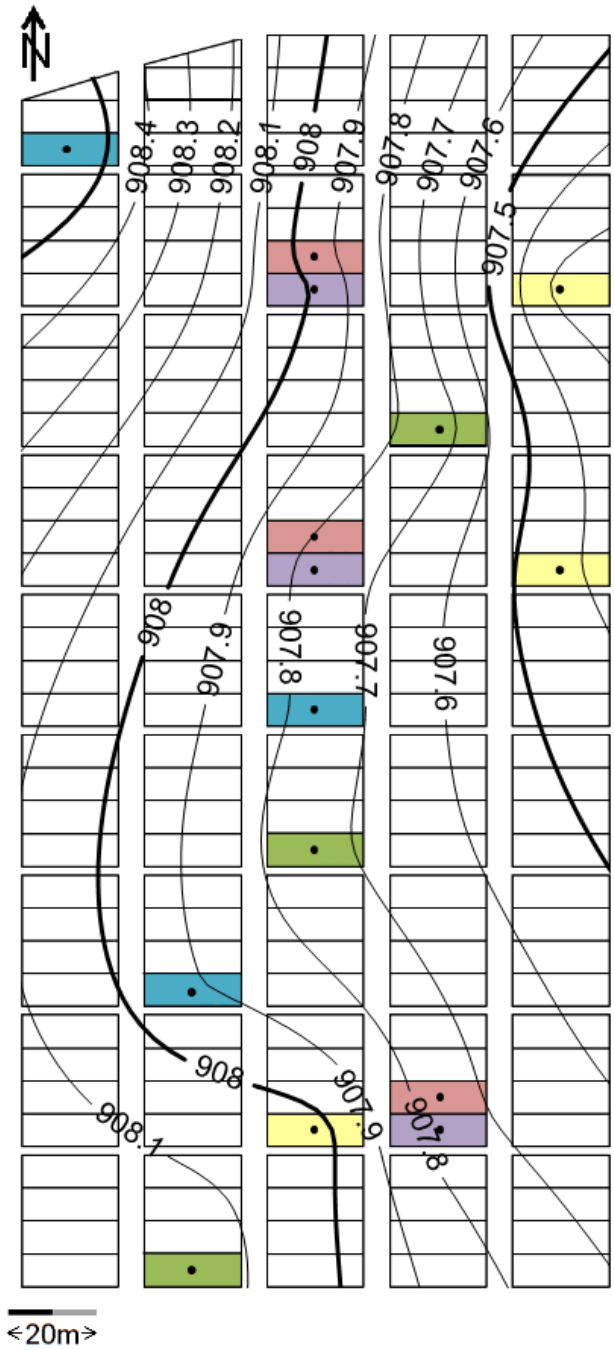

Figure 2.3: Topographic map of Rotation U plotted using unpublished soil elevation data provided by AAFC. Contour interval is 0.1m. Well locations are represented with black circles. Green = Alfalfa treatment, pink = CRN+m treatment, purple = CRN treatment, yellow = CLTN treatment, blue = CF200 treatment.

The groundwater wells on the site are 9′ deep (~2.7m), 2″ (~5cm) diameter Schedule 80 PVC pipes, with the bottom 8′ (~2.4m) perforated with 1/8″ (~0.3cm) diameter holes spaced 6″ (~15cm) apart. The wells are sealed with bentonite 1′ (~0.3m) below the soil surface and are surrounded by one inch (~2.5cm) of coarse silica sand. The wells are commonly buried beneath the soil to facilitate field operations and 2′ well extensions were installed for groundwater sample collection. Fifteen of the thirty-four wells located on Rotation U were sampled for groundwater from July 2006 to October 2009 (see Figure 2.2 for locations of sampled wells). Figure 2.3 is a topographic map of Rotation U drawn using unpublished soil elevation data provided by AAFC. The topography of the agricultural field is gently sloping towards the east at a slope of roughly 0.5° (the difference between maximum and minimum soil elevation was ~1.3m).

2.5.1. Water Table Elevations Under Rotation U

Depths to the water table (WT) were measured by AAFC personnel in July of 2006, 2007, 2008, and 2009. Water table depths ranged from 1.06m to 2.75m, and averaged 1.73±0.32m (AAFC, unpublished data). Water table elevations were calculated from the measured depths to the WT using soil surface elevations (AAFC, unpublished data) and the WT elevations were then averaged according to year to provide the values listed in Table 2.4.

Table 2.4: Water table elevation data (±SD) in meters above sea level (m ASL) for July months of years 2006 through 2009 calculated using unpublished data provided by the AAFC. See Appendix C for data.

Year	Water Table Elevation (m ASL)		
	Average	Max	Min
2006	906.51 ± 0.38	907.18	905.65
2007	905.98 ± 0.43	906.58	904.98
2008	905.94 ± 0.52	906.86	904.51
2009	906.26 ± 0.20	906.59	905.97

The highest average WT elevation of 906.51±0.38mASL was observed in 2006. In 2009, the average WT elevation was 906.26±0.20mASL, while in 2008, the average WT elevation was 905.94±0.52mASL. The water table was the lowest in 2007 (average 905.98±0.43mASL). The largest WT elevation difference (maximum WT elevation – minimum WT elevation) of 2.35m

was measured in 2008, while the smallest difference in WT elevations (0.62m) was measured in 2009.

The shape of the water table beneath Rotation U was mostly unchanged through the years 2006 to 2009 and the WT generally mimicked topography, which was gently sloping towards the east, with a maximum elevation change of approximately 1.3m (see Figure 2.3 for topography and Figure 2.4 for WT elevation maps). General groundwater flow direction was towards the east.

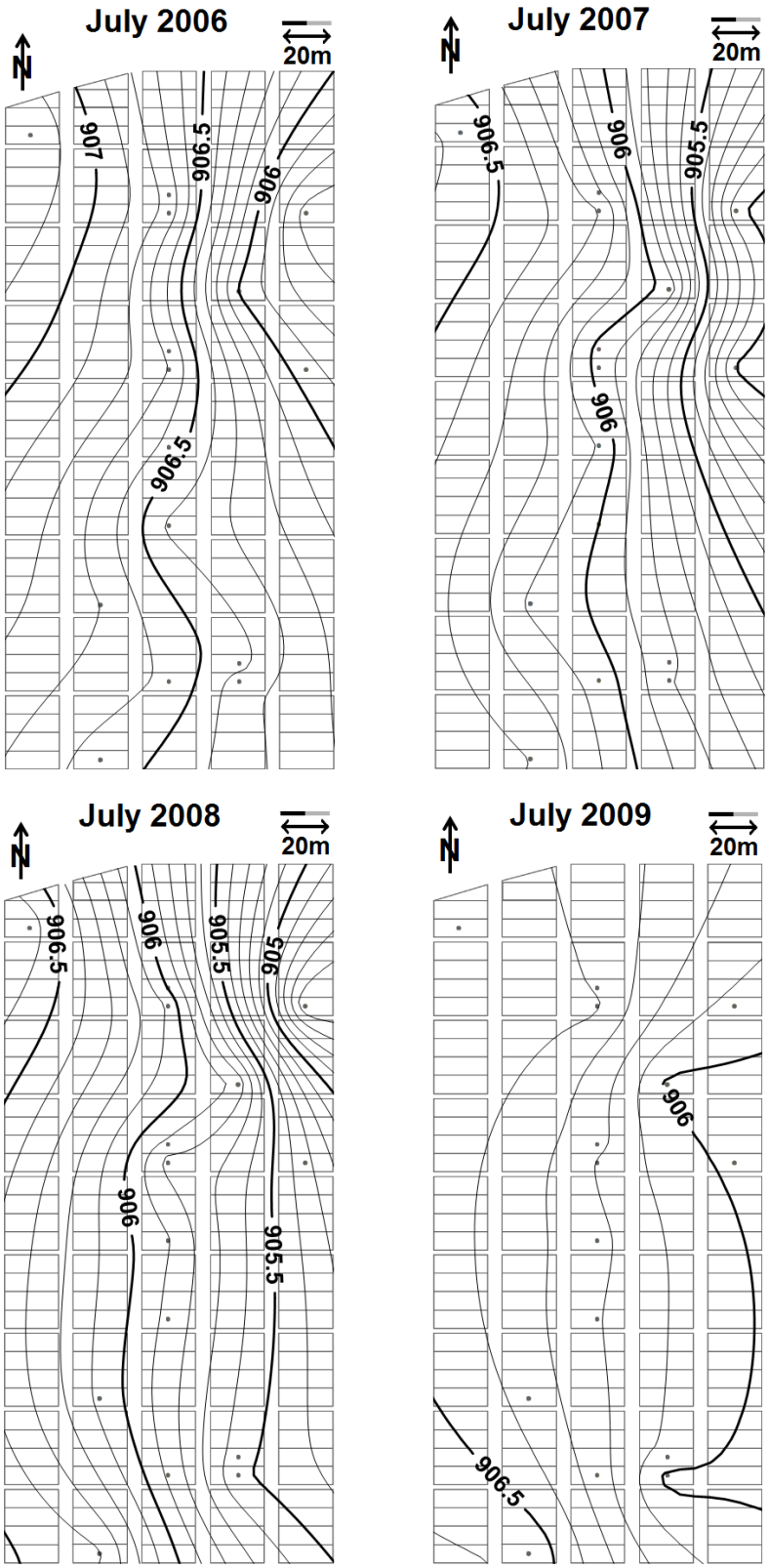

Figure 2.4: Contour maps of WT elevations (wells = black circles, contour interval = 0.1m) drawn using WT elevation data from fifteen wells.

CHAPTER 3: METHODS

3.1. Soils

Soil samples were obtained by AAFC personnel in May of 2008 using a Giddings hydraulically-driven soil sampler (Giddings Machine Co., Fort Collins, Colorado). Two soil cores (38mm in diameter) were collected from each of the 0-15cm, 15-30cm, 30-45cm, 45-60cm, and 60-90cm soil depth intervals for each of the three replicate plots under the alfalfa, CRN+m, CRN, and CF200 treatments. The two soil cores sampled from each soil depth interval and treatment plot were homogenized to provide a total of sixty soil samples (4 treatments x 3 replicate plots x 5 depth intervals = 60 samples). The soil samples were placed into foil trays and transported to the laboratory, where the soil samples were put through a 4mm sieve and were homogenized by mixing. The soil samples were then air-dried at room temperature in aluminum pans to arrest biological activity and crushed to pass a 2mm sieve using a perforated drum mill, before fine-grinding the soils to a diameter of ~0.180mm or less by tumbling in a roller mill for 24 hours. The soil samples were stored in polyethylene-lined paper bags at room temperature until further analysis.

The soils were analyzed for total nitrogen content (Barrie and Prosser, 1996) and isotopic ratios of total nitrogen using an interfaced Costech 4010 elemental analyzer and Finnigan Mat Delta+XL mass spectrometer (Preston and Owens, 1983) at the University of Calgary Isotope Science Laboratory. The samples were weighed into tin cups and flash combusted with an oxygen pulse in a quartz combustion column at a controlled temperature of 1020°C. The eluent gases were carried with a helium gas stream to a 650°C reduction furnace, where NO_x species were converted into N_2 gas. The CO_2 and N_2 gases were separated using a gas chromatograph and were then “leaked” into the isotope ratio mass spectrometer, where the areas of the sample peaks and reference peaks were compared to calculate δ^{15}N-totalN values. USGS-40 with a δ^{15}N value of −4.52±0.2‰ and USGS-41 with a δ^{15}N value of +47.57±0.2 were used as international reference materials. Analytical precision for the total nitrogen contents and δ^{15}N-totalN values were ±5% and ±0.2‰ of the measured values, respectively.

3.2. Plant Root Simulator (PRS) Probes

Plant root simulator probes (anion exchange membranes) were installed in the soils to measure nitrate supply rates (rate of adsorption) and the isotopic compositions of the adsorbed nitrates were used to identify the nitrate source(s). Each probe (3cm x 15cm x 0.5cm) contained a two-sided anion exchange membrane with a total surface area of 17.5cm^2.

To prepare the probes for insertion into the soil, the probes were first submerged in 1.5L of 0.5M $HCl_{(aq)}$ for approximately one hour to remove any residual anions from previous experiments. The probes were then rinsed thoroughly with de-ionized water and soaked with 2L of 0.5M $NaHCO_{3(aq)}$ for roughly twelve hours under constant mixing to regenerate the probes with bicarbonate ions.

Twenty-four PRS-probes were installed in the soils of the alfalfa, CLTN, CRN+m, and CRN treatment plots on August 4th, 2011 (4 treatments x 3 replicate plots x 2 probes per plot = 24 samples). The probes were inserted into the top 10-15cm of the soils at a 45° angle, approximately 2m west of the groundwater wells and 20cm away from the plant stems. For each replicate plot, two probes were installed at a distance of 76cm apart. The probes were left in the soils to adsorb nitrates for two weeks and were removed on August 18th. The probes were then placed into re-sealable Ziploc® plastic bags and kept in a portable cooler before transportation back to the laboratory within the same day. At the laboratory, the probes were immediately cleaned using de-ionized water and a soft toothbrush. The cleaned probes were then placed in new Ziploc® plastic bags and transported in a cooler to the University of Calgary, where they were kept refrigerated before analysis. On August 20th, a second set of twenty-four probes were installed into fresh soil slots approximately 1m west of the first set of probes. The probes were removed four weeks later on September 16th, 2011 and were cleaned and transported the same way as the previous set of probes.

Four days after the removal of the second set of probes, the nitrates were eluted off both sets of probes by adding various volumes of 0.5M $HCl_{(aq)}$ into Ziploc® bags containing the probes and allowing the probes to soak for two days. For the first set of probes (2-week duration PRS-probes), replicate probes installed on the same plot were eluted together in the same Ziploc® bag using ~35mL of $HCl_{(aq)}$, whereas for the second set of probes (4-week duration PRS-probes), each probe was eluted separately using ~20mL of $HCl_{(aq)}$. After soaking the probes in the acid for two days, the probes were removed from the Ziploc® bags and $Ag_2O_{(s)}$ was added

to the elutions to remove excess chloride ions through precipitation of $AgCl_{2(s)}$, in order to minimize interference during nitrate concentration determination using ion chromatography (Fu *et al*., 2007). To maximize precipitation of $AgCl_{2(s)}$, the elutions were stirred three to four times a day and were left to precipitate for approximately one week. The sample elutions were then passed through 0.1μm Millipore filters under a vacuum and were poured into 30mL high density polyethylene (HDPE) plastic bottles rinsed with de-ionized water. All elutions were kept refrigerated until further analysis.

Nitrate concentrations ($NO_2^- + NO_3^-$) of the PRS-probe extractions were determined using ion chromatography (Dionex ICS-2000). The nitrate concentration detection limit was 0.02mg/L and analytical precision was ±5% of the reported values.

The $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3 values were analyzed using the bacterially-mediated "denitrifier method" described by Sigman *et al*. (2001) and Casciotti *et al*. (2002). *Pseudomonas aureofaciens*, a strain of denitrifying bacteria, were grown in a tryptic soy broth for seven days and were then distributed into individual sample vials that were flushed with inert N_2 gas. Specific volumes of sample solution containing approximately 20-50 nmoles of nitrate were then injected into the vials, where the bacteria were allowed to convert the nitrates into N_2O gas for approximately sixteen hours. The bacteria were then lysed using an injection of $NaOH_{(aq)}$ and the samples were subsequently placed into an auto-sampler. Helium gas was used to flush the sample gas out of the vial headspace into a series of gas traps, a PreCon® device, and an HP 6890 gas chromatograph, which removed excess moisture and carbon dioxide, before the N_2O gas entered the isotope ratio mass spectrometer (Finnigan Mat Delta+XL). Peaks of the reference materials and peaks of the samples were compared to calculate the $\delta^{15}N$ and $\delta^{18}O$ values of the sample nitrate. The international reference materials, IAEA NO_3 ($\delta^{15}N$ = +4.69±0.2‰ and $\delta^{18}O$ = +25.6±0.6‰), USGS 34 ($\delta^{15}N$ = −1.80±0.2‰ and $\delta^{18}O$ = −27.9±0.6‰), and USGS 35 ($\delta^{15}N$ = +2.70±0.2‰ and $\delta^{18}O$ = +57.5±0.8‰) were used to maintain analytical precisions of ±0.5‰ for $\delta^{15}N$-NO_3 and ±1.0‰ for $\delta^{18}O$-NO_3.

Three blanks were also used to assess potential nitrate contamination. The first blank consisted of a cleaned and regenerated probe that was not installed in the field but was eluted using $HCl_{(aq)}$, precipitated for $AgCl_{2(s)}$ using $Ag_2O_{(s)}$, and filtered with 0.1μm filter paper. The second blank contained only $HCl_{(aq)}$ and $Ag_2O_{(s)}$ in the plastic bag, and was filtered for $AgCl_{2(s)}$. The third blank contained only $HCl_{(aq)}$ in the plastic bag and was left unfiltered.

Nitrate concentrations of 1.79mg/L and 1.86mg/L (corresponding "supply rates" of 2.05μg/cm^2 and 2.13μg/cm^2) were detected in the probe blank and the $HCl_{(aq)}$ + $Ag_2O_{(s)}$ blank, respectively (see Table 3.1). Potential nitrate contamination by the plastic bag, $Ag_2O_{(s)}$ and/or the filter paper may have occurred; however, the amount of contaminant nitrate from the probe was small and the measured $\delta^{15}N$ and $\delta^{18}O$ values of the contaminant nitrate (−7.5‰ and −6.7‰) were significantly different from the $\delta^{15}N$ and $\delta^{18}O$ values of the probe nitrates. Nitrate was not detected in the HCl(aq) blank. To correct for the nitrate contributed by the plastic bag, silver oxide, and/or filter paper, approximately 1.83mg/L (average nitrate concentration measured in the blanks) was subtracted from the measured nitrate concentrations before calculation of supply rates.

Nitrate supply rates were calculated by multiplying the measured PRS-probe extracted nitrate concentrations by the volume of acid added and then dividing by the total ion exchange membrane surface area (17.5cm^2 per probe eluted). Supply rates are reported in mgNO_3/cm^2 for a specific burial time (i.e., 2 weeks and 4 weeks).

To determine the amount of nitrate remaining on the probes after one extraction and to assess potential isotope fractionation during the elution process, four probes (one from each treatment) from the 4-week set of probes were re-eluted using the same methods. Repeat extractions showed evidence that some nitrate (12.3 to 15.9%) still remained adsorbed on the probes after the first elution and that nitrate isotopic fractionation did occur to a certain extent during the elution process (see Table 3.2).

Table 3.1: Specifications, nitrate concentrations, and nitrate isotopic ratios of blanks.

Blank Test	**Specifications**	**[NO_3^-] (mg/L)**	**$\delta^{15}N$-NO_3 (‰ – AIR)**	**$\delta^{18}O$-NO_3 (‰ - VSMOW)**
Probe	Unused probe, plastic bag, HCl, Ag_2O, filtered	1.79	-	-
HCl + Ag_2O	Plastic bag, HCl, Ag_2O, filtered	1.86	−7.5	−6.7
HCl	HCl only, not filtered	0.00	-	-

Table 3.2: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values of first and second PRS-probe elutions.

Treatment	**$[NO_3^-]$ (mg/L)**		**$\delta^{15}N\text{-}NO_3$ (‰ - AIR)**		**$\delta^{18}O\text{-}NO_3$ (‰ - VSMOW)**	
	First Elution	**Second Elution**	**First Elution**	**Second Elution**	**First Elution**	**Second Elution**
Alfalfa	48.5	9.2	5.8	3.8	−9.0	−12.6
CLTN	74.2	10.4	4.6	3.2	−5.2	−11.3
CRN+m	49.3	8.1	5.2	3.6	−6.7	−10.9
CRN	35.5	6.6	5.7	4.1	−6.5	−9.8

The $\delta^{15}N$ values of nitrate from the second elutions were 1.4 to 2.0‰ lower compared to the first elutions, while the $\delta^{18}O\text{-}NO_3$ values of the second elutions were 3.3 to 6.1‰ lower than the first elutions. This suggests that there is a preference for the heavier isotopes to be eluted off first. Silva *et al*. (2000) observed similar results where ^{15}N was preferentially eluted. Initial extractions had higher $\delta^{15}N\text{-}NO_3$ values than the "true value" (bulk sample) and consecutive elutions progressively decreased in $\delta^{15}N\text{-}NO_3$ values (Silva *et al*., 2000).

3.3. Groundwater

Shallow groundwater samples (n = 235) were collected by AAFC personnel on twenty-one sampling dates between July 18th, 2006 and October 5th, 2009 from fifteen wells (3 wells per treatment x 5 treatments = 15 wells) under the alfalfa, CLTN, CRN+m, CRN, and CF200 treatments. Groundwater samples were obtained using a 50mL bottle-top dispenser (Brand Gmbh. Dispensette) with a 3m long PVC intake tube that was weighted at the bottom end by 0.3m of stainless steel tubing. Before sampling, the dispenser and intake tube were thoroughly rinsed first with distilled water and then with groundwater. The intake tube was lowered to 0.2-0.3m below the water table and then, without purging the wells, groundwater samples were pumped into 30mL HDPE plastic bottles that were rinsed with groundwater. All groundwater samples were frozen and archived until analysis.

The frozen groundwater samples were analyzed at the University of Calgary Applied Geochemistry group laboratory and Isotope Science Laboratory between 2009 and 2012. The groundwater samples were thawed at room temperature for roughly 12 to 24 hours and were filtered with 0.45µm Millipore filter paper. The filtrates were poured into new 30mL HDPE

plastic bottles rinsed with de-ionized water. The filtered groundwater samples were stored in the refrigerator or were frozen until nitrate concentrations and isotopic compositions were analyzed with ion chromatography and mass spectrometry using the methods described in Section 3.2.

3.4. Statistical Methods

One-way analysis of variance (ANOVA) tests, Kruskal-Wallis tests (Kruskal and Wallis, 1952), and Welch's t-tests (Welch, 1951) were conducted using Minitab® 16 to evaluate and compare differences in various geochemical parameters (e.g., total soil nitrogen contents and $\delta^{15}N\text{-}NO_3$ values) caused by varying agricultural treatment. Histograms of the data were plotted to evaluate data normality, and Levene's tests (Levene, 1960) with 95% confidence intervals were performed to assess homoscedasticity (equality of variances). Square-root transformations were applied on non-normally distributed data to achieve normality. Outliers were identified and removed using the method by Grubbs (1969) at a confidence interval of 95% before testing the null hypothesis that there exists no difference between treatments for a specific geochemical parameter. The null hypothesis was rejected if the calculated p-values were less than the alpha value and it was therefore concluded that there was a significant difference between the tested geochemical parameters of various treatments. An alpha value of 0.05 (i.e., a 95% confidence interval) was used for all hypothesis tests.

For data with near normal distributions and equal variances, one-way ANOVA tests were used in conjunction with Tukey-Kramer post-hoc tests (Tukey, 1953; Kramer, 1956) to identify which specific treatments were statistically different. In cases where normality could not be achieved through data transformations (e.g., groundwater nitrate concentration data) non-parametric Kruskal-Wallis tests (Kruskal and Wallis, 1952) were conducted in addition to one-way ANOVA tests to compare results. Welch's t-tests (Welch, 1951) were used to test for treatment differences when data were heteroscedastic. Alpha values were corrected using the Holm-Bonferroni method (Holm, 1979) to ensure family-wise error rates of at least 0.05 for multiple Welch's t-test comparisons. Note that corrected alpha values were not reported when corresponding p-values were greater than 0.05 for simplicity and convenience, since corrected alpha values were always less than 0.05.

CHAPTER 4: RESULTS AND DISCUSSION

To accurately determine the source and fate of nitrate in groundwater, it is important to first obtain a good understanding of soil nitrogen transformation processes such as nitrification, since soil nitrogen can heavily influence groundwater nitrate concentrations. In Section 4.1, total nitrogen contents and soil total nitrogen isotopic ratios are reported and discussed. After describing the isotopic ratios of total soil nitrogen (which contains mostly organic nitrogen), the PRS-probe data are discussed in Section 4.2 to identify the sources of inorganic nitrogen (nitrate) in the soils. The $\delta^{15}N$ values of the total soil nitrogen and the $\delta^{15}N$ values of the probe-extracted nitrates are then compared at the end of Section 4.2. Lastly, results of the groundwater nitrate analyses are given in Section 4.3 and the sources of groundwater nitrates are investigated using isotopic ratios.

4.1. Soil Nitrogen

In this section, total soil nitrogen contents and the isotopic compositions of soil total nitrogen are described in detail to determine the source(s) of soil nitrogen and to evaluate any differences caused by varying agricultural treatments. Soil depth profiles are also provided to show changes in total nitrogen contents or $\delta^{15}N$-totalN values with depth.

4.1.1. Total Nitrogen Contents

Total soil nitrogen contents ranged from 0.05% to 0.22% of the dry soil mass (see Appendix A for soil data) and fell into the range of typical total soil nitrogen contents for mineral soils (0.02% to 0.50%; Brady and Weil, 2002). Since there were three replicate plots per treatment, data from replicate plots were averaged to provide a single average total nitrogen content for each treatment and depth interval. The average total nitrogen contents are tabulated in Table 4.1.

At the 0-15cm soil depth interval, the alfalfa and CRN+m treatments had the highest average total soil nitrogen contents of 0.21±0.01%, while the CRN and CF200 treatments had average total nitrogen contents of 0.19±0.00% and 0.19±0.01%, respectively. Within the 15-30cm soil layer, the alfalfa and CRN+m treatments had the highest average total soil nitrogen contents of 0.18±0.00% and 0.18±0.01%, whereas the CRN treatment had an average of

0.17±0.01%, and the CF200 treatment had the lowest average total nitrogen content of 0.16±0.01%. For the 30-45cm soil depth interval, the average total soil nitrogen content was 0.14±0.02% for the alfalfa treatment, 0.12±0.02% for the CRN+m treatment, 0.12±0.01% for the CRN treatment, and 0.11±0.01% for the CF200 treatment. In the 45-60cm soil layer, the alfalfa treatment had the highest average total nitrogen content of 0.11±0.01%, followed by the CRN+m (0.10±0.02%), CF200 (0.10±0.03%), and CRN (0.09±0.02%) treatments. At the 60-90cm soil depth range, all four treatments had identical average total nitrogen contents of 0.07% (±0.01 to 0.02%).

A diagram showing the average total nitrogen contents versus depth for each treatment is shown in Figure 4.1. Total nitrogen contents in the soils decreased gradually with depth for all treatments. Given that the soils had a total soil nitrogen content of 0.2% and a soil bulk density of 1.4g/cm^3, the top 15cm of soil contained roughly ~4200kg of nitrogen per hectare.

Table 4.1: Average total soil nitrogen contents (±SD) for each treatment and soil depth interval.

Depth (cm)	% Total Nitrogen			
	Alfalfa	**CRN+m**	**CRN**	**CF200**
0-15	0.21 ± 0.01	0.21 ± 0.01	0.19 ± 0.00	0.19 ± 0.01
15-30	0.18 ± 0.00	0.18 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
30-45	0.14 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.11 ± 0.01
45-60	0.11 ± 0.01	0.10 ± 0.02	0.09 ± 0.02	0.10 ± 0.03
60-90	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.02

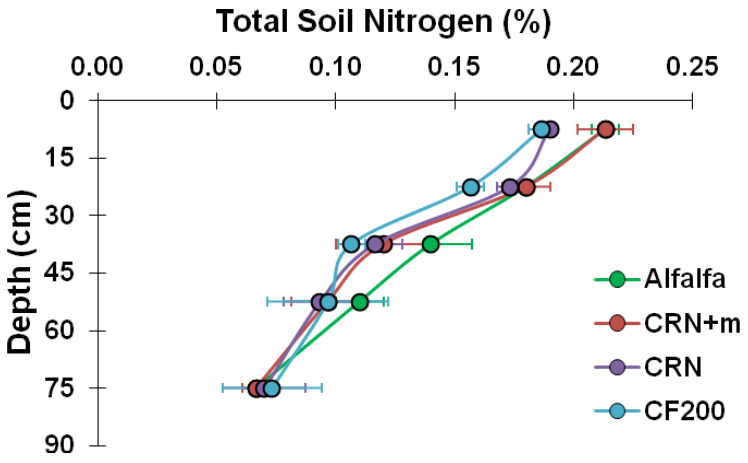

Figure 4.1: Average total soil nitrogen contents (±SD) with depth for each treatment. Note that the data are plotted at the mid-points of each soil depth interval.

For each soil depth interval, average total nitrogen contents were very similar for all four treatments, varying by only 0.02-0.03% at most. Results of multiple Welch's t-tests with 95% confidence intervals showed that there were no statistical differences between the average total soil nitrogen contents of each treatment for every given soil depth range (see Appendix D for statistical test results), with the exception of the 0-15cm depth interval, where the alfalfa treatment had a significantly higher average total nitrogen content than the CF200 treatment (corrected $\alpha = 0.0167$, $t = 5.66$, $p = 0.005$). It must be noted, however, that sample sizes were small ($n = 3$ for each treatment and soil depth range), so the statistical results should be interpreted with caution.

Treatment-specific average total soil nitrogen contents were calculated using data from all five soil depth intervals. The alfalfa (0.14±0.05%) and CRN+m (0.14±0.06%) treatments had slightly higher average total soil nitrogen contents compared to the CRN (0.13±0.05%) and CF200 (0.12±0.04%) treatments. A one-way ANOVA test with a 95% confidence interval was used to determine any statistical differences amongst the four treatments; results showed that there were no significant differences between the average total soil nitrogen contents of the four treatments ($F_{3, 56} = 0.35$, $p = 0.79$). Results from a Kruskal-Wallis test also showed that the mean ranks of total soil nitrogen contents were not statistically different between the four treatments ($\alpha = 0.05$, $H = 1.04$, $DF = 3$, $p = 0.79$).

The alfalfa, CRN+m, CRN, and CF200 treatments had very similar total soil nitrogen contents despite having received different inorganic fertilizer and manure amendments. This suggests that inorganic fertilizer and manure application did not significantly affect total soil nitrogen contents in the short term (fertilizers were applied for less than two decades). In the next section, isotopic ratios of soil total nitrogen are used to help further evaluate the effect of inorganic fertilizers and manure on soil nitrogen, and to identify the source of soil total nitrogen.

4.1.2. Isotopic Ratios of Total Nitrogen

The individual isotopic compositions of total soil nitrogen for all sixty soil samples ranged between 4.8‰ and 9.0‰ (see Appendix A for complete dataset). Data from the three replicate plots of each treatment were used to calculate an average δ^{15}N-totalN value for each treatment and depth range (listed in Table 4.2).

For the uppermost soil layer (0-15cm depth), the CRN+m treatment had the highest

average soil δ^{15}N-totalN value of 7.9±0.2‰, followed by CF200 with 7.5±0.9‰, CRN with 7.2±0.4‰, and alfalfa with 7.1±0.6‰. At the 15-30cm depth range, soils from the alfalfa treatment had the highest average δ^{15}N-totalN value of 7.8±0.8‰, while soils from the CRN+m and CRN treatments had identical average δ^{15}N-totalN values of 7.7‰ (±0.6‰ and ±0.4‰), and soils under the CF200 treatment had the lowest average value of 7.6±0.9‰. For the 30-45cm depth interval, soils under the alfalfa (7.6±0.9‰) and CF200 (7.5±1.3‰) treatments were slightly more enriched in ^{15}N compared to soils from the CRN+m (7.0±0.4‰) and CRN (6.9±0.4‰) treatments. Average soil δ^{15}N-totalN values were 7.1±0.6‰ for alfalfa, 7.0±1.3‰ for CF200, 6.7±0.3‰ for CRN+m, and 6.6±0.6‰ for CRN at the 45-60cm soil depth range. For the deepest soil layer (60-90cm depth), the CRN+m and CF200 treatments both had average soil δ^{15}N-totalN values of 6.3‰ (±1.8‰ and ±1.4‰), while soils from the CRN treatment had 5.9±0.8‰, and the alfalfa treatment had an average soil δ^{15}N-totalN value of 5.5±0.8‰.

Table 4.2: Average soil δ^{15}N-totalN (±SD) values for each treatment and soil depth interval.

Depth (cm)	**δ^{15}N-totalN (‰ - AIR)**			
	Alfalfa	**CRN+m**	**CRN**	**CF200**
0-15	7.1 ± 0.6	7.9 ± 0.2	7.2 ± 0.4	7.5 ± 0.9
15-30	7.8 ± 0.8	7.7 ± 0.6	7.7 ± 0.4	7.6 ± 0.9
30-45	7.6 ± 0.9	7.0 ± 0.4	6.9 ± 0.4	7.5 ± 1.3
45-60	7.1 ± 0.6	6.7 ± 0.3	6.6 ± 0.6	7.0 ± 1.3
60-90	5.5 ± 0.8	6.3 ± 1.8	5.9 ± 0.8	6.3 ± 1.4

At each specific depth range, variability in the average soil δ^{15}N-totalN values between treatments was small. The largest difference in average soil δ^{15}N-totalN values between treatments was only 0.8‰, which was observed in both the 0-15cm (between the alfalfa and CRN+m treatments) and 60-90cm soil depth intervals (between the alfalfa and CRN+m treatments, and between the alfalfa and CF200 treatments). Isotopic compositions of total soil nitrogen were the most similar in the 15-30cm soil depth range, since the δ^{15}N-totalN values varied by only 0.2‰ at this depth. Multiple Welch's t-tests indicated that the differences between the average soil δ^{15}N-totalN values of various treatments were not statistically significant at any given depth interval ($\alpha = 0.05$, $p = 0.08$ to 1.00; see Appendix D for statistical test results).

With respect to the average soil δ^{15}N-totalN values for each treatment calculated using data from all five depth intervals, the CF200 treatment had the highest average soil δ^{15}N-totalN

value of 7.2±1.1‰, followed by the CRN+m (7.1±1.0‰), alfalfa (7.0±1.1‰), and CRN (6.9±0.8‰) treatments. The differences between the average soil δ^{15}N-totalN values of each treatment were not larger than the measurement uncertainty of 0.2‰. A one-way ANOVA test was used to determine any statistical differences between the average soil δ^{15}N-totalN values of the four treatments using a confidence interval of 95%. Results indicated that there were no significant differences between the various treatments and their corresponding average soil δ^{15}N-totalN values ($F_{3,56} = 0.27$, $p = 0.85$). Similarly, a Kruskal-Wallis test also showed that there were no significant differences between the mean ranks of soil δ^{15}N-totalN values of each treatment ($\alpha = 0.05$, $H = 0.88$, $DF = 3$, $p = 0.83$).

The average δ^{15}N-totalN values of soils under the four treatments were quite similar, despite having received different agricultural amendments. This implies that agricultural practices, such as fertilizer and manure application, had relatively little short-term (few decades or less) impact on the δ^{15}N-totalN values of soils. If the effects of synthetic fertilizer inputs were more substantial, then the CRN treatment should have theoretically yielded an average soil δ^{15}N-totalN value closer to 0‰ (similar to the δ^{15}N value of synthetic fertilizer) rather than the measured 6.9±0.8‰. Likewise, the CRN+m treatment should have had a higher average soil δ^{15}N-totalN value than the other treatments, since manure is typically enriched in ^{15}N, and it was applied on the CRN+m treatment plots in the fall of 2007, shortly before soil sampling in the spring of 2008. However, the average soil δ^{15}N-totalN value for the CRN+m treatment (7.1±1.0‰) was similar to that of the CF200 (7.2±1.1‰) and alfalfa (7.0±1.1‰) treatments, which did not receive fertilizer or manure.

Research by Meints *et al*. (1975) indicated that increases in synthetic fertilization rates did not decrease the soil δ^{15}N-totalN of continuous corn (*Zea mays* L.) and soybean (*Glycine max* L.) plots, and it was concluded that fertilizer nitrogen inputs were not significant enough to overcome ^{15}N-enriching soil processes, such as nitrification and ammonia volatilization. The authors also noted that fertilizer effects were relatively minor, since fertilization rates were small compared to the large pool of soil total nitrogen. In another study by Gormly and Spalding (1979), the soil δ^{15}N-totalN values of unfertilized corn fields (7.6±1.3‰) were found to be similar to the soil δ^{15}N-totalN values of recently fertilized (within three weeks) corn fields (7.7±0.5‰).

The 100-200kgN/ha of nitrogen supplied annually to Rotation U through synthetic

fertilizer application was relatively small (< 5%) compared to the ~4200kgN/ha of total nitrogen in the soils. Manure was applied approximately once every five years at a rate of 33.5Mg/ha (wet weight) on the CRN+m treatment plots. The amount of nitrogen supplied by the manure was roughly 426.5kgN/ha, assuming that the manure was approximately 67% dry matter, and that the total nitrogen content was 1.9% of the dry matter (Ellert and Janzen, 2008). Therefore, the amount of additional nitrogen supplied by each manure application (circa once every five years) was only ~10% of the total nitrogen present in the soils. Application of inorganic fertilizers and manure likely did not affect the short-term average δ^{15}N-totalN values of the soil total nitrogen pool due to the fact that the nitrogen supplied through agricultural amendments was only ~5-10% of the soil total nitrogen. Over longer periods of time (i.e., several decades or more), however, the soil total nitrogen pool will likely be influenced by synthetic fertilizer and manure application more considerably.

Another possible explanation for the lack of difference in average δ^{15}N-totalN values between treatments is that the original δ^{15}N-totalN source signals were significantly altered by nitrogen transformation processes during the cycling of fertilizer-derived nitrogen in the soil zone. The average δ^{15}N-totalN value of 7.0±1.0‰ (entire dataset average) was likely a long-term cumulative value predominantly caused by recurring denitrification, nitrification (and subsequent leaching of the produced nitrates), and/or ammonia volatilization, since these processes cause the remaining soil nitrogen to be enriched in ^{15}N.

It is also possible that the soil nitrogen was relatively old and was sourced from manure that was applied in years up to 1989, which would explain the elevated δ^{15}N-totalN values. Relatively recent nitrogen sources (i.e., synthetic fertilizers) likely affected the δ^{15}N-totalN value of the soils to a lesser extent.

For all treatments except CRN+m, average soil δ^{15}N-totalN values increased slightly from the 0-15cm depth zone to the 15-30cm depth zone, before decreasing at depths greater than 30cm (see Figure 4.2). Delwiche and Steyn (1970) observed similar results, where the δ^{15}N-totalN values peaked at roughly 20cm depth for Yolo sandy loam soils obtained from an agricultural field near Davis, California. The authors suggested that the shape of the δ^{15}N-totalN curve in the soil depth profile may have been a function of soil texture, since coarse soil particles tend to be relatively depleted in ^{15}N compared to finer particles (Ledgard *et al*. 1984; Tiessen *et al*., 1984). Therefore, the soil at 15-30cm depth may have been relatively finer than soils both

above and below, causing δ^{15}N-totalN values to first increase, and then decrease with depth.

Another potential cause of the particular increase and subsequent decrease in the average δ^{15}N-totalN values with depth could be mineralization of organic nitrogen, followed by leaching of the inorganic nitrogen, assimilation of the inorganic nitrogen by plants, and/or denitrification. Mineralization of organic nitrogen causes the remaining organic nitrogen pool to be enriched in ^{15}N (Kendall, 1998); therefore, if the produced inorganic nitrogen (ammonium and nitrate) is leached, assimilated by plants, and/or denitrified afterwards, then the remaining total nitrogen pool will be enriched in the heavier nitrogen isotope. The average δ^{15}N-totalN peak at 15-30cm depth may have been caused by high mineralization rates within that soil zone, followed by leaching of the mineral nitrogen.

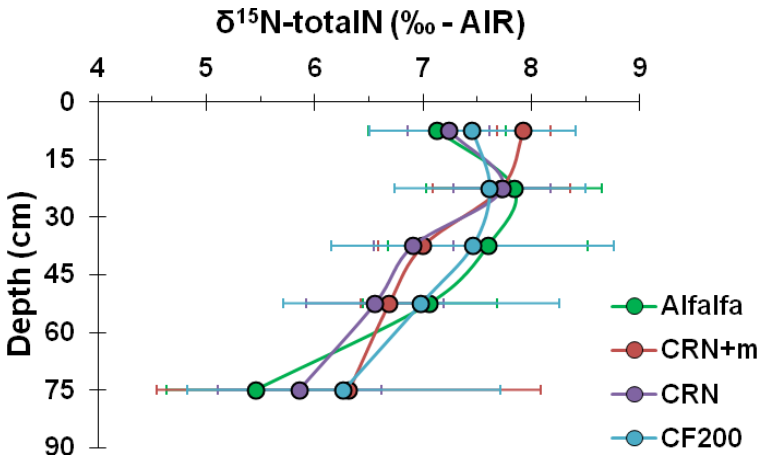

Figure 4.2: Average δ^{15}N-totalN values (±SD) with depth for each treatment. Note that the data are plotted at the mid-points of each soil depth interval.

Isotopic data suggested that the relatively recent application of synthetic fertilizers (since 1991) and manure did not significantly impact the isotopic composition of the soil nitrogen pool in the short term (treatment differences in soil δ^{15}N-totalN values may be observable after several decades of manure and synthetic fertilizer application, however). The amount of total nitrogen in the soil reservoirs is large; hence, the effects of synthetic fertilizer application were not clearly observed in the isotopic ratios of soil total nitrogen. Soil nitrogen conversion processes, such as nitrification, likely had more influence on the soil δ^{15}N-totalN values.

Although the soil δ^{15}N-totalN values were quite similar across various agricultural treatments and did not provide much insight into the sources of soil nitrogen, treatment

differences may be detectible in inorganic soil nitrogen. In the next section, soil inorganic nitrogen (nitrate) is investigated using PRS-probe data.

4.2. Plant Root Simulator (PRS) Probes

Nitrate supply rates, along with the $\delta^{15}N$ and $\delta^{18}O$ values of soil nitrates extracted from the PRS-probes, are described in Section 4.2.1 (2-week duration PRS-probes) and Section 4.2.2 (4-week duration PRS-probes) to identify potential sources of soil nitrate. Treatment differences in the soil nitrate supply rates, as well as in the $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values, are also investigated to determine the effect of various agricultural amendments on soil nitrate. The PRS-probe data are then compared to the 2008 soil data in Section 4.2.3 to determine any differences between the $\delta^{15}N$ values of total nitrogen and the $\delta^{15}N$ values of soil nitrate.

4.2.1. Two-week Duration PRS-probes

For the 2-week duration set of PRS-probes, two probes were installed on each of the twelve plots and the two probes were eluted together to provide a single nitrate sample (twelve samples in total). Individual nitrate supply rates for the twelve samples were highly variable and ranged from 0.046mgNO_3/cm^2/2wks to 0.25mgNO_3/cm^2/2wks, with an overall average of 0.11±0.056mgNO_3/cm^2/2wks (see Appendix B for PRS-probe data).

Data from replicate plots were averaged together to provide an average supply rate, average $\delta^{15}N\text{-}NO_3$ value, and average $\delta^{18}O\text{-}NO_3$ value for each treatment (tabulated in Tables 4.3a-d). Soils under the CRN treatment had the highest average supply rate of 0.13±0.11mgNO_3/cm^2/2wks, followed by soils from the CRN+m (0.11±0.040mgNO_3/cm^2/2wks), alfalfa (0.11±0.0058mgNO_3/cm^2/2wks), and CLTN (0.079±0.045mgNO_3/cm^2/2wks) treatments.

The differences between the average nitrate supply rates of the various treatments were not statistically significant, as indicated by multiple Welch's t-tests ($\alpha = 0.05$, $p = 0.38$ to 0.87; see Appendix D for statistical test results); however, the statistical results should be interpreted with caution, since sample sizes were small ($n = 3$ for each treatment). Agricultural treatment likely did not have a substantial effect on the nitrate supply rates of the soils, since both unfertilized and fertilized plots had similar nitrate supply rates.

Table 4.3a: Statistical data of nitrate supply rates, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values for the 2-week PRS-probe extractions from the alfalfa treatment.

Statistic	**$mgNO_3/cm^2/2wks$**	**$\delta^{15}N\text{-}NO_3$ (‰ - AIR)**	**$\delta^{18}O\text{-}NO_3$ (‰ - VSMOW)**
mean	0.11	3.8	−7.1
max	0.11	5.1	−6.4
min	0.10	2.2	−7.8
stdev	0.0058	1.5	0.7
n	3	3	3
SE	0.0033	0.9	0.4

Table 4.3b: Statistical data of nitrate supply rates, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values for the 2-week PRS-probe extractions from the CRN+m treatment.

Statistic	**$mgNO_3/cm^2/2wks$**	**$\delta^{15}N\text{-}NO_3$ (‰ - AIR)**	**$\delta^{18}O\text{-}NO_3$ (‰ - VSMOW)**
mean	0.11	8.7	−4.7
max	0.16	10.7	−4.0
min	0.085	7.7	−5.8
stdev	0.040	1.8	0.9
n	3	3	3
SE	0.023	1.0	0.5

Table 4.3c: Statistical data of nitrate supply rates, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values for the 2-week PRS-probe extractions from the CRN treatment.

Statistic	**$mgNO_3/cm^2/2wks$**	**$\delta^{15}N\text{-}NO_3$ (‰ - AIR)**	**$\delta^{18}O\text{-}NO_3$ (‰ - VSMOW)**
mean	0.13	6.9	−5.6
max	0.25	7.6	−3.3
min	0.061	5.8	−6.9
stdev	0.11	1.0	2.0
n	3	3	3
SE	0.062	0.6	1.1

Table 4.3d: Statistical data of nitrate supply rates, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values for the 2-week PRS-probe extractions from the CLTN treatment.

Statistic	$mgNO_3/cm^2/2wks$	$\delta^{15}N\text{-}NO_3$ (‰ - AIR)	$\delta^{18}O\text{-}NO_3$ (‰ - VSMOW)
mean	0.079	8.8	−5.3
max	0.13	10.4	−3.4
min	0.046	6.5	−6.9
stdev	0.045	2.0	1.8
n	3	3	3
SE	0.026	1.2	1.0

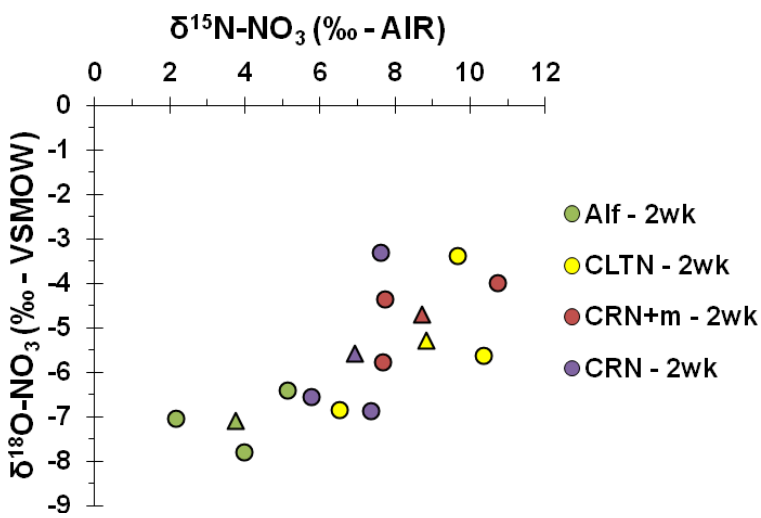

Figure 4.3: Dual isotope diagram ($\delta^{18}O\text{-}NO_3$ versus $\delta^{15}N\text{-}NO_3$ values) of 2-week duration PRS-probe nitrates (n = 12). Average values are plotted as triangles.

Figure 4.3 is a dual isotope diagram showing the $\delta^{18}O$ versus $\delta^{15}N$ values of nitrates eluted from the 2-week duration PRS-probes. Individual $\delta^{15}N$ values of probe-extracted nitrates ranged between 2.2‰ and 10.7‰, while individual $\delta^{18}O\text{-}NO_3$ values ranged between −3.3‰ and −7.8‰.

Nitrates extracted from the CLTN and CRN+m treatment PRS-probes had the highest average $\delta^{15}N$ values of 8.8±2.0‰ and 8.7±1.8‰, respectively. The CRN treatment had an average eluted $\delta^{15}N\text{-}NO_3$ value of 6.9±1.0‰, while the alfalfa treatment had the lowest average eluted $\delta^{15}N\text{-}NO_3$ value of 3.8±1.5‰. Probe extractions from the alfalfa treatment had the lowest

average $\delta^{18}O$-NO_3 value of −7.1±0.7‰. Average $\delta^{18}O$ values of nitrates extracted from the CRN, CLTN, and CRN+m PRS-probes were −5.6±2.0‰, −5.3±1.8‰, and −4.7±0.9‰, respectively.

Statistical analyses indicated that there were no significant differences between the average $\delta^{15}N$ values of probe-extracted nitrates from the CLTN, CRN+m, and CRN treatments (Welch's t-tests, $\alpha = 0.05$, $p = 0.22$ to 0.94; see Appendix D for statistical test results). Eluted nitrates from the alfalfa treatment had distinctively lower $\delta^{15}N$-NO_3 values compared to eluted nitrates from the three corn treatments (see Figure 4.3), which appears logical for a nitrogen-fixing crop such as alfalfa, but this difference was not statistically significant according to Welch's t-tests (corrected $\alpha = 0.008$, 0.01, 0.0125; $p = 0.034$, 0.04, 0.056; see Appendix D).

The average $\delta^{15}N$ and average $\delta^{18}O$ values of nitrates eluted from the CRN+m, CRN, and CLTN treatment PRS-probes were very similar, despite the CRN+m treatment having received manure, whereas the CRN and CLTN treatments did not. Since the last manure application event within the study period was in the fall of 2007 and the probes were installed in 2011, the majority of the mineralized nitrogen derived from manure was likely assimilated by plants, leached, and/or denitrified by 2011. Without the effect of manure, the CRN+m, CRN, and CLTN treatments were essentially the same, since all three treatments received 100kgN/ha of synthetic fertilizers per year since 2004 (CLTN received 200kgN/ha/yr prior to 2004, but this effect may have dissipated by 2011).

Isotopic data showed evidence that the source of soil nitrates under the corn treatments was likely ammonification of non-leguminous soil organic matter, followed by nitrification. According to Oelmann *et al*. (2007), nitrates produced by mineralization of leguminous SOM (soil organic matter) and by mineralization of non-leguminous SOM have distinct $\delta^{15}N$-NO_3 values. An average $\delta^{15}N$-NO_3 value of 9.3±0.9‰ and an average $\delta^{18}O$-NO_3 value of 5.7±0.8‰ was obtained for nitrates derived from mineralization of non-leguminous SOM (Oelmann *et al*., 2007). The CRN+m, CRN, and CLTN treatments had probe-extracted nitrates with average $\delta^{15}N$ values within this range (8.7±1.8‰, 6.9±1.0‰, and 8.8±2.0‰, respectively); however, the average $\delta^{18}O$-NO_3 values (−4.7±0.9‰, −5.6±2.0‰, and −5.3±1.8‰, respectively) were significantly lower than the value reported by Oelmann *et al*. (2007).

During nitrification, two oxygen atoms are taken from water and one oxygen atom is taken from atmospheric O_2 to form NO_3^- (Hollocher *et al*., 1984; Durka *et al*., 1994; Wassenaar, 1995; Mayer *et al*., 2001). Nitrates produced from nitrification should have $\delta^{18}O$-NO_3 values of

−3‰, according to equation [1.7], given that the $\delta^{18}O$ value of water was approximately −16‰ (Rock, 2005) and that the $\delta^{18}O$-O_2 value was roughly +23‰ (Clark and Fritz, 1997; Kendall, 1998). Probe elutions from the CRN+m, CRN, and CLTN treatments had average $\delta^{18}O$-NO_3 values (−4.7±0.9‰, −5.6±2.0‰, and −5.3±1.8‰, respectively) that were similar to the theoretical value of −3‰.

Nitrates eluted from probes under the alfalfa treatment had an average $\delta^{15}N$ value of 3.8±1.5‰ and an average $\delta^{18}O$-NO_3 value of −7.1±0.7‰. The low average $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3 values suggest that the source of soil nitrates in the alfalfa treatment plots was mineralization of leguminous SOM. Nitrates produced from the mineralization of leguminous SOM have $\delta^{15}N$-NO_3 values of 1.5±0.6‰ and $\delta^{18}O$-NO_3 values of 5.1±0.9‰, according to the incubation experiment conducted by Oelmann *et al*. (2007). Although the nitrate extractions from the alfalfa treatment PRS-probes had an average $\delta^{15}N$-NO_3 value of 3.8±1.5‰ (similar to the 1.5±0.6‰ reported by Oelmann *et al*., 2007), the average eluted $\delta^{18}O$-NO_3 value was lower than the theoretical value of −3‰ for nitrates produced from nitrification.

A potential reason for this discrepancy is that the $\delta^{18}O$-H_2O value of the 2011 soil waters were more negative than the −16‰ measured by Rock (2005). According to equation [1.7], the soil waters needed to have a $\delta^{18}O$-H_2O of −22.4‰ in order to have produced nitrates with a $\delta^{18}O$-NO_3 value of -7.1‰. This is not likely, considering that soil waters tend to have higher $\delta^{18}O$-H_2O than groundwater (Kendall, 1998).

Another possible explanation for the low average $\delta^{18}O$-NO_3 value is biochemical oxygen exchange. Kool *et al*. (2007) stated that during intermediate steps in nitrification, particularly during the step where NO_2^- is oxidized to NO_3^-, oxygen exchange with H_2O can occur if the step is reversible. If oxygen exchange with water had occurred, then the $\delta^{18}O$ values of the nitrates derived from nitrification could have potentially been much lower than the estimated −3‰, depending on the extent of oxygen exchange. Isotopic fractionation during adsorption may have also caused the $\delta^{18}O$-NO_3 values to be lower than the expected value of −3‰ if ^{14}N was preferentially adsorbed onto the PRS-probes.

Isotopic data from the 2-week set of PRS-probes indicated that soil nitrates under the corn treatment plots were likely sourced from the mineralization of non-leguminous SOM, while the soil nitrates under the alfalfa treatment plots were likely sourced from the mineralization of

leguminous SOM. In the next section, results of the 4-week duration PRS-probe field experiment are discussed.

4.2.2. Four-week Duration PRS-probes

For the 4-week duration PRS-probes, two probes were installed on each replicate treatment plot, for a total of twenty-three probes (one broke during removal). Each probe was eluted separately, providing six nitrate samples for each treatment (CRN had five).

Nitrate supply rates ranged from 0.0049 to 0.17mgNO_3/cm^2/4wks (individual data), and averaged 0.050±0.039mgNO_3/cm^2/4wks (see Appendix B for full dataset). Supply rate data for the 4-week duration were quite variable; probes located less than one meter apart on the same plot had very different nitrate supply rates. For example, one plot from under the CLTN treatment had replicate probes with supply rates of 0.025mgNO_3/cm^2/4wks and 0.17mgNO_3/cm^2/4wks.

The nitrate supply rate data were averaged according to treatment to provide the values listed in Tables 4.4a-d. Note that only the first set of elutions were used to calculate the average δ^{15}N-NO_3 values. The highest average nitrate supply rate of 0.066±0.057mgNO_3/cm^2/4wks was from the CLTN treatment. The CRN treatment had a lower average nitrate supply rate of 0.060±0.049mgNO_3/cm^2/4wks, while the alfalfa and CRN+m treatments had average supply rates of 0.044±0.022mgNO_3/cm^2/4wks and 0.030±0.018mgNO_3/cm^2/4wks, respectively. Results from a one-way ANOVA test with a 95% confidence interval showed that there were no statistically significant differences in the nitrate supply rates of the various treatments ($F_{3,19}$ = 0.98, p = 0.42). A Kruskal-Wallis test indicated that there were no significant differences between the mean ranks of nitrate supply rates of each treatment (α = 0.05, H = 2.38, DF = 3, p = 0.50). These results further suggest that fertilizer and manure amendments had little effect on the nitrate supply rates of soils.

Although supply rates were highly variable, there was comparatively low variability in the δ^{15}N and δ^{18}O values of nitrates extracted from the PRS-probes. Nitrate δ^{15}N values (n = 23) ranged between 2.1‰ and 9.0% (see Figure 4.4) and averaged 5.8±1.6‰ for all treatments. The δ^{18}O values of the eluted nitrates ranged between −14.9‰ and −3.0‰ and averaged −7.6±2.9‰ for all treatments.

Table 4.4a: Statistical data of nitrate supply rates, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values for the 4-week PRS-probe extractions from the alfalfa treatment.

Statistic	**$mgNO_3/cm^2/4wks$**	**$\delta^{15}N\text{-}NO_3$ (‰ – AIR)**	**$\delta^{18}O\text{-}NO_3$ (‰ – VSMOW)**
mean	0.044	5.9	−9.3
max	0.064	8.7	−7.1
min	0.076	4.4	−14.9
stdev	0.022	1.5	2.8
n	6	6	6
SE	0.0089	0.6	1.1

Table 4.4b: Statistical data of nitrate supply rates, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values for the 4-week PRS-probe extractions from the CRN+m treatment.

Statistic	**$mgNO_3/cm^2/4wks$**	**$\delta^{15}N\text{-}NO_3$ (‰ – AIR)**	**$\delta^{18}O\text{-}NO_3$ (‰ – VSMOW)**
mean	0.030	6.3	−9.0
max	0.054	7.8	−6.2
min	0.0049	4.9	−13.9
stdev	0.018	1.2	3.6
n	6	6	6
SE	0.0074	0.5	1.5

Table 4.4c: Statistical data of nitrate supply rates, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values for the 4-week PRS-probe extractions from the CRN treatment.

Statistic	**$mgNO_3/cm^2/4wks$**	**$\delta^{15}N\text{-}NO_3$ (‰ – AIR)**	**$\delta^{18}O\text{-}NO_3$ (‰ – VSMOW)**
mean	0.060	5.7	−6.7
max	0.14	6.8	−5.3
min	0.015	3.2	−8.3
stdev	0.049	1.4	1.4
n	5	5	5
SE	0.022	0.6	0.6

Table 4.4d: Statistical data of nitrate supply rates, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values for the 4-week PRS-probe extractions from the CLTN treatment.

Statistic	$mgNO_3/cm^2/4wks$	$\delta^{15}N\text{-}NO_3$ (‰ – AIR)	$\delta^{18}O\text{-}NO_3$ (‰ – VSMOW)
mean	0.066	5.1	−5.2
max	0.17	9.0	−3.0
min	0.014	2.1	−6.7
stdev	0.057	2.3	1.3
n	6	6	6
SE	0.023	1.0	0.5

Eluted nitrates from PRS-probes under the CRN+m treatment had the highest average $\delta^{15}N$ value of 6.3±1.2‰, followed by nitrates extracted from the alfalfa (5.9±1.5‰), CRN (5.7±1.4‰), and CLTN (5.1±2.3‰) treatment PRS-probes. Probes from the alfalfa, CRN+m, CRN, and CLTN treatment plots yielded extracted nitrates with average $\delta^{18}O\text{-}NO_3$ values of −9.3±2.8‰, −9.0±3.6‰, −6.7±1.4‰, and −5.2±1.3‰, respectively. A one-way ANOVA test with a 95% confidence interval indicated that there were no significant differences between the average $\delta^{15}N\text{-}NO_3$ values of the four treatments ($F_{3,19} = 0.51$, $p = 0.68$). Results of a Kruskal-Wallis test also showed that there were no significant differences in the mean ranks of $\delta^{15}N\text{-}NO_3$ values between treatments ($\alpha = 0.05$, $H = 2.14$, $DF = 3$, $p = 0.54$).

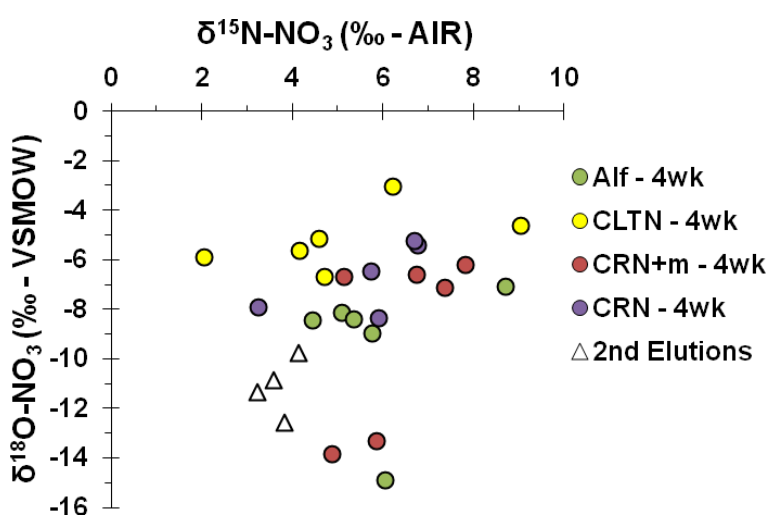

Figure 4.4: Dual isotope diagram ($\delta^{18}O\text{-}NO_3$ versus $\delta^{15}N\text{-}NO_3$ values) of 4-week duration PRS-probe nitrates (n = 23) and second nitrate extractions (n = 4).

The isotopic composition data of the eluted nitrates suggest that the soil nitrates adsorbed on the PRS-probes were sourced from mineralization of SOM, particularly of non-leguminous SOM ($\delta^{15}N\text{-}NO_3$ of 9.3±0.9‰, from Oelmann *et al*., 2007), for all four treatments. The alfalfa treatment, despite being a legume treatment, had soil $\delta^{15}N\text{-}NO_3$ values that implied that mineralization of non-leguminous SOM, rather than mineralization of leguminous SOM, was the source of soil nitrates. Oelmann *et al*. (2005) explained that SOM could be separated into two pools: (1) readily available nitrogen compounds, and (2) less readily available organic nitrogen compounds. During the first two weeks of their 8-week soil incubation experiment, mainly nitrogen sourced from leguminous SOM was mineralized, while during later weeks, mineralization of less labile, non-leguminous SOM was dominant. Therefore, since the probes remained in the soils for four weeks, the adsorbed nitrates under the alfalfa treatment may have been derived from a combination of both mineralization of leguminous SOM (possibly to a lesser extent) and mineralization of non-leguminous SOM (possibly with a larger contribution).

The average $\delta^{18}O$ values for nitrates eluted from the PRS-probes ranged from −5.2 to −9.3‰. These average $\delta^{18}O\text{-}NO_3$ values were considerably lower than the theoretical value of −3‰ for nitrification-derived nitrates. This may be due to oxygen exchange with water or isotopic fractionation during nitrate adsorption, as discussed in Section 4.2.1.

4.2.3. Comparison of Probe-Extracted Nitrates with Soil Total Nitrogen

In this section, the average $\delta^{15}N\text{-}NO_3$ values of the 2-week and 4-week PRS-probe extractions are compared to the average $\delta^{15}N$-totalN values of the 2008 soils sampled from the 0-15cm depth interval (see Table 4.5).

For the alfalfa treatment, results from a Welch's t-test with a 95% confidence interval ($t = 3.59$, $p = 0.07$) indicated that the average $\delta^{15}N$ value of total nitrogen in the 2008 soil samples (7.1±0.6‰) was not significantly different from the average $\delta^{15}N$ value of eluted nitrates from the 2-week PRS-probes (3.8±1.5‰). Similarly, results ($t = 1.73$, $p = 0.13$) showed that the average $\delta^{15}N$-totalN value for the 2008 soils sampled from the alfalfa treatment was not significantly different from the average $\delta^{15}N$ value of nitrates eluted from the alfalfa treatment 4-week PRS-probes (5.9±1.5‰).

For the CRN treatment, there was no statistical difference between the average $\delta^{15}N$-totalN value of the 2008 soils (7.2±0.4‰) and the average eluted $\delta^{15}N\text{-}NO_3$ value of the 2-week

PRS-probes (6.9±1.0‰), according to a Welch's t-test ($\alpha = 0.05$, $t = 0.52$, $p = 0.66$). The average δ^{15}N-totalN value of the 2008 soil samples from the CRN treatment was not significantly different from the average δ^{15}N value (5.7±1.4‰) of nitrates extracted from the CRN treatment 4-week PRS-probes ($t = 2.30$, $p = 0.08$).

For the CRN+m treatment, the average δ^{15}N-totalN value of 7.9±0.2‰ for the 2008 soils was was not statistically different from the average δ^{15}N-NO_3 value of 8.7±1.8‰ for the 2-week probe elutions (Welch's t-test, $\alpha = 0.05$, $t = -0.77$, $p = 0.52$). However, statistical test results (corrected $\alpha = 0.025$, $t = 3.20$, $p = 0.024$) indicated that the 2008 soils sampled from the CRN+m treatment had an average δ^{15}N-totalN value that was significantly higher than the average δ^{15}N-NO_3 value of 6.3±1.2‰ for the 4-week PRS-probe extractions.

In general, the δ^{15}N values of the probe-extracted nitrates were similar to the δ^{15}N values of the soil total nitrogen (~7‰), which is composed of mostly organic nitrogen, possibly because the soil nitrates adsorbed by the 2-week and 4-week PRS-probes were sourced from mineralization of SOM.

Table 4.5: Averages (±SD) and ranges of δ^{15}N values for the 2008 soils (total nitrogen) and 2011 PRS-probes (nitrates).

Treatment	**2-Week δ^{15}N-NO_3 (‰ - AIR)**		**4-Week δ^{15}N-NO_3 (‰ - AIR)**		**Soil δ^{15}N-totalN (‰ - AIR)**	
	average	**range**	**average**	**range**	**average**	**range**
Alfalfa	3.8 ± 1.5	2.2 - 5.1	5.9 ± 1.5	4.4 - 8.7	7.1 ± 0.6	6.5 - 7.8
CRN+m	8.7 ± 1.8	7.7 - 10.7	6.3 ± 1.2	4.9 - 7.8	7.9 ± 0.2	7.8 - 8.2
CRN	6.9 ± 1.0	5.8 - 7.6	5.7 ± 1.4	3.2 - 6.8	7.2 ± 0.4	6.8 - 7.6

The average δ^{15}N values of the total soil nitrogen and the average δ^{15}N values of the soil nitrates imply that mineralization of SOM was a dominant, recurring process in the soils of all three treatments. If leaching of soil nitrates produced from mineralization of SOM was also the primary source of groundwater nitrates, then the δ^{15}N-NO_3 values of groundwater nitrate should also theoretically be in the range of +2 to +11‰ (similar to probe-eluted nitrates and total soil nitrogen). This range of probe-extracted δ^{15}N-NO_3 values is used to help identify mineralization of SOM as a potential source of groundwater nitrates in Section 4.3.2.3.

4.3. Groundwater

In this section, nitrate concentrations, along with the $\delta^{15}N$ and $\delta^{18}O$ values of groundwater nitrate, are reported in an attempt to determine potential source(s) of nitrate in the shallow groundwater, and to establish any differences in groundwater nitrate concentration and/or groundwater nitrate isotopic composition caused by varying agricultural treatments. In addition, nitrogen conversion processes, such as nitrification and denitrification, are identified by correlating nitrate isotopic composition data with groundwater nitrate concentrations.

4.3.1. Nitrate Concentrations

4.3.1.1. Data Overview

Nitrate concentrations ranged from 0.7mg/L to 1276.6mg/L for all shallow groundwater samples (n = 235) obtained from all fifteen wells under the alfalfa, CLTN, CRN+m, CRN, and CF200 treatments (5 treatments x 3 wells per treatment = 15 wells; see Table 4.6 for summary statistics and Appendix C for groundwater data). The samples had an average nitrate concentration of 187.7mg/L and a standard deviation of 193.1mg/L. The groundwater nitrate concentration data was skewed to the right (see Figure 4.5), and a significant number of samples (n = 143) had nitrate concentrations higher than 100mg/L. Only fifty-three samples had nitrate concentrations below the WHO (2008) nitrate drinking water limit of 45mg/L, indicating that the groundwater beneath Rotation U was severely contaminated. Two samples under the CLTN treatment had groundwater nitrate concentrations > 1000mg/L.

Table 4.6: Descriptive statistics of nitrate concentrations for all groundwater samples from all fifteen wells.

Statistic	**Groundwater [NO_3^-] (mg/L)**
mean	187.7
median	139.8
max	1276.6
min	0.7
stdev	193.1
n	235
SE	12.6

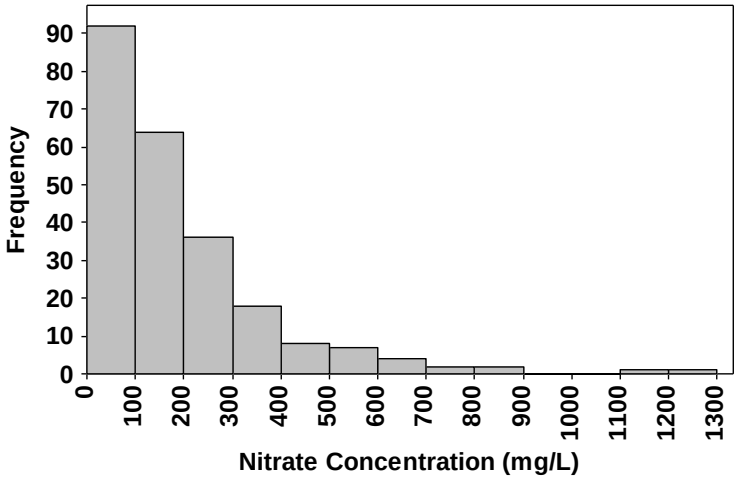

Figure 4.5: Histogram of all groundwater nitrate concentration data (n = 235) obtained from all fifteen wells.

4.3.1.2. Temporal Variations

In this section, temporal changes in the groundwater nitrate concentrations beneath Rotation U are discussed to evaluate the effect of fertilizer rate changes and manure application on groundwater nitrate concentrations. Figure 4.6 shows the groundwater nitrate concentrations for all samples obtained from all fifteen wells (n = 235) from July of 2006 to October of 2009 for each specific treatment.

The individual groundwater data from all five treatments were then grouped and averaged together with respect to year, and the corresponding statistical data are summarized in Table 4.7. Data from fourteen wells were used in the statistical calculations, because groundwater data from Well 22 of the alfalfa treatment were excluded, since groundwater from Well 22 consistently had significantly lower nitrate concentrations (6.9±5.7mg/L) than the other two wells under the alfalfa treatment. Note that the two outliers with nitrate concentrations > 1000mg/L were also excluded from the statistical calculations to prevent skewing of average values.

The average groundwater nitrate concentration (averaged over all treatments) was 275.6±205.6mg/L in 2006, 210.9±178.5mg/L in 2007, 141.9±92.4mg/L in 2008, and 183.4±169.1mg/L in 2009. Average groundwater nitrate concentrations decreased from 2006 to 2008, and subsequently increased from 2008 to 2009. Overall, the average groundwater nitrate concentration decreased approximately 92mg/L from 2006 to 2009.

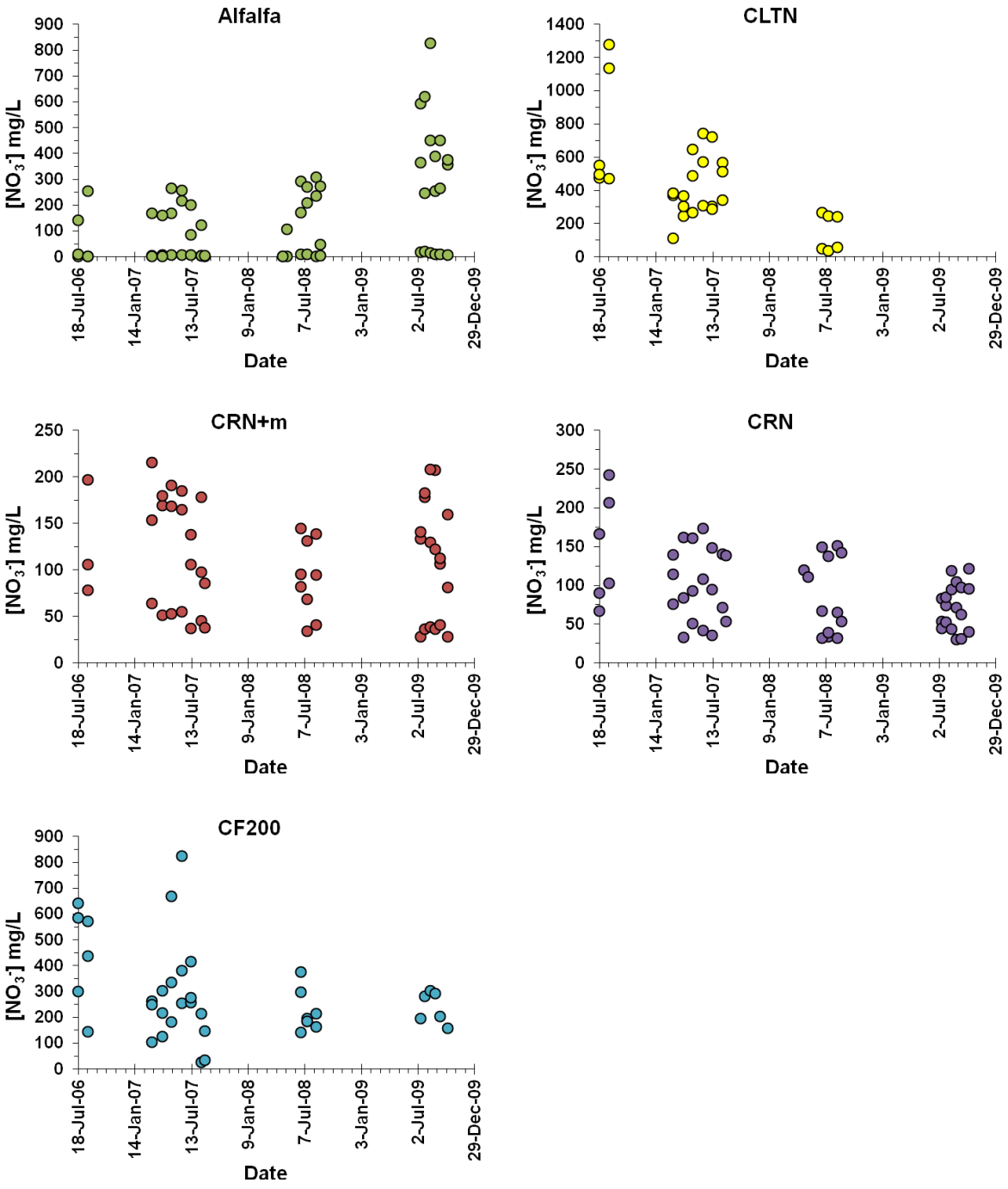

Figure 4.6: Groundwater nitrate concentrations with time for each treatment (includes data from all wells). For more detailed graphs of groundwater nitrate concentrations with time for each specific well, see Appendix E.

Table 4.7: Nitrate concentration statistical data for years 2006 through 2009. Concentrations >1000mg/L and groundwater data from Well 22 of the alfalfa treatment were excluded.

Statistic	Groundwater [NO_3^-] (mg/L)			
	2006	**2007**	**2008**	**2009**
mean	275.6	210.9	141.9	183.4
median	207.0	167.1	138.0	122.0
max	642.0	825.6	375.3	827.9
min	0.7	0.9	1.3	27.8
stdev	205.6	178.5	92.4	169.1
n	23	89	46	54
SE	42.9	18.9	13.6	23.0

Year 2008 had the lowest average groundwater nitrate concentrations out of all four years, in spite of the manure application in fall of 2007. One possible reason is that the groundwater did not reflect the nitrogen input from the recent 2007 manure application yet in 2008. Another potential reason is that the application rate of manure was not high enough to have affected overall groundwater nitrate concentrations. In addition, the nitrates mineralized from the manure may have been denitrified or rapidly assimilated by plants/microorganisms. The effect of manure on average nitrate concentrations is discussed in more detail later in this section.

The individual groundwater nitrate concentration data were averaged by treatment and by year to provide the statistical data tabulated in Tables 4.8a-e. For each treatment, statistics were calculated using data obtained from three wells, except for the alfalfa treatment, where Well 22 data were excluded and data from only two wells were used. Changes in average groundwater nitrate concentrations over time for each treatment are shown in Figure 4.7.

Table 4.8a: Nitrate concentration statistical data for years 2006 through 2009 – Alfalfa treatment.

Statistic	Groundwater [NO_3^-] (mg/L)			
	2006	**2007**	**2008**	**2009**
mean	101.5	127.7	191.4	433.2
median	75.1	160.6	222.0	381.9
max	255.1	265.4	307.5	827.9
min	0.7	0.9	1.3	247.5
stdev	120.9	98.8	107.3	172.5
n	4	13	10	12
SE	60.4	27.4	33.9	49.8

Table 4.8b: Nitrate concentration statistical data for years 2006 through 2008 – CLTN treatment.

Statistic	Groundwater [NO_3^-] (mg/L)		
	2006	2007	2008
mean	499.1	418.6	149.0
median	487.4	368.2	149.6
max	548.7	745.2	264.5
min	472.9	110.2	36.2
stdev	34.9	176.0	111.6
n	4	18	6
SE	17.4	41.5	45.6

Table 4.8c: Nitrate concentration statistical data for years 2006 through 2009 – CRN+m treatment.

Statistic	Groundwater [NO_3^-] (mg/L)			
	2006	2007	2008	2009
mean	126.9	118.9	92.2	109.6
median	105.5	122.2	94.5	117.4
max	197.0	215.4	144.7	208.4
min	78.3	37.3	34.1	27.8
stdev	62.2	61.2	40.5	63.6
n	3	20	9	18
SE	35.9	13.7	13.5	15.0

Table 4.8d: Nitrate concentration statistical data for years 2006 through 2009 – CRN treatment.

Statistic	Groundwater [NO_3^-] (mg/L)			
	2006	2007	2008	2009
mean	145.9	101.0	87.2	72.4
median	134.3	94.3	66.9	72.8
max	242.8	173.2	151.1	121.5
min	67.0	32.9	31.7	30.1
stdev	70.3	46.3	48.6	29.2
n	6	19	13	18
SE	28.7	10.6	13.5	6.9

Table 4.8e: Nitrate concentration statistical data for years 2006 through 2009 – CF200 treatment.

Statistic	Groundwater [NO_3^-] (mg/L)			
	2006	2007	2008	2009
mean	446.6	277.8	219.5	238.7
median	504.6	255.2	190.1	242.7
max	642.0	825.6	375.3	301.8
min	143.6	24.7	141.3	156.8
stdev	193.1	197.2	77.9	60.8
n	6	19	8	6
SE	78.8	45.2	27.5	24.8

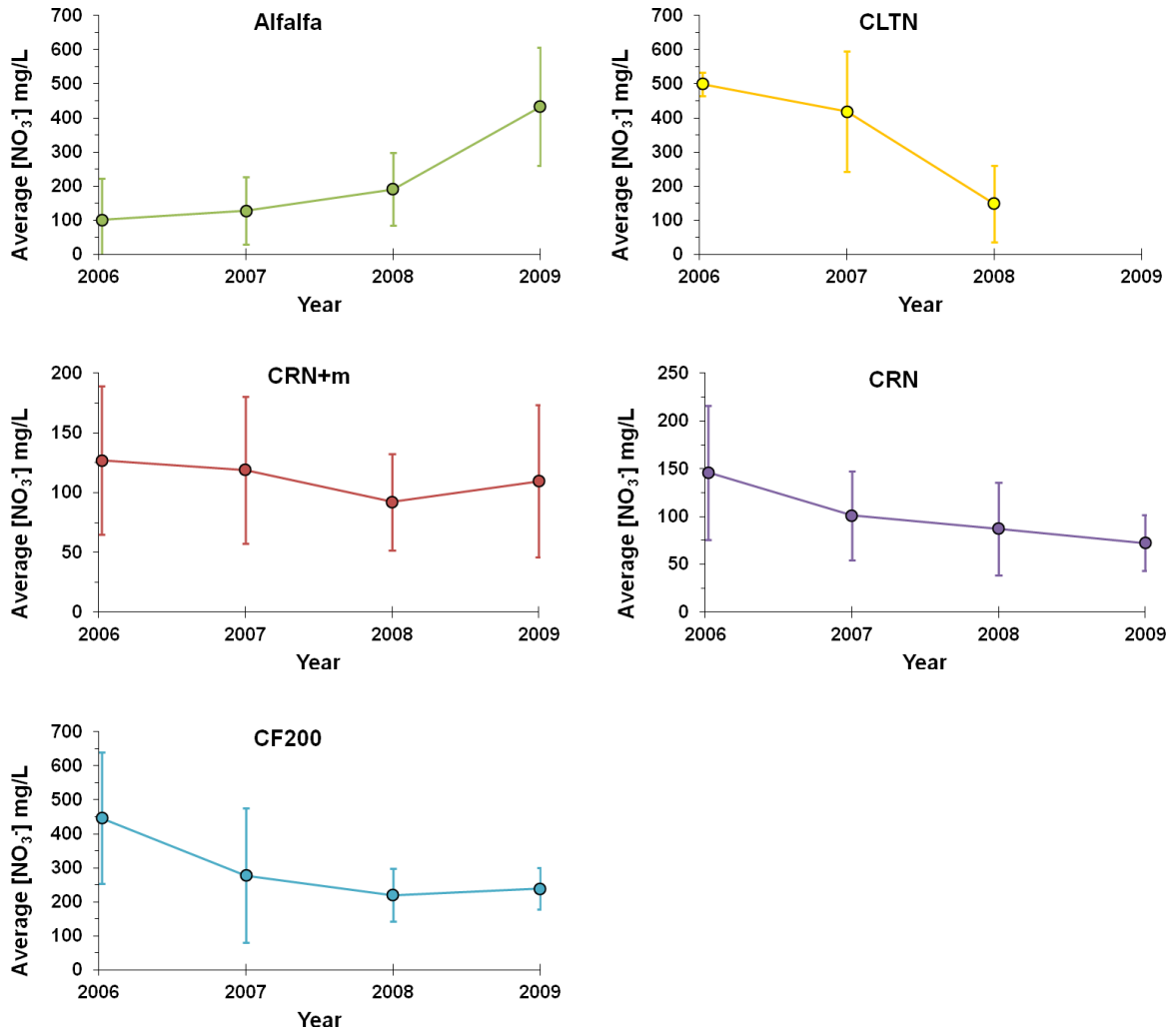

Figure 4.7: Average groundwater nitrate concentrations (±SD) from 2006 to 2009 for each treatment. Averages were calculated using nitrate concentration data obtained from three wells for each treatments except the alfalfa treatment, where data from only two wells were used.

Average groundwater nitrate concentrations under the alfalfa treatment continuously increased from 101.5±120.9mg/L in 2006 to 127.7±98.8mg/L in 2007, and from 191.4±107.3mg/L in 2008 to 433.2±172.5mg/L in 2009. The average groundwater nitrate concentration beneath the alfalfa treatment was significantly higher in 2009 than in 2006, 2007, or 2008, according to a one-way ANOVA with a 95% confidence interval ($F_{3,35} = 14.02$, $p = 0.00$; see Appendix D for statistical test results).

For the CLTN treatment, average groundwater nitrate concentrations constantly decreased from 2006 to 2008 (from 499.1±34.9mg/L to 418.6±176.0mg/L, and then to 149.0±111.6mg/L). The average groundwater nitrate concentration below the CLTN treatment was significantly higher in 2006 than in 2008 (Welch's t-test, $\alpha = 0.05$, $t = 7.17$, $p = 0.00$). The 2007 average groundwater nitrate concentration under the CLTN treatment was also significantly higher than in 2008 (Welch's t-test, $t = 4.38$, $p = 0.001$).

For groundwater below the CF200 treatment, average nitrate concentrations decreased from 446.6±193.1mg/L in 2006 to 277.8±197.2mg/L in 2007. A Welch's t-test indicated that there was a significant difference between the 2006 and 2007 average groundwater nitrate concentrations under the CF200 treatment ($t = 2.69$, $p = 0.036$). Average groundwater nitrate concentrations under the CF200 treatment further decreased to 219.5±77.9mg/L in 2008, but increased slightly to 238.7±60.8mg/L in 2009. The average groundwater nitrate concentration below the CF200 treatment was significantly higher in 2006 than in 2008 (Welch's t-test, $t = 2.72$, $p = 0.035$), but the CF200 average groundwater nitrate concentration was not significantly higher in 2006 than in 2009 ($t = 2.51$, $p = 0.054$).

The CRN treatment had average groundwater nitrate concentrations of 145.9±70.3mg/L in 2006, 101.0±46.3mg/L in 2007, 87.2±48.6mg/L in 2008, and 72.4±29.2mg/L in 2009. A Welch's t-test indicated that there was no statistical difference between the 2006 and 2009 average groundwater nitrate concentrations under the CRN treatment ($t = 2.49$, $p = 0.055$).

The average groundwater nitrate concentrations for the CRN+m treatment were relatively constant; average groundwater nitrate concentrations were 126.9±62.2mg/L, 118.9±61.2mg/L, 92.2±40.5mg/L, and 109.6±63.6mg/L, for each respective year from 2006 to 2009. The average groundwater nitrate concentrations below the CRN+m treatment in years 2006, 2007, 2008, and 2009 were not significantly different, according to statistical results from a one-way ANOVA ($\alpha = 0.05$, $F_{3,46} = 0.50$, $p = 0.69$).

The most probable cause for the increase in average nitrate concentrations with time for groundwater underneath the alfalfa treatment was nitrogen fixation, followed by ammonification of the SOM, nitrification, and leaching of the nitrates into the underlying groundwater, since the alfalfa treatment did not receive any inorganic fertilizers. The groundwater below the CLTN treatment seemed to have lost 70% of its original nitrate from 2006 to 2008; this drastic decrease in average nitrate concentrations (total decrease of about 350mg/L) may have been caused by the decrease in fertilization rate initiated in 2004 from 200kgN/ha per year to 100kgN/ha per year, and/or denitrification.

Fertilizer application had ceased by 2004 for the CF200 treatment (fertilizer rate was 200kgN/ha/yr in years preceding 2004) and the average groundwater nitrate concentrations of the CF200 treatment seemed to reflect this change in fertilization rate. Over time, the residual nitrates that had accumulated in the groundwater below the CF200 treatment from previous fertilization events may have been denitrified and/or consumed by plants or microorganisms, which would have caused the average nitrate concentration to decrease from 2006 to 2008. In 2009, the average nitrate concentration in groundwater under the CF200 treatment increased to 238.7±60.8mg/L, potentially due to nitrogen fixation and subsequent leaching of the mineralized nitrates, as the crop grown on CF200 plots that year was alfalfa.

Groundwater beneath the CRN treatment decreased in average nitrate concentrations continuously over time, with a total decrease of 73.5mg/L from 2006 to 2009. The low average nitrate concentrations may have been due to the fact that the CRN treatment did not receive any fertilizers in years up to 2003 (synthetic fertilizers were applied at rate of 100kgN/ha/yr starting in 2004). The steady decrease in average groundwater nitrate concentrations under the CRN treatment suggested that denitrification may have occurred and that there was no significant loading or build-up of nitrogen in the groundwater as a result of the 100kgN/ha/yr fertilization rate.

Like the CRN treatment, the CRN+m treatment had relatively low and constant average groundwater nitrate concentrations, possibly due to the fact that synthetic fertilizers were not applied until 2004 at a rate of 100kgN/ha/yr. There was no substantial increase in the average groundwater nitrate concentration from 2007 to 2008 under CRN+m, despite manure application in the fall of 2007. In fact, average nitrate concentrations actually decreased from 118.9±61.2mg/L in 2007 to 92.2±40.5mg/L in 2008. It was not until 2009 that the average

groundwater nitrate concentration for the CRN+m treatment increased slightly to 109.6±63.6mg/L. Slow recharge water transit times may have partly caused the groundwater nitrate concentrations beneath the CRN+m treatment to not have been immediately affected by recent manure applications.

Recharge water transit times may have potentially been long (decades), considering that the hydraulic conductivities of the fine-grained glacio-lacustrine deposits and underlying till are 1.3×10^{-7}m/s and 6.7×10^{-9}m/s, respectively (Rodvang *et al.*, 2004). The vertical groundwater flow velocity for the weathered till in southern Alberta was estimated at ~0.1m/yr (Hendry, 1988). Assuming that the overlying soils had a similar vertical water flow velocity, recharge waters would have taken approximately 10 to over 20 years to reach the water table, given that there is no preferential flow via fractures or plant-root systems. Therefore, it would have taken a considerable amount of time before the effect of manure application could have been detectable in the groundwater. However, during irrigation season, the water table was occasionally detected less than a meter below the soil surface and water-logging was also known to sometimes occur; therefore, recharge water transit times may have actually been on the order of only days to months (Ellert, pers. communication).

Assuming that recharge water transit times were on the order of months, the effects of the 2007 manure application should have been observed within the study period. Given this, the lack of an increase in the groundwater nitrate concentrations may have been partly due to the fact that each manure application (circa once every five years) provided an additional nitrogen input of only ~10% of the total soil nitrogen. If the groundwater had similar proportions of manure-derived nitrogen as did the soils, then the amount of groundwater nitrate sourced from manure on a yearly basis was likely small. In addition, manure consists primarily of organic nitrogen; hence mineralization of the manure nitrogen and leaching of the nitrates must occur before manure application can be reflected in the groundwater nitrate concentrations. The inorganic nitrogen provided by the manure may have also been denitrified or assimilated into the biomass of plants and microorganisms, which left little inorganic nitrogen to be leached into the groundwater.

Denitrification, changes in fertilization rate, nitrogen fixation followed by leaching, and several other factors could have caused the temporal changes in groundwater nitrate concentrations. Trends in groundwater nitrate concentrations over time may provide some insight into land-use history, but the source(s) of the nitrate and transformation processes, such as

denitrification, cannot be easily identified with concentration data alone. In Section 4.3.2, the isotopic ratios of nitrate are used to investigate the accuracy of the hypotheses mentioned in this section. The next section describes variations in average groundwater nitrate concentrations between treatments in attempt to further assess the influence of synthetic fertilizer and manure usage on groundwater nitrate concentrations.

4.3.1.3. Treatment Variations

The average groundwater nitrate concentrations for each specific treatment and year are plotted together for comparison in Figure 4.8. In general, groundwater from the alfalfa treatment displayed increasing average nitrate concentrations with time, while the groundwaters from the CLTN, CF200, CRN+m, and CRN treatments showed overall decreases in average nitrate concentrations over time except in 2009.

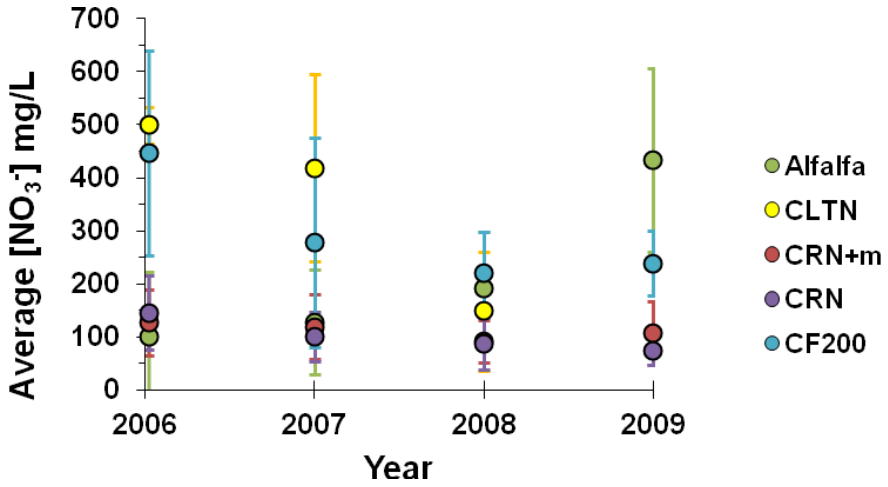

Figure 4.8: Changes in average nitrate concentrations (±SD) with time for all treatments.

The alfalfa, CRN+m, and CRN treatments had respective average groundwater nitrate concentrations of 101.5±120.9mg/L, 126.9±62.2mg/L, and 145.9±70.3mg/L, in year 2006. Average groundwater nitrate concentrations were also similar in 2007 under the alfalfa (127.7±98.8mg/L), CRN+m (118.9±61.2mg/L), and CRN treatments (101.0±46.3mg/L). In year 2008, average nitrate concentrations were 191.4±107.3mg/L, 92.2±40.5mg/L, and 87.2±48.6mg/L for groundwater below the alfalfa, CRN+m, and CRN treatments, respectively.

Welch's t-tests with 95% confidence intervals showed that the unfertilized alfalfa treatment did not have significantly different average groundwater nitrate concentrations than the fertilized CRN+m or CRN treatments in year 2006 ($t = -0.80$, $p = 0.47$ and $t = -1.02$, $p = 0.38$; see Appendix D for statistical test results), year 2007 ($t = -0.41$, $p = 0.69$ and $t = 0.01$, $p = 0.99$), or year 2008 ($t = 1.91$, $p = 0.08$ and $t = 2.12$, $p = 0.06$), although the CRN+m and CRN treatments had both received 100kgN/ha/yr of synthetic fertilizers since 2004.

In 2009, however, results from Welch's t-tests ($t = 8.03$, $p = 0.00$, and $t = 11.12$, $p = 0.00$) indicated that the alfalfa treatment (433.2±172.5mg/L) had significantly higher average groundwater nitrate concentrations than the CRN+m (109.6±63.6mg/L) and CRN treatments (72.4±29.2mg/L). Groundwater beneath the alfalfa treatment also had a higher average nitrate concentration (191.4±107.3mg/L) than groundwater below the fertilized CLTN treatment (149.0±111.6mg/L) in 2008, but the difference was not statistically significant ($t = 0.59$, $p = 0.57$).

The CLTN average groundwater nitrate concentration of 499.1±34.9mg/L was not significantly higher than that of the CF200 treatment (446.6±193.1mg/L) in 2006, according to a Welch's t-test ($\alpha = 0.05$, $t = 0.81$, $p = 0.46$). But in 2008, groundwater under the CF200 treatment plots (219.5±77.9mg/L) had a considerably higher average nitrate concentration compared to the groundwater under the CLTN treatment (149.0±111.6mg/L), even though the difference was not statistically significant ($t = -1.50$, $p = 0.19$).

Statistical results ($\alpha = 0.05$, $t = 8.36$, $p = 0.00$) showed that the 2006 average groundwater nitrate concentration beneath the CRN treatment plots (145.9±70.3mg/L) was significantly lower than under the CLTN treatment plots (499.1±34.9mg/L). Results ($t = 8.73$, $p = 0.00$) also indicated that the CRN treatment (101.0±46.3mg/L) had a significantly lower average groundwater nitrate concentration than the CLTN treatment (418.6±176.0mg/L) in 2007. Statistical evidence ($t = 7.73$, $p = 0.00$) showed that the groundwater beneath the CRN+m treatment (118.9±61.2mg/L) in 2007 had significantly lower average nitrate concentrations than the CLTN treatment (418.6±176.0mg/L). In addition, the 2007 average groundwater nitrate concentrations were significantly lower under the CRN+m and CRN treatment plots (118.9±61.2mg/L and 101.0±46.3mg/L) relative to the groundwater beneath the CF200 treatment plots (277.8±197.2mg/L), as indicated by Welch's t-tests (corrected $\alpha = 0.01$, $t = -3.28$, $p = 0.003$ and corrected $\alpha = 0.007$, $t = -3.98$, $p = 0.001$).

The alfalfa, CRN+m, and CRN treatments had similar average groundwater nitrate concentrations in years 2006 through 2008, possibly due to the fact that synthetic fertilizer applications for the CRN+m and CRN treatments did not start until 2004, and that all three treatments were unfertilized in years up to and including 2003. In 2009, however, the alfalfa treatment had a substantially higher average nitrate concentration than the fertilized CRN+m and CRN treatments. This implies that nitrogen fixation by the alfalfa plants and subsequent mineralization of the leguminous plant residue may have provided a greater source of nitrogen input than the 100kgN/ha/yr of ammonium-nitrate applied on the corn plots. According to Smil (1999, and references therein), annual nitrogen fixation rates can range from 65 to 600kgN/ha for alfalfa plants.

Average groundwater nitrate concentrations were similar beneath the CLTN and CF200 treatment plots in 2006, likely because the CLTN and CF200 treatments both received 200kgN/ha/yr of inorganic fertilizers in years up to and including 2003. The CF200 treatment no longer received synthetic fertilizers starting in 2004, but in 2008, the CF200 plots had a higher average groundwater nitrate concentration than the CLTN plots, which had continued receiving fertilizers at a rate of 100kgN/ha/yr after 2003. This suggests that synthetic fertilizer usage was not the only contributing factor that affected groundwater nitrate concentrations; other factors, such as cropping system, nitrification, and/or denitrification, must have had an impact on groundwater nitrate concentrations as well.

The more heavily fertilized treatments (CLTN and CF200 at rates of 200kgN/ha/yr in years preceding 2004) typically had higher average groundwater nitrate concentrations compared to the more recently fertilized CRN+m and CRN treatments (100kgN/ha/yr beginning in 2004), which suggests that synthetic fertilizer application influenced groundwater nitrate concentrations to some degree.

Groundwater nitrate concentration data from all four years were grouped according to treatment and the statistical data is tabulated in Table 4.9. Figure 4.9 is a box-plot of the groundwater nitrate concentration data. The box-plot whiskers represent Q1-(1.5 x IQR) and Q3+(1.5 x IQR), where Q1 and Q3 are the lower and upper quartiles and IQR is the interquartile range (Q3-Q1). The CLTN treatment had the highest overall average groundwater nitrate concentration of 372.3±192.0mg/L, followed by the CF200 (285.8±174.1mg/L), alfalfa (235.3±185.2mg/L), CRN+m (111.2±58.2mg/L), and CRN (93.4±49.0mg/L) treatments.

Table 4.9: Summary statistics of groundwater nitrate concentrations for each treatment, calculated using nitrate concentration data from all four years.

Groundwater $[NO_3^-]$ mg/L	All Treatments	Alfalfa	CLTN	CRN+m	CRN	CF200
mean	187.7	235.3	372.3	111.2	93.4	285.8
median	139.8	236.0	353.3	106.4	87.7	255.2
max	1276.6	827.9	745.2	215.4	242.8	825.6
min	0.7	0.7	36.2	27.8	30.1	24.7
stdev	193.1	185.2	192.0	58.2	49.0	174.1
n	235	39	28	50	56	39
SE	12.6	29.7	36.3	8.2	6.6	27.9

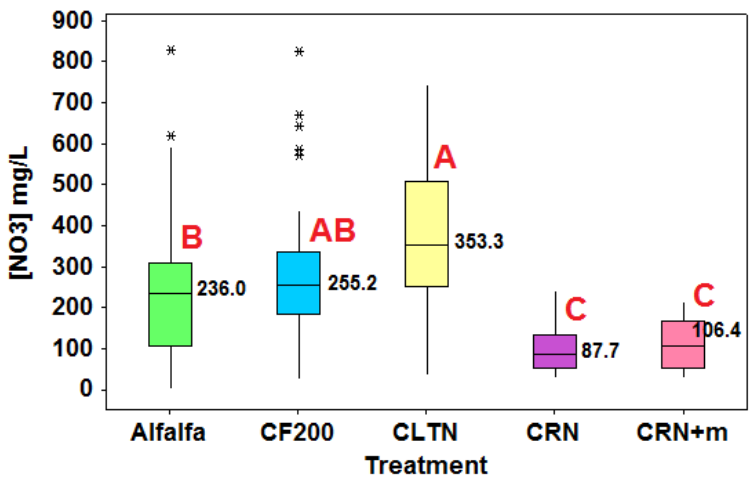

Figure 4.9: Box-plot of groundwater nitrate concentrations with median values labeled. Data points outside the whisker ranges were classified as outliers and are represented with asterisks. Treatments that do not share a letter have significantly different average groundwater nitrate concentrations, according to a one-way ANOVA (see Appendix D for statistical results).

The average groundwater nitrate concentration under the CLTN treatment was significantly higher than under the alfalfa, CRN+m, and CRN treatments, but the average groundwater nitrate concentrations were not statistically different between the CLTN and CF200 treatments, the alfalfa and CF200 treatments, or between the CRN+m and CRN treatments, according to a one-way ANOVA ($\alpha = 0.05$, $F_{4, 205} = 28.82$, $p = 0.00$). Similar results were obtained from multiple Welch's t-tests with 95% confidence intervals, which concluded that the average groundwater nitrate concentrations were not statistically different between the CLTN

and CF200 treatments, or between the CRN+m and CRN treatments, but the average groundwater nitrate concentration under the CLTN treatment was significantly different than the groundwater nitrate concentrations underneath the alfalfa, CRN+m, and CRN treatments (see Appendix D).

Statistical test results indicate that treatment indeed had a significant impact on groundwater nitrate concentrations. Groundwater beneath plots with similar fertilizer amendments (e.g., the CRN+m and CRN treatments) had similar average nitrate concentrations. Also, heavy agricultural nitrogen loading, as a result of long-term fertilization, had likely caused the average nitrate concentration of the groundwater beneath the CLTN treatment to have been substantially higher than under the unfertilized alfalfa treatment or the recently fertilized CRN+m and CRN treatments.

The average groundwater nitrate concentration under the CRN+m treatment (111.2±58.2mg/L) was not significantly higher than under the CRN treatment (93.4±49.0mg/L), despite the CRN+m treatment having received manure. This suggests that the effect of manure on groundwater nitrate concentrations was relatively minor in the short term (few decades or less), which is in agreement with previous conclusions derived from the soil data.

Given that fertilizer application affected groundwater nitrate concentrations to a certain extent, then inorganic fertilizers must have been one of the sources of nitrates in the groundwater. Treatment differences and temporal trends in groundwater nitrate concentrations suggest that nitrogen fixation (followed by ammonification of SOM and nitrification) also likely contributed to groundwater nitrates. In the next section, isotopic ratios of nitrate are used to accurately identify the occurrence of nitrogen transformation processes and to more precisely determine the sources of groundwater nitrate.

4.3.2. Isotopic Compositions of Nitrate

4.3.2.1. Data Overview

Isotopic composition data was generated for two hundred and twenty-nine samples. The $\delta^{15}N$ values of nitrate ranged between 2.1 and 59.6‰, with an average of 14.5±8.8‰. The $\delta^{18}O$ values of nitrate ranged from −7.9 to 40.5‰, and averaged 3.1±6.6‰ (see Table 4.10 for summary statistics and Appendix C for the whole isotopic dataset). Note that average $\delta^{15}N\text{-}NO_3$

and $\delta^{18}O\text{-}NO_3$ values were calculated for statistical purposes only, and that the average values should not be used to interpret source signals, because there were likely contributions of nitrate from multiple sources (as indicated by the wide range of $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values) and transformation processes (e.g., denitrification) can alter the isotopic compositions of nitrate.

Figure 4.10a and Figure 4.10b are histograms of the $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values of all the analyzed groundwater samples, and Figure 4.11 is a dual isotope diagram showing the $\delta^{18}O\text{-}NO_3$ versus $\delta^{15}N\text{-}NO_3$ values of the entire dataset. One hundred and ninety-seven groundwater samples had $\delta^{15}N\text{-}NO_3$ values between +5 and +20‰. Twenty-nine samples had $\delta^{15}N\text{-}NO_3$ values higher than +20‰, the majority of which were sampled from Well 22 (see Figure 4.11). Most of the groundwater samples (n = 210) had $\delta^{18}O\text{-}NO_3$ values that fell between −5 and +10‰.

Table 4.10: Statistical data of $\delta^{15}N$ and $\delta^{18}O$ values of nitrate for all groundwater samples.

Statistic	**$\delta^{15}N\text{-}NO_3$ (‰ – AIR)**	**$\delta^{18}O\text{-}NO_3$ (‰ – VSMOW)**
mean	14.5	3.1
median	12.5	2.0
max	59.6	40.5
min	2.1	−7.9
stdev	8.8	6.6
n	229	229
SE	0.6	0.4

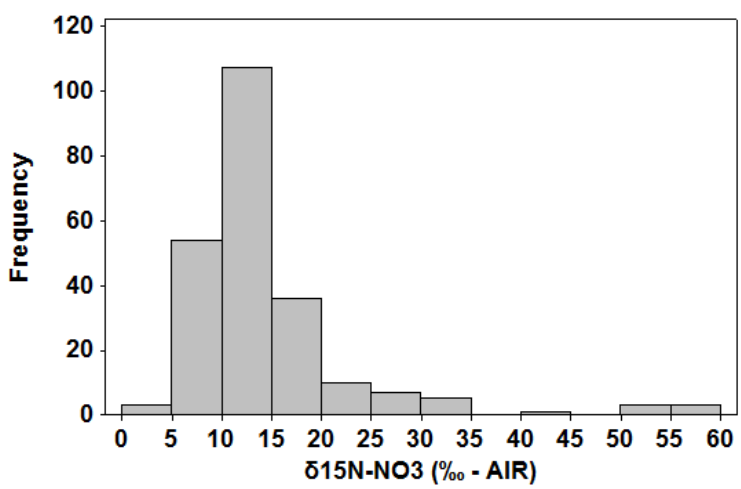

Figure 4.10a: Histogram of $\delta^{15}N\text{-}NO_3$ values for all groundwater samples (n = 229).

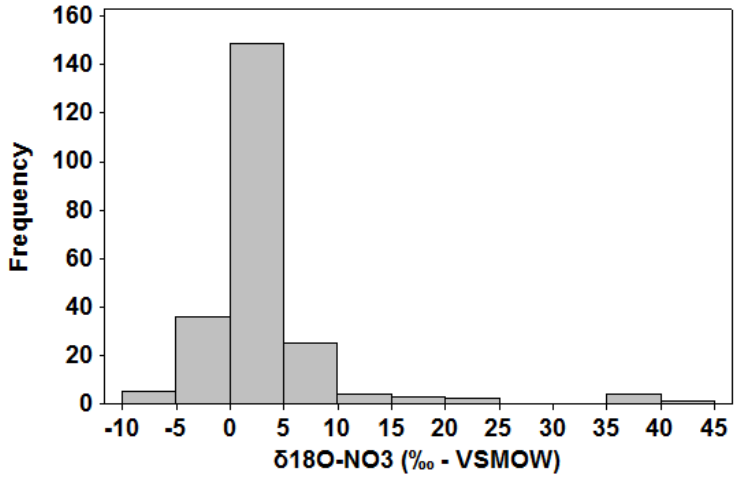

Figure 4.10b: Histogram of $\delta^{18}O\text{-}NO_3$ values for all groundwater samples (n = 229).

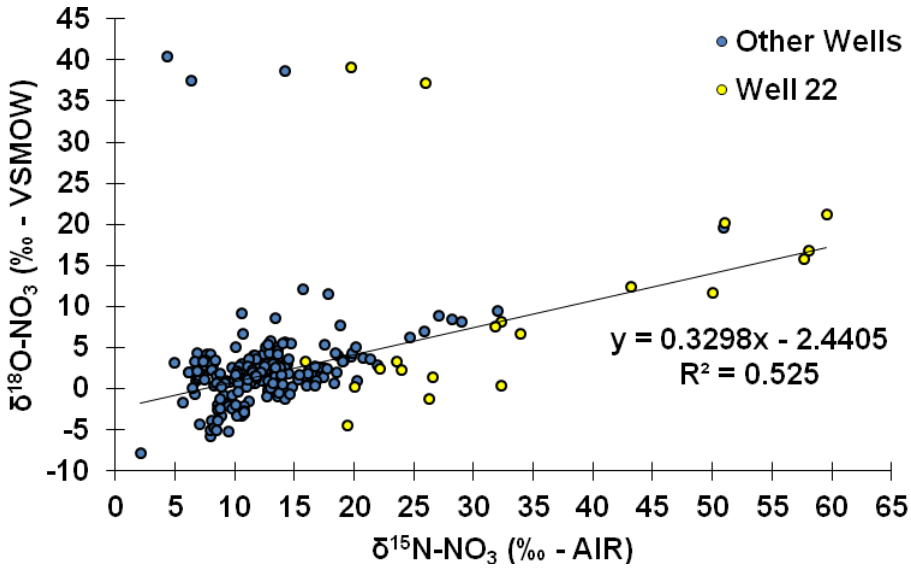

Figure 4.11: Dual isotope diagram ($\delta^{18}O\text{-}NO_3$ versus $\delta^{15}N\text{-}NO_3$) of all groundwater nitrate samples (n = 229).

There were five samples with $\delta^{18}O\text{-}NO_3$ values higher than +30‰, which could be interpreted as evidence of atmospheric deposition (see Figure 4.11). This source seems unlikely, however, because the high $\delta^{18}O\text{-}NO_3$ values were detected only during the August to October months of 2009, and the $\delta^{18}O\text{-}NO_3$ values decreased to between −3.3‰ and +3.1‰ within a couple weeks to about two months. More so, nitrates derived from atmospheric deposition

typically correspond to low nitrate concentrations, but the nitrate concentrations for the five samples in question ranged from 9.26mg/L to 207.5mg/L. Another discrepancy is that two of the five data points had corresponding $\delta^{15}N\text{-}NO_3$ values that were higher than the expected range of −15 to +15‰ for atmospheric deposition (Kendall *et al*., 2007). Therefore, rather than atmospheric deposition, the high $\delta^{18}O\text{-}NO_3$ values may have been a result of another source or nitrogen transformation process. These five data points are considered as outliers for the purpose of this thesis and are not included in any further statistical calculations or graphs.

According to Böttcher *et al*. (1990), enrichment of ^{15}N and ^{18}O during denitrification occurs at a ratio of about 2:1, such that the linear regression line on a dual isotope diagram ($\delta^{18}O\text{-}NO_3$ versus $\delta^{15}N\text{-}NO_3$) should have a slope of about 0.5 for groundwater samples affected by denitrification. Although the slope of the linear regression line was calculated to be 0.33 (R^2 = 0.525) for the groundwater nitrate samples, $\delta^{15}N\text{-}NO_3$ values higher than +20‰ (see Figure 4.11) indicate that ^{15}N-enriching processes (i.e., denitrification) likely occurred during the observation period. In addition, previous studies have found that slopes greater or less than 0.5 are also possible for denitrification (e.g., Cey *et al*., 1999; Singleton *et al*., 2007). The trend of increasing $\delta^{15}N\text{-}NO_3$ values with corresponding increases in $\delta^{18}O\text{-}NO_3$ values suggest that denitrification has likely occurred in the groundwater beneath Rotation U.

Nitrate isotopic composition data can potentially aid in the identification of the sources of the nitrate; however, source identification may be much more difficult and less straightforward in environments where denitrification or nitrification processes are dominant, since denitrification and nitrification are both processes that can alter and mask the isotopic signals of the original nitrate sources. Because of this, the extent of nitrification and denitrification is evaluated first in Section 4.3.2.2, and source determination is discussed later in Section 4.3.2.3.

4.3.2.2. Nitrification and Denitrification

To determine the occurrence of nitrification and denitrification, three sets of graphs were plotted for the groundwater nitrate samples: i) nitrate concentration versus time, $\delta^{15}N\text{-}NO_3$ versus time, and $\delta^{18}O\text{-}NO_3$ versus time graphs; ii) $\delta^{15}N\text{-}NO_3$ versus nitrate concentration and $\delta^{18}O\text{-}NO_3$ versus nitrate concentration graphs; and iii) dual isotope diagrams of $\delta^{18}O\text{-}NO_3$ versus $\delta^{15}N\text{-}NO_3$ values. The process by which nitrification and denitrification was identified is discussed in this section for groundwater samples obtained from select representative wells. See Appendix C for

groundwater data and Appendix E for all graphs. The spatial distributions of nitrate concentrations and the $\delta^{15}N$ values of nitrate are then analyzed in an attempt to identify denitrification with respect to groundwater flow directions.

From July 18th, 2006 to March 9th, 2007, groundwater nitrate concentrations in Well 18 of the CF200 treatment decreased from 300.2mg/L to 103.4mg/L, while $\delta^{15}N\text{-}NO_3$ values increased from 10.0‰ to 14.1‰, and $\delta^{18}O\text{-}NO_3$ values increased from 1.6‰ to 5.5‰ (see Table 4.11 and Figure 4.12). Groundwater nitrate concentrations in Well 18 also decreased from 256.5mg/L on July 11th, 2007 to 148.1mg/L on August 24th, 2007, while $\delta^{15}N\text{-}NO_3$ values increased from 10.5‰ to 12.8‰, and $\delta^{18}O\text{-}NO_3$ values increased from 0.4‰ to 5.3‰. A similar trend was observed in 2009, although the decrease in groundwater nitrate concentrations did not correspond exactly to increases in the $\delta^{15}N$ and $\delta^{18}O$ values of nitrate (concentrations did not decrease until August 10th, 2009 but $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values started increasing on July 24th, 2009).

Denitrification is accompanied by decreases in nitrate concentration and corresponding increases in both the $\delta^{15}N$ and $\delta^{18}O$ values of nitrate, since the denitrification process enriches the remaining nitrates in the heavier isotopes, ^{15}N and ^{18}O (Gormly and Spalding, 1979; Böttcher *et al*., 1990; Kendall, 1998). Therefore, the observed trends suggest that denitrification occurred in the shallow groundwater within the vicinity of Well 18.

During the nitrification process, the lighter nitrogen isotopes are preferentially converted into nitrates, which causes the produced nitrates to be enriched in ^{14}N relative to the reactant ammonium. As a result, increases in nitrate concentration and decreases in both the $\delta^{15}N$ and $\delta^{18}O$ values of nitrate are evidence of nitrification (Kendall, 1998). From March 9th to June 13th, 2007, groundwater nitrate concentrations increased in Well 18 from 103.4mg/L to 255.2mg/L, while $\delta^{15}N\text{-}NO_3$ values decreased from 14.1‰ to 11.1‰, and $\delta^{18}O\text{-}NO_3$ values decreased from 5.5‰ to 1.1‰. Groundwater nitrate concentrations also increased in 2009 from 196.1mg/L to 281.9mg/L between July 10th and July 24th, while $\delta^{15}N\text{-}NO_3$ values decreased from 11.0‰ to 8.8‰, and $\delta^{18}O\text{-}NO_3$ values decreased from 1.2‰ to −1.2‰. These trends imply that nitrification may have occurred.

Table 4.11: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values of groundwater samples obtained from Well 18 (CF200 treatment) between July 2006 and October 2009.

Date	**Well 18 Groundwater**		
	$[NO_3^-]$ (mg/L)	**$\delta^{15}N\text{-}NO_3$ (‰ – AIR)**	**$\delta^{18}O\text{-}NO_3$ (‰ – VSMOW)**
July-18-06	300.2	10.0	1.6
August-18-06	143.6	11.5	2.0
March-09-07	103.4	14.1	5.5
April-12-07	124.8	13.6	4.3
May-09-07	182.9	11.8	1.3
June-13-07	255.2	11.1	1.1
July-11-07	256.5	10.5	0.4
August-13-07	215.1	11.6	1.3
August-24-07	148.1	12.8	5.3
June-25-08	141.3	13.4	2.6
July-15-08	194.4	11.3	0.6
August-11-08	215.1	12.2	2.0
July-10-09	196.1	11.0	1.2
July-24-09	281.9	8.8	−1.2
August-10-09	301.8	11.0	0.3
August-26-09	292.2	11.1	1.0
September-10-09	203.6	11.7	1.5
October-05-09	156.8	11.8	1.5

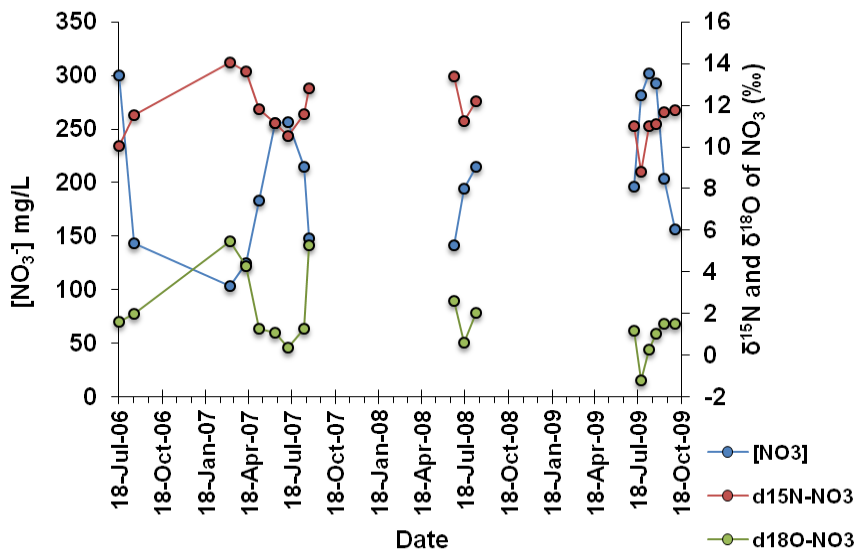

Figure 4.12: Concentrations, $\delta^{15}N$ values, and $\delta^{18}O$ values of groundwater nitrates versus time for Well 18 from the CF200 treatment.

The changes in nitrate concentrations and isotopic compositions over time from 2006 to 2009 indicate that there were trends of alternating nitrification and denitrification. Because nitrification requires aerobic conditions, whereas denitrification requires anaerobic conditions, both could have occurred in the groundwater only if dissolved oxygen concentrations fluctuated with time. This would have been possible if the infiltration of oxygenated recharge waters occurred periodically. During periods of recharge, oxidizing conditions would have been attained and nitrification in groundwater would have been possible. After the consumption of the dissolved oxygen (for example, by the oxidation of organic matter) and establishment of reducing conditions, denitrification would have been possible. This hypothesis, however, assumes that there were sufficient amounts of ammonium present in the groundwater for nitrification to occur. Since groundwater ammonium concentrations and dissolved oxygen concentrations were not measured, the possibility that nitrification occurred in the groundwater cannot be conclusively investigated.

Another potential explanation for the alternating nitrification-denitrification trend is that nitrates derived from nitrification were leached from the oxygenated, unsaturated soil zone above and were denitrified subsequently in the groundwater below, where oxygen concentrations were likely lower. It is uncertain whether nitrification occurred in the soils or in the groundwater, due to lack of groundwater ammonium concentration and dissolved oxygen data; however, the former is probably more likely, since the soil and PRS-probe isotopic data showed evidence of soil nitrification (see Section 4.2).

Groundwater data from Wells 6, 22, 28, and 34 also displayed similar trends of alternating increases and decreases in groundwater nitrate concentrations, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values. This suggests that the soil-nitrified nitrates may have been leached from the soils above and that the nitrates may have been subsequently denitrified in the groundwater within the vicinity of Wells 6, 22, 28, and 34 (see Appendix E for graphs). Although the alternating trends were not as distinct, changes in the chemical and isotopic compositions of nitrate signify that the shallow groundwater in Wells 5, 8, 10, 13, 14, 16, 30, and 32 had also likely undergone some degree of denitrification of soil-nitrified nitrates at particular instances in time.

Table 4.12 shows the nitrate concentrations, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values of groundwater from Well 31 under the CRN+m treatment for each sampling date. Even though

groundwater nitrate concentrations fluctuated over time from March to August of 2007 (the difference between the maximum and minimum nitrate concentrations was 77.2mg/L), the $\delta^{15}N$ values of groundwater nitrate were almost constant ($\delta^{15}N\text{-}NO_3$ values increased by only 0.8‰; see Figure 4.13). In contrast, the changes in groundwater $\delta^{18}O\text{-}NO_3$ values over time were consistent with denitrification; the $\delta^{18}O\text{-}NO_3$ values increased with decreasing nitrate concentrations and decreased with increasing nitrate concentrations. For instance, the $\delta^{18}O\text{-}NO_3$ values increased from 2.7‰ to 5.6‰, while groundwater nitrate concentrations decreased from 191.0mg/L to 138.2mg/L between May 9th, 2007 and July 11th, 2007 (the $\delta^{15}N\text{-}NO_3$ value only increased by 0.4‰ during this time interval).

Table 4.12: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values of groundwater samples obtained from Well 31 (CRN+m treatment) between August 2006 and October 2009.

Date	**Well 31 Groundwater**		
	$[NO_3^-]$ (mg/L)	**$\delta^{15}N\text{-}NO_3$ (‰ – AIR)**	**$\delta^{18}O\text{-}NO_3$ (‰ – VSMOW)**
August-18-06	105.5	11.3	3.7
March-09-07	215.4	12.4	4.1
April-12-07	180.1	12.6	5.1
May-09-07	191.0	12.5	2.7
June-13-07	185.0	12.8	5.3
July-11-07	138.2	12.9	5.6
August-13-07	178.1	13.2	4.0
June-25-08	144.7	14.3	5.6
July-15-08	131.0	14.7	5.4
August-11-08	138.7	14.5	3.6
July-10-09	141.2	12.9	4.3
July-24-09	183.1	9.6	0.9
August-10-09	208.4	11.6	4.0
August-26-09	122.5	11.7	4.1
September-10-09	112.2	11.8	3.7
October-05-09	159.7	9.8	−0.8

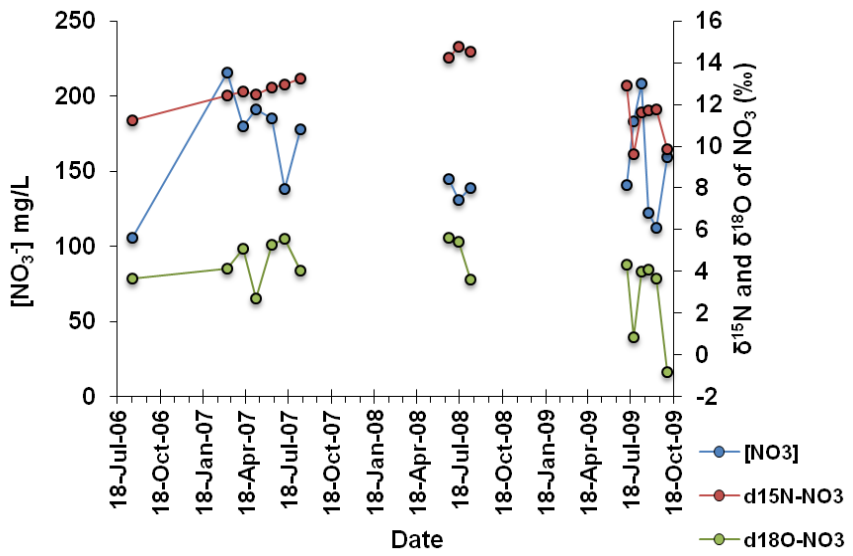

Figure 4.13: Concentrations, $\delta^{15}N$ values, and $\delta^{18}O$ values of groundwater nitrates versus time for Well 31 from the CRN+m treatment.

The trends in nitrate concentrations, $\delta^{15}N$-NO_3 values, and $\delta^{18}O$-NO_3 values with time for groundwater sampled from Well 31 between March and August, 2007 are not clearly indicative of soil nitrification and groundwater denitrification. A potential explanation for this is that multiple processes, such as input of nitrates derived from fertilizer or manure, nitrification, and denitrification, were simultaneously affecting the nitrogen pool, which caused the isotopic signals to fluctuate for $\delta^{18}O$ but not $\delta^{15}N$. Even though clear patterns of soil nitrification and groundwater denitrification could not be identified in 2007, trends observed in year 2009 suggest that soil nitrification and subsequent leaching of the produced nitrates may have occurred. From July 10th to July 24th, 2009, nitrate concentrations increased from 141.2mg/L to 183.1mg/L, while the $\delta^{15}N$ and $\delta^{18}O$ values of nitrate decreased from 12.9‰ to 9.6‰ and from 4.3‰ to 0.9‰, respectively.

Similarly, the soil nitrification and groundwater denitrification trend could not be confidently identified from the groundwater data of Well 2; when nitrate concentrations decreased, $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3 values remained relatively constant, and when nitrate concentrations increased, the $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3 values fluctuated by roughly 2‰ and 3.5‰, respectively (see Appendix E). It was only during the period between June 25th and July 15th, 2008 that denitrification may have occurred, according to the temporal data trends.

The $\delta^{15}N$ and $\delta^{18}O$ values of nitrate are plotted as a function of groundwater nitrate concentration in Figure 4.14a and Figure 4.14b for groundwater samples from Well 5 and Well 31, respectively (both wells are from the CRN+m treatment). If denitrification occurred in the groundwater, or if nitrates derived from soil nitrification were leached into the groundwater, then the $\delta^{15}N$ and $\delta^{18}O$ values of groundwater nitrates should increase with decreasing nitrate concentrations, and the $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values should decrease with increasing nitrate concentrations. This negatively proportional relationship was apparent in the groundwater data of Well 5, as indicated by the linear regression lines with slopes of −0.102 (R^2 = 0.774) and −0.019 (R^2 = 0.273) on graphs of $\delta^{15}N\text{-}NO_3$ versus nitrate concentration and $\delta^{18}O\text{-}NO_3$ versus nitrate concentration, respectively (see Figure 4.14a). Groundwater samples from Wells 13, 14, 18, 28, 32, and 34 also had nitrate concentrations that were negatively correlated with the $\delta^{15}N\text{-}NO_3$ values (slope of linear regression line = −0.017 to −0.185, R^2 = 0.428 to 0.699; see Appendix E) and the $\delta^{18}O\text{-}NO_3$ values (slope = −0.0097 to −0.075, R^2 = 0.125 to 0.762). High $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values corresponded to low nitrate concentrations for groundwater sampled from Well 22 (slopes of regression lines were −1.071 and −0.552), but the data were more scattered (R^2 = 0.184 and 0.179). Results of the linear regression analyses (R^2 values and negative slopes) of the $\delta^{15}N\text{-}NO_3$ versus nitrate concentration and $\delta^{18}O\text{-}NO_3$ versus nitrate concentration graphs suggest that the groundwater within the vicinity of Wells 5, 13, 14, 18, 22, 28, 32, and 34 had likely undergone denitrification.

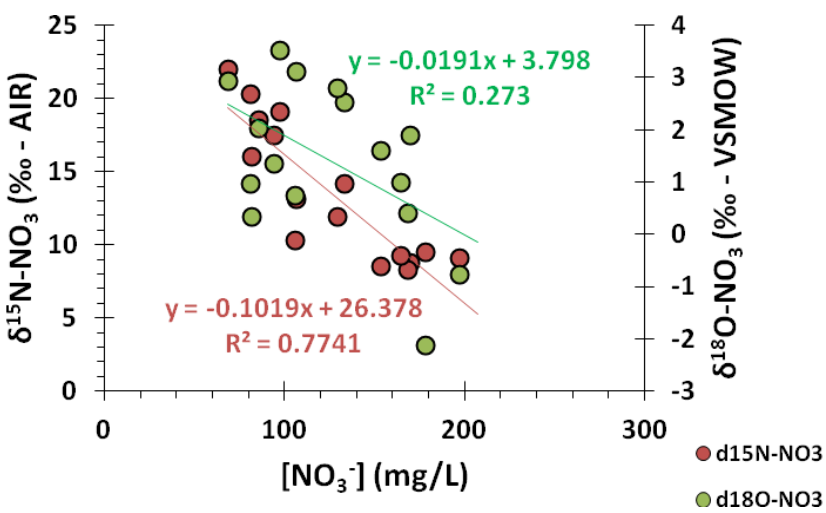

Figure 4.14a: Changes in $\delta^{15}N$ and $\delta^{18}O$ values with nitrate concentration for groundwater samples obtained from Well 5 of the CRN+m treatment.

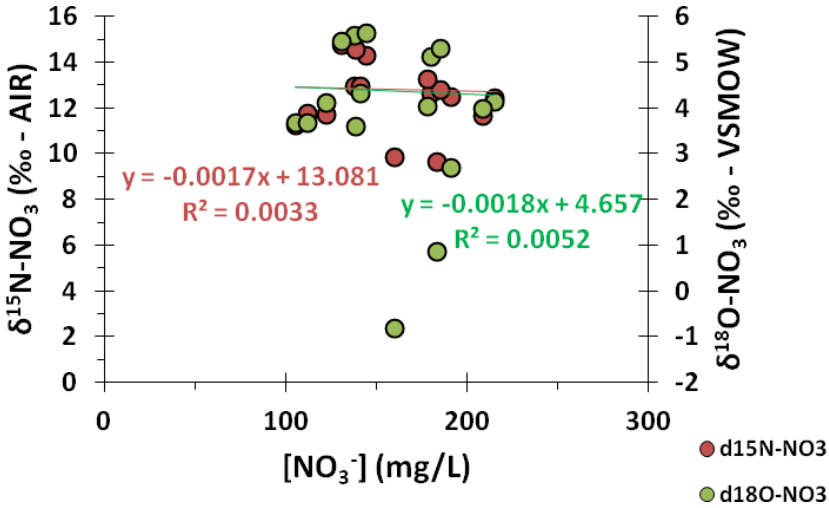

Figure 4.14b: Changes in $\delta^{15}N$ and $\delta^{18}O$ values with nitrate concentration for groundwater samples obtained from Well 31 of the CRN+m treatment.

Denitrification likely did not occur in the groundwater of Well 31, since the expected trend of increasing $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values with decreasing nitrate concentrations was not observed in the groundwater data (slope of linear regression line = −0.0017 and −0.0018, R^2 = 0.0033 and 0.0052; see Figure 4.14b). Denitrification could not be identified in the groundwater of Well 8 or Well 16, because the $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values did not increase considerably with decreasing nitrate concentrations (slope of linear regression lines were less than −0.010). Groundwater nitrates from Wells 6, 10, and 30 likely also did not undergo denitrification, since the $\delta^{18}O\text{-}NO_3$ values were not negatively correlated to groundwater nitrate concentrations, as indicated by positive linear regression slopes of 0.006 to 0.0076 (see Appendix E).

Dual isotope diagrams ($\delta^{18}O\text{-}NO_3$ versus $\delta^{15}N\text{-}NO_3$ values) for groundwater nitrate samples obtained from Well 22 (alfalfa treatment) and Well 30 (CLTN treatment) are shown in Figure 15a and Figure 15b (see Appendix E for all dual isotope diagrams). Table 4.13 lists the slopes of the linear regression lines on the dual isotope diagrams of groundwater nitrates sampled from all fifteen wells. A linear regression line with a slope of approximately 0.5 on a dual isotope diagram is evidence for denitrification (Böttcher *et al*., 1990), although previous studies have also measured linear regression slopes higher than 0.5. Specifically, Cey *et al*. (1999) calculated a linear regression slope of approximately 0.59 and Mengis *et al*. (1999) calculated a

linear regression slope of about 0.67. Singleton *et al*. (2007) plotted linear regression lines with slopes ranging between 0.47 and 0.66, while Fukada *et al*. (2003) reported a slope of 0.76.

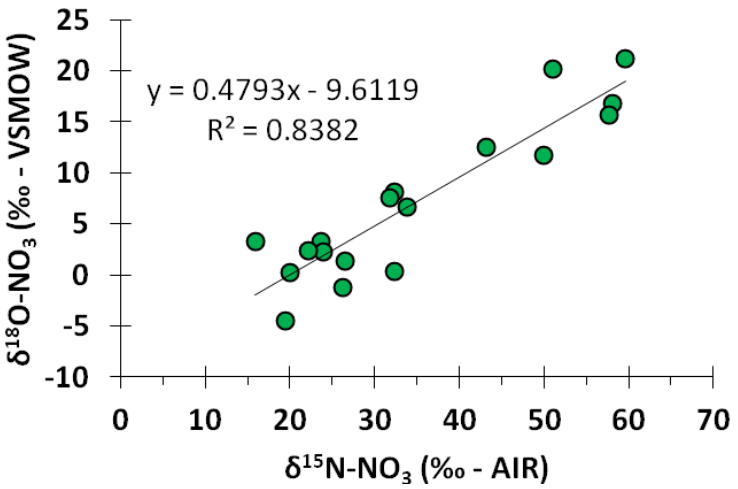

Figure 4.15a: Dual isotope diagram of groundwater nitrates obtained from Well 22 of the alfalfa treatment.

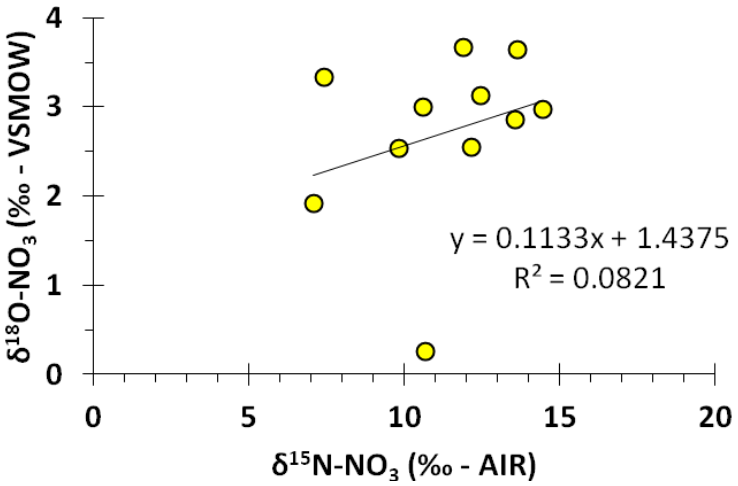

Figure 4.15b: Dual isotope diagram of groundwater nitrates obtained from Well 30 of the CLTN treatment.

Table 4.13: Slopes and R^2 values of linear regression lines on dual isotope diagrams ($\delta^{18}O\text{-}NO_3$ versus $\delta^{15}N\text{-}NO_3$) of groundwater nitrates sampled from various wells and treatments.

Treatment	Well	Slope	R^2
Alfalfa	10	0.75	0.693
	22	0.48	0.838
	34	0.65	0.792
CLTN	8	0.18	0.165
	16	1.11	0.626
	30	0.11	0.082
CRN+m	5	0.15	0.217
	13	0.75	0.597
	31	0.91	0.583
CRN	6	1.31	0.750
	14	0.43	0.872
	32	0.44	0.743
CF200	2	0.38	0.691
	18	1.16	0.756
	28	0.43	0.970

Denitrification was evident in the groundwater of Well 22, as indicated by a linear regression line with a slope of 0.48 (R^2 = 0.838; see Figure 4.15a) on the dual isotope diagram. The dual isotope diagrams of groundwater nitrates obtained from Wells 2, 14, 28, and 32 had respective linear regression lines with slopes of 0.38 (R^2 = 0.691), 0.43 (R^2 = 0.872), 0.43 (R^2 = 0.970), and 0.44 (R^2 = 0.743), which imply that denitrification also occurred in the groundwater within the vicinity of Wells 2, 14, 28, and 32.

Dual isotope diagrams of groundwater nitrates sampled from Wells 6, 10, 13, 16, 18, 31, and 34 showed trends of increasing $\delta^{18}O\text{-}NO_3$ with higher $\delta^{15}N\text{-}NO_3$ values, although the slopes of the regression lines were higher than 0.5 (slopes ranged from 0.65 to 1.31). Well 6 and Well 18 groundwater isotopic data had linear regression lines with the highest slopes of 1.31 and 1.16, respectively. Linear regression lines of dual isotope diagrams with slopes higher than 1.0 may have been a consequence of the open-system nature of the research site. Addition of new nitrates through fertilizer, nitrification, and manure may have affected the isotopic signals typical of denitrification, and thereby caused the slope to be significantly different than the values reported in previous studies.

Denitrification likely did not occur in the groundwater of Well 30, since the dual isotope diagram of groundwater nitrates sampled from Well 30 had a linear regression line with a slope of 0.11 (R^2 = 0.082; see Figure 4.15b). Likewise, groundwater nitrate isotopic data from Well 5 (slope of linear regression line = 0.15 and R^2 = 0.217; see Appendix E) and Well 8 (slope of linear regression line = 0.18 and R^2 = 0.165) did not display trends of increasing $\delta^{18}O$-NO_3 values with higher $\delta^{15}N$-NO_3 values; therefore, denitrification could not be identified confidently for groundwater sampled from Well 5 or Well 8.

Denitrification likely occurred in the groundwater of Wells 6, 10, 13, 14, 18, 22, 28, 32, and 34, given the conclusions drawn from the three sets of graphs. Although the groundwater data from Wells 2, 5, 8, 16, 30, and 31 did not display clear trends indicative of denitrification, partial denitrification may have still occurred at particular instances in time, since the graphs of changes in nitrate concentration with time, and the graph of changes in isotopic composition with time showed trends (i.e., nitrate concentrations decreased, while the $\delta^{15}N$ and $\delta^{18}O$ values of nitrate increased) that are indicative of denitrification at certain time intervals. For example, Well 31 groundwater data showed trends that were not clearly indicative of denitrification (nitrate concentrations fluctuated, while $\delta^{15}N$-NO_3 values increased constantly; see Figure 4.13), but denitrification was suspected to have occurred during some time periods (e.g., July to August of 2009).

Contour maps of the groundwater nitrate concentrations are shown in Figure 4.16. Note that the contour maps should be interpreted with caution, since the study site is highly complex (Rotation U consists of one hundred and eighty plots with several different agricultural treatments), and only groundwater data from fifteen wells were used to draw the contour maps.

In general, the lowest groundwater nitrate concentrations were detected beneath the center plots of Rotation U, and groundwater nitrate concentrations typically increased outwards from the center plots. Given that denitrification occurred in the groundwater, then nitrate concentrations should have decreased along the groundwater flow direction (towards the east), but this was not observed in the groundwater beneath Rotation U. For instance, groundwater nitrate concentrations increased, rather than decreased, towards the east on July 18th, 2006; August 18th, 2006; July 11th, 2007; July 11th, 2009; and August 10th, 2009 (see Figure 4.16).

Groundwater nitrate concentrations may have been influenced by plot location, since groundwater nitrate concentrations down flow of plots with high fertilization rates in the past

were typically higher (see Figure 4.17). For instance, the northernmost CRN+m treatment plot was located directly east of a plot that had received 150kgN/ha/yr of synthetic fertilizers from 1991 to 2003, whereas the CRN+m treatment plot located nearest to the center of Rotation U was east of a plot that had a previous fertilization rate of 50kgN/ha/yr. The average nitrate concentration of groundwater beneath the CRN+m treatment plot east of the plot that had received 50kgN/ha/yr of fertilizer was considerably lower than the average groundwater nitrate concentration under the CRN+m treatment plot located east of the plot with a past fertilizer application rate of 150kgN/ha/yr (47.7±18.3mg/L and 126.1±41.0mg/L, respectively). However, groundwater located down flow of plots with high fertilization rates in the past did not always have corresponding high average nitrate concentrations. For example, the southernmost CF200 treatment plot was located east of a plot that did not receive any synthetic fertilizer before 2004, but the average groundwater nitrate concentration (341.5±258.6mg/L) was substantially higher than the average nitrate concentration in groundwater below the CRN+m treatment plot located east of a plot with a previous fertilization rate of 150kgN/ha/yr (206.3±63.3kgN/ha/yr). This suggests that fertilization rates and groundwater nitrate input from adjacent plots were not the only factors that affected the groundwater nitrate concentrations beneath agricultural plots; denitrification of groundwater nitrates may have also contributed to differences in average groundwater nitrate concentrations under plots with the same agricultural treatment.

High $\delta^{15}N\text{-}NO_3$ values were detected in the groundwater of specific wells (e.g., Well 10 and Well 22) and the $\delta^{15}N\text{-}NO_3$ values decreased outward from the well locations (see Figure 4.18). If denitrification occurred in the groundwater, then the $\delta^{15}N$ values of the groundwater nitrates should have increased down flow, since denitrification enriches the remaining nitrates in ^{15}N (Kendall, 1998); however, the groundwater $\delta^{15}N\text{-}NO_3$ values decreased eastwards on July 18th, 2006; August 18th, 2006; July 15th, 2008; July 11th, 2009; and August 10th, 2009 (see Figure 4.18). In addition, regions with high $\delta^{15}N\text{-}NO_3$ values did not always correspond to regions with low concentrations of groundwater nitrates, and regions with high groundwater nitrate concentrations did not always correspond to regions with low $\delta^{15}N\text{-}NO_3$ values. For example, on August 18th, 2006, the groundwater within the vicinity of Well 10 had nitrate concentrations less than 50mg/L, but the corresponding $\delta^{15}N\text{-}NO_3$ values were less than +10‰, which implies that the low nitrate concentrations in the groundwater from Well 10 may not have been caused by denitrification, since denitrification enriches residual nitrates in ^{15}N.

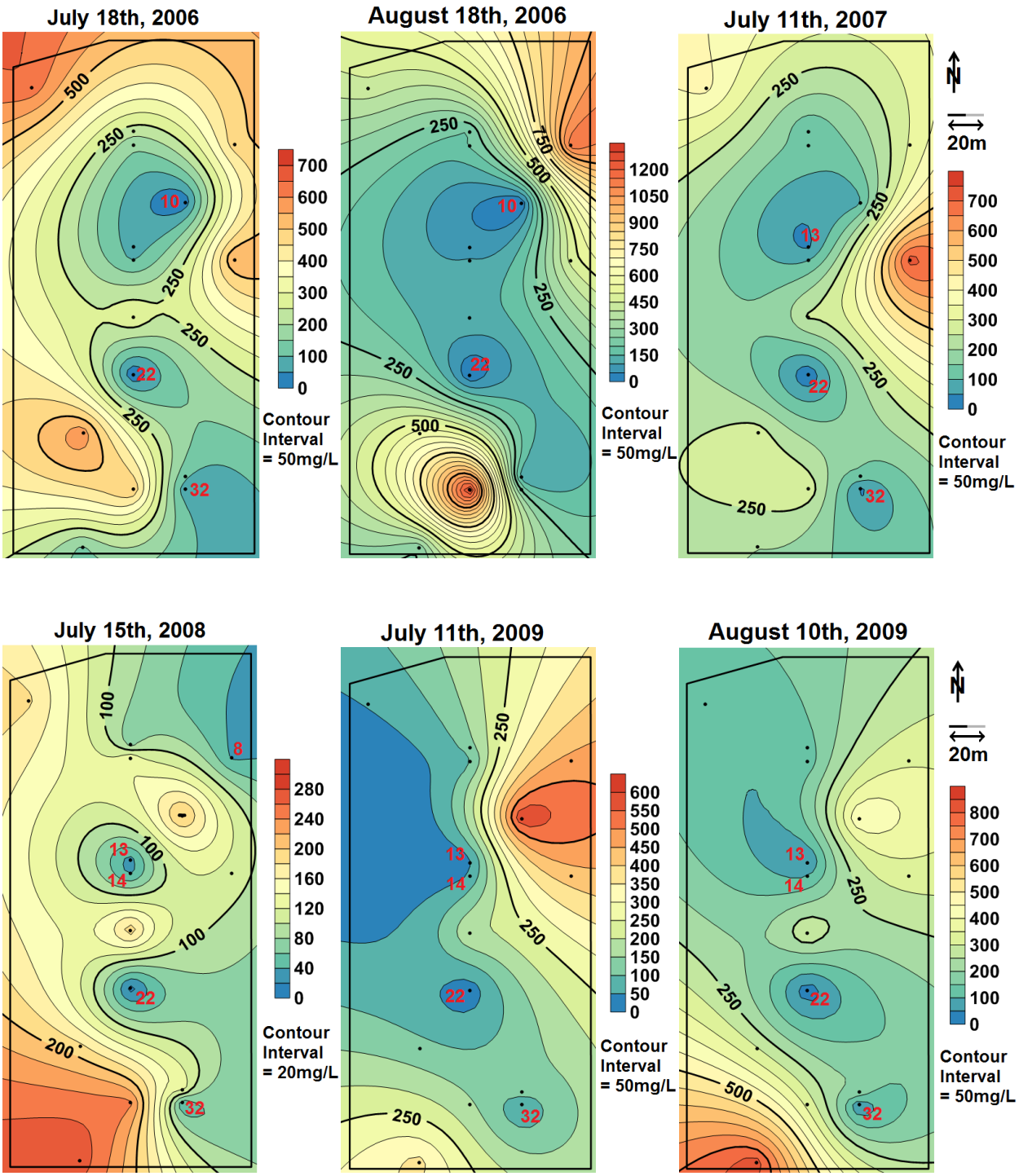

Figure 4.16: Contour maps of groundwater nitrate concentrations (mg/L) showing locations of wells (black circles). Select well numbers with low groundwater nitrate concentrations potentially caused by denitrification are identified in red. Note the difference in contour intervals.

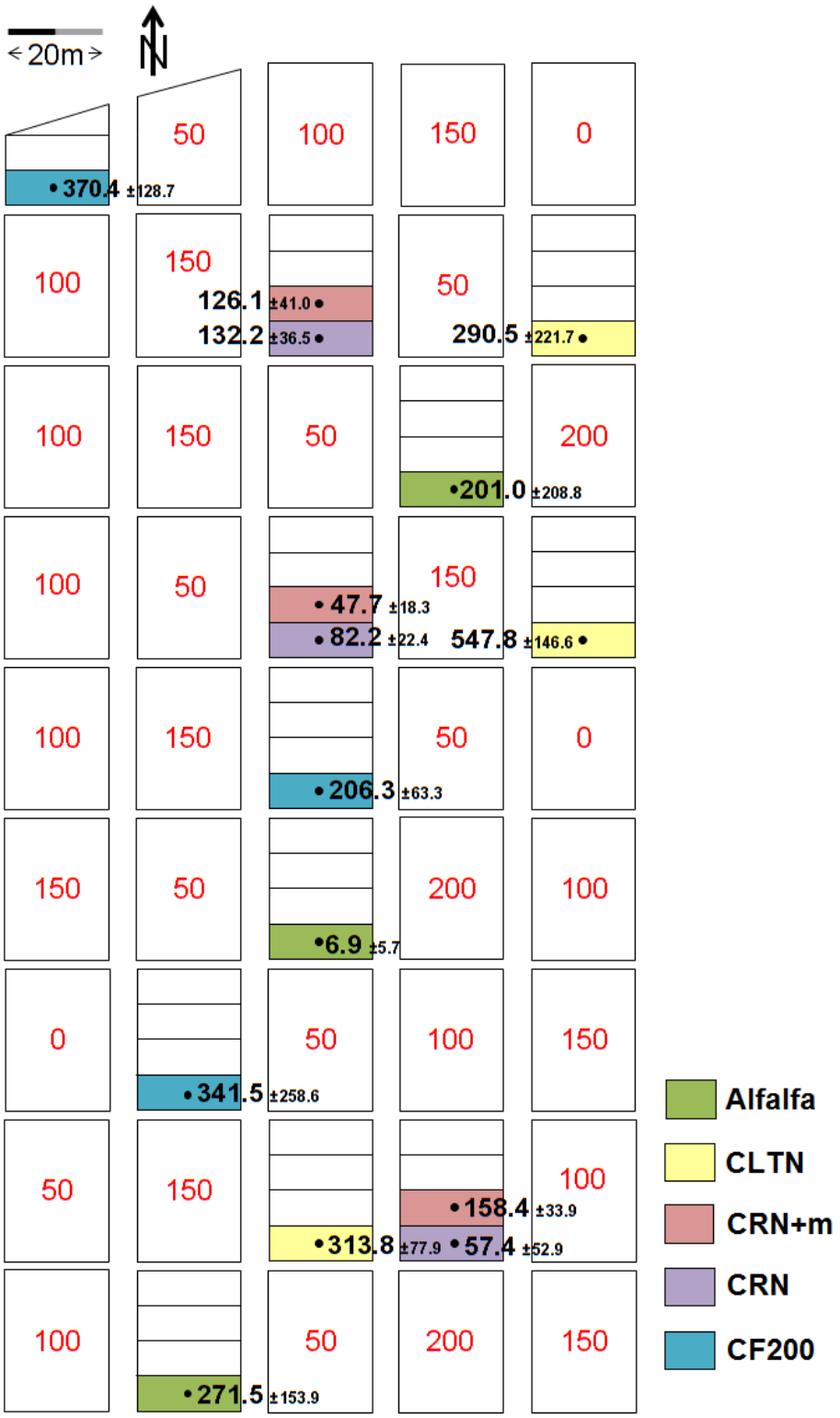

Figure 4.17: Plot plan of Rotation U showing well locations (black circles) and fertilization rates from 1991 to 2003 in kgN/ha/yr (red text). Average nitrate concentrations (±SD) for groundwater sampled from each well are labeled in black text.

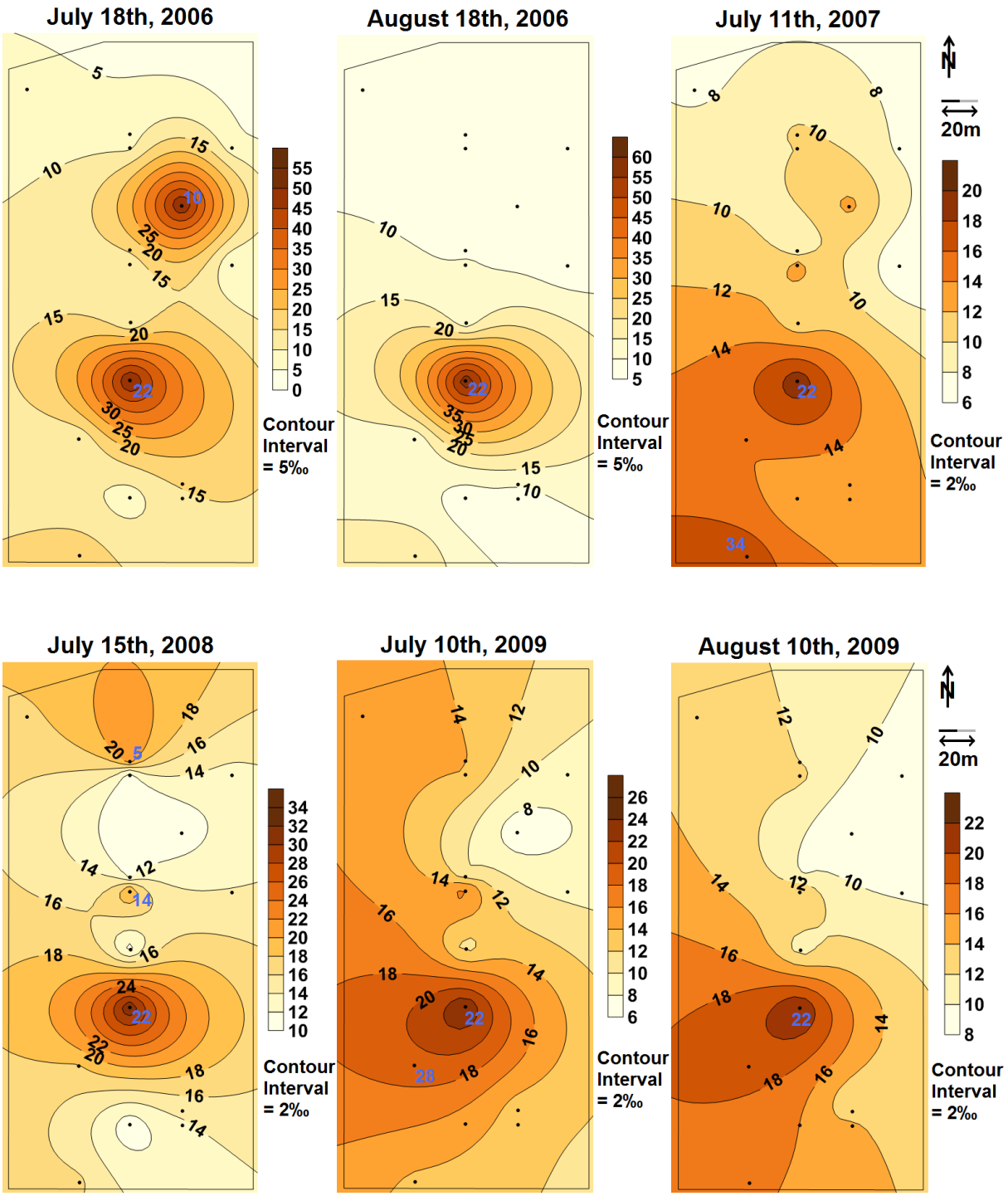

Figure 4.18: Contour maps of groundwater $\delta^{15}N\text{-}NO_3$ values (‰ – AIR) showing locations of wells (black circles). Select well numbers with high groundwater $\delta^{15}N\text{-}NO_3$ values are identified in blue. Note the difference in contour intervals.

Groundwater nitrate concentrations should have theoretically decreased towards the eastern edge of the agricultural field and the $\delta^{15}N$ values of groundwater nitrate should have increased towards the east, if denitrification occurred in the groundwater along its eastward-flowing pathway, but this was not the case, as both low nitrate concentrations and high $\delta^{15}N\text{-}NO_3$ values were restricted to groundwater from specific wells and did not correlate with groundwater flow direction. This may have been in part due to insufficient data for contouring, since only a few wells were located along the groundwater flow-path. Also, "new" nitrates from fertilizers and/or from mineralization of SOM may have been potentially added along the groundwater flow pathway, and caused the isotopic signals of denitrification to have been obscured.

Even though denitrification could not be clearly identified along the flow direction of groundwater, previously discussed evidence (changes in groundwater nitrate concentrations with time, changes in isotopic ratios of nitrate with time, and slopes of the linear regression lines on $\delta^{18}O\text{-}NO_3$ versus $\delta^{15}N\text{-}NO_3$ diagrams) suggest that denitrification likely occurred in the groundwater beneath Rotation U at certain sites. Specifically, groundwater nitrates from nine out of the fifteen wells likely underwent denitrification and the groundwater nitrates from Well 22 in particular must have undergone extensive denitrification, as indicated by a linear regression line with a slope of 0.48 ($R^2 = 0.838$), consistently low nitrate concentrations (< 20mg/L), and by $\delta^{15}N\text{-}NO_3$ values as high as +59.6‰ (see Figure 4.16 and Figure 4.18).

4.3.2.3. Sources of Nitrate

Potential sources of nitrate in groundwater beneath Rotation U include nitrogen fixation, mineralization of SOM, synthetic fertilizers, manure, and nitrification of till ammonium. The isotopic signals were mainly dictated by nitrogen transformation processes, rather than sources of nitrate; hence, the sources are evaluated in this section using soil PRS-probe data and through extrapolation of the linear regression lines on the dual isotope diagrams (see Figure 4.19).

Nitrification of till ammonium was likely not a significant source of groundwater nitrates, since most data points did not fall within the expected range for geologic nitrates ($\delta^{15}N\text{-}NO_3$ = +8 to +26‰, $\delta^{18}O\text{-}NO_3$ = +5 to +9‰; Hendry *et al*., 1984 and Mayer *et al*., 2004). The isotopic source signals typical of fertilizer ($\delta^{15}N\text{-}NO_3$ = −4 to +4‰; Kendall, 1998) and fixed nitrogen ($\delta^{15}N$ of ~0‰) were also not clearly observed in the groundwater below Rotation U.

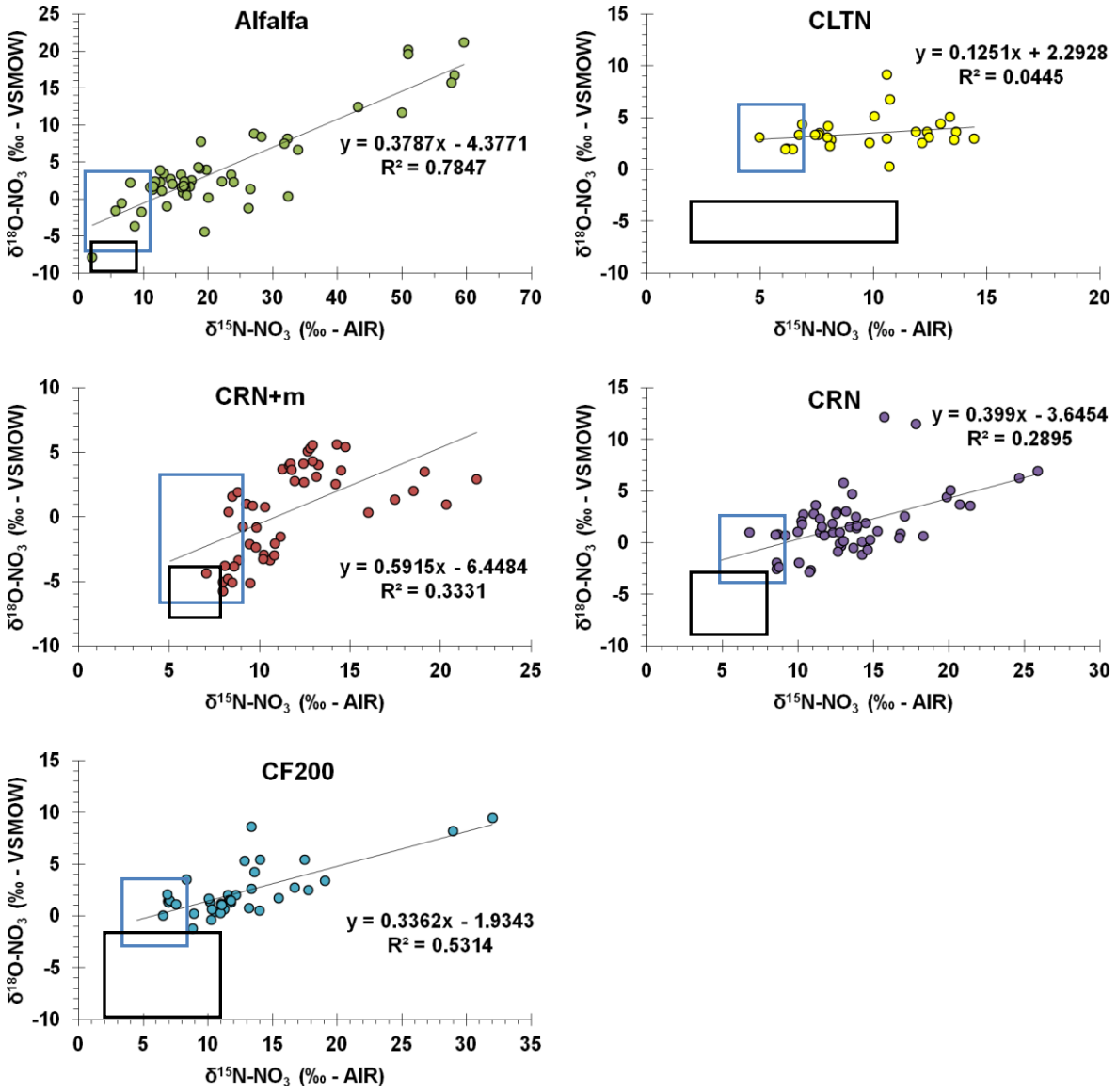

Figure 4.19: Dual isotope diagrams with linear regression lines for groundwater nitrate samples from each treatment. Blue boxed areas represent predicted $\delta^{15}N$ and $\delta^{18}O$ ranges of original nitrate sources. Black boxed areas represent $\delta^{15}N$ and $\delta^{18}O$ ranges of probe-eluted nitrates. Note that the $\delta^{15}N$ and $\delta^{18}O$ ranges of probe-eluted nitrates for the CF200 treatment were estimated using PRS-probe data from the alfalfa, CLTN, CRN+m, and CRN treatments, since PRS-probes were not installed in the CF200 treatment plots.

According to statistical analyses conducted on the total soil nitrogen contents and δ^{15}N-totalN values, fertilizer and manure amendments did not substantially affect the total nitrogen contents of the soils in a single year, which led to the hypothesis in Section 4.1 that synthetic fertilizers and manure were not significant sources of nitrogen input in the short term (soil total nitrogen contents were increased by only ~5-10% through each fertilizer and manure application). Similar conclusions were drawn in Section 4.2 from the isotopic compositions of the probe-extracted nitrates; the soil nitrates were most probably sourced from mineralization of SOM rather than from nitrogen fixation or inorganic fertilizers.

In Section 4.3, however, the differences in groundwater nitrate concentrations between treatments indicated that agricultural amendments had some influence on groundwater nitrate concentrations, since the groundwater beneath heavily fertilized plots had higher average nitrate concentrations than groundwater below plots fertilized at lower rates. It was therefore concluded that synthetic fertilizers, possibly after nitrogen cycling in the soil, likely contributed to the groundwater nitrate pool. Differences in groundwater nitrate concentrations between treatments suggested that nitrogen fixation may have also been a significant source of nitrogen input, since the average groundwater nitrate concentration below the unamended alfalfa treatment was significantly higher than the average groundwater nitrate concentrations below the fertilized CRN+m and CRN treatments.

Although it is likely that some groundwater nitrates were derived from synthetic fertilizers and fixed nitrogen, groundwater δ^{15}N-NO_3 values suggest that synthetic fertilizers and fixed nitrogen were not significant, direct sources of groundwater nitrate. The original isotopic source signals of the synthetic fertilizer and of the fixed nitrogen are likely not observed, possibly due to alteration of the original δ^{15}N and δ^{18}O values by nitrogen transformation processes such as nitrification and denitrification during nitrogen cycling in the soils.

It was previously determined from the PRS-probe data that the source of soil nitrates under the alfalfa treatment was ammonification of leguminous and non-leguminous SOM (δ^{15}N-NO_3 values of eluted nitrates ranged between approximately +2 and +9‰), followed by nitrification. The source of groundwater nitrates under the alfalfa treatment was most probably mineralization of SOM as well, assuming that the groundwater nitrates were leached from the soils above. This hypothesis of nitrate leaching and subsequent denitrification in the groundwater below is supported by conclusions drawn from the graphs of groundwater nitrate concentration

versus time, $\delta^{15}N\text{-}NO_3$ values versus time, and $\delta^{18}O\text{-}NO_3$ values versus time. Extrapolation of the dual isotope linear regression line (see Figure 4.19) also suggests that the groundwater nitrates below the alfalfa treatment were likely produced from mineralization of SOM, given that the initial $\delta^{15}N\text{-}NO_3$ values (before the enrichment of ^{15}N by denitrification) were likely less than +10‰.

Mineralization of non-leguminous SOM was likely the source of soil nitrates under the CLTN treatment, as indicated by probe-eluted $\delta^{15}N\text{-}NO_3$ values between roughly +2 and +11‰. If the groundwater nitrates were leached from the overlying soils, then the $\delta^{15}N$ values of the groundwater nitrates should be similar to the $\delta^{15}N$ values of the soil nitrates. The $\delta^{15}N$ values ranged between approximately +5 and +15‰ (see Figure 4.19) for groundwater nitrates under the CLTN treatment plots; therefore, the groundwater nitrates were likely soil-nitrified nitrates that were leached from the overlying soils. In addition, the extrapolated groundwater $\delta^{15}N\text{-}NO_3$ values fall within the range of expected values for mineralization of SOM (less than +10‰), which implies that the groundwater nitrates were leached from the soils above.

Even though CLTN was not a manured treatment, it had high groundwater $\delta^{15}N\text{-}NO_3$ values (greater than +8‰) which are indicative of manure influence. A potential explanation is that the groundwater incorporated nitrates from past manure application events (manure was applied on all plots up to 1989), or that groundwater mixing caused the nitrates from the manured plots to be intermixed with nitrates from the non-manured CLTN plots, since two of the CLTN treatment plots were located down-flow of the CRN+m treatment plots (see Figure 4.17). This is not as probable, considering that groundwater from beneath the CRN+m treatment did not have elevated $\delta^{15}N\text{-}NO_3$ values even though manure was applied directly on the CRN+m plots in 2007. Hence, a more likely explanation is that the groundwater nitrates below the CLTN treatment plots underwent partial denitrification, which caused the $\delta^{15}N\text{-}NO_3$ values to increase.

Probes from the CRN+m, CRN, and CF200 treatments had extracted nitrates with $\delta^{15}N$ values that ranged from +5 to +8‰, from +3 to +8‰, and from +2 to +11‰, respectively. Mineralization of SOM was identified as the source of soil nitrate under the CRN+m, CRN, and CF200 treatment plots in Section 4.2. Since the extrapolated groundwater $\delta^{15}N\text{-}NO_3$ values for the CRN+m, CRN, and CF200 treatments were approximately +10‰ or less, the groundwater nitrates beneath the CRN+m, CRN, and CF200 treatments were also likely nitrified-nitrates that were sourced from the overlying soils.

The $\delta^{15}N$ values of groundwater nitrate suggest that mineralization of SOM was the main source of groundwater nitrates, and that synthetic fertilizers and fixed nitrogen were not significant, direct sources of nitrate input. However, the original isotopic signal of the synthetic fertilizer and the original isotopic signal of the fixed nitrogen may have been lost during nitrogen transformation processes (i.e., during nitrification/denitrification).

CHAPTER 5: SUMMARY AND CONCLUSIONS

In this study, nitrogen in the soil and in shallow groundwater beneath an agricultural research field, identified as "Rotation U," was studied extensively to i) evaluate the extent of shallow groundwater nitrate contamination, ii) better comprehend the fate of anthropogenic nitrogen in an agroecosystem, iii) determine the sources of soil and groundwater nitrate, iv) identify the occurrence of nitrogen transformation processes, and v) assess any differences in soil or groundwater nitrogen caused by varying agricultural treatments through the use of stable isotopes. The five treatments investigated in this study were the unamended alfalfa treatment, the long-term fertilized corn treatment (CLTN), the recently fertilized corn treatment (CRN), the recently fertilized and manured corn treatment (CRN+m), and the previously fertilized corn treatment (CF200).

5.1. Soils

Sixty air-dried soil samples were obtained in May of 2008 from the 0-15cm, 15-30cm, 30-45cm, 45-60cm, and 60-90cm soil depth intervals under the alfalfa, CRN, CRN+m, and CF200 treatments. The soils were analyzed for total nitrogen content and nitrogen isotopic composition.

Total nitrogen contents within the upper 15cm of soil averaged 0.20±0.01% and the soils were estimated to contain ~4200kgN/ha of nitrogen. Average total nitrogen contents were very similar and did not differ significantly between treatments, indicating that relatively recent agricultural amendments within two decades of the study period did not substantially impact the soil total nitrogen pool in the short term. This may have been in part due to the fact that each annual synthetic fertilizer application and each manure application circa once every five years increased the total nitrogen contents in the soils by less than 5% and less than 10%, respectively. Over longer periods of time (several decades), however, synthetic fertilizer and manure inputs likely had a larger cumulative effect on the pool of soil total nitrogen.

Similarly, there was no significant difference in the average soil δ^{15}N-totalN values of the alfalfa (7.0±1.1‰), CRN (6.9±0.8‰), CRN+m (7.1±1.0‰), and CF200 (7.2±1.1‰) treatment plots. The original source signals of fixed nitrogen and of synthetic fertilizer (δ^{15}N values of ~0‰) were not detected in any of the soil samples, despite nitrogen fixation and synthetic

fertilizer having been the main sources of nitrogen input on the alfalfa and corn treatments plots since 1991. This may have been a result of the pool of nitrogen present in the soils having been considerably larger than the amount of nitrogen provided through synthetic fertilizer application. All four treatments had average soil δ^{15}N-totalN values of approximately +7‰, which is likely a long-term cumulative value resulting from nitrogen sources (possibly manure applied before 1989) and recurring nitrogen transformation processes in the soil.

5.2. Plant Root Simulator (PRS) Probes

Plant root simulator probes are ion exchange membranes that were used to measure the nitrate supply rates of soils. Twenty-four PRS-probes were installed on August 4th, 2011 and on August 20th, 2011 (two days after the removal of the first set of probes) on plots under the alfalfa, CRN, CRN+m, and CLTN treatments. The adsorbed nitrates were eluted and analyzed isotopically to determine the source(s) of soil nitrate.

Statistical analyses conducted on the 2-week and 4-week duration PRS-probe data suggested that fertilizer and manure application likely did not have a large impact on soil nitrate supply rates, since plots under various agricultural treatments did not have significantly different average nitrate supply rates.

Isotopic data (δ^{15}N-NO_3 = +2 to +11‰, δ^{18}O-NO_3 = −2 to −10‰) from the 2-week and 4-week duration PRS-probe extractions implied that the source of nitrates in the soil was mineralization of SOM. More specifically, soil nitrates from the plots under the CRN, CRN+m, and CLTN treatments were produced by mineralization of non-leguminous SOM, as indicated by average eluted δ^{15}N-NO_3 values of less than 10‰. This finding is in agreement with the research of Oelmann *et al*. (2007), who calculated an average δ^{15}N-NO_3 value of 9.3±0.9‰ for nitrates derived from the mineralization of non-leguminous SOM.

Soil nitrates from the alfalfa treatment plots were likely sourced from a combination of both mineralization of leguminous SOM and mineralization of non-leguminous SOM, since the soil nitrates extracted from the 2-week PRS-probes had an average δ^{15}N-NO_3 value of 3.8±1.5‰, (similar to the average δ^{15}N-NO_3 value of 1.5±0.6‰ reported by Oelmann *et al*., 2007 for nitrates derived from the mineralization of leguminous SOM), while the 4-week probes had extracted nitrates with an average δ^{15}N-NO_3 value of 5.9±1.5‰. The average δ^{18}O-NO_3 values

were slightly lower than the expected −3‰ for soil nitrification, possibly due to oxygen isotope exchange with water.

5.3. Groundwater

Two hundred and thirty-five shallow groundwater samples were collected between July 18th, 2006 and October 5th, 2009. All groundwater samples were analyzed for nitrate concentrations and most of the groundwater samples (n = 229) were analyzed for isotopic compositions of nitrate. The average groundwater nitrate concentration for the entire dataset was 187.7±193.1mg/L, and only fifty-three samples had nitrate concentrations below the WHO (2008) nitrate limit of 45mg/L for drinking water, signifying severe nitrate contamination of the shallow groundwater beneath Rotation U.

Decreasing average groundwater nitrate concentrations under the CLTN treatment were suspected to have been caused by the decrease in fertilizer application rates in year 2004 and denitrification. The alfalfa treatment had increasing average groundwater nitrate concentrations, which were hypothesized to have been a result of nitrogen fixation and subsequent leaching of mineralized nitrogen. Manure that was applied relatively recently (since 1991, only on the CRN+m plots) did not appear to have been a major source of groundwater nitrogen input in the short term (less than a few decades), since the CRN+m treatment had average groundwater nitrate concentrations similar to the non-manured CRN treatment. Application of manure likely did not affect groundwater nitrate concentrations considerably due to the fact that manure contains mostly organic nitrogen; groundwater nitrate concentrations would not reflect changes caused by manure application until the organic nitrogen is converted into nitrate through ammonification and nitrification. Additionally, the inorganic nitrogen derived from the manure may have been assimilated by plants and microorganisms in the soil zone, which might have left little to be leached into the groundwater below.

Statistical analyses conducted on the average groundwater nitrate concentrations of the five treatments suggested that fertilizer amendments did indeed have an effect on average groundwater nitrate concentrations. The CLTN treatment resulted in the highest average groundwater nitrate concentration, most likely because it had received synthetic fertilizers since 1991, whereas the CRN and CRN+m treatments had the lowest average groundwater nitrate concentrations, likely because synthetic fertilizers were only applied relatively recently as of

2004. However, the average groundwater nitrate concentration beneath the alfalfa treatment was significantly higher than the average groundwater nitrate concentrations beneath the synthetically-fertilized CRN and CRN+m treatments. This suggested that synthetic fertilizer application was not the only factor that influenced average groundwater nitrate concentrations; nitrogen transformation processes, such as nitrogen fixation, nitrification, and denitrification, also affected the nitrate concentrations of groundwater.

Although treatment differences in average groundwater nitrate concentrations suggested that synthetic fertilizer and nitrogen fixation (followed by ammonification of the SOM, nitrification, and leaching of the nitrates) likely contributed to groundwater nitrates, the isotopic signals of synthetic fertilizer and fixed nitrogen were not clearly observed in the groundwater nitrate isotopic data, due to the occurrence of nitrification in the soils and extensive denitrification in the aquifer, which altered the original isotopic nitrate source signals.

Mineralization of SOM in soils and subsequent transport to the groundwater was determined to have been the main source of groundwater nitrates, given the isotopic compositions of probe-extracted nitrates and the extrapolation of linear regression lines on the dual isotope diagrams ($\delta^{15}N\text{-}NO_3$ values were estimated to have been less than +10‰, identifying mineralization of SOM as the main source of nitrates). Temporal data trends (changes in nitrate concentration, $\delta^{15}N\text{-}NO_3$ values, and $\delta^{18}O\text{-}NO_3$ values over time) also suggested that nitrates sourced from mineralization of SOM were leached into the groundwater below, where denitrification subsequently occurred.

Groundwater nitrate isotopic data showed strong evidence that groundwater nitrates sampled from nine out of the fifteen wells had likely undergone denitrification. In particular, groundwater nitrate samples obtained from Well 22 of the alfalfa treatment had undergone substantial denitrification, as suggested by an average $\delta^{15}N\text{-}NO_3$ value of +34.9±14.6‰ and a linear regression line with a slope of 0.48 (R^2 = 0.838) on a $\delta^{18}O\text{-}NO_3$ versus $\delta^{15}N\text{-}NO_3$ diagram. As a result, groundwater obtained from Well 22 had an average groundwater nitrate concentration of only 6.9±5.7mg/L, the lowest of all the wells.

Denitrification could not be confidently identified along groundwater flow pathways, due to the complex nature of the agroecosystem. Nitrate concentrations should have theoretically decreased along the groundwater flow-path, whereas the $\delta^{15}N$ values of groundwater nitrate should have increase down flow, but $\delta^{15}N\text{-}NO_3$ values and nitrate concentrations were not

always correlated with groundwater flow direction. Additional nitrates leached from the soils may have been added to the groundwater during flow and caused the $\delta^{15}N\text{-}NO_3$ values to be altered. Plot location (being in close proximity to other plots with historically high fertilization rates) may have also affected the groundwater nitrate concentrations beneath particular treatment plots.

Although the groundwater nitrate $\delta^{15}N$ values predominantly reflected nitrogen transformation processes and did not conclusively identify synthetic fertilizers or manure as direct sources of groundwater nitrate, from the measured groundwater nitrate concentrations, it was clear that anthropogenic nitrogen inputs had a significant impact on the shallow groundwater. Nitrate concentrations as high as 1276.6mg/L were measured under the long-term fertilized corn treatment. Groundwater nitrate concentrations had decreased significantly since the 2004 fertilization rate reduction (the average groundwater nitrate concentrations under the CLTN treatment decreased from 499.1±34.9mg/L in 2006 to 149.0±111.6mg/L in 2008), but the nitrate concentrations in the groundwater were still considerably higher than the WHO (2008) nitrate concentration limit of 45mg/L for drinking water. As a result of the unconfined nature of the aquifer, risk of further nitrate contamination is high.

Denitrification, which occurred extensively in the groundwater beneath Rotation U, is a process that can significantly reduce groundwater nitrate concentrations through natural means. If denitrification is enhanced by providing sufficient labile organic matter or other electron donors, then denitrification may be a highly efficient and effective method to lower groundwater nitrate concentrations to levels safe enough for drinking.

5.4. Future Research

To better evaluate the differences in soil total nitrogen contents and in $\delta^{15}N\text{-totalN}$ values (caused by varying agricultural amendments) at each soil depth interval, more samples ($n > 5$) should be taken from each soil layer in order to provide sufficient data for more accurate statistical results. Because isotopic fractionation occurred during the elution process, complete elution of the nitrates is necessary and several successive elutions should be combined into a bulk sample before performing isotopic analysis. Further research may also be required to evaluate the potential for isotopic fractionation during probe adsorption of soil nitrates. In future studies, the wells should be purged before groundwater sampling to ensure that the water within

the wells are representative of formation water and not stagnant well-water. Correlation studies of water table depth with groundwater nitrate concentration may provide additional insight into the possibility of groundwater nitrates being sourced from soil nitrates. Recharge water transit times can also provide more insight into the potential effects of fertilizer amendments on groundwater geochemistry and would aid in more accurate risk assessments of groundwater nitrate contamination; this can be done through multiple methods, such as tracer tests. In addition, dissolved oxygen contents and groundwater ammonium concentrations should be measured in the future to better evaluate the potential for nitrification and/or denitrification in groundwater. More wells should also be drilled if possible, to provide better spatial resolution for the identification of denitrification along groundwater flow pathways.

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APPENDIX A: SOIL DATA

Table A-1: Total nitrogen contents and δ^{15}N-totalN values for the 2008 soils sampled from the 0-15cm, 15-30cm, 30-45cm, 45-60cm, and 60-90cm soil depth intervals under the alfalfa, CRN+m, CRN, and CF200 treatment plots.

Well #	Plot	Soil Depth (cm)	δ^{15}N – totalN (‰ – AIR)	% N	Treatment
2	206S	0-15	6.4	0.19	CF200
2	206S	15-30	6.9	0.16	CF200
2	206S	30-45	6.5	0.10	CF200
2	206S	45-60	6.0	0.07	CF200
2	206S	60-90	4.8	0.05	CF200
5	308N	0-15	7.8	0.22	CRN+m
5	308N	15-30	7.3	0.18	CRN+m
5	308N	30-45	7.1	0.14	CRN+m
5	308N	45-60	6.7	0.10	CRN+m
5	308N	60-90	5.6	0.07	CRN+m
6	308S	0-15	7.3	0.19	CRN
6	308S	15-30	7.7	0.17	CRN
6	308S	30-45	7.0	0.13	CRN
6	308S	45-60	5.9	0.09	CRN
6	308S	60-90	5.3	0.06	CRN
10	409S	0-15	7.1	0.21	Alfalfa
10	409S	15-30	7.7	0.18	Alfalfa
10	409S	30-45	7.6	0.16	Alfalfa
10	409S	45-60	7.3	0.12	Alfalfa
10	409S	60-90	5.2	0.07	Alfalfa
13	508N	0-15	7.8	0.22	CRN+m
13	508N	15-30	7.5	0.19	CRN+m
13	508N	30-45	6.6	0.12	CRN+m
13	508N	45-60	6.4	0.11	CRN+m
13	508N	60-90	5.0	0.07	CRN+m
14	508S	0-15	6.8	0.19	CRN
14	508S	15-30	7.3	0.18	CRN
14	508S	30-45	6.5	0.11	CRN
14	508S	45-60	6.7	0.11	CRN
14	508S	60-90	5.5	0.09	CRN
18	608S	0-15	8.0	0.19	CF200
18	608S	15-30	7.3	0.15	CF200
18	608S	30-45	6.9	0.11	CF200
18	608S	45-60	6.5	0.12	CF200
18	608S	60-90	6.3	0.09	CF200
22	708S	0-15	6.5	0.22	Alfalfa

Well #	Plot	Soil Depth (cm)	$\delta^{15}N$ – totalN (‰ – AIR)	% N	Treatment
22	708S	15-30	7.1	0.18	Alfalfa
22	708S	30-45	6.7	0.13	Alfalfa
22	708S	45-60	6.4	0.10	Alfalfa
22	708S	60-90	4.8	0.06	Alfalfa
28	807S	0-15	8.0	0.18	CF200
28	807S	15-30	8.6	0.16	CF200
28	807S	30-45	9.0	0.11	CF200
28	807S	45-60	8.4	0.10	CF200
28	807S	60-90	7.7	0.08	CF200
31	909N	0-15	8.2	0.20	CRN+m
31	909N	15-30	8.5	0.17	CRN+m
31	909N	30-45	7.4	0.10	CRN+m
31	909N	45-60	7.0	0.08	CRN+m
31	909N	60-90	8.3	0.06	CRN+m
32	909S	0-15	7.6	0.19	CRN
32	909S	15-30	8.2	0.17	CRN
32	909S	30-45	7.3	0.11	CRN
32	909S	45-60	7.1	0.08	CRN
32	909S	60-90	6.7	0.06	CRN
34	1007S	0-15	7.8	0.21	Alfalfa
34	1007S	15-30	8.7	0.18	Alfalfa
34	1007S	30-45	8.5	0.13	Alfalfa
34	1007S	45-60	7.5	0.11	Alfalfa
34	1007S	60-90	6.4	0.07	Alfalfa

APPENDIX B: PRS-PROBE DATA

Table B-1: Supply rates and $\delta^{15}N$ values of extracted nitrates from the 2-week PRS-probes.

Well #	Date	$mgNO_3/cm^2/2wks$	$\delta^{15}N_{nitrate}$ (‰)	$\delta^{18}O_{nitrate}$ (‰)	Treatment
10	04-Aug-11	0.12	4.0	-7.8	Alfalfa
22	04-Aug-11	0.11	2.2	-7.0	Alfalfa
34	04-Aug-11	0.10	5.1	-6.4	Alfalfa
5	04-Aug-11	0.10	7.7	-4.4	CRN+m
13	04-Aug-11	0.09	7.7	-5.8	CRN+m
31	04-Aug-11	0.16	10.7	-4.0	CRN+m
6	04-Aug-11	0.25	7.6	-3.3	CRN
14	04-Aug-11	0.06	5.8	-6.6	CRN
32	04-Aug-11	0.07	7.4	-6.9	CRN
8	04-Aug-11	0.14	9.7	-3.4	CLTN
16	04-Aug-11	0.06	10.4	-5.6	CLTN
30	04-Aug-11	0.05	6.5	-6.9	CLTN

Table B-2: Supply rates and $\delta^{15}N$ values of extracted nitrates from the 4-week PRS-probes.

Well #	Date	$mgNO_3/cm^2/4wks$	$\delta^{15}N_{nitrate}$ (‰)	$\delta^{18}O_{nitrate}$ (‰)	Treatment
10	20-Aug-11	0.03	8.7	-7.1	Alfalfa
10	20-Aug-11	0.07	4.4	-8.4	Alfalfa
22	20-Aug-11	0.05	5.1	-8.1	Alfalfa
22	20-Aug-11	0.07	5.4	-8.4	Alfalfa
34	20-Aug-11	0.06	5.8	-9.0	Alfalfa
34	20-Aug-11	0.01	6.0	-14.9	Alfalfa
5	20-Aug-11	0.06	5.2	-6.7	CRN+m
5	20-Aug-11	0.01	5.9	-13.3	CRN+m
13	20-Aug-11	0.03	4.9	-13.9	CRN+m
13	20-Aug-11	0.03	6.7	-6.6	CRN+m
31	20-Aug-11	0.03	7.4	-7.1	CRN+m
31	20-Aug-11	0.05	7.8	-6.2	CRN+m
6	20-Aug-11	0.04	5.7	-6.5	CRN
6	20-Aug-11	0.07	6.8	-5.4	CRN
14	20-Aug-11	0.02	3.2	-7.9	CRN
32	20-Aug-11	0.15	6.7	-5.3	CRN
32	20-Aug-11	0.04	5.9	-8.3	CRN
8	20-Aug-11	0.08	4.6	-5.2	CLTN

Well #	Date	$mgNO_3/cm^2/4wks$	$\delta^{15}N_{nitrate}$ (‰)	$\delta^{18}O_{nitrate}$ (‰)	Treatment
8	20-Aug-11	0.02	4.2	-5.6	CLTN
16	20-Aug-11	0.05	6.2	-3.0	CLTN
16	20-Aug-11	0.05	9.0	-4.6	CLTN
30	20-Aug-11	0.03	4.7	-6.7	CLTN
30	20-Aug-11	0.18	2.1	-5.9	CLTN

APPENDIX C: GROUNDWATER DATA

Table C-1: Water table elevations beneath Rotation U in meters above sea level (m ASL) for years 2006 to 2009.

Well	Water Table Elevation (m ASL)			
	July 2006	July 2007	July 2008	July 2009
2	907.182	906.582	906.662	-
5	906.753	906.203	905.993	906.383
6	906.753	906.303	906.093	906.423
8	905.65	905.02	904.51	-
10	905.98	905.96	905.88	905.98
13	906.669	905.959	905.889	906.239
14	906.699	905.959	905.769	906.189
16	905.938	904.978	-	-
18	906.563	906.093	905.913	906.233
22	906.377	905.997	905.877	906.207
28	906.701	906.081	906.081	-
30	906.58	906.06	905.89	-
31	906.446	905.826	905.556	906.076
32	906.326	905.806	905.516	905.966
34	906.549	906.299	906.419	906.589

Table C-2: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values for groundwater sampled from fifteen wells on various sampling dates.

Well	Date	$[NO_3]$ mg/L	$\delta^{15}N_{nitrate}$ (‰)	$\delta^{18}O_{nitrate}$ (‰)	Treatment
22	July 18, 2006	2.7	51.0	20.2	Alfalfa
22	August 18, 2006	2.1	59.6	21.2	Alfalfa
22	March 9, 2007	5.2	58.1	16.8	Alfalfa
22	April 12, 2007	6.0	57.7	15.7	Alfalfa
22	May 9, 2007	7.0	32.4	0.3	Alfalfa
22	June 13, 2007	7.2	26.3	-1.2	Alfalfa
22	July 11, 2007	7.1	19.5	-4.4	Alfalfa
22	August 13, 2007	5.0			Alfalfa
22	August 24, 2007	1.0	50.0	11.7	Alfalfa
22	April 28, 2008	1.2	15.9	3.3	Alfalfa
22	May 12, 2008	1.5	23.6	3.3	Alfalfa
22	June 25, 2008	10.2	32.3	8.2	Alfalfa
22	July 15, 2008	10.7	31.8	7.5	Alfalfa

Well	Date	$[NO_3]$ mg/L	$\delta^{15}N_{nitrate}$ (‰)	$\delta^{18}O_{nitrate}$ (‰)	Treatment
22	August 11, 2008	0.7	33.9	6.7	Alfalfa
22	August 25, 2008	4.4	43.2	12.5	Alfalfa
22	July 10, 2009	19.1	24.0	2.3	Alfalfa
22	July 24, 2009	19.2	20.0	0.2	Alfalfa
22	August 10, 2009	15.3	22.2	2.4	Alfalfa
22	August 26, 2009	9.3	26.0	37.2	Alfalfa
22	September 10, 2009	9.4	19.7	39.1	Alfalfa
22	October 5, 2009	6.3	26.6	1.4	Alfalfa
10	July 18, 2006	9.1	50.9	19.6	Alfalfa
10	August 18, 2006	0.7	8.7	-3.6	Alfalfa
10	March 9, 2007	0.9	11.5	1.3	Alfalfa
10	April 12, 2007	1.8	2.1	-7.9	Alfalfa
10	May 9, 2007	167.1	13.1	3.5	Alfalfa
10	June 13, 2007	217.9	12.6	3.9	Alfalfa
10	July 11, 2007	86.3	12.6	2.3	Alfalfa
10	August 13, 2007	5.4	14.2	2.7	Alfalfa
10	August 24, 2007	5.0	14.5	2.0	Alfalfa
10	April 28, 2008	1.3	9.8	-1.7	Alfalfa
10	June 25, 2008	171.0	11.8	2.4	Alfalfa
10	July 15, 2008	208.1	11.1	1.6	Alfalfa
10	August 11, 2008	236.0	11.5	1.7	Alfalfa
10	August 25, 2008	47.2	12.9	1.1	Alfalfa
10	July 10, 2009	593.5	6.7	-0.6	Alfalfa
10	July 24, 2009	619.6	5.7	-1.6	Alfalfa
10	August 10, 2009	451.7	8.0	2.2	Alfalfa
10	August 26, 2009	388.5	-	-	Alfalfa
10	September 10, 2009	451.4	-	-	Alfalfa
10	October 5, 2009	357.5	-	-	Alfalfa
34	July 18, 2006	141.0	18.9	7.7	Alfalfa
34	August 18, 2006	255.1	19.8	4.0	Alfalfa
34	March 9, 2007	168.5	18.7	4.1	Alfalfa
34	April 12, 2007	160.6	18.5	4.3	Alfalfa
34	May 9, 2007	265.4	16.5	2.4	Alfalfa
34	June 13, 2007	257.0	17.1	2.0	Alfalfa
34	July 11, 2007	201.3	17.5	2.6	Alfalfa
34	August 13, 2007	123.4	27.1	8.9	Alfalfa
34	May 12, 2008	105.9	28.2	8.4	Alfalfa
34	June 25, 2008	291.9	17.2	1.7	Alfalfa
34	July 15, 2008	270.7	16.5	1.3	Alfalfa

Well	Date	$[NO_3]$ mg/L	$\delta^{15}N_{nitrate}$ (‰)	$\delta^{18}O_{nitrate}$ (‰)	Treatment
34	August 11, 2008	307.5	16.2	0.8	Alfalfa
34	August 25, 2008	273.9	16.7	0.5	Alfalfa
34	July 10, 2009	365.6	13.6	-1.0	Alfalfa
34	July 24, 2009	247.5	16.2	1.7	Alfalfa
34	August 10, 2009	827.9	16.4	1.9	Alfalfa
34	August 26, 2009	254.0	16.0	1.6	Alfalfa
34	September 10, 2009	265.6	16.3	2.3	Alfalfa
34	October 5, 2009	375.4	16.2	1.8	Alfalfa
8	July 18, 2006	476.7	6.9	4.3	CLTN
8	August 18, 2006	1136.2	7.7	4.2	CLTN
8	March 9, 2007	110.2	10.0	5.1	CLTN
8	April 12, 2007	243.2	10.7	6.7	CLTN
8	May 9, 2007	487.4	8.0	4.2	CLTN
8	June 13, 2007	572.9	7.6	3.5	CLTN
8	July 11, 2007	304.5	7.6	3.4	CLTN
8	August 13, 2007	567.9	8.1	2.9	CLTN
8	June 25, 2008	48.8	13.0	4.4	CLTN
8	July 15, 2008	36.2	13.4	5.1	CLTN
8	August 11, 2008	57.4	12.4	3.7	CLTN
16	July 18, 2006	548.7	5.0	3.1	CLTN
16	August 18, 2006	472.9	6.2	2.0	CLTN
16	March 9, 2007	370.5	8.0	3.1	CLTN
16	April 12, 2007	365.9	8.1	2.3	CLTN
16	May 9, 2007	645.8	6.4	2.0	CLTN
16	June 13, 2007	745.2	6.7	3.3	CLTN
16	July 11, 2007	721.9	6.1	2.0	CLTN
16	August 13, 2007	511.2	10.6	9.2	CLTN
30	July 18, 2006	498.0	7.4	3.3	CLTN
30	August 18, 2006	1276.6	7.1	1.9	CLTN
30	March 9, 2007	381.8	10.6	3.0	CLTN
30	April 12, 2007	304.4	11.9	3.7	CLTN
30	May 9, 2007	268.0	13.6	3.6	CLTN
30	June 13, 2007	307.3	12.4	3.1	CLTN
30	July 11, 2007	286.4	12.1	2.6	CLTN
30	August 13, 2007	340.7	9.8	2.5	CLTN
30	June 25, 2008	264.5	13.6	2.9	CLTN
30	July 15, 2008	245.0	10.7	0.3	CLTN
30	August 11, 2008	241.8	14.5	3.0	CLTN
5	August 18, 2006	197.0	9.1	-0.8	CRN+m

Well	Date	$[NO_3]$ mg/L	$\delta^{15}N_{nitrate}$ (‰)	$\delta^{18}O_{nitrate}$ (‰)	Treatment
5	March 9, 2007	153.8	8.5	1.6	CRN+m
5	April 12, 2007	169.6	8.8	1.9	CRN+m
5	May 9, 2007	168.6	8.3	0.4	CRN+m
5	June 13, 2007	164.6	9.3	1.0	CRN+m
5	July 11, 2007	106.2	10.3	0.7	CRN+m
5	August 13, 2007	97.7	19.1	3.5	CRN+m
5	August 24, 2007	85.8	18.5	2.0	CRN+m
5	June 25, 2008	81.8	16.0	0.3	CRN+m
5	July 15, 2008	68.8	22.0	2.9	CRN+m
5	August 11, 2008	94.5	17.5	1.4	CRN+m
5	July 10, 2009	133.4	14.2	2.5	CRN+m
5	July 24, 2009	178.4	9.5	-2.1	CRN+m
5	August 10, 2009	129.6	11.9	2.8	CRN+m
5	August 26, 2009	207.5	6.4	37.5	CRN+m
5	September 10, 2009	106.6	13.1	3.1	CRN+m
5	October 5, 2009	81.5	20.3	1.0	CRN+m
13	August 18, 2006	78.3	7.1	-4.4	CRN+m
13	March 9, 2007	64.0	8.1	-3.8	CRN+m
13	April 12, 2007	51.6	8.8	-3.4	CRN+m
13	May 9, 2007	52.7	8.0	-5.8	CRN+m
13	June 13, 2007	55.1	8.0	-5.1	CRN+m
13	July 11, 2007	37.3	8.3	-4.8	CRN+m
13	August 13, 2007	45.3	9.5	-5.1	CRN+m
13	August 24, 2007	37.6	10.6	-3.3	CRN+m
13	June 25, 2008	95.3	8.6	-3.8	CRN+m
13	July 15, 2008	34.1	11.2	-1.5	CRN+m
13	August 11, 2008	40.7	10.8	-3.0	CRN+m
13	July 10, 2009	27.8	10.2	-2.9	CRN+m
13	July 24, 2009	36.4	8.5	-5.1	CRN+m
13	August 10, 2009	38.4	9.8	-2.4	CRN+m
13	August 26, 2009	36.2	4.3	40.5	CRN+m
13	September 10, 2009	41.1	10.2	-3.3	CRN+m
13	October 5, 2009	28.2	10.8	-2.0	CRN+m
31	August 18, 2006	105.5	11.3	3.7	CRN+m
31	March 9, 2007	215.4	12.4	4.1	CRN+m
31	April 12, 2007	180.1	12.6	5.1	CRN+m
31	May 9, 2007	191.0	12.5	2.7	CRN+m
31	June 13, 2007	185.0	12.8	5.3	CRN+m
31	July 11, 2007	138.2	12.9	5.6	CRN+m

Well	Date	$[NO_3]$ mg/L	$\delta^{15}N_{nitrate}$ (‰)	$\delta^{18}O_{nitrate}$ (‰)	Treatment
31	August 13, 2007	178.1	13.2	4.0	CRN+m
31	June 25, 2008	144.7	14.3	5.6	CRN+m
31	July 15, 2008	131.0	14.7	5.4	CRN+m
31	August 11, 2008	138.7	14.5	3.6	CRN+m
31	July 10, 2009	141.2	12.9	4.3	CRN+m
31	July 24, 2009	183.1	9.6	0.9	CRN+m
31	August 10, 2009	208.4	11.6	4.0	CRN+m
31	August 26, 2009	122.5	11.7	4.1	CRN+m
31	September 10, 2009	112.2	11.8	3.7	CRN+m
31	October 5, 2009	159.7	9.8	-0.8	CRN+m
6	July 18, 2006	166.0	8.7	0.8	CRN
6	August 18, 2006	207.0	9.1	0.7	CRN
6	March 9, 2007	139.8	13.0	5.8	CRN
6	April 12, 2007	161.9	10.4	2.7	CRN
6	May 9, 2007	160.8	10.2	2.1	CRN
6	June 13, 2007	173.2	10.3	1.8	CRN
6	July 11, 2007	148.1	10.0	1.0	CRN
6	August 13, 2007	140.2	12.6	3.0	CRN
6	August 24, 2007	138.6	13.2	3.1	CRN
6	April 28, 2008	120.1	17.8	11.5	CRN
6	May 12, 2008	111.0	13.6	4.7	CRN
6	June 25, 2008	149.2	11.2	3.6	CRN
6	July 15, 2008	137.3	11.1	2.8	CRN
6	August 11, 2008	151.1	11.5	2.3	CRN
6	August 25, 2008	141.8	12.3	1.9	CRN
6	July 10, 2009	82.7	13.8	2.5	CRN
6	July 24, 2009	74.1	11.4	1.0	CRN
6	August 10, 2009	118.7	12.5	2.8	CRN
6	August 26, 2009	71.5	8.6	-1.9	CRN
6	September 10, 2009	62.2	10.1	-2.0	CRN
6	October 5, 2009	121.5	15.7	12.2	CRN
14	July 18, 2006	90.4	11.7	0.7	CRN
14	August 18, 2006	102.5	11.6	1.5	CRN
14	March 9, 2007	114.6	8.5	0.7	CRN
14	April 12, 2007	84.4	8.6	-2.6	CRN
14	May 9, 2007	93.3	12.8	-0.3	CRN
14	June 13, 2007	108.4	12.3	1.0	CRN
14	July 11, 2007	94.3	12.8	1.0	CRN
14	August 13, 2007	71.2	19.9	4.4	CRN

Well	Date	$[NO_3]$ mg/L	$\delta^{15}N_{nitrate}$ (‰)	$\delta^{18}O_{nitrate}$ (‰)	Treatment
14	August 24, 2007	53.4	24.7	6.3	CRN
14	July 15, 2008	34.1	20.1	5.1	CRN
14	June 25, 2008	66.9	20.7	3.7	CRN
14	August 11, 2008	65.4	21.4	3.6	CRN
14	August 25, 2008	53.5	25.9	7.0	CRN
14	July 10, 2009	53.1	17.1	2.5	CRN
14	July 24, 2009	85.1	12.8	-0.1	CRN
14	August 10, 2009	94.6	14.5	1.9	CRN
14	August 26, 2009	104.5	13.4	1.6	CRN
14	September 10, 2009	97.0	13.9	1.4	CRN
14	October 5, 2009	95.7	13.9	1.7	CRN
32	July 18, 2006	67.0	14.2	0.1	CRN
32	August 18, 2006	242.8	6.8	1.0	CRN
32	March 9, 2007	76.3	8.8	-2.4	CRN
32	April 12, 2007	32.9	16.8	0.9	CRN
32	May 9, 2007	50.9	10.8	-2.6	CRN
32	June 13, 2007	41.5	14.2	-1.2	CRN
32	July 11, 2007	35.3	12.7	-0.9	CRN
32	June 25, 2008	32.3	18.3	0.7	CRN
32	July 15, 2008	38.9	13.7	-0.5	CRN
32	August 11, 2008	31.7	16.7	0.4	CRN
32	July 10, 2009	44.8	13.0	0.1	CRN
32	July 24, 2009	52.8	10.8	-2.9	CRN
32	August 10, 2009	43.2	14.8	0.3	CRN
32	August 26, 2009	30.1	14.2	38.7	CRN
32	September 10, 2009	30.6	15.3	1.1	CRN
32	October 5, 2009	40.4	14.6	-0.7	CRN
2	July 18, 2006	642.0	6.9	1.4	CF200
2	August 18, 2006	436.4	7.0	1.3	CF200
2	March 9, 2007	261.5	7.1	1.5	CF200
2	April 12, 2007	303.3	6.9	2.1	CF200
2	May 9, 2007	334.7	8.4	3.5	CF200
2	June 13, 2007	380.7	6.5	0.0	CF200
2	July 11, 2007	415.6	7.5	1.1	CF200
2	June 25, 2008	375.3	10.2	1.4	CF200
2	July 15, 2008	184.4	17.5	5.4	CF200
18	July 18, 2006	300.2	10.0	1.6	CF200
18	August 18, 2006	143.6	11.5	2.0	CF200
18	March 9, 2007	103.4	14.1	5.5	CF200

Well	Date	$[NO_3]$ mg/L	$\delta^{15}N_{nitrate}$ (‰)	$\delta^{18}O_{nitrate}$ (‰)	Treatment
18	April 12, 2007	124.8	13.6	4.3	CF200
18	May 9, 2007	182.9	11.8	1.3	CF200
18	June 13, 2007	255.2	11.1	1.1	CF200
18	July 11, 2007	256.5	10.5	0.4	CF200
18	August 13, 2007	215.1	11.6	1.3	CF200
18	August 24, 2007	148.1	12.8	5.3	CF200
18	June 25, 2008	141.3	13.4	2.6	CF200
18	July 15, 2008	194.4	11.3	0.6	CF200
18	August 11, 2008	215.1	12.2	2.0	CF200
18	July 10, 2009	196.1	11.0	1.2	CF200
18	July 24, 2009	281.9	8.8	-1.2	CF200
18	August 10, 2009	301.8	11.0	0.3	CF200
18	August 26, 2009	292.2	11.1	1.0	CF200
18	September 10, 2009	203.6	11.7	1.5	CF200
18	October 5, 2009	156.8	11.8	1.5	CF200
28	July 18, 2006	584.4	10.3	0.6	CF200
28	August 18, 2006	572.7	10.3	-0.4	CF200
28	March 9, 2007	249.5	15.5	1.7	CF200
28	April 12, 2007	217.2	16.7	2.8	CF200
28	May 9, 2007	669.2	13.4	8.6	CF200
28	June 13, 2007	825.6	8.9	0.2	CF200
28	July 11, 2007	276.7	14.0	0.5	CF200
28	August 13, 2007	24.7	32.0	9.4	CF200
28	August 24, 2007	32.9	29.0	8.2	CF200
28	June 25, 2008	296.4	13.2	0.7	CF200
28	July 15, 2008	185.8	17.8	2.5	CF200
28	August 11, 2008	163.4	19.1	3.4	CF200

APPENDIX D: STATISTICAL RESULTS

Treatment Variations in Soil Total Nitrogen Contents (Welch's t-tests)

Table D-1a: Statistical results of multiple Welch's t-tests comparing average total nitrogen contents between treatments for the 0-15cm soil depth interval. Alpha values were corrected using the Holm-Bonferroni method. Average total nitrogen contents could not be compared for certain treatments, since variance was zero.

Treatment	CRN+m	CRN	CF200
Alfalfa	$t = 0.00$, $p = 1.00$, $\alpha = 0.05$	N/A	$t = 5.66$, $p = 0.005$, $\alpha = 0.0167$
CRN+m	-	N/A	$t = 3.58$, $p = 0.07$, $\alpha = 0.025$
CRN	-	-	N/A

Table D-1b: Statistical results of multiple Welch's t-tests comparing average total nitrogen contents between treatments for the 15-30cm soil depth interval. Alpha values were corrected using the Holm-Bonferroni method. Average total nitrogen contents could not be compared for certain treatments, since variance was zero.

Treatment	CRN+m	CRN	CF200
Alfalfa	N/A	N/A	N/A
CRN+m	-	$t = 1.00$, $p = 0.391$, $\alpha = 0.05$	$t = 3.50$, $p = 0.039$, $\alpha = 0.025$
CRN	-	-	$t = 3.54$, $p = 0.024$, $\alpha = 0.0167$

Table D-1c: Statistical results of multiple Welch's t-tests ($\alpha = 0.05$) comparing average total nitrogen contents between treatments for the 30-45cm soil depth interval.

Treatment	CRN+m	CRN	CF200
Alfalfa	$t = 1.31$, $p = 0.282$	$t = 1.94$, $p = 0.148$	$t = 3.16$, $p = 0.087$
CRN+m	-	$t = 0.25$, $p = 0.819$	$t = 1.11$, $p = 0.383$
CRN	-	-	$t = 1.34$, $p = 0.312$

Table D-1d: Statistical results of multiple Welch's t-tests (α = 0.05) comparing average total nitrogen contents between treatments for the 45-60cm soil depth interval.

Treatment	CRN+m	CRN	CF200
Alfalfa	t = 1.26, p = 0.295	t = 1.58, p = 0.212	t = 0.85, p = 0.484
CRN+m	-	t = 0.27, p = 0.802	t = 0.00, p = 1.000
CRN	-	-	t = -0.20, p = 0.857

Table D-1e: Statistical results of multiple Welch's t-tests (α = 0.05) comparing average total nitrogen contents between treatments for the 60-90cm soil depth interval.

Treatment	CRN+m	CRN	CF200
Alfalfa	t = 0.00, p = 1.000	t = -0.32, p = 0.782	t = -0.53, p = 0.646
CRN+m	-	t = -0.32, p = 0.782	t = -0.53, p = 0.646
CRN	-	-	t = -0.21, p = 0.845

Treatment Variations in Soil Total Nitrogen Contents (One-way ANOVA from Minitab)

```
Source     DF       SS       MS     F      P
Treatment   3  0.00278  0.00093  0.35  0.788
Error      56  0.14735  0.00263
Total      59  0.15013

S = 0.05130   R-Sq = 1.85%   R-Sq(adj) = 0.00%

                               Individual 95% CIs For Mean Based on
                               Pooled StDev
Level     N     Mean    StDev   -+---------+---------+---------+--------
Alfalfa  15  0.14200  0.05388            (------------*------------)
CF200    15  0.12400  0.04485   (------------*------------)
CRN      15  0.12867  0.04868     (------------*-------------)
CRN+m    15  0.13533  0.05693        (-------------*------------)
                                -+---------+---------+---------+--------
                               0.100     0.120     0.140     0.160

Pooled StDev = 0.05130


Grouping Information Using Tukey Method

Treatment   N     Mean  Grouping
Alfalfa    15  0.14200  A
CRN+m      15  0.13533  A
CRN        15  0.12867  A
CF200      15  0.12400  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment
Individual confidence level = 98.94%
```

```
Treatment = Alfalfa subtracted from:

Treatment     Lower    Center    Upper
CF200      -0.06753  -0.01800  0.03153
CRN        -0.06287  -0.01333  0.03620
CRN+m      -0.05620  -0.00667  0.04287

Treatment  ---------+---------+---------+---------+
CF200      (-------------*-------------)
CRN         (-------------*-------------)
CRN+m         (-------------*-------------)
           ---------+---------+---------+---------+
                 -0.035     0.000     0.035     0.070

Treatment = CF200 subtracted from:

Treatment     Lower   Center    Upper
CRN        -0.04487  0.00467  0.05420
CRN+m      -0.03820  0.01133  0.06087

Treatment  ---------+---------+---------+---------+
CRN              (-------------*-------------)
CRN+m              (-------------*-------------)
           ---------+---------+---------+---------+
                 -0.035     0.000     0.035     0.070

Treatment = CRN subtracted from:

Treatment     Lower   Center    Upper
CRN+m      -0.04287  0.00667  0.05620

Treatment  ---------+---------+---------+---------+
CRN+m             (-------------*-------------)
           ---------+---------+---------+---------+
                 -0.035     0.000     0.035     0.070
```

Treatment Variations in Soil Total Nitrogen Contents (Kruskal-Wallis Test from Minitab with α = 0.05)

```
Kruskal-Wallis Test on total N

Treatment   N  Median  Ave Rank      Z
Alfalfa    15  0.1300      33.7   0.83
CF200      15  0.1100      27.6  -0.73
CRN        15  0.1100      29.3  -0.32
CRN+m      15  0.1200      31.4   0.22
Overall    60              30.5

H = 1.03  DF = 3  P = 0.794
H = 1.04  DF = 3  P = 0.793  (adjusted for ties)
```

Treatment Variations in Soil δ^{15}N-totalN Values (Welch's t-tests)

Table D-2a: Statistical results of multiple Welch's t-tests ($\alpha = 0.05$) comparing the average δ^{15}N-totalN values between treatments for the 0-15cm soil depth interval.

Treatment	CRN+m	CRN	CF200
Alfalfa	t = -2.05, p = 0.177	t = -0.25, p = 0.818	t = -0.50, p = 0.654
CRN+m	-	t = 2.66, p = 0.076	t = 0.84, p = 0.491
CRN	-	-	t = -0.37, p = 0.745

Table D-2b: Statistical results of multiple Welch's t-tests ($\alpha = 0.05$) comparing the average δ^{15}N-totalN values between treatments for the 15-30cm soil depth interval.

Treatment	CRN+m	CRN	CF200
Alfalfa	t = 0.20, p = 0.853	t = 0.21, p = 0.850	t = 0.33, p = 0.764
CRN+m	-	t = -0.02, p = 0.984	t = 0.17, p = 0.876
CRN	-	-	t = 0.20, p = 0.857

Table D-2c: Statistical results of multiple Welch's t-tests ($\alpha = 0.05$) comparing the average δ^{15}N-totalN values between treatments for the 30-45cm soil depth interval.

Treatment	CRN+m	CRN	CF200
Alfalfa	t = 1.03, p = 0.412	t = 1.21, p = 0.351	t = 0.15, p = 0.889
CRN+m	-	t = 0.28, p = 0.797	t = -0.58, p = 0.619
CRN	-	-	t = -0.70, p = 0.554

Table D-2d: Statistical results of multiple Welch's t-tests ($\alpha = 0.05$) comparing the average δ^{15}N-totalN values between treatments for the 45-60cm soil depth interval.

Treatment	CRN+m	CRN	CF200
Alfalfa	t = 0.96, p = 0.439	t = 0.98, p = 0.401	t = 0.09, p = 0.934
CRN+m	-	t = 0.32, p = 0.780	t = -0.40, p = 0.73
CRN	-	-	t = -0.52, p = 0.657

Table D-2e: Statistical results of multiple Welch's t-tests ($\alpha = 0.05$) comparing the average δ^{15}N-totalN values between treatments for the 60-90-cm soil depth interval.

Treatment	CRN+m	CRN	CF200
Alfalfa	t = -0.76, p = 0.526	t = -0.62, p = 0.57	t = -0.84, p = 0.461
CRN+m	-	t = 0.41, p = 0.721	t = 0.04, p = 0.972
CRN	-	-	t = -0.43, p = 0.695

Treatment Variations in Soil δ^{15}N-totalN Values (One-way ANOVA from Minitab)

```
Source     DF     SS    MS     F      P
Treatment   3   0.82  0.27  0.27  0.845
Error      56  56.35  1.01
Total      59  57.17

S = 1.003   R-Sq = 1.44%   R-Sq(adj) = 0.00%

                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level     N   Mean  StDev  ---------+---------+---------+---------+
Alfalfa  15  7.018  1.081       (--------------*-------------)
CF200    15  7.157  1.127           (-------------*--------------)
CRN      15  6.860  0.798  (--------------*--------------)
CRN+m    15  7.131  0.974          (--------------*--------------)
                           ---------+---------+---------+---------+
                                  6.65      7.00      7.35      7.70

Pooled StDev = 1.003

Grouping Information Using Tukey Method

Treatment   N   Mean  Grouping
CF200      15  7.157  A
CRN+m      15  7.131  A
Alfalfa    15  7.018  A
CRN        15  6.860  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment

Individual confidence level = 98.94%


Treatment = Alfalfa subtracted from:

Treatment   Lower  Center  Upper  --------+---------+---------+---------+-
CF200      -0.830   0.139  1.107        (-------------*-------------)
CRN        -1.127  -0.158  0.811    (-------------*-------------)
CRN+m      -0.855   0.113  1.082        (-------------*------------)
                                  --------+---------+---------+---------+-
                                       -0.70      0.00      0.70      1.40
```

```
Treatment = CF200 subtracted from:

Treatment   Lower  Center  Upper  --------+---------+---------+---------+-
CRN        -1.265  -0.297  0.672  (-------------*-------------)
CRN+m      -0.994  -0.025  0.943      (-------------*------------)
                                  --------+---------+---------+---------+-
                                       -0.70      0.00      0.70      1.40

Treatment = CRN subtracted from:

Treatment   Lower  Center  Upper  --------+---------+---------+---------+-
CRN+m      -0.697   0.271  1.240          (-------------*-------------)
                                  --------+---------+---------+---------+-
                                       -0.70      0.00      0.70      1.40
```

Treatment Variations in Soil δ^{15}N-totalN Values (Kruskal-Wallis Test from Minitab with α = 0.05)

```
Kruskal-Wallis Test on δ15N-totalN

Treatment   N  Median  Ave Rank      Z
Alfalfa    15   7.120      30.3  -0.04
CF200      15   6.910      31.7   0.31
CRN        15   6.960      27.2  -0.85
CRN+m      15   7.250      32.8   0.59
Overall    60              30.5

H = 0.88  DF = 3  P = 0.831
H = 0.88  DF = 3  P = 0.830  (adjusted for ties)
```

Treatment Variations in 2-Week PRS-Probe Supply Rates (Welch's t-tests)

Table D-3: Statistical results of multiple Welch's t-tests (α = 0.05) comparing the average nitrate supply rates of the 2-week duration PRS-probes from plots under the alfalfa, CLTN, CRN+m, and CRN treatments.

Treatment	CLTN	CRN+m	CRN
Alfalfa	t = 1.07, p = 0.397	t = -0.33, p = 0.774	t = -0.32, p = 0.777
CLTN	-	t = -1.03, p = 0.381	t = -0.72, p = 0.548
CRN+m	-	-	t = -0.19, p = 0.869

Treatment Variations in 2-Week PRS-Probe $\delta^{15}N\text{-}NO_3$ Values (Welch's t-tests)

Table D-4: Statistical results of multiple Welch's t-tests comparing the average eluted $\delta^{15}N\text{-}NO_3$ values of the 2-week duration PRS-probes from plots under the alfalfa, CLTN, CRN+m, and CRN treatments. Alpha values were corrected using the Holm-Bonferroni method.

Treatment	CLTN	CRN+m	CRN
Alfalfa	t = -3.47 p = 0.04 α = 0.01	t = -3.72 p = 0.034 α = 0.008	t = -3.03 p = 0.056 α = 0.0125
CLTN	-	t = 0.08 p = 0.942 α = 0.05	t = 1.46 p = 0.281 α = 0.025
CRN+m	-	-	t = 1.54 p = 0.220 α = 0.0167

Treatment Variations in 4-Week PRS-Probe Supply Rates (One-way ANOVA from Minitab)

```
Source     DF       SS       MS     F      P
Treatment   3  0.00457  0.00152  0.98  0.422
Error      19  0.02946  0.00155
Total      22  0.03402

S = 0.03937   R-Sq = 13.43%   R-Sq(adj) = 0.00%

                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level    N     Mean    StDev   -+---------+---------+---------+--------
Alfalfa  6  0.04410  0.02168       (-----------*----------)
CLTN     6  0.06550  0.05654               (----------*----------)
CRN      5  0.06020  0.04872            (-----------*-----------)
CRN+m    6  0.02998  0.01806   (----------*----------)
                               -+---------+---------+---------+--------
                              0.000     0.030     0.060     0.090
Pooled StDev = 0.03937

Grouping Information Using Tukey Method

Treatment  N     Mean  Grouping
CLTN       6  0.06550  A
CRN        5  0.06020  A
Alfalfa    6  0.04410  A
CRN+m      6  0.02998  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment
```

```
Individual confidence level = 98.89%
Treatment = Alfalfa subtracted from:

Treatment     Lower    Center    Upper
CLTN       -0.04258   0.02140  0.08538
CRN        -0.05100   0.01610  0.08320
CRN+m      -0.07809  -0.01412  0.04986

Treatment     +---------+---------+---------+---------
CLTN                     (------------*------------)
CRN                     (------------*-------------)
CRN+m             (------------*------------)
              +---------+---------+---------+---------
           -0.100    -0.050     0.000     0.050


Treatment = CLTN subtracted from:

Treatment     Lower    Center    Upper
CRN        -0.07240  -0.00530  0.06180
CRN+m      -0.09949  -0.03552  0.02846

Treatment     +---------+---------+---------+---------
CRN                 (------------*------------)
CRN+m         (------------*------------)
              +---------+---------+---------+---------
           -0.100    -0.050     0.000     0.050


Treatment = CRN subtracted from:

Treatment     Lower    Center    Upper
CRN+m      -0.09731  -0.03022  0.03688

Treatment     +---------+---------+---------+---------
CRN+m          (------------*------------)
              +---------+---------+---------+---------
           -0.100    -0.050     0.000     0.050
```

Treatment Variations in 4-Week PRS-Probe Supply Rates (Kruskal-Wallis Test from Minitab with α = 0.05)

```
Kruskal-Wallis Test on Supply Rates

Treatment   N   Median  Ave Rank      Z
Alfalfa     6  0.04850      12.6   0.25
CLTN        6  0.05050      13.8   0.77
CRN         5  0.03800      13.4   0.52
CRN+m       6  0.02500       8.4  -1.51
Overall    23               12.0

H = 2.37  DF = 3  P = 0.499
H = 2.38  DF = 3  P = 0.498  (adjusted for ties)
```

Treatment Variations in 4-Week PRS-Probe $\delta^{15}N\text{-}NO_3$ Values (One-way ANOVA from Minitab)

```
Source     DF     SS    MS     F      P
Treatment   3   4.33  1.44  0.51  0.679
Error      19  53.62  2.82
Total      22  57.95

S = 1.680   R-Sq = 7.48%   R-Sq(adj) = 0.00%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level    N   Mean  StDev  ---------+---------+---------+---------+
Alfalfa  6  5.909  1.479        (-----------*-----------)
CLTN     6  5.130  2.336  (-----------*-----------)
CRN      5  5.675  1.438     (------------*------------)
CRN+m    6  6.305  1.195            (-----------*-----------)
                          ---------+---------+---------+---------+
                                 4.8       6.0       7.2       8.4

Pooled StDev = 1.680


Grouping Information Using Tukey Method

Treatment  N   Mean  Grouping
CRN+m      6  6.305  A
Alfalfa    6  5.909  A
CRN        5  5.675  A
CLTN       6  5.130  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment

Individual confidence level = 98.89%

Treatment = Alfalfa subtracted from:

Treatment   Lower  Center  Upper     +---------+---------+---------+---------
CLTN       -3.508  -0.779  1.951       (-------------*-------------)
CRN        -3.097  -0.234  2.629          (-------------*-------------)
CRN+m      -2.333   0.397  3.126             (-------------*-------------)
                                     +---------+---------+---------+---------
                                  -4.0      -2.0       0.0       2.0
Treatment = CLTN subtracted from:

Treatment   Lower  Center  Upper     +---------+---------+---------+---------
CRN        -2.318   0.545  3.408             (--------------*-------------)
CRN+m      -1.554   1.175  3.905                 (-------------*-------------)
                                     +---------+---------+---------+---------
                                  -4.0      -2.0       0.0       2.0

Treatment = CRN subtracted from:

Treatment   Lower  Center  Upper     +---------+---------+---------+---------
CRN+m      -2.232   0.630  3.493              (-------------*-------------)
                                     +---------+---------+---------+---------
                                  -4.0      -2.0       0.0       2.0
```

Treatment Variations in 4-Week PRS-Probe δ^{15}N-NO_3 Values (Kruskal-Wallis Test from Minitab with α = 0.05)

```
Kruskal-Wallis Test on δ15N-NO3

Treatment   N  Median  Ave Rank      Z
Alfalfa     6   5.572      11.8  -0.07
CLTN        6   4.660       9.0  -1.26
CRN         5   5.904      12.6   0.22
CRN+m       6   6.304      14.7   1.12
Overall    23              12.0

H = 2.14  DF = 3  P = 0.543
```

Comparing 2008 Soil δ^{15}N-totalN values with the 2-Week and 4-Week PRS-Probe δ^{15}N-NO_3 Values (Welch's t-tests)

Table D-5: Statistical results from multiple Welch's t-tests comparing the average δ^{15}N values of total soil nitrogen from the 2008 soil samples and of eluted nitrates from the 2-week and 4-week PRS-probes under the alfalfa, CRN+m, and CRN treatments. Alpha values were corrected with the Holm-Bonferroni method.

Treatment	δ^{15}N (totalN and NO_3)	
	2008 Soils versus 2-Week Probes	2008 Soils versus 4-Week Probes
Alfalfa	t = 3.59, p = 0.070, α = 0.025	t = 1.73, p = 0.134, α = 0.05
CRN+m	t = -0.77, p = 0.522, α = 0.05	t = 3.20, p = 0.024, α = 0.025
CRN	t = 0.52, p = 0.657, α = 0.05	t = 2.30, p = 0.083, α = 0.025

Temporal Variations in Average Groundwater Nitrate Concentrations for the Alfalfa Treatment (One-way ANOVA from Minitab)

```
Source  DF       SS      MS      F      P
Factor   3   711203  237068  14.02  0.000
Error   35   591894   16911
Total   38  1303097

S = 130.0   R-Sq = 54.58%   R-Sq(adj) = 50.68%

                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level   N   Mean  StDev  --+---------+---------+---------+-------
2006    4  101.5  120.9  (--------*--------)
2007   13  127.7   98.8        (----*---)
2008   10  191.4  107.3           (-----*----)
2009   12  433.2  172.5                            (----*----)
                         --+---------+---------+---------+-------
                           0       150       300       450
```

```
Pooled StDev = 130.0


Grouping Information Using Tukey Method

       N   Mean  Grouping
2009  12  433.2  A
2008  10  191.4    B
2007  13  127.7    B
2006   4  101.5    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.92%


2006 subtracted from:

       Lower  Center  Upper  --------+---------+---------+---------+-
2007  -174.0    26.3  226.6            (-------*-------)
2008  -117.4    89.9  297.2              (--------*-------)
2009   129.4   331.7  534.0                        (-------*-------)
                            --------+---------+---------+---------+-
                                 -250         0       250       500


2007 subtracted from:

      Lower  Center  Upper  --------+---------+---------+---------+-
2008  -83.7    63.6  211.0                (-----*----)
2009  165.2   305.4  445.7                          (----*-----)
                            --------+---------+---------+---------+-
                                 -250         0       250       500


2008 subtracted from:

      Lower  Center  Upper  --------+---------+---------+---------+-
2009   91.8   241.8  391.8                       (-----*-----)
                            --------+---------+---------+---------+-
                                 -250         0       250       500
```

Temporal Variations in Average Groundwater Nitrate Concentrations for the CRN+m Treatment (One-way ANOVA from Minitab)

```
Source  DF      SS    MS     F      P
Factor   3    5228  1743  0.50  0.685
Error   46  160864  3497
Total   49  166091

S = 59.14   R-Sq = 3.15%   R-Sq(adj) = 0.00%
```

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level   N    Mean  StDev  -------+---------+---------+---------+--
2006    3  126.93  62.19    (----------------*----------------)
2007   20  118.88  61.24            (------*-----)
2008    9   92.18  40.50  (---------*---------)
2009   18  109.57  63.60         (------*------)
                          -------+---------+---------+---------+--
                                 80       120       160       200

Pooled StDev = 59.14


Grouping Information Using Tukey Method

       N    Mean  Grouping
2006   3  126.93  A
2007  20  118.88  A
2009  18  109.57  A
2008   9   92.18  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 98.94%


2006 subtracted from:

        Lower  Center  Upper  ---+---------+---------+---------+------
2007  -105.65   -8.05  89.55       (----------------*---------------)
2008  -139.85  -34.75  70.34  (----------------*-----------------)
2009  -115.67  -17.36  80.94      (---------------*---------------)
                              ---+---------+---------+---------+------
                              -120       -60         0        60


2007 subtracted from:

       Lower  Center  Upper  ---+---------+---------+---------+------
2008  -89.98  -26.71  36.57          (----------*---------)
2009  -60.53   -9.32  41.90               (-------*--------)
                            ---+---------+---------+---------+------
                            -120       -60         0        60


2008 subtracted from:

       Lower  Center  Upper  ---+---------+---------+---------+------
2009  -46.97   17.39  81.75                (----------*----------)
                            ---+---------+---------+---------+------
                            -120       -60         0        60
```

Treatment Variations in Groundwater [NO_3] for Year 2006 (Welch's t-tests)

Table D-6: Statistical results from multiple Welch's t-tests comparing the average groundwater nitrate concentrations under the alfalfa, CLTN, CRN+m, CRN, and CF200 treatments in year 2006. Alpha values were corrected with the Holm-Bonferroni method. Note that the groundwater nitrate concentration data were square-root transformed.

Treatment	CLTN	CRN+m	CRN	CF200
Alfalfa	t = -3.99 p = 0.028 α = 0.0083	t = -0.80 p = 0.468 α = 0.025	t = -1.02 p = 0.383 α = 0.0125	t = -3.05 p = 0.028 α = 0.01
CLTN	-	t = 7.07 p = 0.019 α = 0.0071	t = 8.36 p = 0.000 α = 0.005	t = 0.81 p = 0.455 α = 0.0167
CRN+m	-	-	t = -0.37 p = 0.730 α = 0.05	t = -3.67 p = 0.010 α = 0.0063
CRN	-	-	-	t = -3.66 p = 0.008 α = 0.0056

Treatment Variations in Groundwater [NO_3] for Year 2007 (Welch's t-tests)

Table D-7: Statistical results from multiple Welch's t-tests comparing the average groundwater nitrate concentrations under the alfalfa, CLTN, CRN+m, CRN, and CF200 treatments in year 2007. Alpha values were corrected with the Holm-Bonferroni method. Note that the groundwater nitrate concentration data were square-root transformed.

Treatment	CLTN	CRN+m	CRN	CF200
Alfalfa	t = -5.29 p = 0.000 α = 0.005	t = -0.41 p = 0.686 α = 0.025	t = 0.01 p = 0.99 α = 0.05	t = -2.57 p = 0.017 α = 0.0125
CLTN	-	t = 7.73 p = 0.000 α = 0.0056	t = 8.73 p = 0.000 α = 0.0063	t = 3.23 p = 0.003 α = 0.0083
CRN+m	-	-	t = 0.85 p = 0.401 α = 0.0167	t = -3.28 p = 0.003 α = 0.01
CRN	-	-	-	t = -3.98 p = 0.001 α = 0.0071

Treatment Variations in Groundwater [NO_3] for Year 2008 (Welch's t-tests)

Table D-8: Statistical results from multiple Welch's t-tests comparing the average groundwater nitrate concentrations under the alfalfa, CLTN, CRN+m, CRN, and CF200 treatments in year 2008. Alpha values were corrected with the Holm-Bonferroni method. Note that the groundwater nitrate concentration data were square-root transformed.

Treatment	CLTN	CRN+m	CRN	CF200
Alfalfa	t = 0.59 p = 0.570 α = 0.025	t = 1.91 p = 0.081 α = 0.0071	t = 2.12 p = 0.055 α = 0.0063	t = -0.93 p = 0.371 α = 0.0125
CLTN	-	t = 0.92 p = 0.394 α = 0.0167	t = 1.10 p = 0.312 α = 0.01	t = -1.50 p = 0.185 α = 0.0083
CRN+m	-	-	t = 0.38 p = 0.711 α = 0.05	t = -4.59 p = 0.000 α = 0.005
CRN	-	-	-	t = -4.93 p = 0.000 α = 0.0056

Treatment Variations in Groundwater [NO_3] for Year 2009 (Welch's t-tests)

Table D-9: Statistical results from multiple Welch's t-tests comparing the average groundwater nitrate concentrations under the alfalfa, CLTN, CRN+m, CRN, and CF200 treatments in year 2009. Alpha values were corrected with the Holm-Bonferroni method. Note that the groundwater nitrate concentration data were square-root transformed.

Treatment	CRN+m	CRN	CF200
Alfalfa	t = 8.03 p = 0.000 α = 0.0083	t = 11.12 p = 0.000 α = 0.01	t = 3.51 p = 0.003 α = 0.025
CRN+m	-	t = 1.86 p = 0.074 α = 0.05	t = -4.76 p = 0.000 α = 0.0125
CRN	-	-	t = -7.62 p = 0.000 α = 0.0167

Treatment Variations in Groundwater [NO_3] (One-way ANOVA from Minitab)

```
Source   DF      SS     MS      F      P
Factor    4  2285.3  571.3  28.82  0.000
Error   205  4063.7   19.8
Total   209  6349.1

S = 4.452   R-Sq = 35.99%   R-Sq(adj) = 34.75%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level        N    Mean  StDev  ---+---------+---------+---------+------
sqrt Alf    38  13.274  6.687               (---*----)
sqrt CLTN   28  18.485  5.636                                (-----*----)
sqrt CRN+m  50  10.140  2.923     (---*---)
sqrt CRN    56   9.336  2.522  (---*---)
sqrt CF200  38  15.842  4.601                       (----*----)
                              ---+---------+---------+---------+------
                               9.0      12.0      15.0      18.0

Pooled StDev = 4.452


Grouping Information Using Tukey Method

             N    Mean  Grouping
sqrt CLTN   28  18.485  A
sqrt CF200  38  15.842  A B
sqrt Alf    38  13.274    B
sqrt CRN+m  50  10.140      C
sqrt CRN    56   9.336      C

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals

All Pairwise Comparisons

Individual confidence level = 99.35%


sqrt Alf subtracted from:

             Lower  Center   Upper
sqrt CLTN    2.161   5.211   8.261
sqrt CRN+m  -5.769  -3.134  -0.498
sqrt CRN    -6.512  -3.939  -1.365
sqrt CF200  -0.242   2.568   5.377

                +---------+---------+---------+---------
sqrt CLTN                             (----*----)
sqrt CRN+m                (----*---)
sqrt CRN                 (---*----)
sqrt CF200                          (---*----)
                +---------+---------+---------+---------
            -12.0      -6.0       0.0       6.0
```

```
sqrt CLTN subtracted from:

Lower  Center   Upper
sqrt CRN+m  -11.235  -8.345  -5.454
sqrt CRN    -11.984  -9.149  -6.315
sqrt CF200   -5.693  -2.643   0.407

                +---------+---------+---------+---------
sqrt CRN+m       (----*----)
sqrt CRN        (----*---)
sqrt CF200                  (----*----)
                +---------+---------+---------+---------
            -12.0      -6.0       0.0       6.0


sqrt CRN+m subtracted from:

             Lower  Center  Upper      +---------+---------+---------+---------
sqrt CRN    -3.188  -0.805  1.578                     (---*---)
sqrt CF200   3.066   5.701  8.337                               (----*---)
                                       +---------+---------+---------+---------
                                   -12.0      -6.0       0.0       6.0


sqrt CRN subtracted from:

            Lower  Center  Upper      +---------+---------+---------+---------
sqrt CF200  3.932   6.506  9.080                               (---*---)
                                       +---------+---------+---------+---------
                                   -12.0      -6.0       0.0       6.0
```

Treatment Variations in Groundwater [NO_3] (Welch's t-tests)

Table D-10: Statistical results of multiple Welch's t-tests comparing average groundwater nitrate concentrations between the alfalfa, CLTN, CRN+m, CRN, and CF200 treatments. Alpha values were corrected using the Holm-Bonferroni method.

Treatment	CLTN	CRN+m	CRN	CF200
Alfalfa	t = -3.42 p = 0.001 α = 0.0167	t = 3.99 p = 0.000 α = 0.005	t = 4.73 p = 0.000 α = 0.0063	t = 4.73 p = 0.000 α = 0.0083
CLTN	-	t = 7.02 p = 0.000 α = 0.0056	t = 7.57 p = 0.000 α = 0.0071	t = 2.30 p = 0.026 α = 0.025
CRN+m	-	-	t = 1.69 p = 0.094 α = 0.05	t = -6.18 p = 0.000 α = 0.01
CRN	-	-	-	t = -6.99 p = 0.000 α = 0.0125

APPENDIX E: NITRIFICATION/DENITRIFICATION GRAPHS

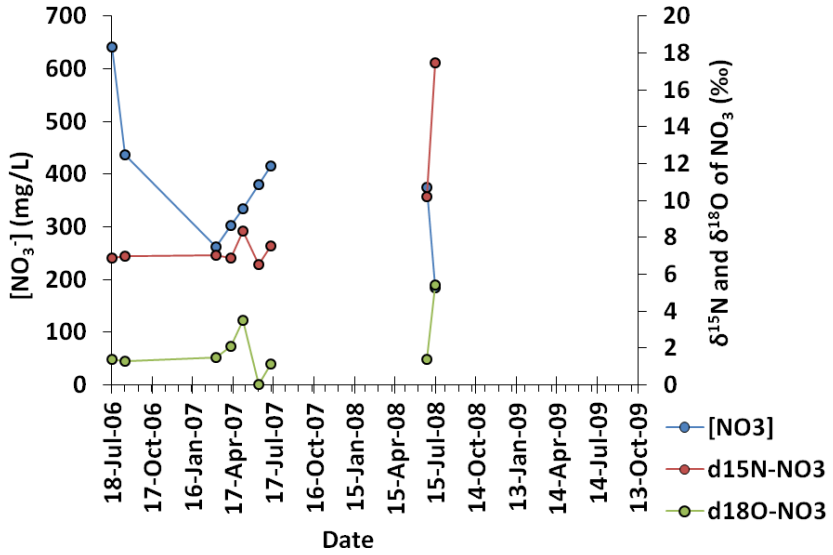

Figure E-1: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 2.

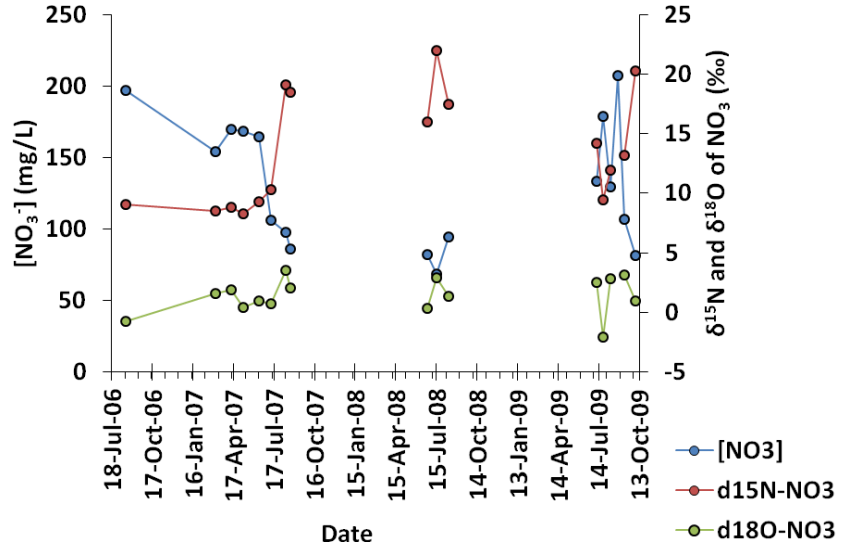

Figure E-2: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 5.

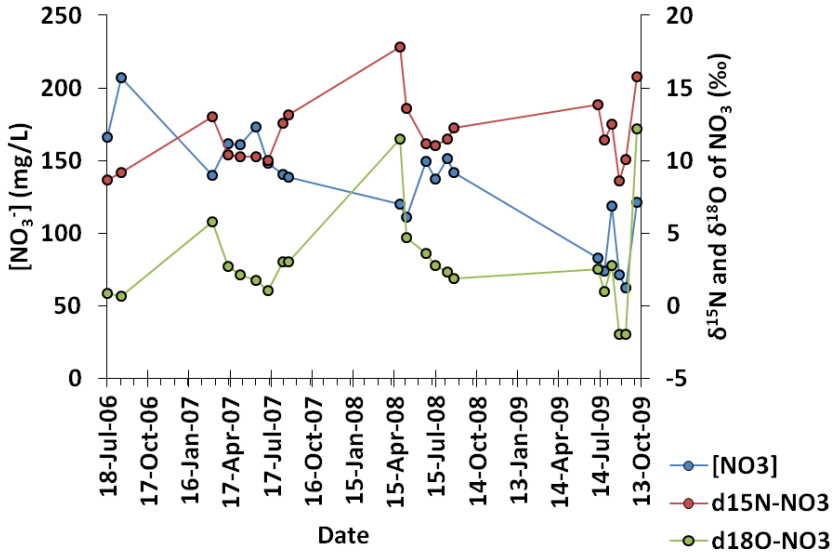

Figure E-3: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 6.

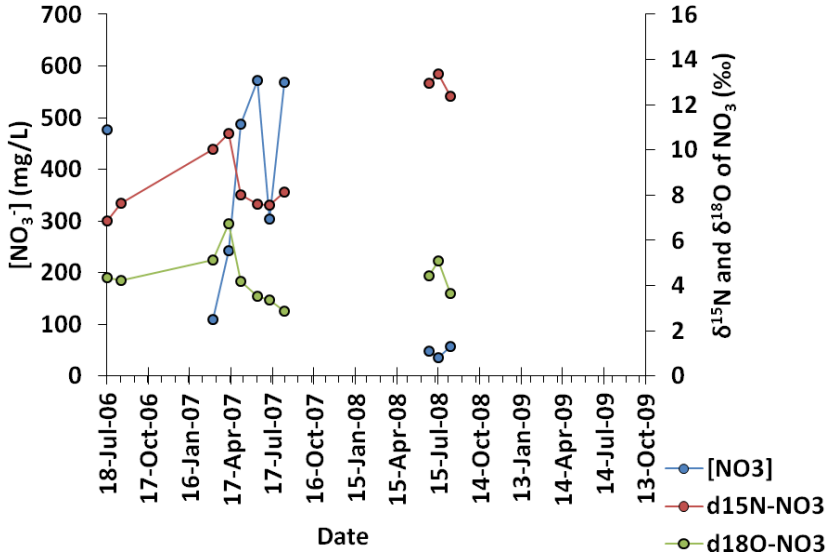

Figure E-4: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 8.

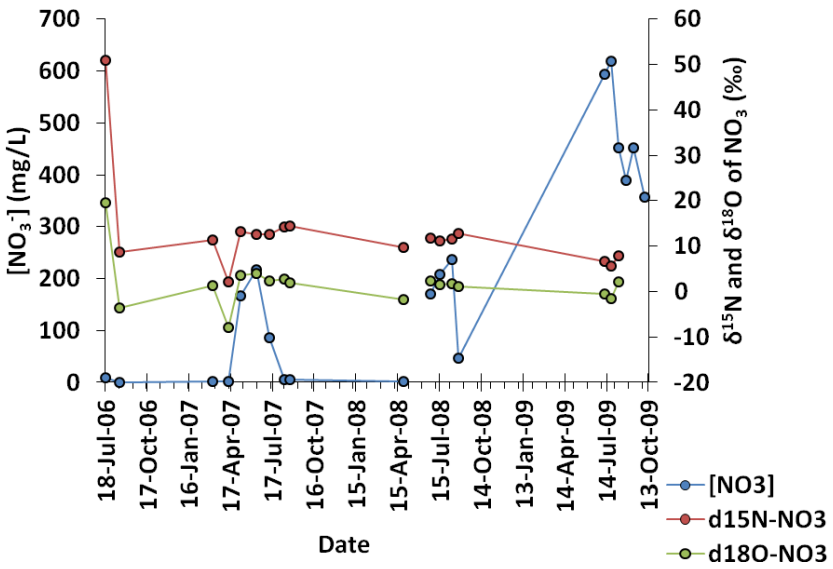

Figure E-5: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 10.

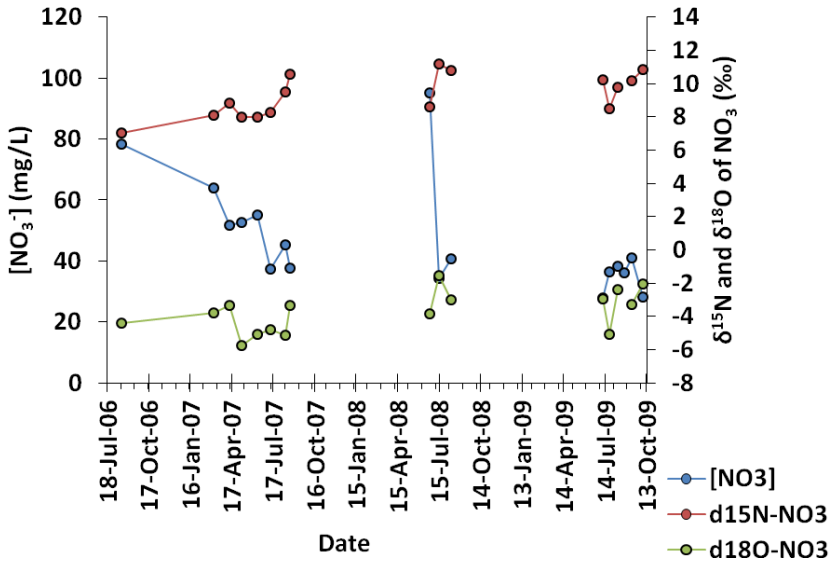

Figure E-6: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 13.

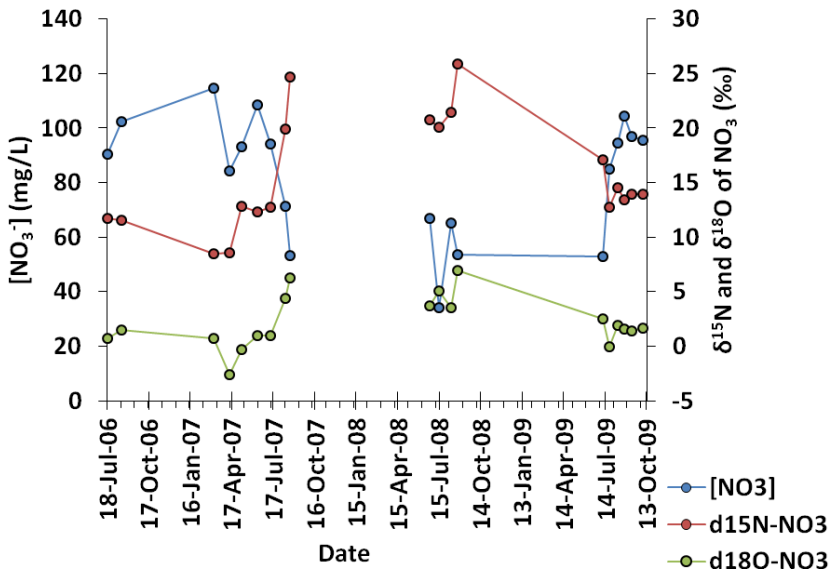

Figure E-7: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 14.

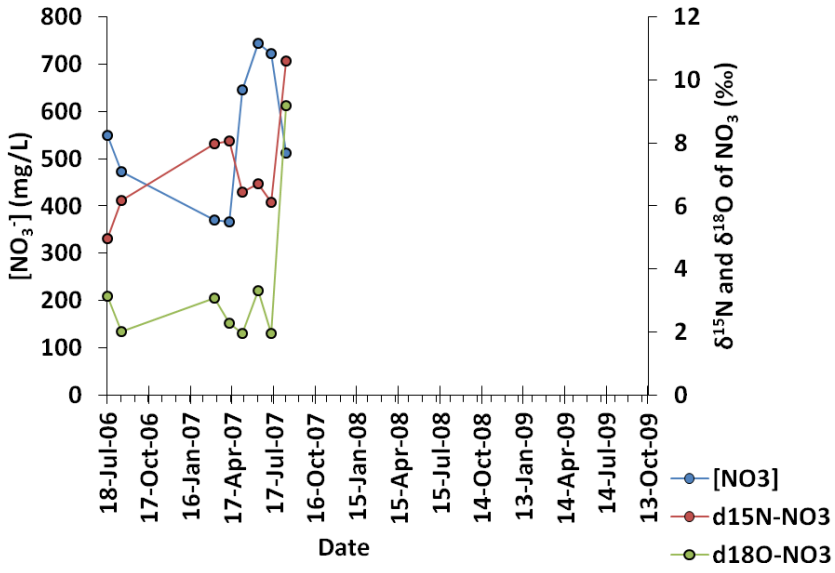

Figure E-8: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 16.

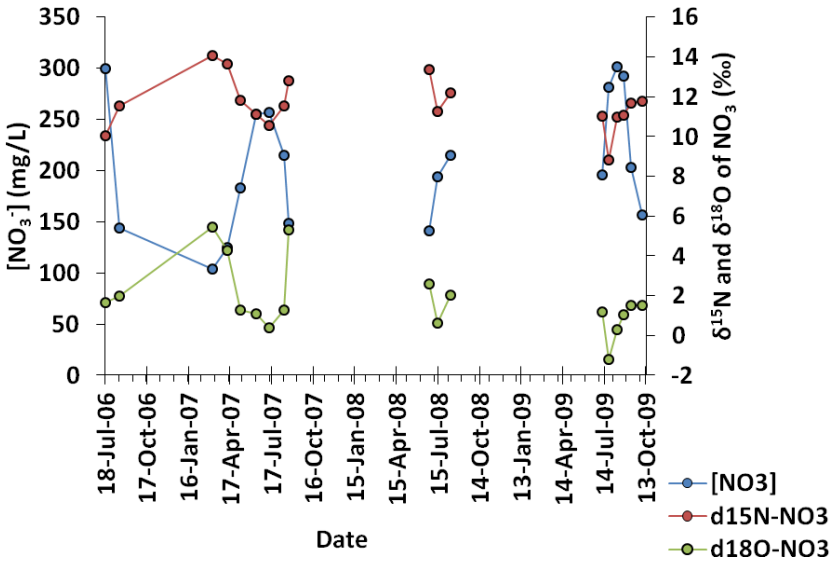

Figure E-9: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 18.

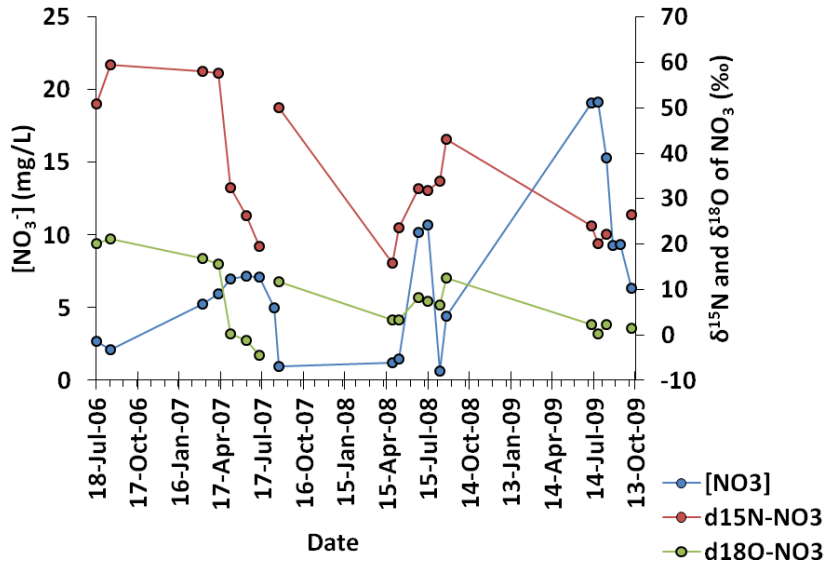

Figure E-10: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 22.

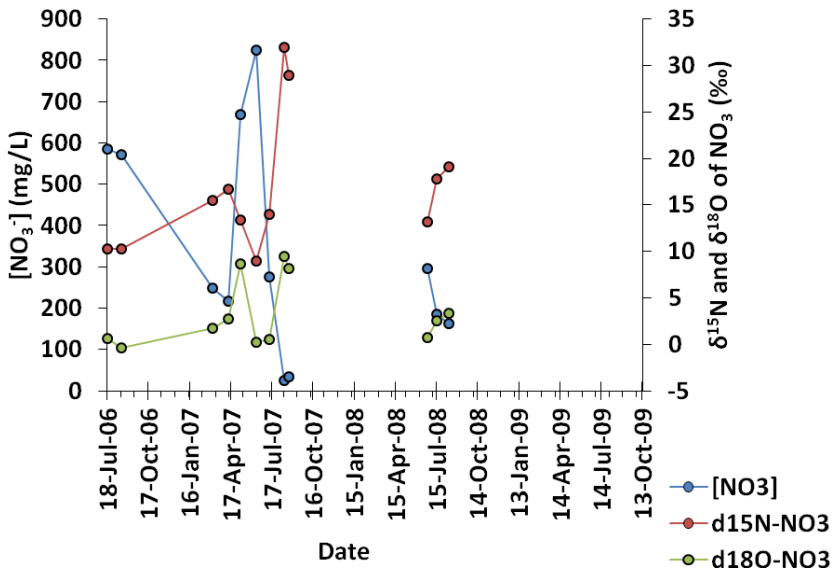

Figure E-11: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 28.

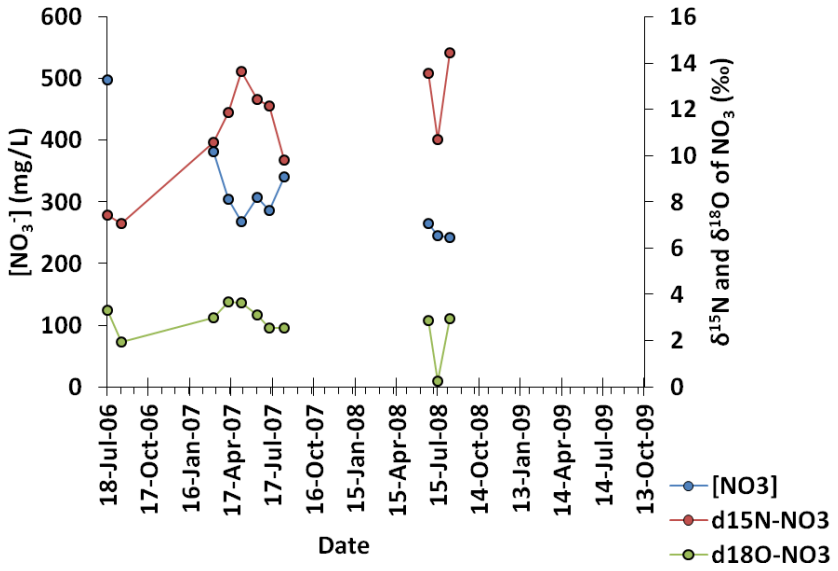

Figure E-12: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 30.

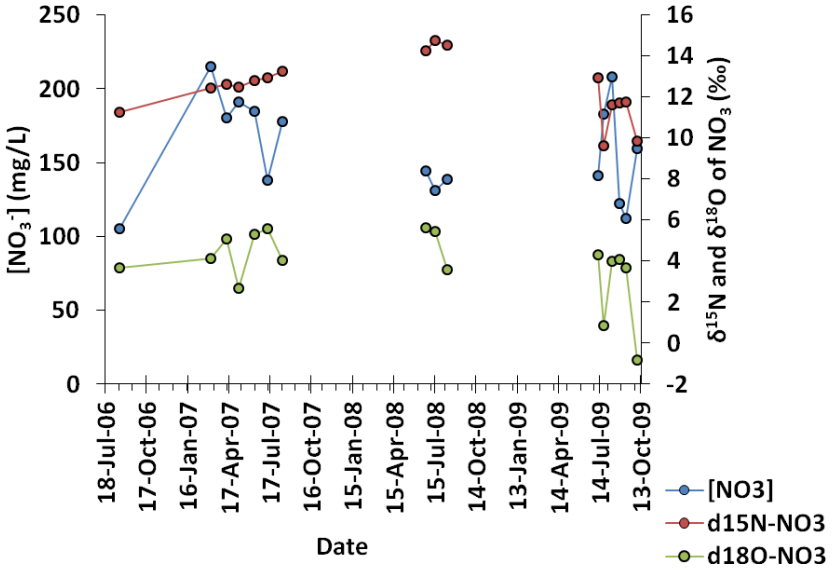

Figure E-13: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 31.

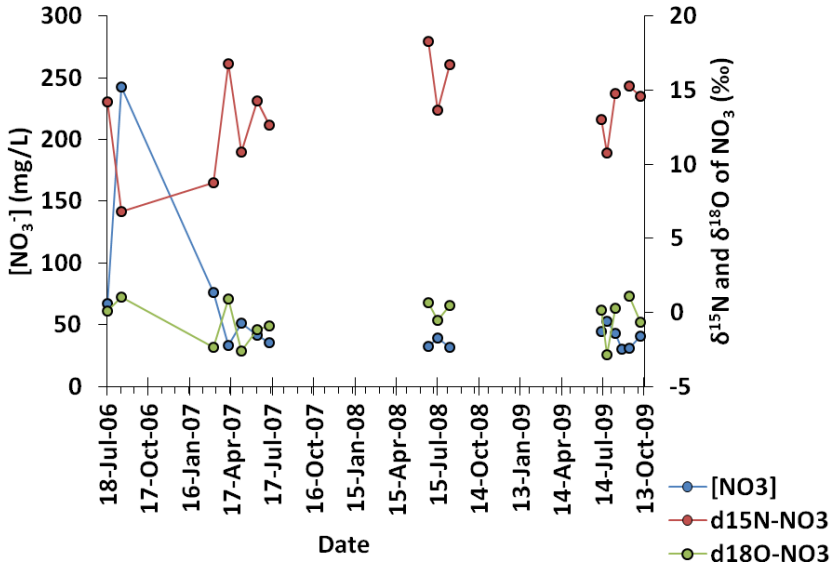

Figure E-14: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 32.

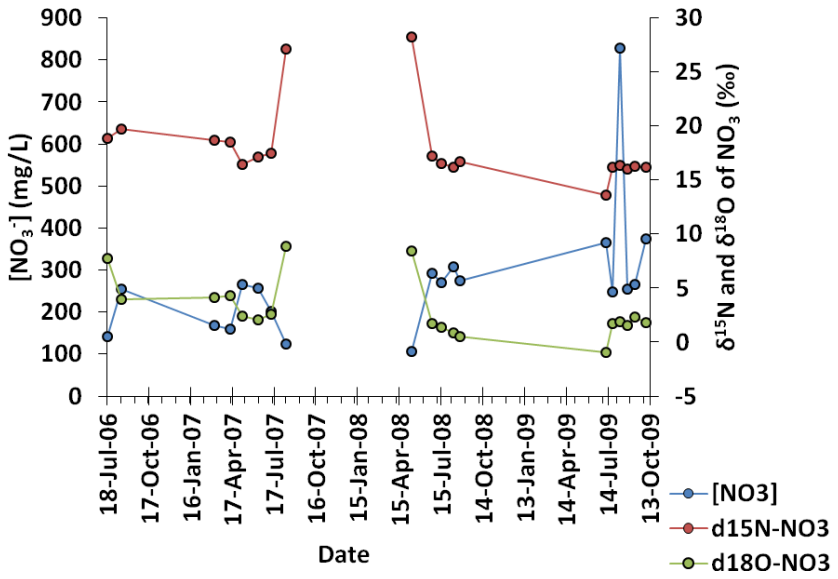

Figure E-15: Nitrate concentrations, $\delta^{15}N\text{-}NO_3$, and $\delta^{18}O\text{-}NO_3$ values over time for groundwater sampled from Well 34.

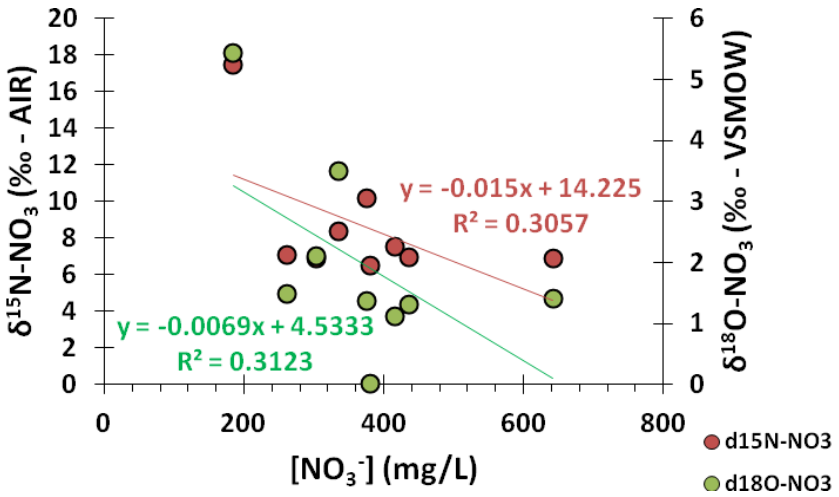

Figure E-16: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 2.

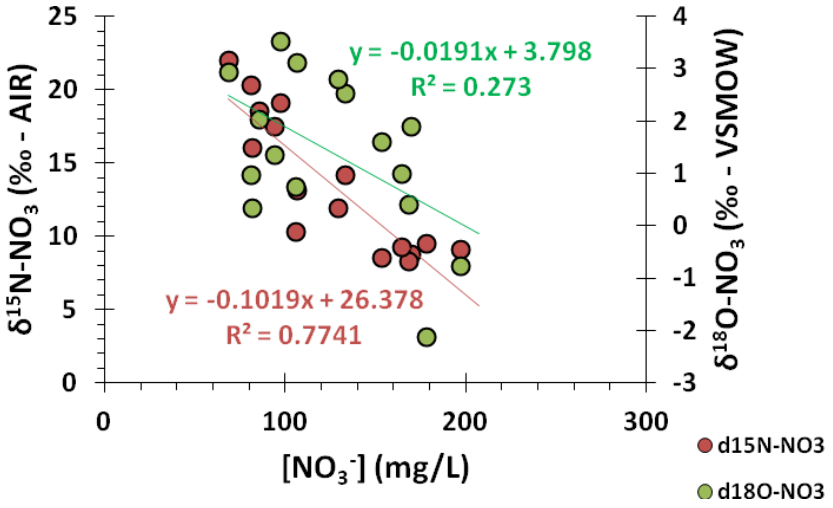

Figure E-17: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 5.

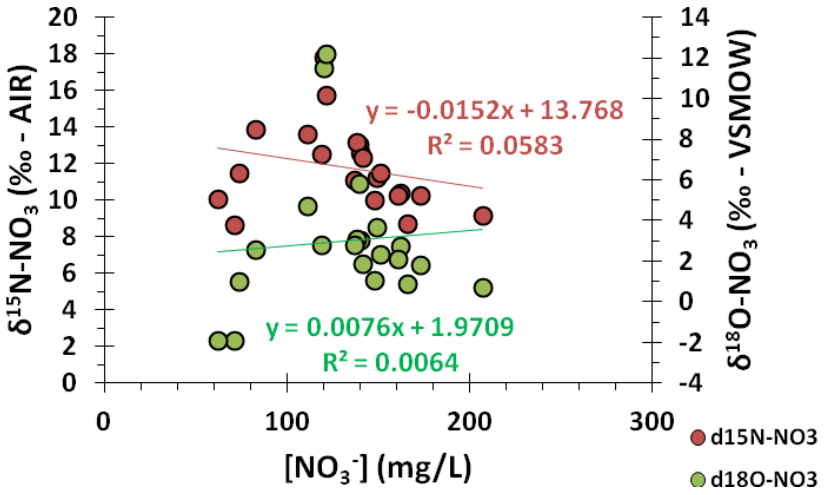

Figure E-18: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 6.

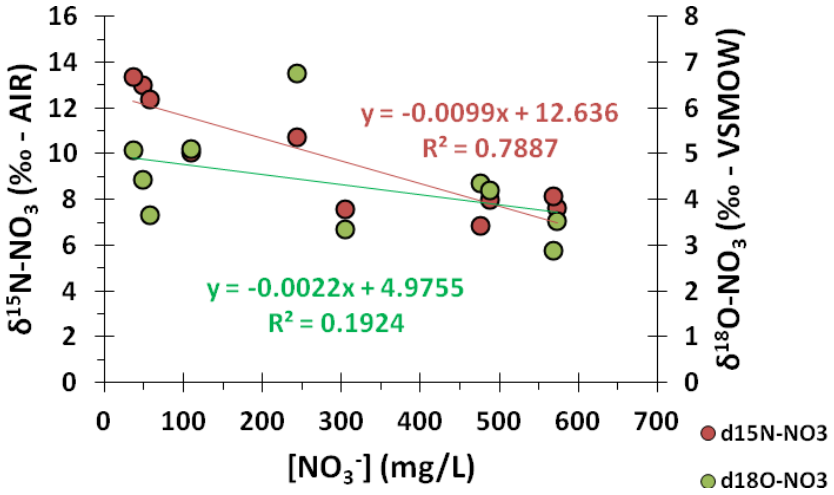

Figure E-19: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 8.

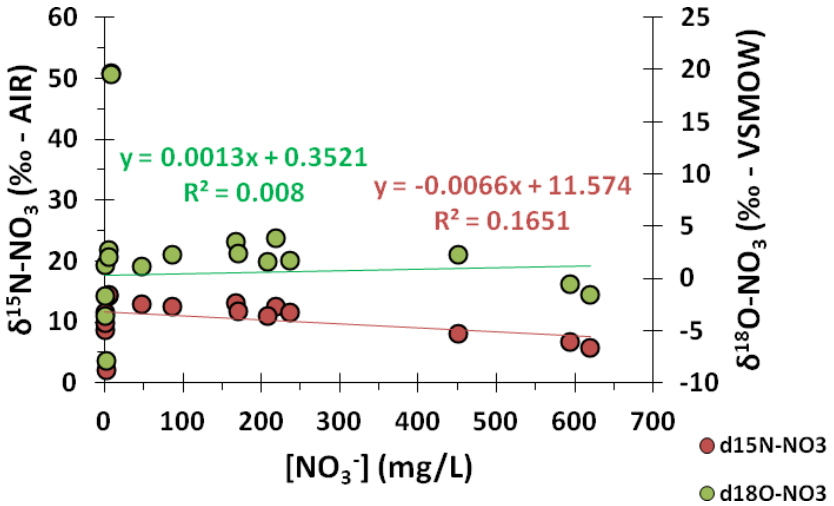

Figure E-20: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 10.

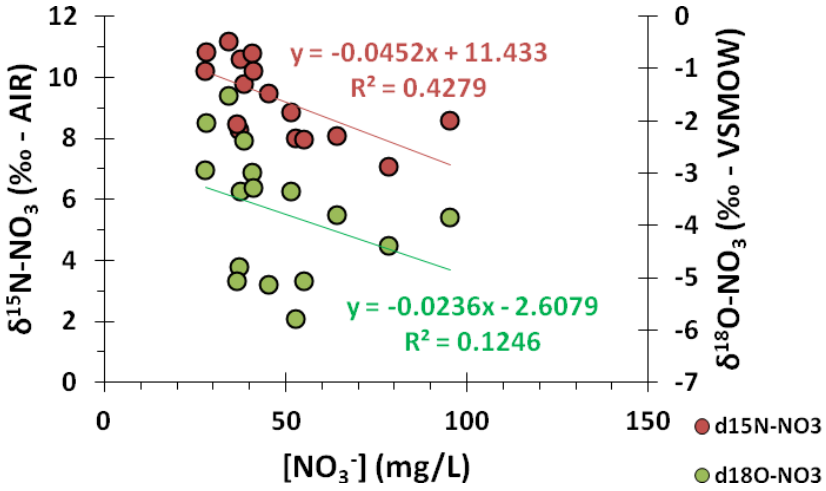

Figure E-21: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 13.

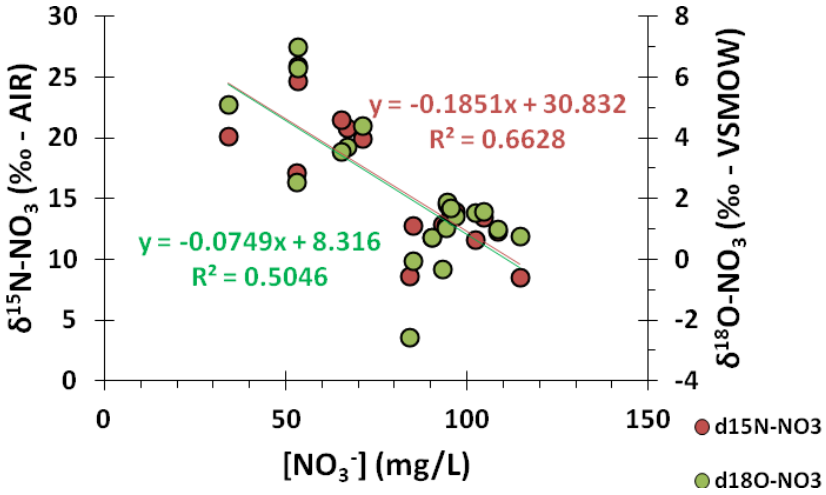

Figure E-22: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 14.

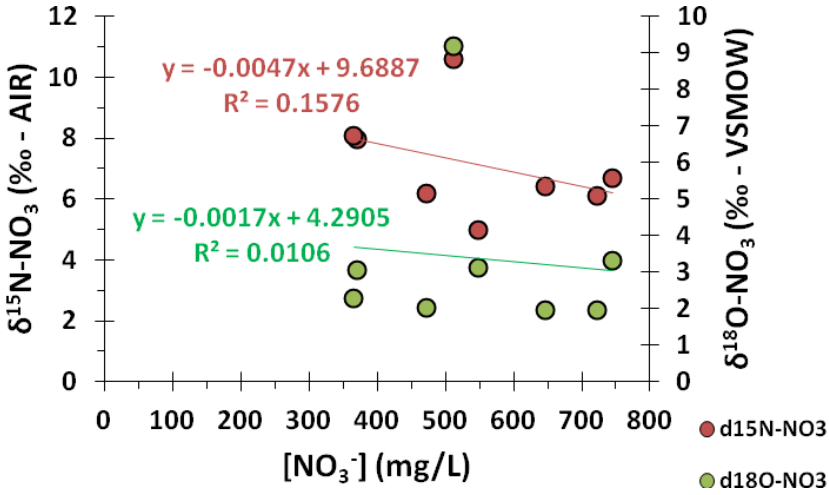

Figure E-23: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 16.

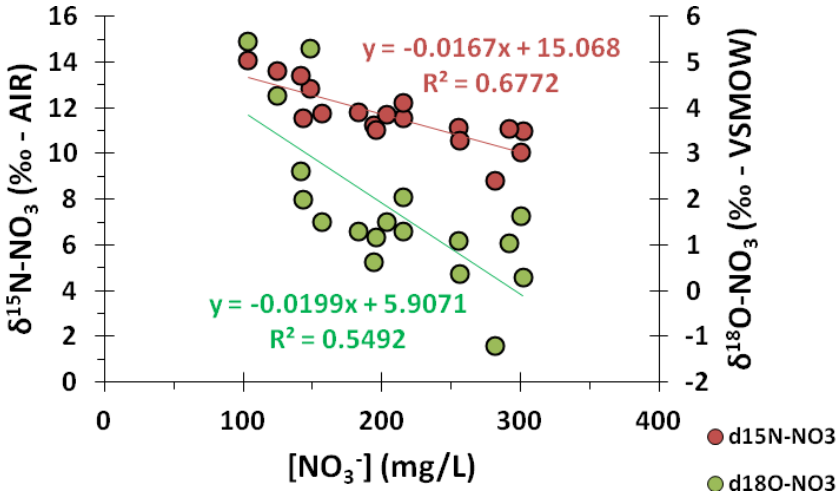

Figure E-24: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 18.

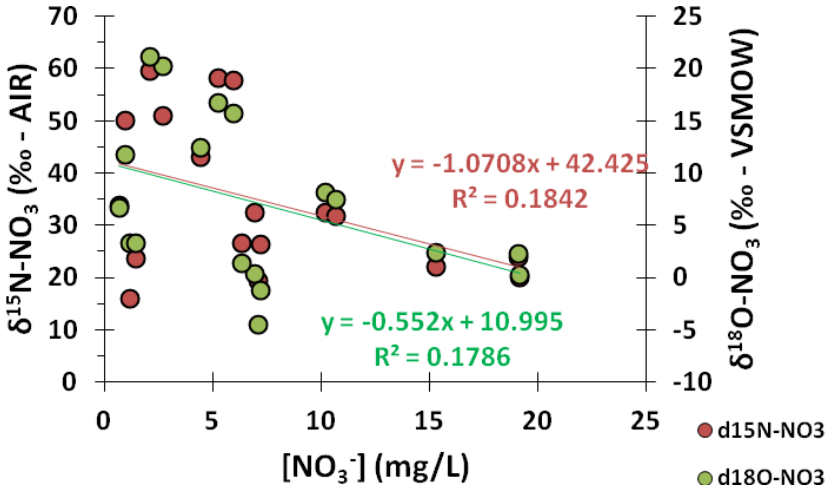

Figure E-25: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 22.

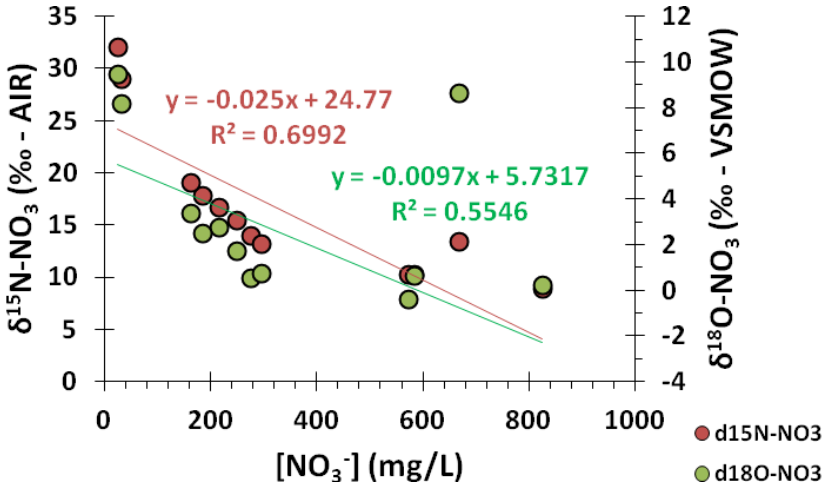

Figure E-26: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 28.

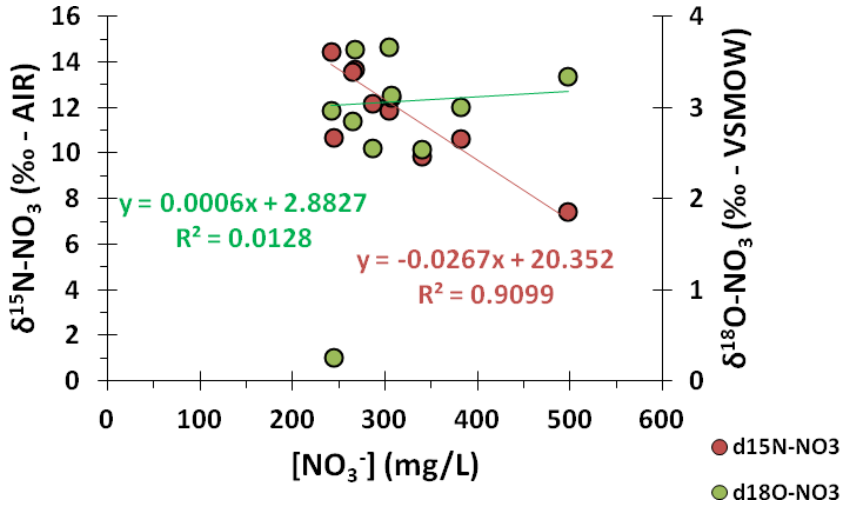

Figure E-27: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 30.

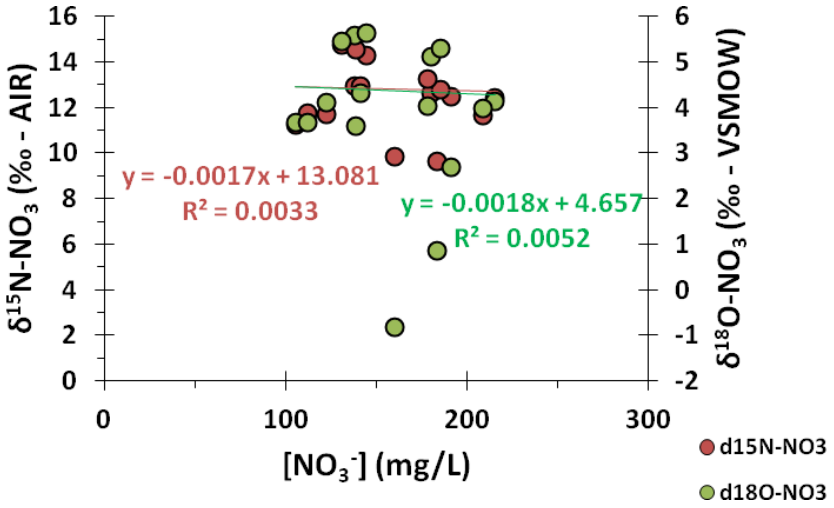

Figure E-28: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 31.

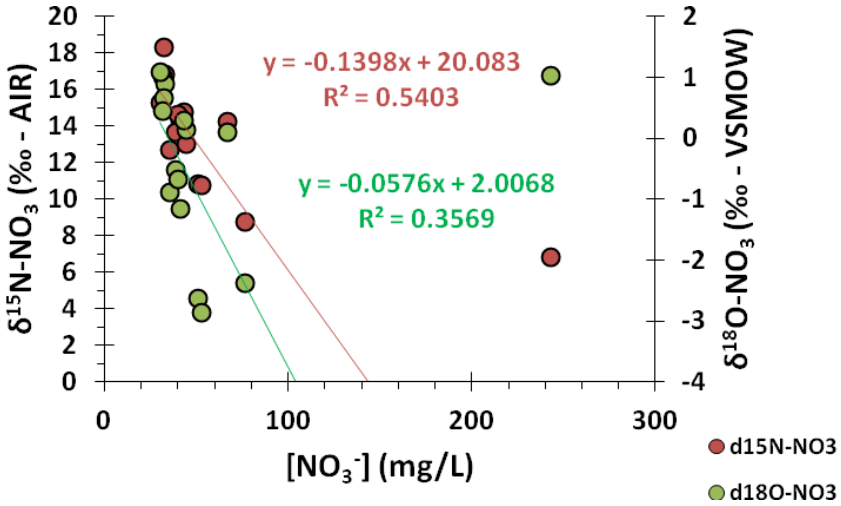

Figure E-29: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 32.

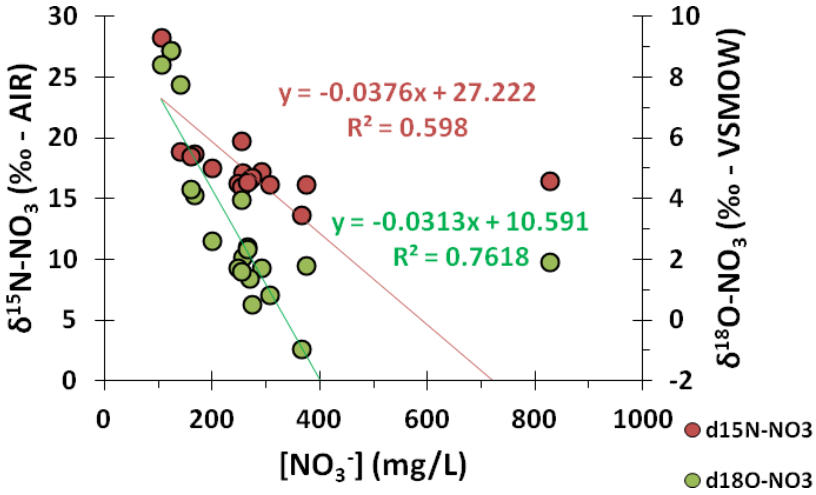

Figure E-30: $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ values versus nitrate concentrations for groundwater sampled from Well 34.

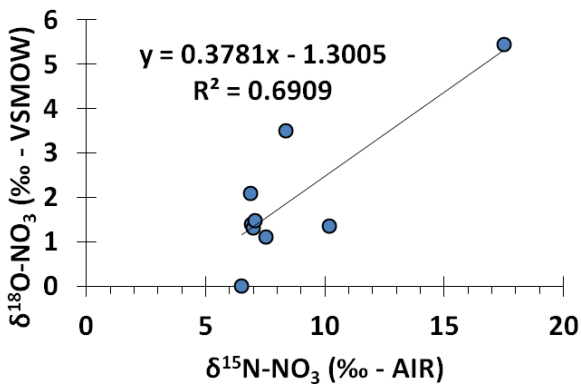

Figure E-31: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 2.

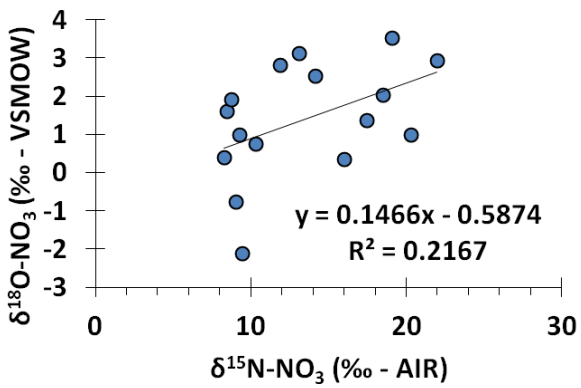

Figure E-32: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 5.

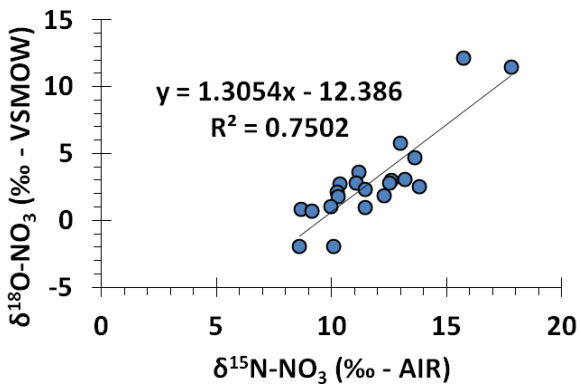

Figure E-33: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 6.

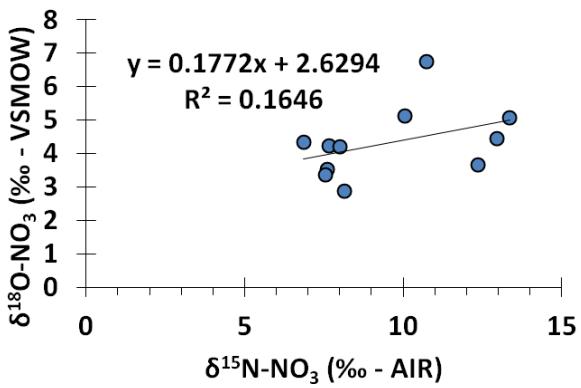

Figure E-34: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 8.

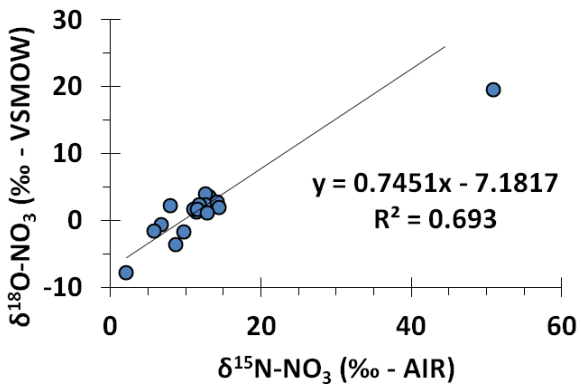

Figure E-35: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 10.

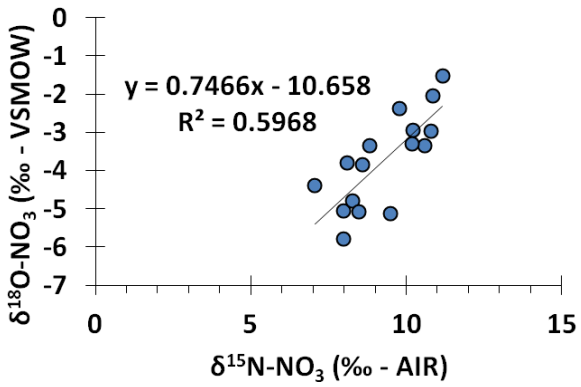

Figure E-36: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 13.

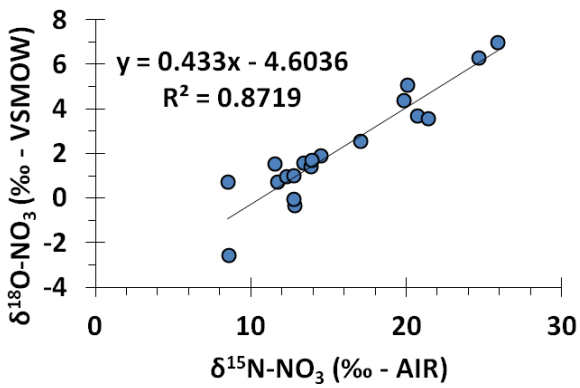

Figure E-37: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 14.

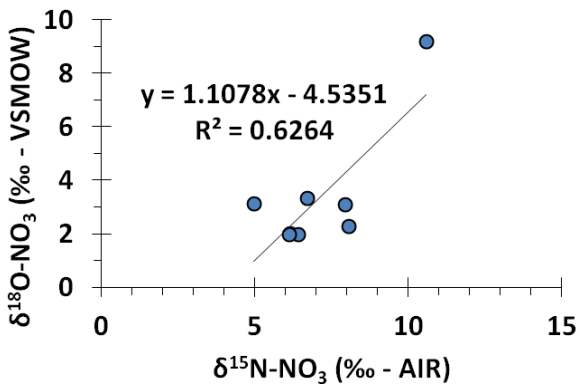

Figure E-38: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 16.

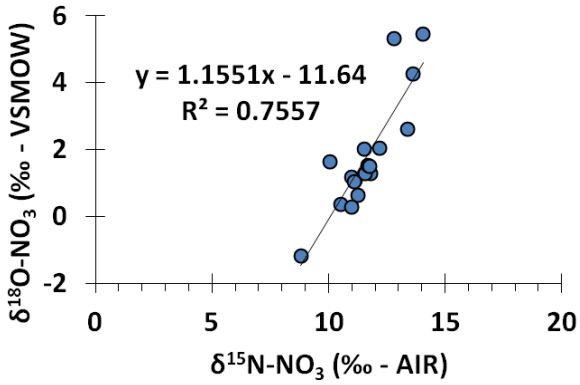

Figure E-39: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 18.

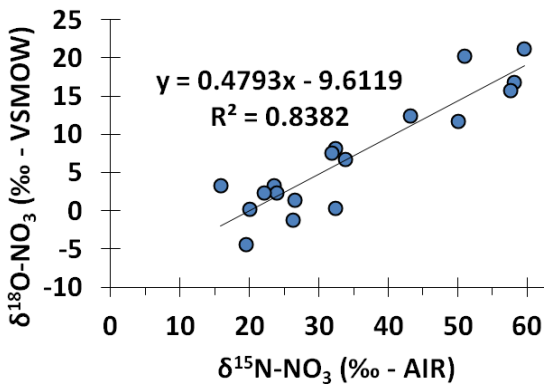

Figure E-40: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 22.

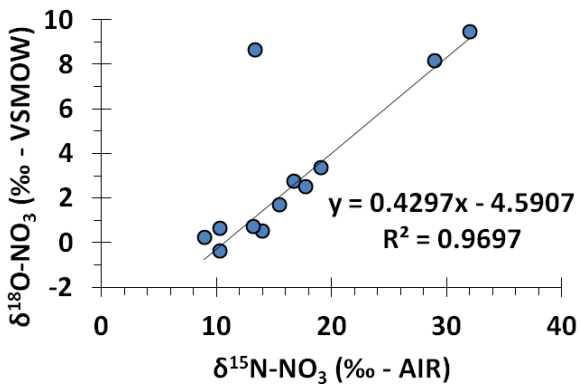

Figure E-41: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 28.

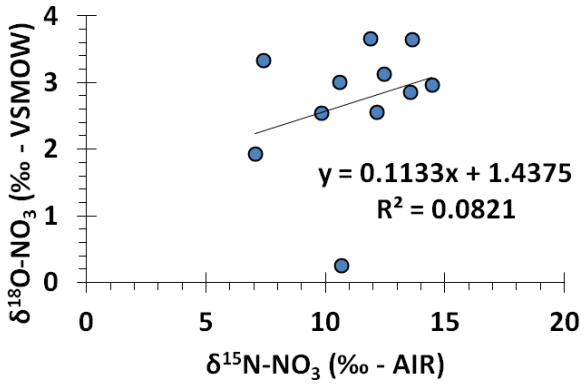

Figure E-42: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 30.

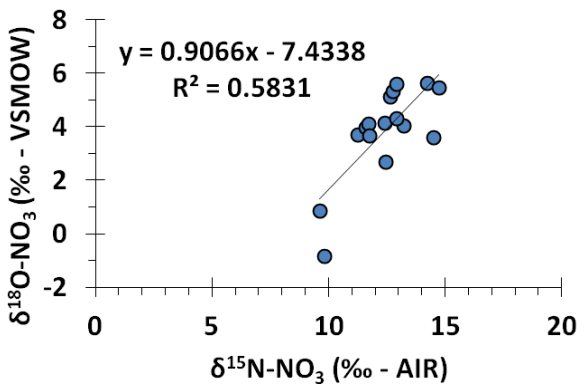

Figure E-43: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 31.

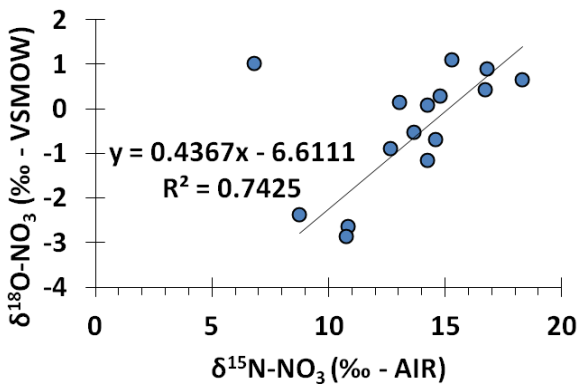

Figure E-44: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 32.

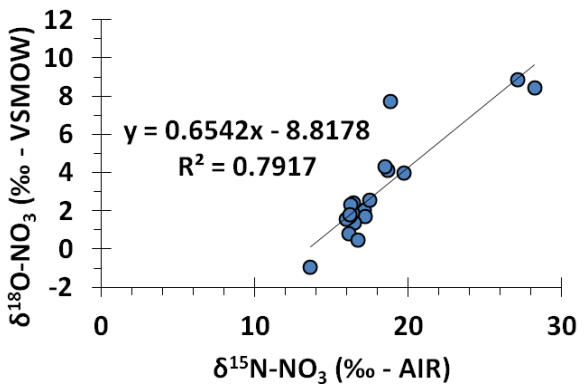

Figure E-45: Dual isotope diagram ($\delta^{15}N$-NO_3 versus $\delta^{18}O$-NO_3 values) of groundwater nitrates sampled from Well 34.