# Multi-decade comparison of groundwater nitrate in the southern Nottawasaga River Watershed

by

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## AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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## ABSTRACT

The Nottawasaga River Watershed is host to the Lake Algonquin Sand Aquifer (LASA), an unconfined aquifer that consists of glaciolacustrine sands and provides water supply to rural residents, for household use, and for applications in agriculture. Previous studies, conducted between 1979 and 1983, measured nitrate in the LASA, and found concentrations that greatly exceeded the drinking water limit (10 mgN/L) and were measured as high as 103 mgN/L (Hill, 1982; Starr, et al., 1987). In these studies, groundwater was tested in domestic wells, groundwater seeps, and multi-level wells situated among agricultural fields. The historically high nitrate in the LASA was linked to agricultural activities, specifically those most focused on potato farming (Hill, 1982). Further to this, a lack of labile organic carbon (LOC) in the aquifer sediments, which acts as an electron donor for denitrification, the natural N cycling process responsible for converting nitrate to harmless $N_2$ gas (Starr & Gillham, 1993).

As part of the current study, nitrate was measured in domestic wells, groundwater seeps, and multi-level wells in 2010 and 2011, to compare the relative change in nitrate in the LASA over that past ~30 years. Statistical differences between the historical and current sample groups were evaluated using a Mann-Whitney rank-sum test for the domestic wells and groundwater seeps. Both of the groups were statistically different between the two dates, however, domestic wells were lower and groundwater seeps were higher, in nitrate. Multi-level wells showed varied results for comparing nitrate with those sampled in 1982 and 1983. Samples within a few meters of the water table had the most distinct changes in nitrate, whereas samples at depth were relatively similar. Increases in nitrate in MC2 could be attributed to the removal of a woodlot and subsequent cultivation which occurred in 2005. The depression of the water table also resulted in a change in the source for set sample points in MC4 and resulted in lower groundwater nitrate than historically measured. The depression of the water table also introduced oxygen and nitrate rich waters to greater depths below the ground surface than what was previously measured. Overall, the mean concentration of nitrate in the multi-level wells was less than the historical data. The decrease in nitrate for the domestic wells and multi-level wells might be related to changes in land management practices over the past three decades.

Source identification for nitrate was constrained with the use of nitrate isotope ratios and artificial sweeteners. Domestic wells, groundwater seeps, and multi-level wells were all dominantly identified as nitrified chemical fertilizer, according to the dual isotope plot of nitrate. Artificial sweeteners were detected in 31% and 35% of the domestic wells and groundwater seeps, respectively, and were excellent tracers for septic wastewater. Further investigation into the proportion of nitrate derived from wastewater showed that septic systems contributed poorly to the nitrate pool in the LASA.

Oxic conditions extended several meters below the water table in the LASA and provided conditions where nitrate could exist at high concentrations. Nitrate removal via denitrification was evident in all datasets, however it was often incomplete and did not remove nitrate entirely. The production of $N_2O$ in domestic wells and multi-level wells was generally attributed to denitrification in oxygenated groundwaters. In domestic wells, where nitrate was not present, and at the deepest sample points in the multi-level wells, redox conditions were classified having undergone nitrate, sulfate, and iron reduction. However, multi-level well data outlines that this occurs at depths of at least 15m below the water table. Groundwater seeps were less reduced and did not show evidence for sulfate or iron reduction. Therefore, the capacity of the aquifer to remove nitrate is not sufficient to cope with the amount of nitrate leached from agricultural practices.

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## 1.1 Study site

### 1.1.1 Location and description

The Nottawasaga River Watershed is located in southern Ontario, about 70 km north-northeast of the city of Toronto. The area of focus for this study consists of a parcel of land within the Simcoe Lowlands; approximately 10 x 15 km, that includes the Nottawasaga River and some of its main tributaries and the town of Alliston (Figure 1.1). Here, the Lake Algonquin Sand Aquifer (LASA) makes up a large portion of the unconfined overburden and is the main water supply for rural households and local farms.

The thickness of the LASA varies between 5 and 30 meters, and is comprised of a sand plain with extremely flat topography (Sibul & Choo-Ying, 1971). Interrupting the very flat topography are a few drumlins and the drainage network of the LASA, which consists of the Nottawasaga River and its major tributaries; namely, the Boyne River, Innisfil Creek, and the Upper Nottawasaga River (Figure 1.1). Flow from the Nottawasaga River is directed northward, eventually draining at Wasaga Beach in the Georgian Bay area of Lake Huron. Riparian zone width varies from being non-existent, along the steeply eroded banks, to several hundreds of meters. The Nottawasaga River Watershed is host to many fish species, including both brown and rainbow trout that travel up the river into the headwaters to spawn at various times of the year.

### 1.1.2 Geology and hydrogeology

The sand and silt deposits of the LASA are glaciolacustrine in origin and were deposited in glacial Lake Algonquin during the previous glacial period. They consist of fine to medium-grained sands with occasional silt and gravel lenses (Figure 1.2), which were deposited on top of an extensive clay till horizon that is impermeable in nature. The sands are unconfined and therefore act as an aquifer for local recharging waters. The sand plain ranges in thickness from less than 1m at the boundaries, to upwards of 30m in various places in the watershed (Sibul & Choo-Ying, 1971). Soils in the area mostly consist of the Tioga and Alliston variety, which are derived directly from the glaciolacustrine sediments, ranging between sandy loams to loamy sands. Soils are generally very well drained, except for near the boundaries, and have a very low organic content. Gravels are present as relic channel deposits, and few organic horizons exist sporadically along the Nottawasaga River and its tributaries

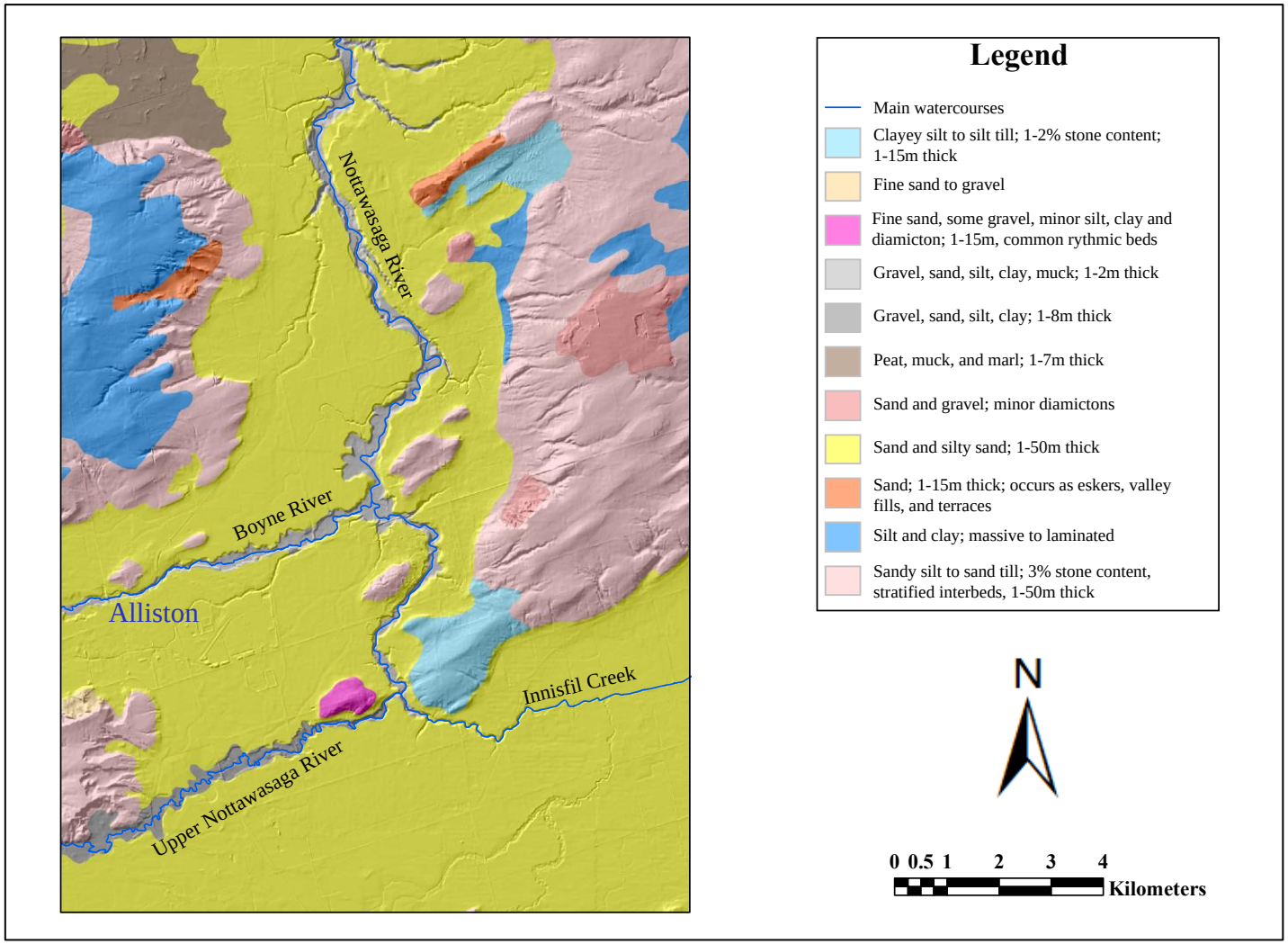



**Figure 1.1** Physiography of the study area and outline of the major watercourses. The extent of the LASA is highlighted by the yellow package of sand and silty sand, noted in the legend.

(Hill, et al., 2000; Devito, et al., 2000).

The water table in the LASA fluctuates seasonally, commonly peaking in the spring and reaching a minimum in the late fall. The aquifer is recharged by precipitation, which infiltrates directly in the spring, summer, and fall, as rain, and falls mostly as snow in the winter, which then melts and contributes to spring melt recharge (Hill, 1986). Water for crop irrigation is commonly drawn from the LASA itself, or from one of the Nottawasaga River and its tributaries. Irrigation waters are also recharged into the underlying aquifer. Annual precipitation rates average approximately 80 cm/year, and recharge into the LASA has been measured to range between 26-36 cm/year (Hill, 1986)

Groundwater flow in the LASA is dominantly lateral and much more significant than vertical flow (Sibul & Choo-Ying, 1971; Hill, 1982). Flow is generally perpendicular to the Nottawasaga River and its main tributaries (Figure 1.3) (Devito, et al., 2000; Hill, 1982; Hill, 1986). Various aquifer measurements (Sibul & Choo-Ying, 1971; MacFarlane, et al., 1983; Devito, et al., 2000) show considerable agreement in the ranges of hydraulic conductivities within the aquifer, which are reported to be between 0.043 and 8.6 m/d (Table 1.1). The residence time of groundwater located south of the Upper Nottawasaga River was estimated with measurements of tritium in multi-level piezometers (Starr, et al., 1987). Evidence showed that all groundwater had recharged post 1953, and therefore was approximately less than 30 years old. No further estimation of residence time could be made from these data.

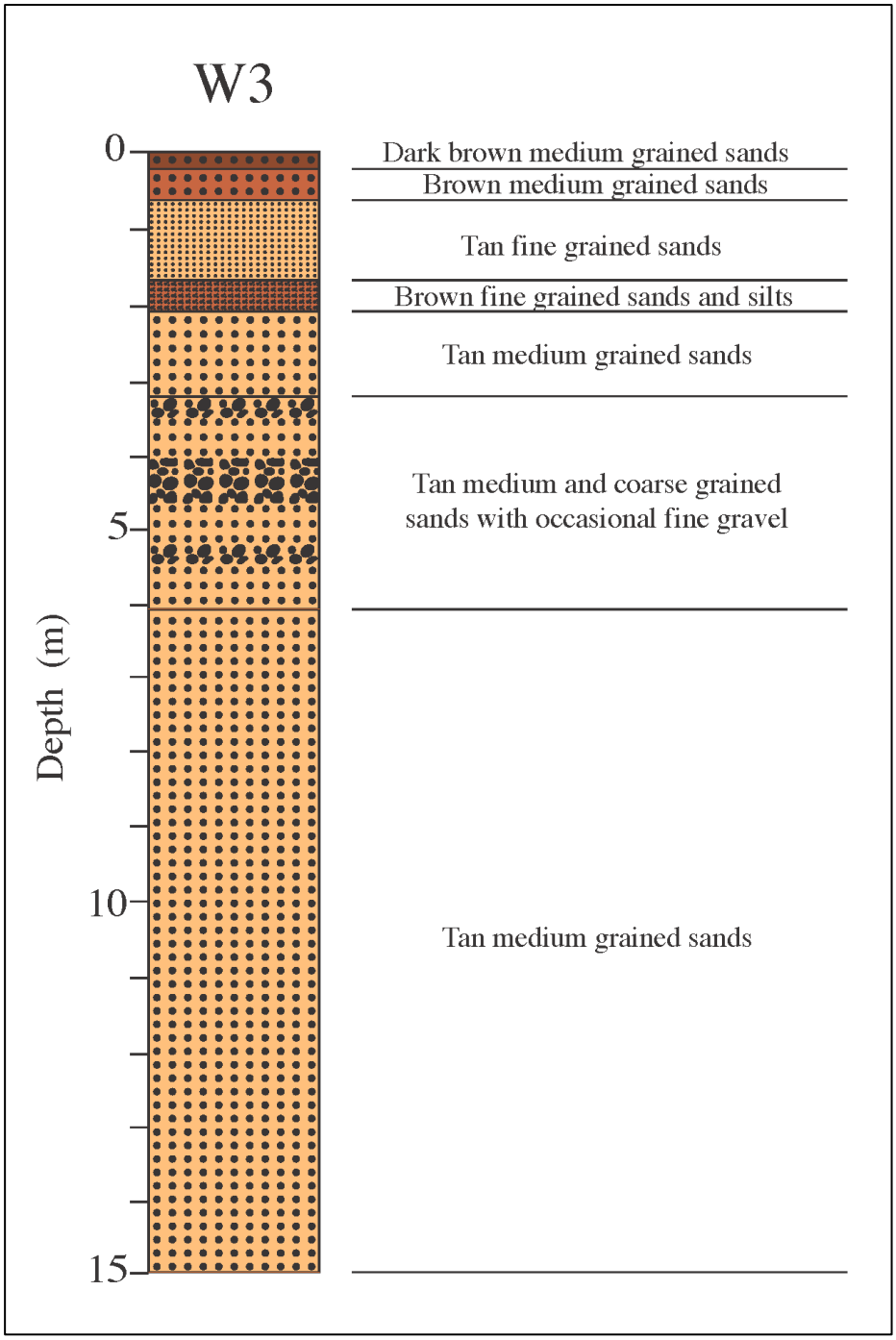



**Figure 1.2** LASA geology profile example from multi-level piezometer W3; revised from Starr et al. (1987).

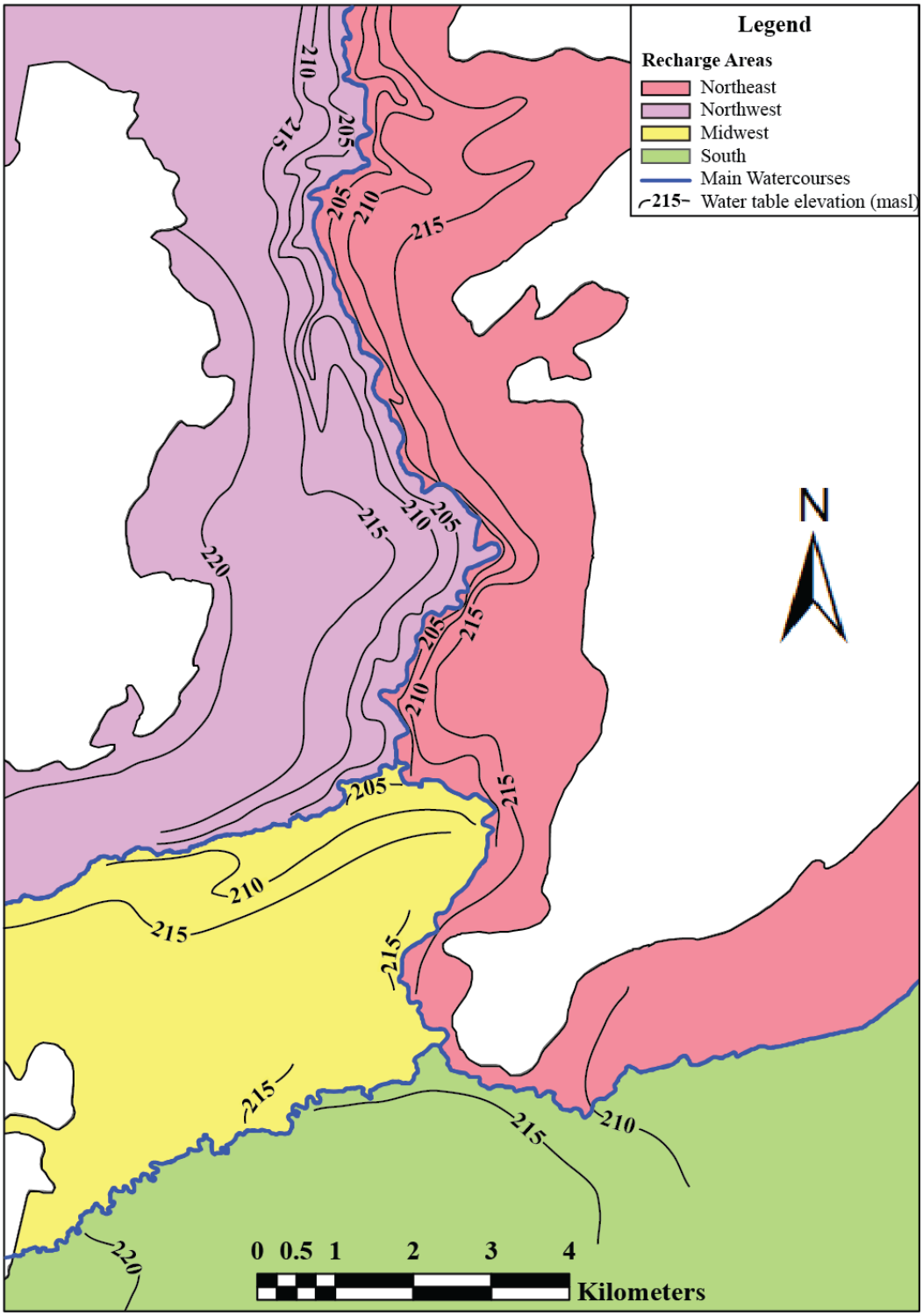



**Figure 1.3** Water table elevations within the LASA as measured by Hill (1982). The aquifer is divided and categorized by each separate recharge area. Groundwater flow is perpendicular to the water table and therefore flows into the creeks and rivers.

**Table 1.1** Hydraulic conductivities measured within the Lake Algonquin Sand Aquifer.

| Site | Material | Test type | K (m/d) | Reference |
|---|---|---|---|---|
| Boyne River | Sand | Hvorslev method | 0.10 – 1 | (Devito et al., 2000) |
| Boyne River | Coarse sand and fine gravel | Hvorslev method | 2.0 – 7.5 | (Devito et al., 2000) |
| Borden | Fine and medium grained sand | Grain-size Correlation | 5.2 – 8.6 | (MacFarlane et al., 1983) |
| Borden | Silty fine grained sand | Grain-size Correlation | 0.043 – 1.7 | (MacFarlane et al., 1983) |
| Borden | Fine and medium grained sand | Permeameter Test | 8.6 | (MacFarlane et al., 1983) |
| Borden | Silty fine grained sand | Permeameter Test | 0.086 | (MacFarlane et al., 1983) |
| Borden | Fine and medium grained sand | Pumping test | 2.6 – 4.3 | (MacFarlane et al., 1983) |
| Borden | Fine and medium grained sand | Piezometer Response | 2.6 | (MacFarlane et al., 1983) |
| Essa – Nottawasaga River Main | Sand | n/a | 1.7 | (Sibul and Choo-Ying, 1971) |
| Tecumseth – Upper Nottawasaga River | Sand | n/a | 2.6 | (Sibul and Choo-Ying, 1971) |

### 1.1.3 Land use

The study site is dominated by agricultural land with some industrial land located close to the town of Alliston. The main crop in the area is potatoes, which is commonly rotated with rye, or rye and then corn. Other favored crops include sod, soybean, corn, and fewer crops of mixed vegetables. A few pastures are scattered throughout the study area but no expansive livestock areas exist. Forested areas are generally concentrated along the Nottawasaga River and its major tributaries. Since the 1980s, major changes in land use include an expansion of the Alliston urban area and its industrial sites, including a Honda plant located to the South of the Boyne River. A golf course and residential area has also been developed on both the east and west sides of the Nottawasaga River, located just south of the Boyne River junction (Figure 1.4). Other changes include an increase in the extent of sod fields and, to a lesser extent, potato fields, although potato crops are spatially the most prevalent.

## 1.2 Water quality in the Lake Algonquin Sand Aquifer

### 1.2.1 Previous research

The southern Nottawasaga River Watershed has been the subject of many studies since the 1970s regarding groundwater nitrate dynamics. Both private wells and groundwater seeps situated within the LASA were sampled for nitrate in 1980 and 1979, respectively (Hill, 1982). Various areas were revealed to contain high nitrate (>10 mgN/L) with maximum concentrations reaching 103 mgN/L. Although nitrate contamination was found to be widespread and closely correlated with potato and other highly fertilized crops, little could be said about the vertical variation of nitrate within the aquifer, and whether or not low nitrate concentrations could be attributed to low N sources or to nitrate removal processes, such as denitrification. In 1982 and 1983, a study was conducted, using multi-level sampling wells, to give insight into this aspect of nitrate variability in the LASA (Starr, et al., 1987) and (Starr & Gillham, 1993). Ratios of nitrate to $Cl^-$ were used to assess whether denitrification was present at depth. It was determined that denitrification was not evident at depth, owing to an insufficient amount of labile organic carbon.

A detailed investigation of nitrate loss beneath areas of continuous potato cropping in the LASA, was also conducted by Hill (1986). Nitrate application rates were used in combination with a monthly climatic water budget for the years of 1977 to 1982. Changes in $NO_3^-$ and $Cl^-$ in the soil profile were

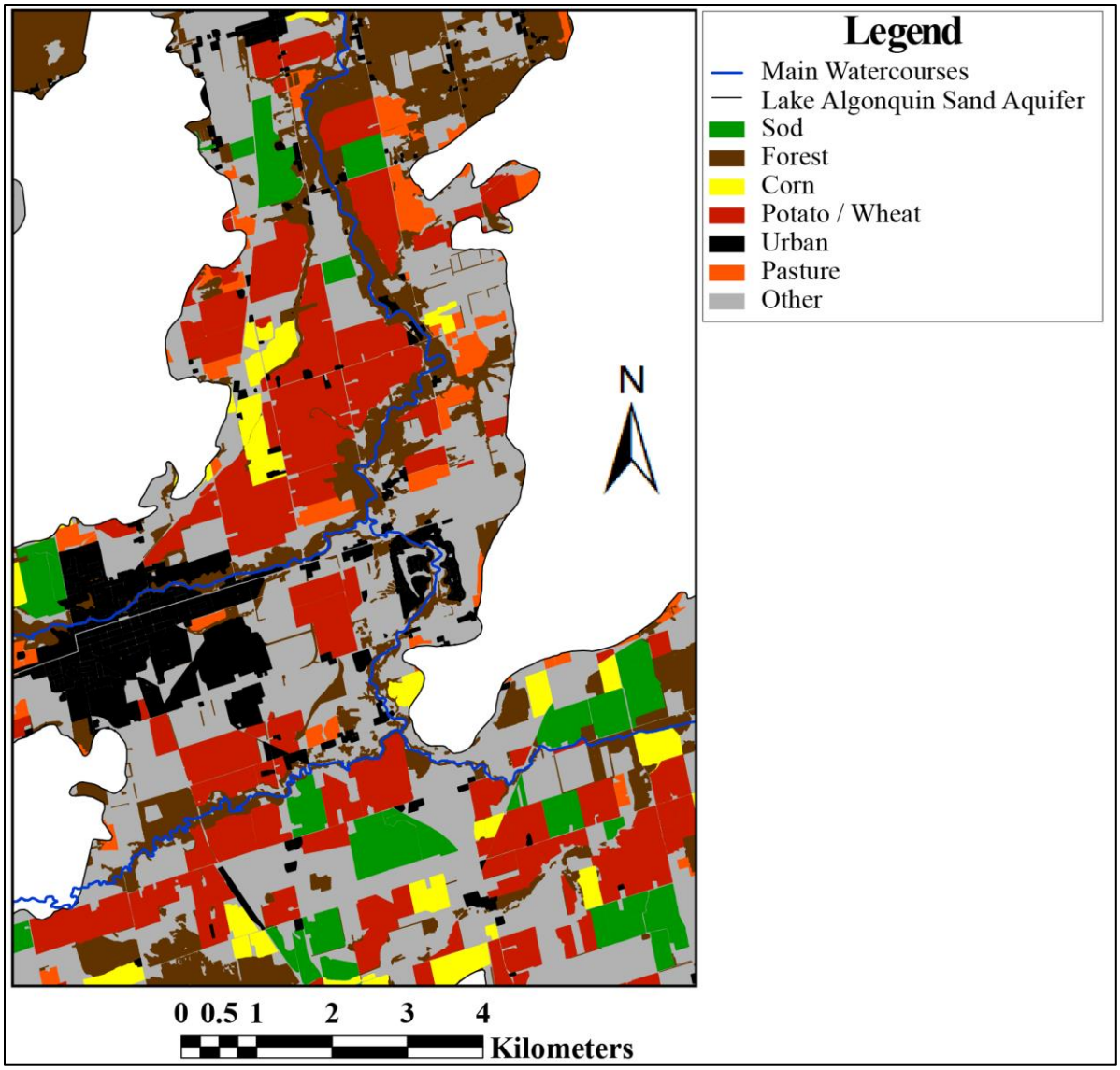



**Figure 1.4** Land use as mapped by Post (2011) in the general study area and around the multi-level piezometers. Potato and wheat are grouped as one category because they are commonly planted in rotation with one another.

attributed to temporal variation of percolation; little leaching occurred during the summer and in the early spring. Groundwater springs located adjacent to the potato fields also had nitrate concentrations greater than 10 mgN/L. It was concluded that increased nitrate leaching might be associated with greater groundwater recharge occurring as large precipitation events.

Further research in the LASA includes an investigation of riparian zone dynamics and nitrate removal capacity within a floodplain that drains an upland sand aquifer, located on the north side of the Boyne River; one of the main tributaries to the Nottawasaga River. Waters draining the adjacent agricultural fields were tracked through the riparian zone, and were analyzed for nitrate, $Cl^-$, DOC, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$, and were studied in conjunction with detailed piezometric head measurements. Nitrate concentrations were largely varied within the floodplain and were closely correlated to the floodplain geometry and lithology, with denitrification having occurred where nitrate-rich groundwater flowed through buried channel sediments and where surface waters recharged through peat deposits on route to the deeper sands. Overall, it was concluded that floodplain geometry and the presence of organic-rich subsurface deposits may have more influence on the removal of nitrate than the width of riparian zone vegetated buffers (Devito, et al., 2000) and (Hill, et al., 2000).

### 1.2.2 Influencing factors for changes in farming practices

Nutrient management programs have been designed for reducing excess fertilizer application and the leaching of nutrients into agricultural aquifers and watersheds. The Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA) oversees these issues and has published a Nutrient Management Act (NMA) (2002) to aid in outlining possible nutrient management strategies. The purpose of the NMA is to regulate the storage, handling and land application of nutrients with the overall goal of protecting water sources from contamination by agricultural activities. Nitrate is the most common contaminant derived from agricultural practices in southern Ontario (Egboka, 1984). Beneficial management programs (BMPs) are included in the NMA and involve several integrated practices such as the use of cover crops to recover excess N, better timing of fertilizer application and irrigation, and the implementation of crop rotations. BMPs are not only aimed at reducing agriculturally derived groundwater contamination, but are also implemented to achieve optimal crop production and provide economic benefits for farmers.

The exact use of BMPs in most agricultural areas in southern Ontario is largely unknown, and it has been suggested that the cost of labor and supplies for implementing BMPs may deter farmers from adopting such voluntary practices (Filson, et al., 2009). The rise in fertilizer prices in the last few decades could be a motivating factor for farmers to adopt more efficient nutrient management practices, at least in the way of optimizing fertilizer application rates. Finally, incidents of groundwater

contamination, such as the Walkterton tragedy, are likely to have increased awareness among farmers and the public regarding the influence of land use on local groundwater quality.

### 1.3 Research objectives

Two research chapters will address specific points of interest with regards to measuring and tracing nitrate cycling and reduction in the LASA. For each chapter, groundwater chemistry is examined in several detailed vertical profiles, after Starr et al. (1987) and at an aquifer-wide scale, after Hill (1982). The evolution of geochemical and stable isotope knowledge will aid in further examining the sources and cycling of nitrate, which have not previously been employed at this study site, and will be of use for formulating future nutrient management programs or policies.

The objective of the first research section, Chapter 3, is to examine changes in nitrate concentrations in the unconfined aquifer since the late 1970s and early 1980s. It is hypothesized that the use of BMPs and changes in land use will have caused an overall decrease in nitrate concentrations in the aquifer. Mapping and statistical analyses are used here to detect changes in nitrate in the LASA compared to those studies of Hill (1982), Starr et al. (1987), and Starr and Gillham (1993).

The objective of the second research chapter, Chapter 4, is to examine the sources, transport and fate of nitrate in the LASA. Hydrogeological observations from the LASA are used to delineate how groundwater flow may distribute and transport N. An investigation of the redox behavior of these agricultural groundwaters involves the examination of a suite of geochemical parameters: anions, cations, ammonium ($NH_4^+$), nitrous oxide ($N_2O$), DOC, soluble reactive phosphorus (SRP), and artificial sweeteners. Novel stable isotope techniques are also employed to better understand mechanisms of nitrate sources and removal; $\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$, $\delta^{18}O\text{-}N_2O$, $\delta^{15}N\text{-}N_2O$, and $\delta^{15}N\text{-}NH_4$ are those considered. These data will be used to support two major goals: 1) determining the sources of nitrate in the LASA, 2) understanding the redox state of the aquifer and main processes controlling nitrate and its removal. Where nitrate concentrations are low, geochemical and isotopic tools will be valuable in distinguishing between attenuation, dilution, and decreased fertilizer loading.

# 2. NITRATE CYCLING, APPLICABLE METHODS FOR DEFINING SOURCES AND PROCESSES, AND STUDY METHODOLOGY

## 2.1 Nitrogen cycling in agricultural watersheds

Nitrogen (N) is naturally occurring in soil, surface water, and groundwater as a derivative of biomass degradation, from nitrogen fixation or from atmospheric deposition. In agricultural areas, elevated concentrations of nitrogen in water, most commonly as either ammonium ($NH_4^+$) or nitrate ($NO_3^-$), can arise from application of nitrogen to crops in the form of organic or inorganic fertilizers. Organic fertilizers are derived from animal, and sometimes, human wastes, whereas inorganic fertilizers are derived from naturally occurring mineral deposits or are manufactured synthetically. Septic tanks, manure storage facilities, and animal feedlots are also some common point sources that may contribute to increased concentrations of nitrate in surface water and groundwater. Currently, agricultural practices are the highest source of N contributing to the environment with nitrate contamination being recognized on a global scale (Schindler, et al., 2006). In agricultural areas of Ontario, nitrate contamination in rural wells can be an issue. A survey of Ontario wells in 1991 and 1992 found nitrate contamination above 10 mgN/L in 14% of the wells sampled (Goss, et al., 1998). The sources of the nitrate were attributed to agricultural activities, either from the application of manure or synthetic fertilizer on overlying crops.

If high nitrate groundwater encounters horizons with low labile organic carbon (LOC), the reduction of nitrate can occur via iron sulfide oxidation. The result is an increase in groundwater sulfate, which can be reduced downgradient to produce dissolved sulfide. The further reaction of dissolved sulfide with iron and particulate iron (hydr)oxides results in highly insoluble iron sulfide minerals, however, phosphates adsorbed to the iron (hydr)oxides or present as iron phosphates become mobilized. Finally, it is the resulting increase in the pool of available P that is thought to be a strong contributor to eutrophication in some environments (Smolders, et al., 2010; Schindler, et al., 2008).

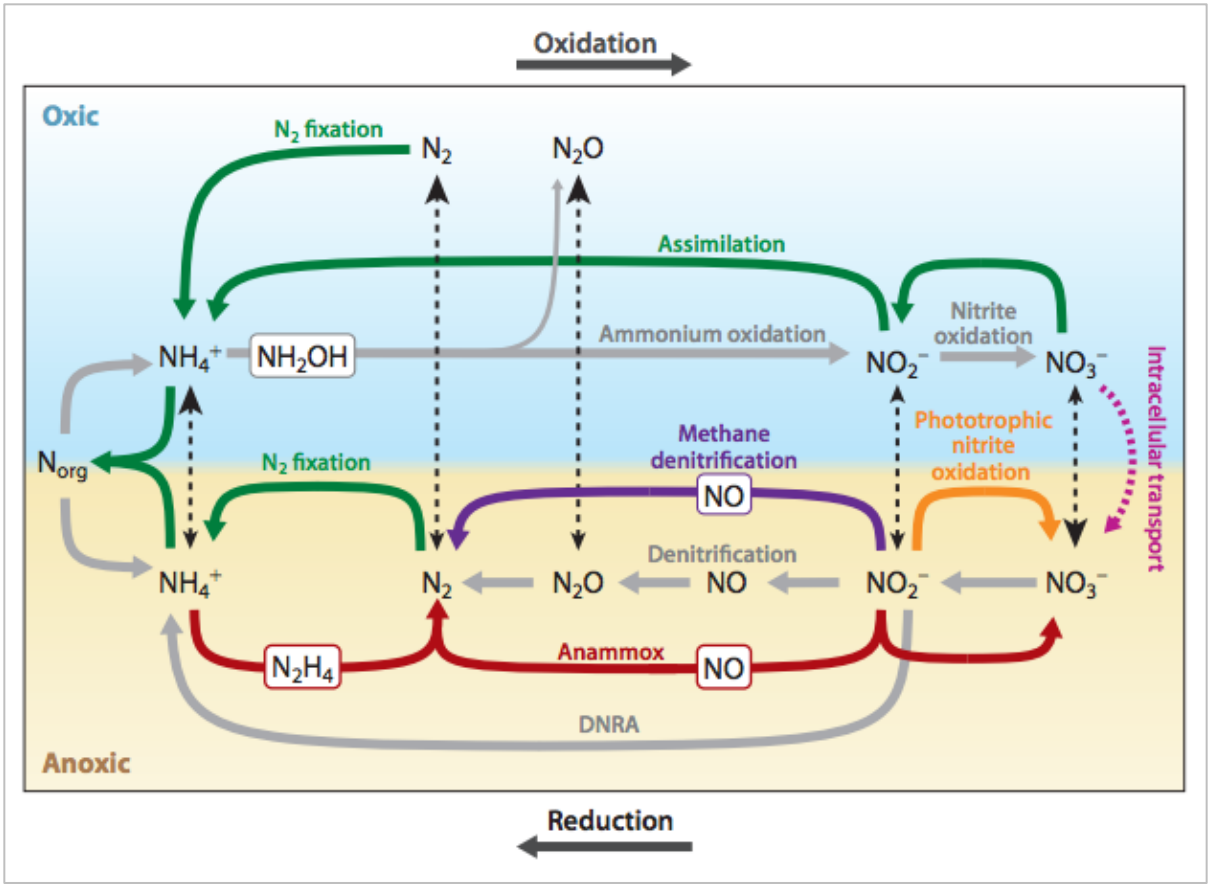



**Figure 2.1** The nitrogen cycle as schematically designed by Thamdrup (2012). Thick arrows represent metabolic transformations. Dashed vertical arrows indicate exchange or transport between oxic and anoxic environments and the relative size of the arrowheads represent the dominant direction of transport.

### 2.1.1 Nitrate production

In agricultural environments, elevated nitrate in surface water and groundwater is commonly derived from the application of fertilizers. Fertilizers are commonly applied as either synthetic forms such as ammonium nitrate ($NH_4NO_3$) or urea, or as manure. Smaller contributions may be derived from the soil N pool, which is formed from plant decay, N assimilation into microbial biomass, and atmospheric deposition. Point sources such as septic systems or manure storage facilities may also be a contributing factor for nitrate accumulation in groundwater (Aravena, et al., 1993). In either case, residual $NH_4^+$ that has not been incorporated into organic forms by assimilation (see the following section; 1.1.2.1 Assimilation) or sorbed to soil, will be converted by the microbial population to nitrate under aerobic conditions. This mechanism of conversion is called nitrification, which involves a stepwise conversion

of $NH_4^+$ to nitrate (ammonium oxidation and nitrite oxidation in Figure 1.1). The overall oxidation of $NH_4^+$ to nitrate is as follows:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad \text{Eq. 1.1}$$

Nitrate is highly mobile in water and can be transported over large distances from its source if the geochemical or microbial conditions do not favor removal. Thus, it is known to be one of the most widespread contaminants of ground and surface waters, globally (Goss, et al., 1998).

### 2.1.2 Nitrate removal

The removal of nitrate in agricultural watersheds involves transformation into either organic or inorganic forms and can generally be summarized by the following four pathways: 1) assimilation, 2) denitrification, 3) dissimilatory nitrate removal to ammonium (DNRA), and 4) anaerobic ammonium oxidation (anammox) (Figure 2.1). The first is a biotic mechanism that fully incorporates nitrate into plant or animal biomass, whereas the latter three involve the reduction of nitrate into different inorganic forms, which depend on biogeochemistry of the environment, and may be biotically or abiotically mediated.

Although nitrate removal can occur through various reaction mechanisms, many of these pathways may negatively impact the environment and disrupt the nitrogen cycle, for example, increasing the production and release of $N_2O$ into the atmosphere. $NH_4^+$ formed via dissimilatory reactions is susceptible nitrification and will be reintroduced into the hydrosphere if not directly assimilated by plants. Denitrification may or may not proceed to completion, as it requires a stepwise conversion of nitrate to dinitrogen ($N_2$) (see section 2.1.2.2 Denitrification). One of the final reactions in this sequence involves the production and consumption of $N_2O$. If $N_2O$ is not completely consumed by the subsequent denitrifying reaction it may be reincorporated into the N cycle, or may be lost to the atmosphere where it acts as a strong greenhouse gas, accounting for nearly 300 times the greenhouse gas effect of carbon dioxide ($CO_2$) on a per molecule basis (Bates, et al., 2008). Generally the removal of nitrate from waters contributes to better the overall water quality, however the pathway and flux of nitrogen to other environments should be considered, as N fluxes may impact downgradient or neighboring hydrological systems, or the atmosphere.

*2.1.2.1 Assimilation*

Assimilation is a purely biotic process that involves the incorporation of nitrate into microbial, plant or animal biomass (Figure 2.1). In agricultural environments, this would translate into direct uptake by the crop to create plant biomass. Microbes in the soil or groundwater also assimilate nitrate in order to increase biomass. In the case of assimilation into crop biomass, this allows temporary removal of nitrate from the environment. If crops are not removed for human consumption they could pose as source of nitrate into the soil and groundwater via mineralization (Burgin & Hamilton, 2007). Farmers commonly take advantage of this cycling by growing cover crops that are efficient at fixing atmospheric $N_2$ and therefore are not completely dependent on supplemental fertilization. The cover crops are grown and then left to decompose naturally, which replenishes the N content of the soil in the form of organic N. This improves soil fertility for the next crop, which then assimilates N directly from the soil as cover crop residues decompose.

*2.1.2.2 Denitrification*

The reduction and removal of nitrate is a naturally occurring mechanism that may be an abiotic or biotic reaction. Denitrification is a stepwise nitrate removal process that results in the eventual production of $N_2$ gas, with intermediates of nitrite ($NO_2^-$), nitric oxide (NO), and $N_2O$ (Figure 2.1). For denitrification to proceed, redox conditions must be suboxic to anoxic. The efficiency of denitrification is best under anoxic conditions, but it does seem to proceed in the presence of some oxygen; a specific or critical oxygen concentration has not been calculated or observed as inhibiting denitrification (Robertson & Kuenen, 1992). Suboxic conditions promote the release of $N_2O$ during denitrification because the nitrous oxide reductase enzyme is inhibited by the presence of $O_2$ (Snider, 2011).

Microbes that use organic carbon (C) as an electron donor utilize the heterotrophic pathway of denitrification. This process is termed respiratory denitrification, and is what is usually considered when the term denitrification is used, without any specification to which pathway is being considered. This mechanism is thought to be the main pathway for nitrate removal in both soil and water. Although this may be true, other forms of denitrification and mechanisms for nitrate removal should be considered when reviewing the N cycling processes in a nitrate-impacted environment. Two other known forms of denitrification use autotrophic processing and involve the coupling of nitrate reduction with: 1) the oxidation of reduced sulfur (S) forms, and 2) the oxidation of iron (Fe) (Burgin & Hamilton, 2007).

Reduced S forms, including free sulfide ($H_2S$ or $S^{2-}$) and elemental S, are used as electron donors by chemolithoautotrophic denitrifiers. This mechanism requires a low concentration of ambient free

sulfide, which is known to inhibit the production of $N_2O$ and $N_2$, and therefore favors DNRA, discussed later (see section 2.1.2.3). These bacteria, without the result of inhibiting complete denitrification, can also oxidize iron sulfide (FeS), and have been found to exist in nitrate contaminated groundwater (Bottcher, et al., 1990; Postma, et al., 1991). In all cases, the oxidized form of S is $SO_4^{2-}$, and in the latter case, is coupled with an increase in Fe, both of which can be used as evidence of denitrification.

Both abiotic and biotic pathways are able to reduce nitrate with the oxidation of Fe (Weber, et al., 2006). The abiotic pathway involves the conversion of nitrate to $NO_2^-$ by ferrous iron ($Fe^{2+}$), which then follows with the rapid reduction of nitrite to $N_2$. The works of Postma et al., (1991) concluded that this reduction pathway is only significant in removing nitrate from groundwater where nitrate inputs were low. The equivalent microbially mediated reaction involves the same steps but is favored at relatively low temperatures and pH ranging between 5.5 and 7.2, and therefore it may be a more favorable reaction in surface waters than its abiotic counterpart (Weber, et al., 2001). Further knowledge pertaining to the controls on these processes remain poorly understood and it is unclear how much they contribute to overall nitrate removal. They may, however, be key in removing nitrate in areas where organic C is limited and reduced Fe is in excess.

*2.1.2.3 DNRA*

The biotic reaction of DNRA involves the consumption of nitrate and the formation of $NH_4^+$ under anaerobic conditions with the aid of an electron donor. Processing of nitrate to $NH_4^+$ with the use of organic C is termed fermentative DNRA. The pathways of respiratory denitrification and fermentative DNRA are supported by the same geochemical variables (anoxia, available nitrate, and LOC) and therefore act as competing processes (Tiedje, et al., 1982). However, it is proposed that fermentative DNRA is favorable in environments where nitrate is limited and LOC is abundant (Kelso, et al., 1997; Silver, et al., 2001; Tiedje, 1998; Kaspar & Tiedje, 1981). It has been argued that DNRA is a more energetically efficient nitrate-reducing pathway than denitrification because the transfer of electrons during DNRA is eight per mole, whereas denitrification transfers only five electrons (Tiedje, 1998). In laboratory conditions, the community of nitrate-reducers in freshwater sediment consisted almost entirely of $NH_4^+$ producing species, such as dissimilatory nitrate-reducers when nitrate was not added to the sediment. The addition of ample amounts of nitrate to the sediment increased the amount of denitrifiers in the sediment to 58% of the total nitrate-reducer community (Nijburg, et al., 1997). In marine sediment, it was observed that dissimilatory nitrate reducers performed approximately 80% of the nitrate reduction, but accounted for slightly over half of the community diversity with 15 types of dissimilatory nitrate-reducers and 13 denitrifiers present. Furthermore, the $NH_4^+$ production was more efficient with the addition of a nitrate-limited culture than with a nitrate-rich culture (Bonin, 1996).

The other main DNRA reaction is chemolithoautotrophic and uses reduced S forms, including $H_2S$ and $S^{2-}$, and metal bound sulfides (e.g. $Fe_2S_3$) as electron donors. As previously discussed, these transformations are competitive with autotrophic denitrification (see section 2.1.2.2).

The occurrence of DNRA by S-oxidizing bacteria is becoming more recognized within freshwater ecosystems (Burgin & Hamilton, 2007). Some research has shown that the addition of nitrate to sulfide-containing, freshwater sediments activated the production and accumulation of $NH_4^+$, and accounted for up to 30% of the nitrate removal (Brunet & Garcia-Gil, 1996).

The DNRA pathway is conservative with respect to ecosystem N cycling, unlike denitrification or anammox (see 2.1.2.4 Anammox) which involve the conversion of nitrate to forms that may be lost to the atmosphere, $N_2O$ and $N_2$ (Burgin & Hamilton, 2007). However, the cycling and fate of $NH_4^+$ beyond its formation via DNRA is not well identified. $NH_4^+$ is more biologically available and less mobile than nitrate; it may be assimilated or converted back to nitrate via nitrification depending on the environmental conditions. The role of DNRA as a viable method of nitrate removal is also largely unverified. In upland tropical forest soils, DNRA was approximated to account for 75% of the nitrate transformation, at least three times greater than denitrification (Silver, et al., 2001). However, the relation of this to temperate soils is unknown; microbial diversity and geochemistry may differ significantly between these environments. The relationship between temperature and DNRA productivity is also noted by Kelso et al. (1997), who examined increases in both stream nitrate and $NH_4^+$, from DNRA during warm summer conditions.

*2.1.2.4 Anammox*

Anammox is a biotic nitrate and ammonium removal process that occurs under anaerobic conditions with the presence of $NH_4^+$, and anammox bacteria (Figure 2.1). $NH_4^+$ acts as the electron donor and the result is the production of $N_2$ and permanent removal of nitrate and $NH_4^+$ at a molar ratio of 3:5, respectively (Mulder, et al., 1995). Although the loss of both nitrate and $NH_4^+$ can occur during coupled nitrification/denitrification reaction sequences, the lack of $N_2O$ production may be a distinguishing characteristic of anammox (Burlow, et al., 2010). The presence of simple organic compounds will inhibit anammox, and will favor denitrification, therefore anammox may be most important where LOC is limited, or nitrate is in excess in comparison with the amount of C inputs.

Anammox is common within marine ecosystems and is thought to be a large contributor in the removal of nitrate in marine ecosystems, producing up to two thirds of the $N_2$ below a depth of 20 m (Burgin & Hamilton, 2007). It is less common in shallow marine and estuarine waters, and its contribution in freshwaters is even less extensively documented, although its detection in various environments within

the past few years is increasing due to recent advances in technology. Current literature identifies anammox as a natural attenuator of N released from septic systems (Robertson, et al., 2011), a manure lagoon (Lazenby, 2011), within a municipal aquifer contaminated by drainage from a fertilizer company, and leakage from the wastewater treatment ponds of a chemical company (Clark, et al., 2008). It has been traced in various freshwater systems, such as the tropical Lake Tanganyika (Schubert, et al., 2008), and the presence of anammox bacteria have been noted in a variety of environments including marshes, lakeshores, a contaminated porous aquifer, permafrost soil, and in agricultural soil (Humbert, et al., 2010). Its presence in a paddy field in southern China accounted for up to 37% of the $N_2$ production. It is expected that the occurrence of anammox will continue to be recorded and quantitatively constrained as a nitrate removal process in contaminated environments.

### 2.1.3 Nitrate toxicity

$NO_3^-$ toxicity has been documented in several species, including humans. In human infants, the ingestion of water containing $NO_3^-$ above the drinking water limit of 10 mgN/L can cause methemoglobinemia, and may even impact fetus development when ingested by pregnant women (Fan & Steinberg, 1996). Methemoglobinemia is a disease that is initiated by the ingestion of highly concentrated nitrate, which is reduced to $NO_2^-$ within the digestive tract. The $NO_2^-$ oxidizes the ferrous iron in hemoglobin to ferric iron. The altered form of hemoglobin is known as methemoglobin, a type that is not able to bind and deliver oxygen (Fan, et al., 1987). Although methemoglobin is naturally occurring in the bloodstream, adverse health effects are distinct when it comprises 10% or more of the total hemoglobin of the body. Symptoms are severe and include cyanosis (the blue discoloration of skin or mucous membranes due to a lack of oxygen), headaches, dizziness, fatigue, coma, shock, convulsions, and, at concentrations exceeding 60% of the total hemoglobin, a high risk of mortality exists (Kross, et al., 1992). The striking blue discoloration of the skin with the initial onset of the disease is the source of its household name, blue baby syndrome. In addition to this syndrome, ingested nitrate and $NO_2^-$ may also contribute to cancer development in the digestive tract, and bladder and ovarian cancers (Camargo & Alonso, 2006).

Nitrate toxicity to aquatic animals has also been documented, and confirms that high nitrate concentrations can cause mortality in freshwater invertebrates, amphibians, and fishes also by decreasing oxygen transport in the blood (Camargo & Alonso, 2006). The sensitivity of freshwater animals is commonly greater than saltwater animals (Camargo, et al., 2005), and therefore a maximum level of 3 mgN/L of nitrate is used as the current Canadian Guideline for the Protection of Aquatic Life.

## 2.2 Parameters for tracing nitrogen sources and cycling processes

### 2.2.1 Stable isotopes

The number of neutrons residing in the nucleus of a given element can vary, and these different nuclides are termed isotopes. Isotopes can be stable or radioactive, which references the stability of the nuclide under natural conditions. Stable isotopes maintain the same number of neutrons throughout chemical processing and over time, whereas radioactive isotopes will decay over time, meaning they transform to other nuclides over time.

For the lighter elements (low atomic number), one isotope is usually dominant, and the other isotopes exist at a small fraction of the natural abundance. The natural abundance of $^{15}N$, for example, is 0.364%, very low compared to $^{14}N$, which makes up 99.636% of nitrogen atoms. The stable isotopes of interest in the current study include $^{15}N$, $^{18}O$, and $^{2}H$, measured in $NO_3^-$, $N_2O$, $NH_4^+$, and $H_2O$, and will be used for tracing biochemical transformations and possible sources affecting the inorganic N pool in the LASA.

The mass difference between two stable isotopes of the same element causes small differences in bond strength, where heavier isotopes provide stronger bonds. This creates a natural preference for light isotopes to react faster in chemical and biological reactions and phase changes (Kendall & Aravena, 2000; Gibson, et al., 2005). Thus, isotopic fractionation during biological, chemical, and physical processes allows them to be used to define sources and the processes in the environment. Reporting of stable isotope signatures usually follows the delta (δ) notation. This method measures isotopic ratios (R = less abundant isotope/most abundant isotope) of the sample and compares that to a known standard, for example:

$$\delta^{15}N,\ \delta^{18}O,\ \text{or}\ \delta^{2}H\ (‰) = [R_{sample}/R_{standard} - 1] \times 1000 \qquad \text{(Eq. 2.1)}$$

The delta value is expressed in units of parts per thousand (permil, ‰). Common isotopic standards are used globally in order to achieve consistency reporting within the stable isotope research community. For $^{15}N/^{14}N$, the primary reference standard is atmospheric air ($N_2$), and for $^{18}O/^{16}O$ and $^{2}H/^{1}H$ the standard is Vienna Standard Mean Ocean Water (VSMOW), which approximates the bulk isotopic composition of the present-day global ocean reservoir (Gibson, et al., 2005). Delta values are reported as either positive or negative, relative to the standard used.

While delta notation reports a relative isotopic abundance with respect to a known standard, fractionation factors ($\alpha_{P\text{-}S}$) and enrichment factors ($\varepsilon_{P\text{-}S}$) are a means to describe the differences in isotopic abundances between the products (P) and substrate (S) of a reaction:

$$\alpha_{P\text{-}S} = R_P/R_S \quad \text{(Eq. 2.2)}$$

$$\varepsilon_{P\text{-}S} = (\alpha_{P\text{-}S} - 1) * 1000 \quad \text{(Eq. 2.3)}$$

$$\varepsilon_{P\text{-}S} \sim \Delta = \delta_P - \delta_S \quad \text{(Eq. 2.4)}$$

In general, enrichment factors ($\varepsilon_{P\text{-}S}$) are more commonly used to define the difference in isotopic values between elements of interest in the substrate and product of a reaction, as they are reported in terms of delta notation. This can be calculated directly (Eq 2.3) or approximated (Eq 2.4) by using big delta ($\Delta$); the difference between the isotopic values of the product ($\delta_P$) and substrate ($\delta_S$). The approximation of $\Delta$ for $\varepsilon$ is most accurate when fractionation is small, and error increases as fractionation increases or the pool size of reactant is limited.

*2.2.1.1 $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$*

Nitrate sources can be separated into four general categories, each which has a varying, yet often distinct, isotopic signatures: 1) Nitrate produced via atmospheric processes and deposition via precipitation; 2) Nitrate produced from nitrification of inorganic fertilizers; 3) Nitrate produced from nitrification of animal and human waste; 4) Synthetic nitrate produced via the Haber-Bosch process. The first source is commonly termed 'atmospheric nitrate' whereas the second and third are both nitrification-derived and referred to as 'microbial nitrate' and the latter, 'synthetic nitrate'. The isotopic signatures resulting from fractionation during reaction pathways for both the production and removal of nitrate (nitrification, denitrification, anammox, and DNRA) are important. The ranges of isotopic signatures for nitrate measured in groundwater and soil have been clearly summarized and are displayed in Figure 2.4. Environments where nitrate isotopes are commonly utilized to provide information on nitrate sources and cycling include forest, agricultural areas (organic and inorganically derived sources), septic systems and wastewater treatment plants.

In the atmosphere, nitrogen is present as derivative of both human and natural activities. Such human activities largely consist of those involving fossil fuel combustion (industrial processes, vehicle emissions, and electricity generation) and natural sources such as biomass burning, soil emissions and lightning. N is released in various gas types, for example, $NH_3$ or $NO_X$, where $NO_X$ sources are largely contributed from fossil fuel combustion. In the atmosphere, $NO_X$ compounds are oxidized to nitric acid ($HNO_3$), which easily dissociates to nitrate; in this form it can be deposited back to the earth via precipitation. Other forms of gaseous N can be deposited through dry deposition, i.e. atmospheric fallout, which collects directly onto the earth's surface and may be mobilized into aquifers or surface waters when precipitation occurs (Kendall, et al., 2007). The isotopic signatures of nitrate deposited

from wet deposition have been measured to range within -15‰ to +15‰ for $\delta^{15}N$ and 60‰ to 94‰ for $\delta^{18}O$, in analyses using the denitrifier method (Kendall, et al., 2007). Results of previous analytical methods, such as the silver nitrate ($AgNO_3^-$) method, presented lower, inaccurate $\delta^{18}O$ values, which is said to be due to reaction with glassware during combustion, causing exchange with oxygen in the glass (Revesz & Bohlke, 2002) or with oxygen-bearing contaminants (e.g. organic matter) (Figure 2.4).

Microbial nitrate is produced from the stepwise conversion of $NH_4$ to nitrate (nitrification) in soil and water; processes that can leave an imprint of the isotopic fractionation process in both N pools involved. Nitrification is favorable and occurs rapidly, under oxic conditions, but may not always proceed to completion. This results in the formation of aqueous and/or gaseous compounds, such as $NH_2OH^-$, $NO_2^-$, NO, and $N_2O$. The N and O fractionations differ because they are derived from different compounds with N originating from $NH_4^+$ and/or $NO_2^-$, and O from $O_2$ and/or $H_2O$ or $NO_2^-$, therefore the behavior of each is discussed separately (Kendall, et al., 2007).

For the $\delta^{15}N$ of microbial nitrate, the effect of fractionation depends on the fraction of the substrate pool that is consumed, the initial $\delta^{15}N$ signature, and the isotopic fractionation factor. For agricultural environments with fertilizer application, determining the final $\delta^{15}N$ of microbial nitrate may be problematic, as fractionation is highly variable before, during, and after a heavy application of ammonium-based fertilizer (Kendall, et al., 2007). For this reason several studies have recorded $\delta^{15}N$ of microbial nitrate in various environments, with varying DIN sources.

The $\delta^{15}N$ value derived from human and animal waste is typically positive; this is due to a small enrichment (2-3‰) that occurs in the digestive tract of all N consumers. However, if N sources are subject to volatilization, such as stockpiles of manure, they can undergo fairly substantial enrichment, and result in $\delta^{15}N$ values between +10‰ and +20‰. Therefore, the $\delta^{15}N$ values of nitrate produced by nitrification of these sources can range between ~+5‰ and +20 ‰, depending on the effects or presence of volatilization. In general, it is difficult to distinguish signatures of animal or human waste in groundwater, where the exact sources are unknown and potential source isotopic compositions have not been measured directly (Kendall, et al., 2007). For inorganic $NH_4^+$ sources, such as fertilizer, the value of $\delta^{15}N$ is generally around 0‰, reflecting the atmospheric $N_2$ from which it is generated. Overall, values dominantly range between -4‰ to +4‰, with outliers as low as -8‰ and as high as +7‰ (Kendall, et al., 2007). Synthetic nitrate fertilizers have $\delta^{15}N$ values, which are slightly higher than $NH_4^+$ fertilizers, and $\delta^{18}O$ values that commonly range from +17‰+ to +25‰, reflecting atmospheric $O_2$ which is used during production and differing from that of microbial nitrate (Kendall,

et al., 2007). Specific studies measuring nitrogen isotope compositions for several types of sources are summarized in Figure 4.2.

The $\delta^{18}O$ values of microbial nitrate, produced via nitrification of ammonium from various sources, is commonly approximated by a simple linear relationship involving $\delta^{18}O$ of $O_2$ and $H_2O$ (Eq. 2.5). The general interpretation is that the first oxygen is derived from dissolved $O_2$ and the next two each from $H_2O$ (Andersson & Hooper, 1983; Kumar, et al., 1983; Hollocher, 1984).

$$\delta^{18}O\text{-}NO_3^- = 1/3*\delta^{18}O\text{-}O_2 + 2/3*\delta^{18}O\text{-}H_2O \quad \text{(Eq. 2.5)}$$

Although this approximation is broadly used, it is well understood that controls on the $\delta^{18}O$ values of microbial nitrate are more complex than what Eq. 2.5 portrays. The investigation of $\delta^{18}O$ values of microbial nitrate by Snider et al. (2010) better identifies the complexities of the processes involved. The linear relationship of the $\delta^{18}O$ values of microbial nitrate is redefined as including the $\delta^{18}O$ value of $H_2O$, $\delta^{18}O$ value of $O_2$, the fraction of intra- and extracellular abiotic O-exchange ($f_{ABIOTIC}$), and both kinetic and equilibrium isotope effect coefficients, $^{18}\varepsilon_{eq}$ and $^{18}\varepsilon_k$, respectively (Figure 1.2).

$$\delta^{18}O\text{-}NO_3^- = 1/3(2+ f_{ABIOTIC})\delta^{18}O\text{-}H_2O$$

$$+ 1/3[f_{ABIOTIC}(2^{18}\varepsilon_{eq} - \delta^{18}O\text{-}O_2 - {}^{18}\varepsilon_{k,O2} - {}^{18}\varepsilon_{k,H2O,1})$$

$$+ \delta^{18}O\text{-}O_2 - {}^{18}\varepsilon_{k,O2} - {}^{18}\varepsilon_{k,H2O,1} + {}^{18}\varepsilon_{k,H2O,2}] \quad \text{(Eq. 2.6)}$$

The equation outlined by Snider et al. (2010) is more complex and therefore needs much more data to calculate $\delta^{18}O\text{-}NO_3^-$. However, Snider et al. (2010) used controlled laboratory experiments with known $\delta^{18}O\text{-}O_2$ and $\delta^{18}O\text{-}H_2O$ values, and plotted the resulting $\delta^{18}O\text{-}NO_3^-$ value in comparison to that predicted by Eq. 2.5 (Figure 2.3). For agricultural soils with low OM, such as those present in the current study, the $\delta^{18}O\text{-}NO_3^-$ value was as much as 7‰ lower than that predicted by Eq. 2.5 when the $\delta^{18}O\text{-}NO_3^-$ value was less than 5‰.

Using the above methods, the $\delta^{18}O\text{-}NO_3^-$ value of microbial nitrate can be broadly estimated. In Egbert, Ontario, which is located in the southern Nottawasaga River Watershed, the $\delta^{18}O$ value in precipitation has been measured between 1998 and 2002 (Birks, et al., 2004) and has an average value of -10.6‰. Using equation 2.5, and the information on variance from this calculation provided by Snider et al. (2010), the expected average $\delta^{18}O$ value of microbial nitrate would be around +0.77‰. Thus, one would expect a $\delta^{18}O\text{-}NO_3^-$ value of approximately -6 to 2 ‰ for samples collected in the LASA that have nitrate from nitrification, and have not undergone subsequent cycling or partial denitrification.

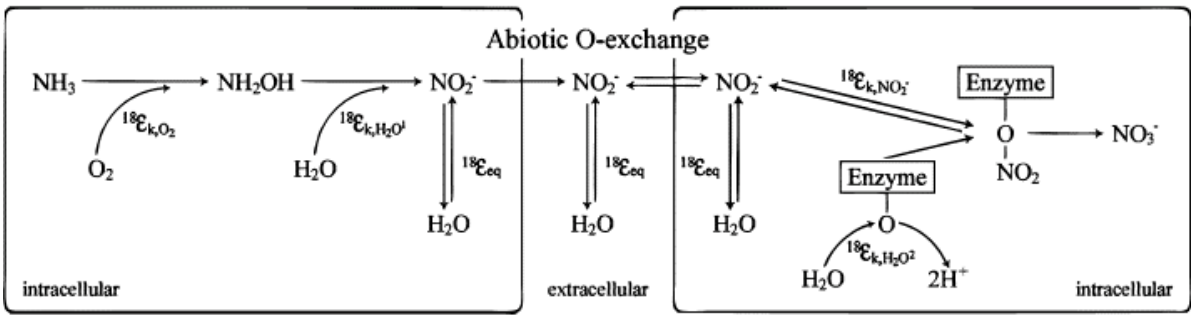



**Figure 2.2** A schematic representation of bacterial nitrification as presented by Snider et al. (2010).

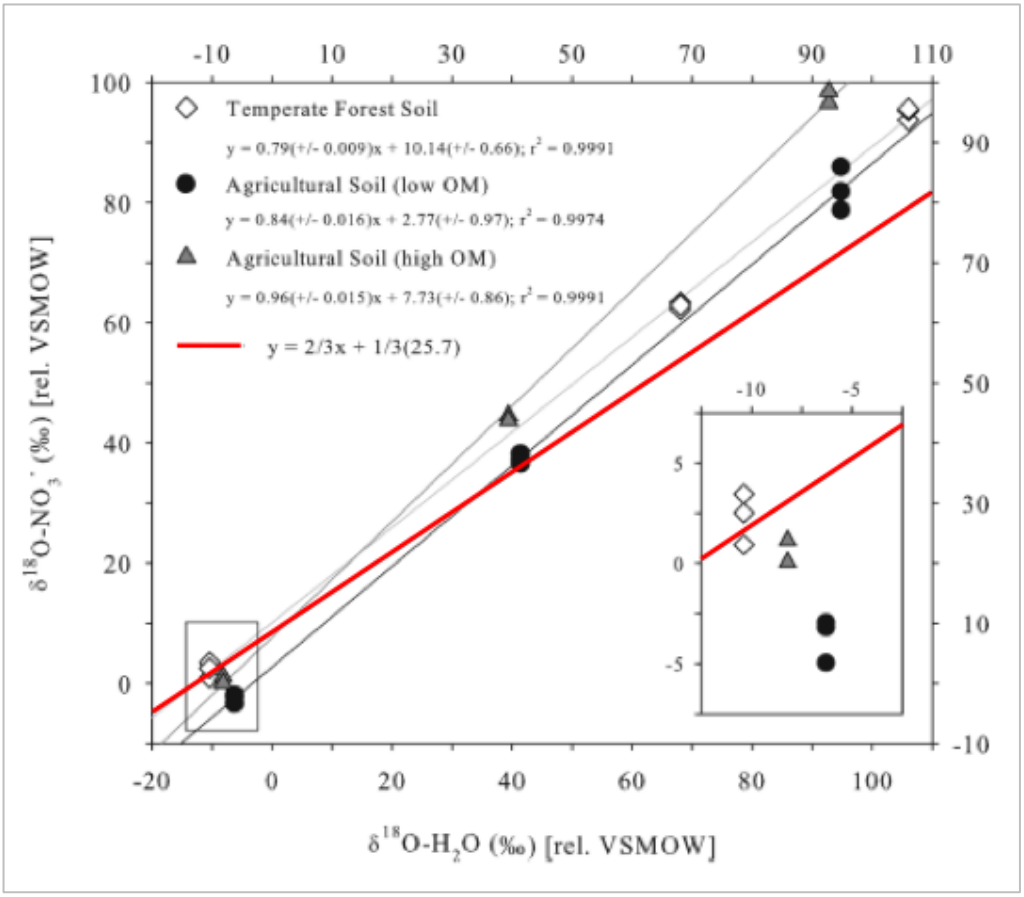



**Figure 2.3** $\delta^{18}O\text{-}NO_3^-$ values produced from nitrification of temperate forest and agricultural soils incubated with waters of varying $\delta^{18}O\text{-}H_2O$ values modified from Snider et al. (2010). Linear regressions ((standard error) of the data and $r^2$ values are shown in the legend. The red line represents $\delta^{18}O\text{-}NO_3^-$ - with varying $\delta^{18}O\text{-}H_2O$ and constant $\delta^{18}O\text{-}O_2$ (commercial gas mixture) +25.7‰), and oxygen sourced from $H_2O$ and $O_2$ (in a ratio of 2:1 ratio). The inset shows an enlargement of the area of the main graph where $\delta^{18}O\text{-}NO_3^-$ is formed from soils incubated with waters at natural abundance for $\delta^{18}O$.

The $\delta^{18}O\text{-}NO_3^-$ vs. $\delta^{15}N\text{-}NO_3^-$ plot, or 'dual nitrate isotope plot', is used for both attributing nitrate sources and for identifying the effects of nitrate removal processes, most specifically denitrification. As denitrification proceeds, the $\delta^{15}N$ and $\delta^{18}O$ values of the residual nitrate pool increase. The dual nitrate isotope plot provides insight to the ratio of enrichment between $\delta^{15}N$ and $\delta^{18}O$ of nitrate, by using the slope of the line plotted through various sample points. Common ratios in groundwater are a little less than 2:1 (Chen & MacQuarrie, 2005) for $\delta^{15}N$:$\delta^{18}O$ when attenuated by denitrification. Additionally, when $\delta^{15}N\text{-}NO_3^-$ is plotted vs. nitrate concentration, the increase in the $\delta^{15}N$ value is exponential as nitrate decreases (Kendall, et al., 2007).

Details of the $\delta^{18}O$ and $\delta^{15}N$ values of groundwater nitrate for a riparian site within the southern Nottawasaga River Watershed were provided by Devito et al., (2000). Samples were collected in a flood plain environment, which was adjacent to a cropped area, directly north of the Boyne River. The $\delta^{18}O$ and $\delta^{15}N$ values of nitrate for samples at the source without denitrification or any form of removal were ~2‰ and ~5‰, respectively. Nitrate in samples collected in the floodplain showed higher of $\delta^{18}O$ and $\delta^{15}N$ values, and dual nitrate isotope plot showed a line slope ($\delta^{15}N$: $\delta^{18}O$ ratio) of 0.56. Plotting the $\delta^{15}N\text{-}NO_3^-$ and nitrate concentration also showed a clear exponential increase in $\delta^{15}N\text{-}NO_3^-$ as nitrate decreases. These data and trends give good indication of the effects of denitrification on $\delta^{15}N$ and $\delta^{18}O$ values of nitrate in the study area.

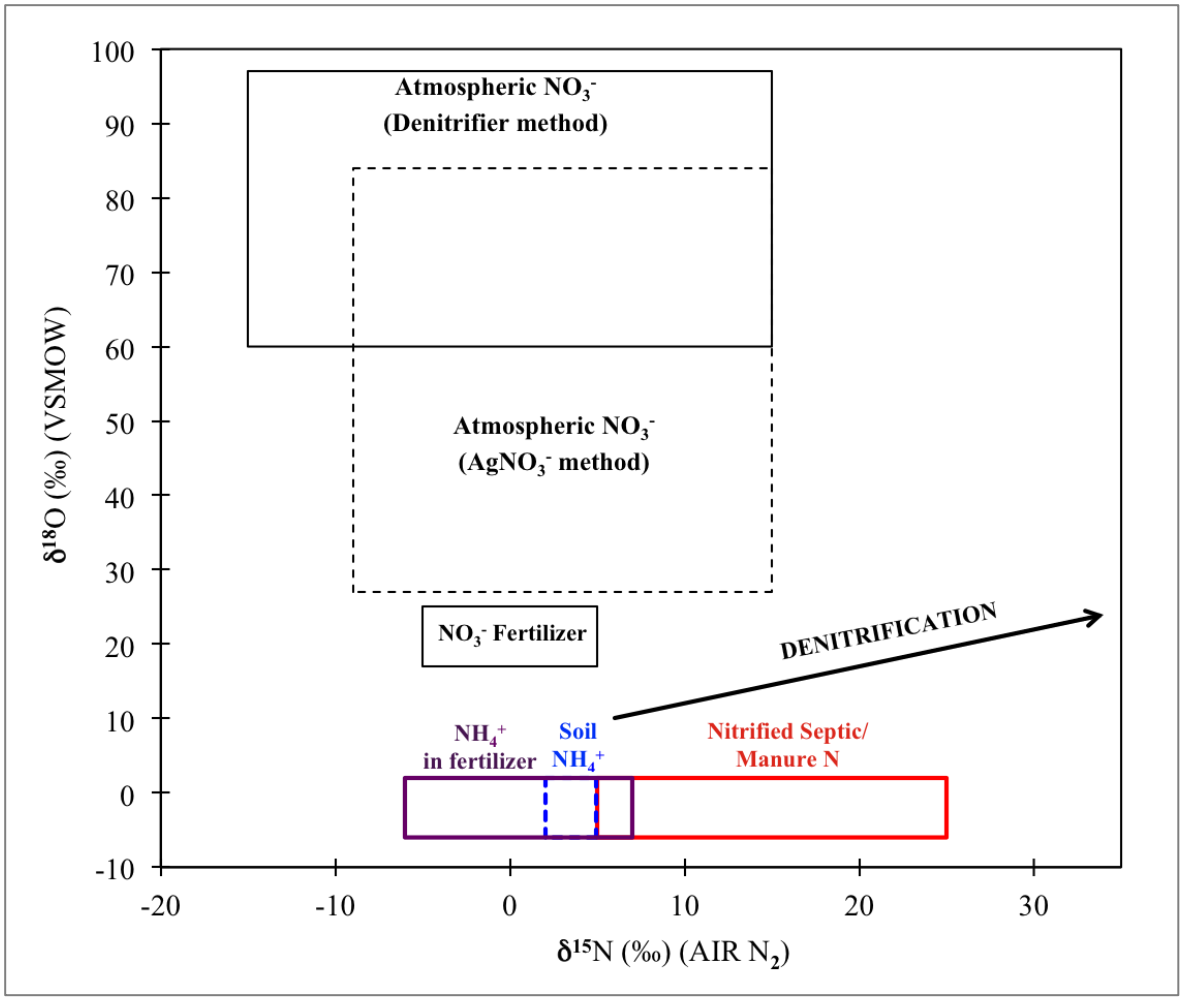



**Figure 2.4** Summary of dual nitrate isotope plot ranges and relationships. $\delta^{18}O\text{-}NO_3^-$ values of nitrification (colored boxes) are estimated to range from -6‰ to +2‰ (based on a local precipitation data with an average $\delta^{18}O\text{-}H_2O$ of -10.6‰, **(Birks, et al., 2004)**). $\delta^{15}N\text{-}NO_3^-$ values from nitrification are derived from the supporting literature are as follows: ***Atmospheric nitrate:*** **(Kendall, et al., 2007)**. ***Manure/septic:*** Aravena, et al., 1993; Wassenaar, 1995; Girard & Hillaire-Marcel, 1997; Kendall, 1998; Choi & Ro, 2002; Griggs & Kump, 2003; Curt, et al., 2004; Kellman, 2005; Katz, et al., 2009 ***Ammonium in fertilizer:*** Wassenaar, 1995; Vitoria, et al., 2004; Bateman & Kelly, 2007; Flood, 2011 ***Soil:*** (Kendall, 1998). The black arrow labeled 'Denitrification' outlines the increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ at a ratio of 2:1, illustrating the possible changes in isotope composition via denitrification. The starting location of the denitrification line is arbitrary.

#### *2.2.1.2 $\delta^{15}N$-$N_2O$ and $\delta^{18}O$-$N_2O$*

Stable isotopes of $N_2O$ provide evidence for discriminating the source of $N_2O$ production, and can be paired with nitrate and $NH_4^+$ stable isotopes to determine isotope effects (ε). Isotope effects may give more information to which cycling processes are present than only the delta values alone. The signatures of stable isotopes in N cycling processes can be complex and overprinted, due to multiple recycling processes and so using both δ and ε values give a greater depth to interpret the sources and processes.

The isotope effect for $\delta^{15}N$-$N_2O$ produced from nitrification ($\varepsilon_{N2O\text{-}NH4^+}$) has been measured and calculated to range from -45‰ to -68.2‰ (Shearer & Kohl, 1986; Yoshida, 1988; Kendall, 1998; Perez, et al., 2001; Stein & Yung, 2003; Sutka, et al., 2006). $\delta^{15}N$-$N_2O$ values from nitrification in soil incubation experiments with agricultural soils and forested peat ranged from -35‰ to -16‰ with isotope effects of -55‰ to -37‰ (Snider, 2011). The enrichment factor for oxygen produced from nitrification is considered to be more complex, and accounts for fractionation from $H_2O$ and $O_2$, and has been studied much less than that of $\delta^{15}N$-$N_2O$. Using laboratory incubation experiments and a Monte Carlo simulation model, Snider et al. (2012) found that values of $\delta^{18}O$-$N_2O$ produced by nitrification ranged between +13 and +35‰ for agricultural and temperate forest soils.

Isotope effects involving the formation of $N_2O$ via denitrification ($\varepsilon_{N2O\text{-}NO3^-}$) have been summarized at length (Remple, 2008) and range from -45.2‰ to -10.4‰ for $\delta^{15}N$. A more highly constrained range of $^{15}N$-$\varepsilon_{N2O\text{-}NO3}$ values documented by Snider et al. (2013) involved controlled incubations with agricultural soils and results ranged from -30‰ to -9‰. Furthermore, the $^{18}O$ isotope effect of denitrification varied between 32‰ and 60‰.

#### *2.2.1.3 $\delta^{18}O$-$H_2O$ and $\delta^{2}H$-$H_2O$*

The main stable isotopes of water, $\delta^{18}O$ and $\delta^{2}H$, undergo natural fractionation during water-cycle processes (e.g. evaporation, condensation, etc.), which vary spatially and temporally at local, regional, and global scales. Isotopic signatures are specifically affected by mass dependent partitioning throughout the hydrologic cycle, which provides a linear relationship between $\delta^{18}O$-$H_2O$ and $\delta^{2}H$-$H_2O$ values of precipitation. This association is defined by the global meteoric water line (GMWL; Craig, 1961) which is based on global $\delta^{18}O$-$H_2O$ and $\delta^{2}H$-$H_2O$ of mean annual amount-weighted precipitation:

$$\delta^{2}H = 8*\delta^{18}O + 10 \qquad \text{(Eq. 2.6)}$$

At the watershed scale, and within hydrogeological studies, variations in the isotopic composition of precipitation can be used to delineate different parcels of water. Precipitation accounts for a large quantity of groundwater recharge and local water isotope effects vary the signatures of $\delta^{18}O\text{-}H_2O$ and $\delta^{2}H\text{-}H_2O$. Local water isotope relationships will be similar to the GMWL with slight deviations due to more local fractionation factors, such as seasonal, altitude, and continental effects. Seasonality commonly results in values of $\delta^{18}O\text{-}H_2O$ and $\delta^{2}H\text{-}H_2O$ being lower in winter and higher in the summer. This is controlled by three main factors: (1) the variation of seasonal temperatures at a location, which induces Rayleigh fractionation in precipitation; (2) seasonal evapotranspiration over the continents alters the atmospheric water balance; (3) changes in the sources of water vapor and storm trajectories over the seasons (Rozanski, et al., 1993). Altitude and continentality effects result in more depleted values as air masses are orographically uplifted or moved substantial distances inland. Both effects are consequent of the same mechanism, which involves the preferential removal of the heavier isotopes during the condensation and rainout process (Rozanski, et al., 1993).

Shallow groundwater isotopic values closely approximate the mean local precipitation signatures of $\delta^{18}O\text{-}H_2O$ and $\delta^{2}H\text{-}H_2O$. The Canadian Network for Isotopes in Precipitation has calculated amount-weighted mean annual values of $\delta^{18}O\text{-}H_2O$ and $\delta^{2}H\text{-}H_2O$ at Egbert, Ontario, which is located within the southern Nottawasaga River Watershed. Between October 1998 and September 2002 the averaged values were -10.6 ‰ and -72.4 ‰, respectively (Birks, et al., 2004). These values can be used to distinguish waters under seasonality or continental effects, or to approximate the $\delta^{18}O\text{-}NO_3^-$ (as mentioned in section 2.1.2.1).

### 2.2.2 Geochemical constituents

#### *2.2.2.1 Cations and anions*

In agricultural areas, potassium ($K^+$) is applied directly to crops with $Cl^-$ as potassium chloride (KCl). Any excess $K^+$ that is not assimilated by the crop or adsorbed onto soil particles can percolate into the aquifer, but may be affected by ion exchange with organic matter or clay particles within the unsaturated or saturated zones (Bohlke, 2002). The associated $Cl^-$ is generally unreactive and, therefore, its presence can be used as a tracer of fertilizer loading. On the other hand, $Cl^-$ has multiple anthropogenic and natural sources. For example, high $Cl^-$ may result from the application of road de-icing salts in the form of NaCl. In that case, an elevated sodium ($Na^+$) signature would likely be associated with the elevated $Cl^-$. An elevation of $Na^+$ and $Cl^-$ concentrations may also indicate a septic system source (Hamilton, et al., 1993). If background concentrations of $Cl^-$ are high, $Na^+$ alone has been used to trace septic system influence (Robertson, et al., 1991). Sulfate ($SO_4^{2-}$) may be applied

with ammonium ($NH_4^+$), calcium ($Ca^{2+}$) or magnesium ($Mg^{2+}$) to crops in the fertilization process. Redox conditions and biogeochemical reactions, such as nitrate reduction (e.g. denitrification or DNRA) coupled to free sulfide or metal-bound sulfide oxidation may increase $SO_4^{2-}$ concentrations in groundwater. If conditions are highly reducing, an increase in $Fe^{2+}$ will also be apparent if $SO_4^{2-}$ is produced specifically from the oxidation of pyrite ($FeS_2$). Sites where $K^+$, $SO_4^{2-}$, and phosphate ($PO_4^{3-}$) are elevated may indicate preferential flow paths within coarse-grained deposits lacking oxides, clays and other charged surfaces and exchange sites. Although high concentrations of $PO_4^{3-}$ can also signal a septic system source, Robertson et al (1991) have reported that concentrations of 5 mg-P/L within the septic plume can dissipate to 1 and 0.1 mg-P/L over very short distances, likely due to adsorption. The presence of $Ca^{2+}$ and $Mg^{2+}$ may become artificially elevated with the application of lime or dolomite to fields (Denver, 1989; Bohlke, 2002), or could occur naturally with dissolution of the minerals in the aquifer matrix. Essentially, the geochemical signature of groundwater influence by agricultural activities or from septic system sites will depend on inputs, processes, and aquifer characteristics.

*2.2.2.2 Nitrogen species: nitrous oxide, ammonium, and nitrite*

Several inorganic forms of N, in addition to nitrate, are fundamental in identifying sources and cycling processes that control the presence of inorganic N. The complexity of these controlling factors can be great, with multiple contributing N sources or biogeochemical mechanisms involving the production and consumption of inorganic N sometimes occurring simultaneously. This compels a need for the measurement of other common inorganic N species; namely $N_2O$, $NO_2^-$, and $NH_4^+$, for identifying the sources and fate of nitrate.

$N_2O$ is largely produced during nitrification and denitrification but is also produced in smaller quantities by some assimilatory bacteria and fungi (Bleakley & Tiedje, 1982) and by some non-denitrifying soil nitrate reducers (Smith & Zimmerman, 1981). $N_2O$ exits as a residual of the incomplete execution of such mechanisms, commonly driven by the result of insufficiently supplied bacteria or electron donors/acceptors. While nitrification and denitrification are primarily restricted to the aerobic and anaerobic parts of the saturated zone, respectively, there has been documentation of denitrification occurring under slightly aerobic conditions (Stein & Yung, 2003), a function which may complicate the use of $N_2O$ for nitrate source and process analysis. Furthermore, when either reaction is efficient at reaching completion, $N_2O$ will be transformed to $N_2$ and will not be detected in the environment. In nitrification, the production of $N_2O$ will normally correlate to a decrease in the concentration of $NH_4^+$, whereas in denitrification, increased $N_2O$ relates to the consumption and therefore decrease in nitrate pool. If either the nitrate or $NH_4^+$ pool is large, the amount of $N_2O$

production can be substantial, without resulting in a large decrease in the concentrations of the substrate N pool. It is, however, perceived that large concentrations of $N_2O$ will be detected if the nitrate pool is large and denitrification is occurring, as high concentrations of nitrate tend to inhibit the reduction of $N_2O$ to $N_2$ (Stein & Yung, 2003). Lastly, a complete lack of $N_2O$ concomitant to a decrease in concentration of both nitrate and $NH_4^+$ may indicate that the anammox process is important (Burlow, et al., 2010).

The measure of $NH_4^+$ is an integral component for understanding N cycling and source inputs. $NH_4^+$ is naturally formed in the soil as an end product of the biotic mechanism ammonification, the transformation of organic N to inorganic N to a form that is readily available as a nutrient for plants and microorganisms. $NH_4^+$ is also present in human and animal waste, as a byproduct of similar transformations occurring within the digestive tract; and is therefore a significant constituent of septic systems, wastewater treatment plants, pastures, and animal feedlots. In anaerobic environments, nitrate can be reduced to $NH_4^+$ via DNRA. As previously mentioned, $NH_4^+$ is stable under reduced conditions, and readily becomes oxidized to nitrate (nitrification) under aerobic conditions.

$NO_2^-$, another important inorganic form of N, shares some common behaviors to those of $N_2O$ in that it is produced as an intermediate in both nitrification and nitrate reductive mechanisms. It is, however, involved in DNRA and anammox reactions that do not involve the production of consumption of $N_2O$.

The concentration of $NO_2^-$ in soil and aquatic environments is commonly low, but elevated $NO_2^-$ can indicate interrupted N processing. In nitrification, the oxidation of $NH_4^+$ must exceed that of $NO_2^-$, and in nitrate reduction (DNRA, denitrification, or Anammox), the reduction of nitrate must exceed that of $NO_2^-$. It is easy to see that elevated $NO_2^-$ concentrations alone give little information as to which N cycling mechanisms are present or out of balance, and the measurement of other inorganic N forms are essential for deriving which N processes are active. High concentrations of $NO_2^-$ in major rivers in Northern Ireland were found to be a result of free ammonia ($NH_3$), which acted to inhibit $NO_2^-$ oxidation by *Nitrobacter* spp. The free $NH_3$ had no effect on the oxidation of $NH_4^+$ to $NO_2^-$, and so the accumulation of $NO_2^-$ persisted at toxic levels in the environment (Smith, et al., 1997). Alternatively, the accumulation of $NO_2^-$ is common within the DNRA mechanism, resulting from an inhibitory effect of nitrate on the $NO_2^-$ reductase or enzyme repression (Smith, 1982; Kaspar & Tiedje, 1981). In the process of denitrification, $NO_2^-$ accumulation is uncommon (Robertson & Kuenen, 1992).

#### *2.2.2.3 Dissolved organic carbon (DOC)*

DOC is derived from the degradation of organic matter into soluble forms, which may then exist in surface water and groundwater. DOC is an electron donor for nitrate removal through both respiratory

denitrification and fermentative DNRA. For such reactions, however, DOC has to be in a structurally appropriate form that can be used by the microbial community, known as labile DOC. The lack of labile DOC has been attributed to the high nitrate concentrations in multi-level piezometers in the LASA, in the early 1980s (Starr, et al., 1987; Starr & Gillham, 1993). If DOC is present in very low concentrations and nitrate removal is evident, then other nitrate removal processes may be occurring. In contrast, DOC in septic effluent plumes has been somewhat elevated and positively correlated to $NH_4^+$ (Robertson, et al., 1991; Verstraeten, et al., 2005). Therefore DOC is necessary for understanding the cycling of inorganic N and for identifying possible sources of N contamination.

*2.2.2.4 Soluble reactive phosphorus*

Phosphorus (P) is naturally occurring in soil, rocks and organic material. Soluble reactive phosphorus (SRP) is a measure of orthophosphate, the soluble and inorganic fraction of P, the form directly taken up by plant cells. SRP occurs naturally in sediment deposits and can be mobilized into surface water and groundwater, triggering eutrophication in lakes and rivers (Schindler, et al., 2006). The presence of SRP at high levels (>50 μg/L) has been found to correlate with redox conditions that favor microbial reduction of $Fe^{3+}$ to $Fe^{2+}$ (Carlyle & Hill, 2001) and has been negatively correlated to DO (Lapoint & Clark, 1992). Since the reduction of $Fe^{3+}$ to $Fe^{2+}$ requires a more chemically reducing environment than needed for the reduction of nitrate to $N_2$, it may be possible to use SRP to distinguish between waters having low nitrate concentrations due to microbial reduction and waters that have low nitrate resulting from low N loading. Where nitrate exists in concentrations too low to measure the isotopes of nitrate, SRP may aid in highlighting reducing waters, especially where DO is not measured. Although it is possible to have reducing waters where nitrate is naturally low. SRP will therefore be used with other parameters to determine nitrate sources and redox states in the LASA.

*2.2.2.5 Artificial sweeteners*

Artificial sweeteners are commonly used in processed food and beverage products and easily bypass absorption in the digestive system. They are subsequently found where human waste resides, such as septic systems or wastewater treatment plants. Four types of sweeteners, acesulfame, saccharin, cyclamate and sucralose, are often used in conjunction with one another to detect wastewater. These sweeteners have been found in urban areas as a marker of wastewater treatment plant effluent (Scheurer, et al., 2009; Van Stempvoort, et al., 2011a; Buerge, et al., 2009), in rural septic systems (Van Stempvoort, et al., 2011b), and as a tracer of pig manure application in farmland (Buerge, et al., 2011). Acesulfame is the most consistently detected sweetener in urban wastewater (Buerge, et al., 2009). It has also been strongly correlated with $Cl^-$ in a campground septic plume (Van Stempvoort,

et al., 2011b). In Canada, both acesulfame and sucralose have been introduced fairly recently into food and beverages; in 1988 and 1992, respectively, and therefore can be good indicators of relative water ages (Gougeon, et al., 2004; Van Stempvoort, et al., 2011a). On the other hand, cyclamate and saccharin have shown susceptibility to biodegradation in soil, water, and in activated sludge from a wastewater treatment plant (Buerge, et al., 2009; Van Stempvoort, et al., 2011b), as has sucralose to a lesser extent (Soh, et al., 2011). Saccharin has been also detected in piglet feed and has been traced in source waters having pig manure applied to the overlying farmland (Buerge, et al., 2011). Half-life values measured in aerobic soil incubation experiments for acesulfame, saccharin, cyclamate and sucralose were 3-49 days, 3-12 days, 0.4-6 days, and 8-124 days, respectively. No correlation between half-life values and organic content or texture of the soils (Buerge, et al., 2011). The presence and rate of degradation of each sweetener may provide evidence for separating nitrate contaminant sources in rural areas (Robertson, et al., 2016), and for determining whether such sources have undergone either biodegradation or dilution.

## 2.3 Methods

### 2.3.1 Site selection

#### *2.3.1.1 Multi-level wells*

Some of the multi-level sampling wells (MLWs) installed on the MC and W properties as part of the Starr et al (1987) study were found and repaired in the summer of 2010. Landowners of the two sites were contacted and permission was obtained to locate, repair, and sample the existing wells on each of the properties. Vegetation overgrowth and agricultural activity on nearby land had concealed or damaged some of the wells, leaving 6 of the 13 wells in acceptable condition for sampling; 4 of them on the MC site (MC2, MC4, MC6, and MC7) and 2 on the W site (W4 and W5). The general location of the MLWs is shown in Figure 2.5 and 2.6.

#### *2.3.1.2 Groundwater seeps*

The locations of samples taken by Hill (1982) were used as a template to locate groundwater seeps discharging along the banks of the Nottawasaga River, in the summer of 2010. The historical sample sites could not be exactly replicated due to uncertainty in the initial sample location and the high frequency of seeps occurring along several stretches of the river. Groundwater discharged into the Nottawasaga River as diffuse seeps, pipes, tile drainage, and small streams; the latter of which were assumed to be largely or wholly groundwater fed during base flow conditions (Hill, 1982). Four river water samples were also collected in May 2011. Sample locations are presented on Figure 2.6.

#### *2.3.1.3 Domestic wells*

The domestic well survey map illustrated by Hill (1982) was digitally overlaid onto a Google map of the area and sample sites were traced into a dataset. The Nottawasaga Valley Conservation Authority (NVCA) provided mailing addresses for the dataset and letters were sent to request participation for private water well sampling. Upon receiving only a few letters back, more letters were hand delivered to the mailboxes of most of the residents within the study area. Finally, homeowners were contacted directly by going door to door. Therefore, sample locations were, for the most part, confined to areas dependent on the residents' willingness to participate, or the chance of the resident being home at the time of inquiry. A good amount of agreement between historical and current sample locations was obtained in the study area. The approximate area sampled is outlined in Figure 2.6; exact locations of the domestic well sites cannot be shown to maintain anonymity of the participants.

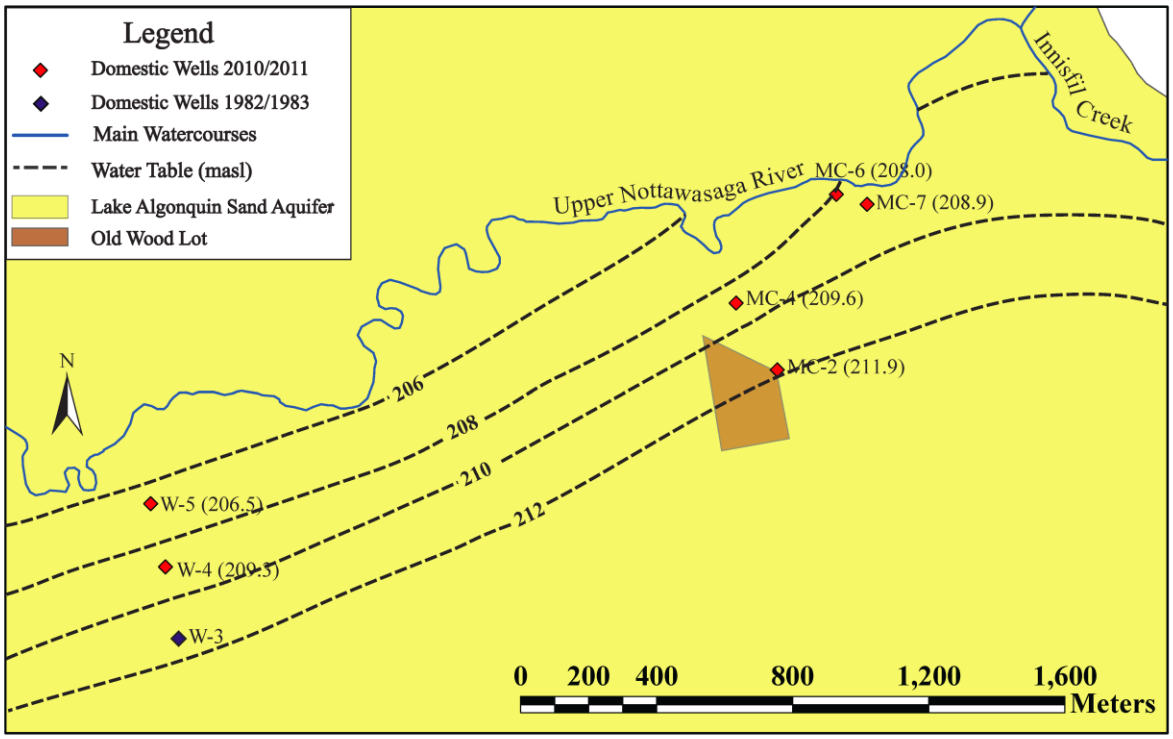



**Figure 2.5** Locations of MC and W multi-level piezometers that were located during reconnaissance, (includes the location of W3, which was not located, but is used to illustrate the cross sectional geology of the LASA, in Figure 1.2). The water table in the area of the multi-level wells is provided from measurement at each well in the summer of 2010, noted in brackets beside each site.

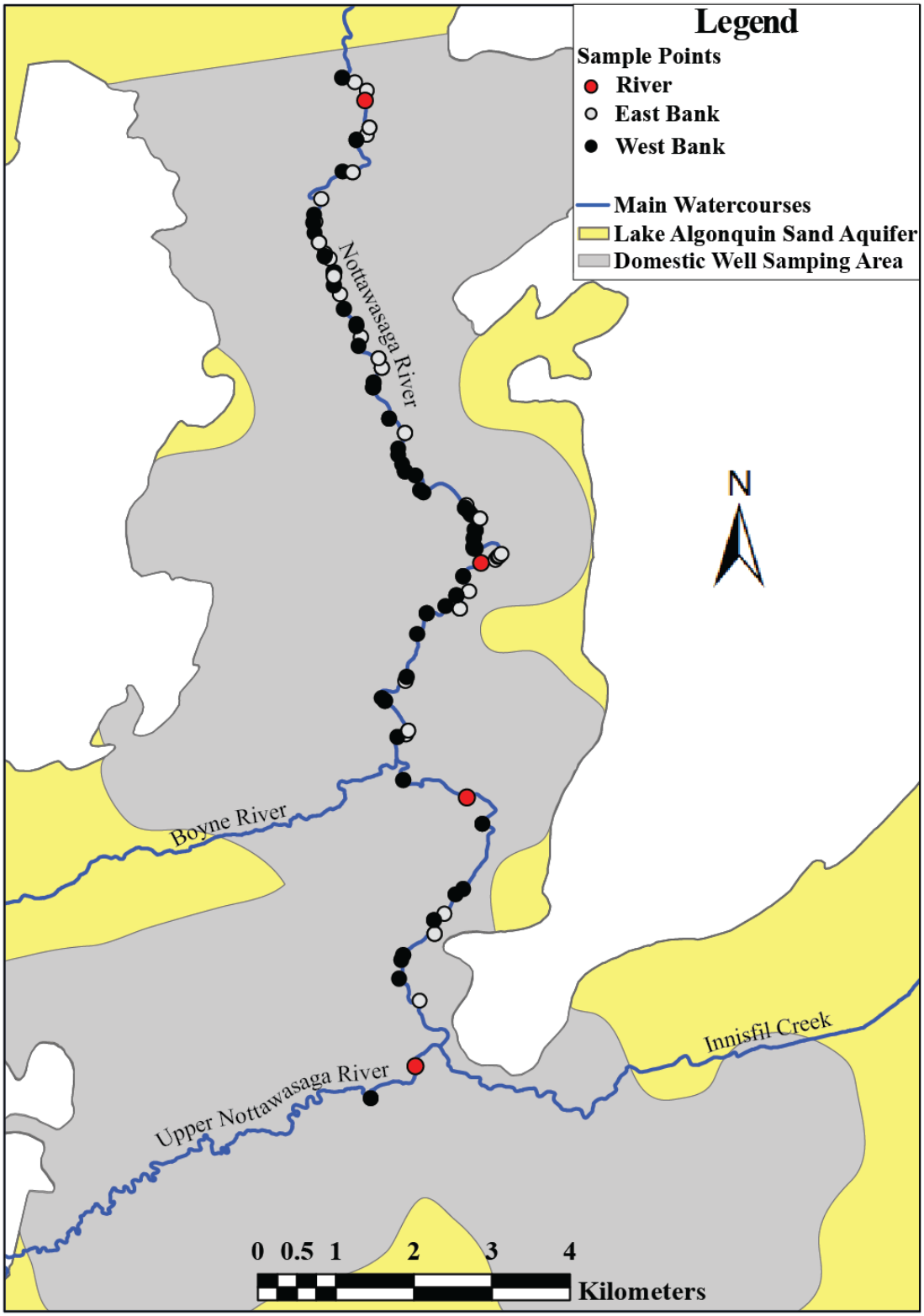



**Figure 2.6** Seep sample locations collected in July and September 2010, river samples collected in May 2010, and properties having multi-level wells.

### 2.3.2 Field procedures

#### *2.3.2.1 General sample collection*

Samples for several parameters were collected in assorted bottle types with preservation techniques depending on the intended analysis, listed accordingly in Table 2.1. Samples collected at the multi-level piezometer and domestic well sites were analyzed with a YSI 600QS Sonde for field parameters (pH, temperature, conductivity, dissolved oxygen (DO)) prior to collection of water for chemical analyses. Sample water was pumped through the flow-through cell containing the YSI Sonde for several minutes until all parameters appeared stable, which indicated that stagnant water residing in the piezometers had been removed and new formation water was being sampled. Samples for the stable isotopes of $N_2O$ (both collected for multi-level piezometer and domestic wells) were collected by pumping directly into the sample bottles, leaving no headspace, and sealing with a Vaccutainer serum stopper which was ventilated with a hypodermic needle, allowing any possible trapped air and overflow to escape. Caps were then sealed with PVC tape. Following this step one of two procedures followed: 1) samples were collected in 2-250 mL Starplex bottles to be stored in a cooler and filtered, partitioned, and preserved accordingly in the laboratory, or 2) samples for SRP, $H_2O$ isotopes, and nitrate isotopes were collected, and then the remaining samples were collected following filtration to 0.45μm either using an in-line filter, syringe tip, or a vacuum filter apparatus.

#### *2.3.2.2 Multi-level wells*

Each multi-level piezometer bundle consists of between 6 and 12, 0.96 cm I.D. polyethylene tubes that are fastened to a central 1.27 cm I.D schedule 80 PVC pipe, which acts as the bottom most sampling point (Starr, et al., 1987). For each sampling depth, a length of sample tubing was inserted into the piezometer and pushed to the bottom, or at least below the depth of the water table, when the bottom could not be reached due to friction between the piezometer and the sampling tube. The bottom end of each piece of tubing was cut at a high angle to prevent the tube from being suctioned to the screen during pumping. Each piezometer depth was measured and labeled accordingly. For the initial cleaning and repair of each sample tube, a peristaltic pump was used to purge the standing water and at least three well volumes of water in each piezometer tube. For all sampling dates, pumping prolonged until YSI Sonde parameters reached stabilization and were recorded, with sample collection following immediately. Samples were collected during the summer of 2010, December 2010, and April 2011. Samples were not collected at all piezometer bundles and sample points at each sampling date due to insufficient recharge volumes as a result of a low water table. Hydraulic head was measured for each piezometer in the summer of 2010 for MC and W site piezometer bundles. Measurements at MC site

bundles included each sample tube, whereas head measurements at the W site were restricted to the first few sample tubes in the groundwater profile.

*2.3.2.3 Groundwater seeps*

Samples of groundwater seeps were taken approximately every few hundred meters along the banks of the Nottawasaga River with greater frequency along the west side bank, as to be similar to the historical sampling methods of Hill (1982). Samples were taken over shorter intervals where riparian zone conditions changed drastically and were taken over larger intervals where flow from the aquifer discharged below the river's surface. A total of 85 samples were collected in the summer of 2010, 12 of which were taken by walking through a short section of the riparian zone, and 73 at a later date by canoeing the length of the river over a two-day period. Of the 85 samples, 26 were chosen for resampling during the spring of 2011, with the objective of examining seasonal difference in groundwater chemistry in the LASA. River water samples from 4 locations along the Nottawasaga River were also collected in May 2011. The YSI Sonde was not used in this procedure because seeps often presented as a seepage face that was not conductive to use. Water samples were collected in 2-250 mL Starplex bottles that were stored in a cooler and filtered, partitioned, and preserved accordingly in the laboratory. Each container was sufficiently rinsed with sample water, and filled, leaving room for expansion during freezing for the nitrate isotope sample. All sample were stored in a cooler, on ice, for the duration of the sampling period, until they reached the laboratory within 12 hours of collection.

*2.3.2.4 Domestic wells*

Each participating household agreeing to the domestic well sampling was asked to complete a questionnaire pertaining to general information about their well. Few of these questionnaires were returned to the researcher after the well sample had been obtained. In one case the well sample was collected and later omitted from the database, as the questionnaire indicated that the well depth was located in the aquifer below the LASA, which is not of interest in the current study. Samples were collected from an outlet that had not been subjected to any water treatment, such as an outside tap or kitchen sink faucet. The YSI Sonde was used to measure temperature, conductivity, pH, DO, and establish that these parameters had stabilized, indicating that the system had been sufficient purged prior to sampling. Samples were collected and stored according to Table 2.1. One sample was not measured with the YSI Sonde because it was collected as a bulk sample by the homeowner, as a matter of convenience. Samples for each parameter were then subsampled from the bulk sample. Filtering for necessary parameters was always completed immediately in the field, using a vacuum filter apparatus, which was rinsed well with DI between collecting each of the samples.

**Table 2.1** List of bottle types and preservation techniques for samples.

| Parameter | Bottle Type | Preservation Method |
|---|---|---|
| [1]Anions | 40 mL Starplex or glass vial | Refrigerated at 4°C |
| [1]Cations | 20 mL Starplex or glass vial | $HNO_3$ added to pH of 2, refrigerated at 4°C |
| [1]$NH_4^+$ | 20 mL Starplex or glass vial | HCl added to pH of 5, frozen |
| [1]DOC | 40 mL Starplex or glass vial | Refrigerated at 4°C |
| $N_2O$ | 60 mL Wheaton serum bottle | 1 mL of saturated $HgCl_2$ solution, refrigerated at 4°C |
| SRP | 50 mL centrifuge | Refrigerated at 4°C |
| [1]Sweeteners | 40 mL Starplex | Frozen |
| [1]Anionic Herbicides | 40 mL Starplex | Frozen |
| [1]Perchlorate | 40 mL Starplex | Frozen |
| $\delta^{15}N\text{-}NO_3^-$ & $\delta^{18}O\text{-}NO_3^-$ | 125 or 250 mL Starplex | Frozen |
| $\delta^{15}N\text{-}N_2O$ & $\delta^{18}O\text{-}N_2O$ | 120 mL Wheaton serum bottle | 2 mL of saturate $HgCl_2$ solution, refrigerated at 4°C |
| $\delta^{18}O\text{-}H_2O$ and $\delta^2H\text{-}H_2O$ | 20 mL Starplex | Stored at room temperature |

[1]Required filtration to 0.45μm

2.3.3 Laboratory analysis

Geochemical and isotopic parameters were measured at the University of Waterloo Environmental Isotope Lab (UW-EIL), and Environmental Geochemistry Lab (UW-EGL), Waterloo, Ontario, the Canadian Center for Inland Waters (CCIW), Burlington, Ontario, and the Stable Isotope Facility (SIF) of UC Davis, Davis, California. Known values of the precision, method detection limit (MDL) and practical quantification limit (PQL) are provided in Table 2.3.

*2.3.3.1 Anions*

Major anions ($F^-$, $Cl^-$, $Br^-$, $NO_2^-$, $NO_3^-$, $SO_4^{2+}$, and $PO_4^{3+}$) were analyzed at CCIW. A Dionex 2500 ion chromatograph was used; sample concentrations were calibrated against multi-ion standards that were analyzed with the samples. Sample concentrations were kept within the working range of the standard by diluting with Milli-Q water when necessary.

*2.3.3.2 Cations*

Major cations ($Al^{3+}$, $Ca^{2+}$, Fe, $K^+$, $Mg^{2+}$, $Na^+$) were analyzed at CCIW, with the use of an inductively coupled plasma-atomic emission spectroscopy using a Horiba Jobin Yvon Ultima 2 ICP (Horiba Jobin Yvon). Sample concentrations were calibrated against multi-ion standards that were included with each run of samples.

#### *2.3.3.3 $NH_4^+$*

$NH_4^+$ concentrations were analyzed at CCIW with a colorimetric method (Spoelstra & Post, 2012) by measuring absorbance at 640nm on a Beckman-Coulter DU720 UV/visible spectrophotometer. Multiple $NH_4^+$ standards were run with the samples for the calibration of the sample values.

#### *2.3.3.4 DOC*

DOC was measured at UW-EGL using a Dorhmann DC-190 High Temperature Total Carbon analyzer equipped with an autosampler system (Rosemount Analytical Inc. Santa Clara, CA). Samples were acidified with 20% phosphoric acid to remove inorganic C and DOC was then measured and corrected to a calibration curve created from a set of three potassium hydrogen phthalate (KPH) standards analyzed during the same run as the samples.

#### *2.3.3.5 Dissolved $N_2O$*

Dissolved $N_2O$ was analyzed at UW-EGL using a headspace equilibrium technique and a gas chromatograph (Thuss, 2008). 12mL Exetainers® were capped and weighed, then inverted and flushed with helium, and re-capped. 6mL of solution was removed from the sample bottle and injected into the exetainer, to create a positive pressure. Exetainers® were re-weighed to determine the total mass of liquid. Samples were then gently shaken for 90 minutes to allow $N_2O$ to reach equilibrium with the headspace. Concentrations of $N_2O$ in the headspace were determined with an Electron Capture Detector (ECD) on a Varian CP 3800 greenhouse gas analyzer (Varian Canada, Inc.). $N_2O$ concentrations were calibrated against gas standards that were included in each sample run. Dissolved $N_2O$ concentrations were then calculated using Henry's Law using the *in situ* temperature and pressure that was recorded during sampling.

#### *2.3.3.6 SRP*

Soluble reactive phosphorus (SRP) was analyzed at CCIW using a modified version of the orthophosphate colorimetric method, measuring absorbance at 885nm on a Beckman-Coulter DU720 UV/visible spectrophotometer. For this procedure, a mixed reagent containing ammonium molybdenate and antimony potassium tartrate was used (Van Stempvoort, et al., 2011a). The method detection limit is noted in Table 2.2.

#### *2.3.3.7 Artificial sweeteners*

All samples were measured for four artificial sweeteners, acesulfame, sucralose, cyclamate and saccharin, at CCIW. Analysis was performed using a Dionex (Sunnyvale, CA, USA) 2500 ion chromatography system coupled to a QTRAP 5500 (AB Sciex, Concord, ON, CAN) triple quadrupole

tandem mass-spectrometer, which was set to negative electrospray ionization (ESI) mode (Van Stempvoort, et al., 2011a). Samples that were detected below the MDL were reported as 'not detected' (N.D.) and are then given a value of 0 ng/L, for data evaluation and graphing purposes.

#### *2.3.3.8 $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$*

Samples for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were determined using two different methods, each performed at SIF-UC Davis or UW-EIL, respectively. The first method used the bacterial denitrification method (Singman, et al., 2001; Casciotti, et al., 2002), using a ThermoFinnigan GasBench + Precon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany), calibrated to the nitrate standards USGS 32, USGS 34 and USGS 35. This method has an analytical precision of ± 0.4 ‰ and ± 0.8 ‰ for $\delta^{15}N$ and $\delta^{18}O$, respectively.

The second method used at UW-EGL (Spoelstra, et al., 2014) followed a modified version of the chemical denitrification method (McIlvin & Altabet, 2005). In brief, samples containing 2µg N were frozen in glass vials, freeze dried, and then reconstituted with 2 mL of 0.75M NaCl. Samples were shaken for 30 minutes on an orbital shaker at 290 RPM. 1 mL of Imidazole solution was then added to each sample, followed by 0.1 mL of cadmium (Cd) powder. Samples were shaken again, on the orbital shaker, for 24 hours to ensure complete reduction of nitrate to nitrite, and then filtered (to remove Cd) and injected into the helium (He)-filled headspace vials. Addition of $NaN_3$ and glacial acetic acid buffer induced the conversion to $N_2O$. Finally, 1 mL of 6M NaOH was added to quench the reaction. Samples were over-pressurized with He and shaken for 1 hour. Sample values were determined in the UW-EILAB, using a GV Trace Gas pre-concentrator system (GV instruments, Thermo Electron Corp., Manchester, UK), attached to a GV Isoprime mass spectrometer. Internal and internationally recognized reference standards (USGS-34, USGS-35, EGC-1, EGC-17) were used in the calibration of the final results and $\delta^{15}N$ and $\delta^{18}O$ were reported relative to atmospheric $N_2$ and Vienna Standard Mean Ocean Water (VSMOW), respectively. The analytical precision for $\delta^{15}N$ and $\delta^{18}O$ is approximately ± 0.3‰ and ± 1‰, respectively. Samples tested at the two different laboratories are listed in Table 2.3.

#### *2.3.3.9 $\delta^{15}N\text{-}N_2O$ and $\delta^{18}O\text{-}N_2O$*

Samples for stable isotope analysis of dissolved $N_2O$ were prepared by removing 60 mL of the sample from the 120mL serum bottle while replacing and over pressurizing with 120 mL of helium. Samples were then gently shaken on an orbital shaker at approximately 90 RPM for one hour to equilibrate the headspace gas with the sample. Sample isotopic values were determined at UW-EILAB, using a GV Trace Gas pre-concentrator system (GV instruments, Thermo Electron Corp., Manchester, UK), attached to a GV Isoprime mass spectrometer. Corrections and calibration were determined with the

use of an internal $N_2O$ isotope standard (EGL-5) (Thuss, 2008). $\delta^{15}N$ and $\delta^{18}O$ have been reported relative to atmospheric $N_2$ and VSMOW, and have an analytical precision of ± 0.1‰ and ± 0.2‰, respectively.

**Table 2.2** Detection limit and precision values for various geochemical parameters.

| Parameter | MDL | PQL | Precision |
|---|---|---|---|
| Anions | 0.003-0.02 mg/L | - | |
| Cations | 0.005-0.01 mg/L | - | |
| $NH_4^+$ | 0.016 mg/L | - | |
| DOC | 0.5 mg/L | - | 0.2 mg/L |
| $N_2O$ | 0.2 μg/L | - | 5% |
| SRP | 2 μg/L | - | |
| Acesulfame | 8 ng/L | 20 ng/L | |
| Saccharin | 21 ng/L | 60 ng/L | |
| Cyclamate | 3 ng/L | 10 ng/L | |
| Sucralose | 5000 ng/L | 15000 ng/L | |

**Table 2.3** Method of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ analysis for groundwater seep, domestic well, and multi-level well samples.

| SIF-UC Davis | UW-EIL |
|---|---|
| Groundwater seeps: September 2010 – all | Groundwater seeps: September 2010 – select data |
| Multi-level wells: Spring samples | Multi-level wells: Summer and fall samples |
| Groundwater seeps: May 2011 samples | Domestic wells: Summer 2011 – all |
| Stream samples: May 2011 | |
| Domestic wells: Summer 2011 – select data | |

*2.3.3.10 $\delta^{18}O\text{-}H_2O$ and $\delta^{2}H\text{-}H_2O$*

Samples for $\delta^{18}O\text{-}H_2O$ and $\delta^{2}H\text{-}H_2O$ were determined using two types of analyses, performed at the SIF- UC Davis, and UW-EIL. The first method used a Laser Water Isotope Analyzer V2 (Los Gatos Research, Inc., Mountain View, CA, USA). Sample isotope ratios were standardized using a range of working standards that were calibrated against IAEA standard reference materials (VSMOW, GISP, and SLAP). The precision is typically ≤ 0.3‰ for $\delta^{18}O$ and ≤ 0.8‰ for $\delta^{2}H$.

The second method used an on-line chromium reduction technique for the analysis of $\delta^2$H-$H_2O$ (Morrison, et al., 2001) and $CO_2$ equilibrium technique for $\delta^{18}$O-$H_2O$ (Epstein & Mayeda, 1953). Both isotopes detected on an IsoPrime/Eurovector-EA-isotope ratio mass spectrometer (EA-IRMS) with a general precision of ± <1‰ for $\delta^2$H and ± 0.1‰ for $\delta^{18}$O. Both analyses report $\delta^{18}$O-$H_2O$ and $\delta^2$H-$H_2O$ relative to VSMOW. Samples run at the two different laboratories are listed in Table 2.4.

**Table 2.4** Method of $\delta^{18}$O-$H_2O$ and $\delta^2$H-$H_2O$ analysis for groundwater seep and multi-level well samples.

| SIF, UC Davis | UW-EIL |
|---|---|
| Groundwater seeps: September 2010 – select data[1] | Groundwater seeps: September 2010 – select data[1] |
| Multi-level wells: Spring samples | Multi-level wells: Summer and fall samples |
| Groundwater seeps: May 2011 samples | |

[1]A portion of the data was run at each laboratory, but no data were run at both. The results and interpretation of these data will include values from both methods.

*2.3.3.11 $\delta^{15}$N-$NH_4^+$*

Stable isotope analysis of $\delta^{15}$N-$NH_4^+$ was performed at UW-EGL using the method of Spoelstra et al., (2011), a modified version of the acidified disk diffusion method (Brooks, et al., 1989) (Sorensen & Jensen, 1991). In brief, dissolved $NH_4^+$ was converted to $NH_3$ gas and trapped on an acidified quartz disk, enclosed in a gas permeable, polytetrafluorethylene (PTFE) membrane. The required sample volume was pipetted into each diffusion jar, and deionized water (DI) added to bring the total volume to 20mL. KCl was added to obtain a concentration of approximately 4M. A phenolphthalein pH indicator solution was added and NaOH solution was added to obtain a pH between 8 and 9, which is indicated by a change in color, to pink. 2mL of tetraborate pH buffer solution was added to stabilize the pH at approximately 9.5. Diffusion jars were shaken on an orbital shaker for 10 days to allow complete diffusion of the $NH_3$ onto the acidified disks. When shaking was complete, the PTFE traps were removed and the quartz disks were transferred into clean vials, then frozen and freeze dried. $\delta^{15}$N-$NH_4^+$ was determined using a Carlo Erba 1108 elemental Analyzer interfaced with a Thermo Instruments Deltaplus isotope ratio mass spectrometer (EA-IRMS). The precision is generally better than ±0.2‰.

# 3. THE CHANGE IN NITRATE CONCENTRATIONS IN THE LAKE ALGONQUIN SAND AQUIFER OVER A 30 YEAR PERIOD

## 3.1 Introduction

Nitrate contamination of shallow aquifers is commonly associated with agricultural lands that overlie highly permeable soils (Bottcher, et al., 1990; Postma, et al., 1991; Bohlke, 2002; Wassenaar, 1995). Ingestion of nitrate contaminated waters, those concentrated above 10 mgN/L, may induce Blue Baby Syndrome in infants, and nitrate can initiate adverse health effects and mortality in effected aquatic ecosystems, at much lower levels. The southern Nottawasaga River Watershed is an area that has been under intense agricultural use for at least the past 150 years. The shallow aquifer system, the Lake Algonquin Sand Aquifer (LASA) underlies much of the agricultural land and consists of sands to loamy sands with few gravel lenses. Previous studies conducted in the area have defined agricultural activity as the main contributor to groundwater nitrate concentrations that exceeded the drinking water limit of 10 mgN/L. Research conducted by Hill (1982) consisted of domestic well and groundwater seep sampling to identify the distribution and source of nitrate in the LASA. Distinct areas of high nitrate were found to exist to the south of the Upper Nottawasaga River, between the Upper Nottawasaga and Boyne Rivers, and to the North of the Boyne River, along the west side of the Nottawasaga River. Positive correlations were made between the concentrations of chloride and nitrate, and land use, which attributed high groundwater nitrate with potato farming. Further research conducted by Starr et al. (1987) studied the vertical profile of nitrate under agricultural fields that had potato farming within their crop rotation. The consistent presence of nitrate at high concentrations, far below the water table, was explained by a lack of labile organic carbon that could otherwise support respiratory denitrification within the aquifer. Although low nitrate did exist in all wells, near the base of the aquifer, it was unclear whether a change in source or nitrate cycling was responsible for this.

The current investigation aims to measure changes in groundwater nitrate since the studies of Hill (1982) and Starr et al (1987) by measuring nitrate in multi-level wells, groundwater seeps, and domestic wells in the LASA. Since the early 1980s there have been changes in land use, including an expansion of the Alliston industrial area, and the development of a golf course along the Nottawasaga River. Changes in farming include crop diversification away from potato and towards sod and mixed vegetables, which have different fertilizer application practices. The application of nutrient management practices and increased knowledge among farmers regarding fertilizer optimization and groundwater contamination may have a direct impact on local groundwater nitrate by way of reducing

the amount of fertilizers being leached to groundwater by reducing fertilizer application quantities and timing.

## 3.2 Methods of statistical analysis and group comparisons

### 3.2.1 Domestic wells and groundwater seeps

A series of Mann-Whitney rank-sum tests were employed to assess the tendency for LASA nitrate concentrations between 1979/80 and the current study to differ. This rank-sum test is used to determine whether one group tends to produce larger observations than a second group, and is preferred for independent, unpaired sample groups (Helsel & Hirsch, 2002). It is anticipated that concentrations of groundwater nitrate collected in the current study will be different from those collected in 1979/80. For both the domestic wells and groundwater seeps, samples were divided into groups depending on the recharge area in which they were located (recharge areas are outlined in Figure 2.3). Each dataset was also compared on a total aquifer basis. The test hypotheses for groundwater seep and domestic well datasets are two sided tests and are set up as follows:

$$H_0: \text{1980 nitrate} = \text{2010 nitrate} \qquad \text{(Eq. 3.1)}$$

$$H_1: \text{1980 nitrate} \neq \text{2010 nitrate} \qquad \text{(Eq. 3.2)}$$

For this test it is not necessary to make assumptions about how the data are distributed, as this does not affect the outcome of the test. If both groups of data come from the same population ($H_0$ retained) then it can be said that there is a 50% probability that a sample from one group could be higher than that from the other group. For this study, the alternate hypothesis is accepted when the p-value is less than an alpha ($\alpha$) of 0.05. Both methods for the exact test and the approximation for large sample datasets were used appropriately and are noted accordingly

### 3.2.2 Multi-level wells

A comparison of LASA nitrate concentrations of the 1982/83 and the current study has been illustrated with respect to point depth locations (Figures 3.4 through 3.6) and with the use of box and whisker type plots (Figure 3.7). Nitrate concentrations from 1982/83 were extracted from the figures of Starr et al (1987) using g3data software (http://frantz.fi/software/g3data/php, (Bauer & Raynolds, 2008)). Each well bundle is compared by the sampling season, involving only the data points that were sampled at both dates. The explanation for this being that the range in nitrate concentration varies so drastically (between 0 and 103 mgN/L) within the vertical profile, that one extra point has the ability to drastically

effect the distribution of the data. The lower and upper extent of the boxes outline the $25^{th}$ and $75^{th}$ percentiles, the horizontal line within the box delineates the median, and the lines extending vertically from the box represent the $5^{th}$ and $95^{th}$ percentiles. A statistical test was not performed on these data, as the dependency of the data within each profile varies at each depth. For example, redox state, land use, and nitrate loading are all factors that affect the nitrate concentration and each varies within the vertical profile. Therefore, for this study, concentration profiles and box and whisker plots will suffice to analyze the trends in groundwater nitrate between the historical and current study.

## 3.3 Results

### 3.3.1 Changes in groundwater nitrate

#### *3.3.1.1 Domestic wells and groundwater seeps*

The distribution and magnitude of nitrate in groundwater seep collected in the summer of 2010 have been compared to those samples collected in 1979 (Hill, 1982) (Figure 3.1). In the northwest section, it is visually apparent that nitrate concentrations for samples collected in 2010 are higher than in 1979. The northeast section also appears to have higher 2010 nitrate values than in 1979. For the midwest section it is more difficult to visually compare the differences between the 2010 and 1979 data, and in the south there is only one data point that happens to be slightly higher in 1979.

Results of the Mann-Whitney rank-sum tests indicate that there is a significant difference between the groundwater seep nitrate concentrations in the northwest recharge area, supported with a p-value of 0.037 (Table 3.1). This is also illustrated with the use of box and whisker plots (Figure 3.3), which in 2010, display higher overall percentile points for the northwest recharge area. Again, the visual comparison of nitrate in the northeast looks as though 2010 data could be higher than in 1980, however the statistics presented a p-value of 0.162 and therefore the null hypothesis is retained and sample sets are classified as not being significantly different. The same result is true for the midwest section; the box and whisker plot has higher $5^{th}$, $25^{th}$, $50^{th}$, and $75^{th}$ percentiles, but a lower $95^{th}$ percentile for 2010 data. The rank-sum test also indicates that the midwest seep sample sets are not significantly different. When all sample points for both datasets are considered, the 2010 nitrate concentrations are significantly different, and more specifically, are higher than the 1979 data for groundwater seeps. The reported p-value for this test is 0.002 (Table 3.1).

Domestic well data and groundwater seep data are combined in Figure 3.2 to outline areas in the LASA where nitrate concentrations exceed 10 mgN/L for 1980 and 2011. High nitrate plumes in the northwest portion of the aquifer are somewhat similar in spatial extent. Box and whisker plots for the domestic well data (Figure 3.3) show that 2011 northwest samples are slightly lower in the $75^{th}$ and $25^{th}$ percentiles, with the $50^{th}$ percentile having an even greater decrease in comparison to the 1980 data. The $95^{th}$ and $5^{th}$ percentiles are however mostly the same. Statistically, the two datasets are not significantly different, and report a p-value of 0.16 (Table 3.1). The northeast section of the aquifer appears to have some changes in the location of high nitrate groundwater (Figure 3.2). The box and whisker plots show that nitrate is lower overall in the northeast in 2011 than in 1980 (Figure 3.3). This difference between datasets is however not statistically significant, as measured by the Mann-Whitney

rank-sum test (Table 3.1), has a p-value of 0.06. The midwest area shows a distinct spatial change in high nitrate distribution (Figure 3.2). In 2011, high nitrate existed in the southern portion of the midwest section, bordering the Upper Nottawasaga River. In contrast, the 1980 data shows high nitrate concentrations in the middle and northern areas of the midwest (Figure 3.2). Box and whisker plots show a similar range in nitrate concentrations for both sample dates, but the 25$^{th}$, 50$^{th}$, and 75$^{th}$ percentiles are moderately lower in the 2011 data. Statistics do not support a significant difference in these data (Table 3.1). In the south section, the existence of high nitrate in 2011 is spatially consistent with the historical data (Figure 3.2). Box and whisker plots for domestic well samples (Figure 3.3) show that 2011 data have slightly decreased compared to the 1980 dataset. Again the Mann-Whitney rank-sum test shows a lack of significance (p-value = 0.06) for the measured difference between these data. A statistical analysis of all data points for each dataset shows that the data are, as a whole, significantly different, and have a reported p-value of 0.011 (Table 3.1).

**Table 3.1** Results of the Mann-Whitney rank-sum test for detecting whether changes exist in nitrate concentrations between 1982/83 and 2010/11, for groundwater seeps and domestic wells.

| Comparison | p-value | $Z_{rs}$ | Hypothesis accepted : Result |
|---|---|---|---|
| **Seeps: Northwest[2]** | **0.037** | **$Z_{rs}$ = -2.08** | **$H_1$ : 1979 ≠ 2010** |
| Seeps: Northeast[2] | 0.162 | $Z_{rs}$ = -1.39 | $H_0$ : 1979 = 2010 |
| Seeps: Midwest[1] | 0.152 | $Z_{rs}$ = -1.52 | $H_0$ : 1979 = 2010 |
| **Seeps: Total aquifer[2]** | **0.002** | **$Z_{rs}$ = -3.07** | **$H_1$ : 1979 ≠ 2010** |
| Domestic Wells: Northwest[2] | 0.16 | $Z_{rs}$ = -1.39 | $H_0$ : 1980 = 2011 |
| Domestic Wells: Northeast[1] | 0.06 | $Z_{rs}$ = -1.88 | $H_0$ : 1980 = 2011 |
| Domestic wells: Midwest[1] | 0.25 | $Z_{rs}$ = -1.15 | $H_0$ : 1980 = 2011 |
| Domestic Wells: South[2] | 0.24 | $Z_{rs}$ = -1.17 | $H_0$ : 1980 = 2011 |
| **Domestic Wells: Total aquifer[2]** | **0.011** | **$Z_{rs}$ = -2.52** | **$H_1$ : 1980 ≠ 2011** |

[1]: Exact rank-sum test.
[2]: Rank-sum test with large sample approximation.

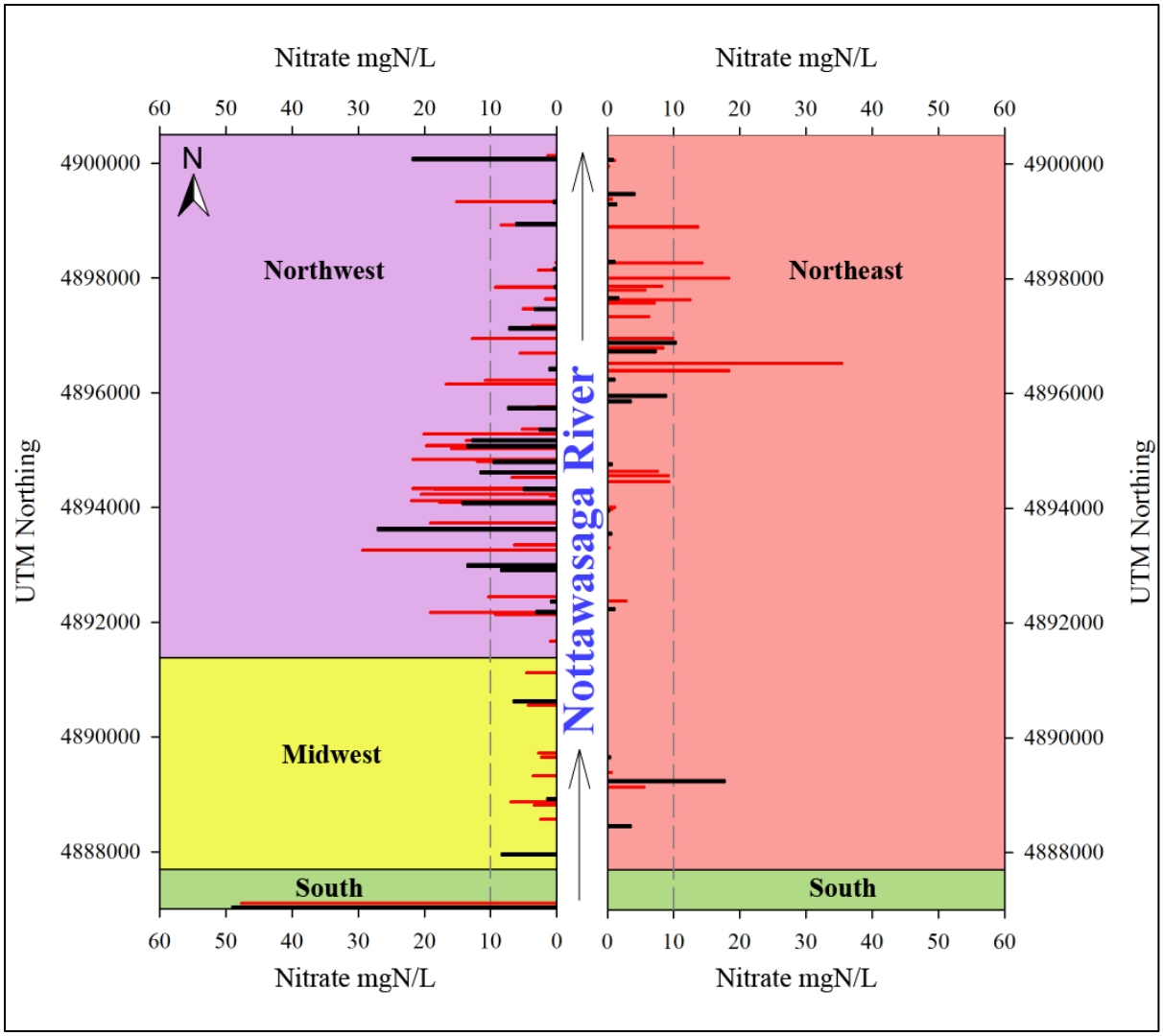



**Figure 3.1** Illustration of the spatial distribution of nitrate concentrations in groundwater seeps in 1979 (black; Hill, 1982) and 2010 (red), with colors indicating recharge areas as outlined in Figure 1.3.

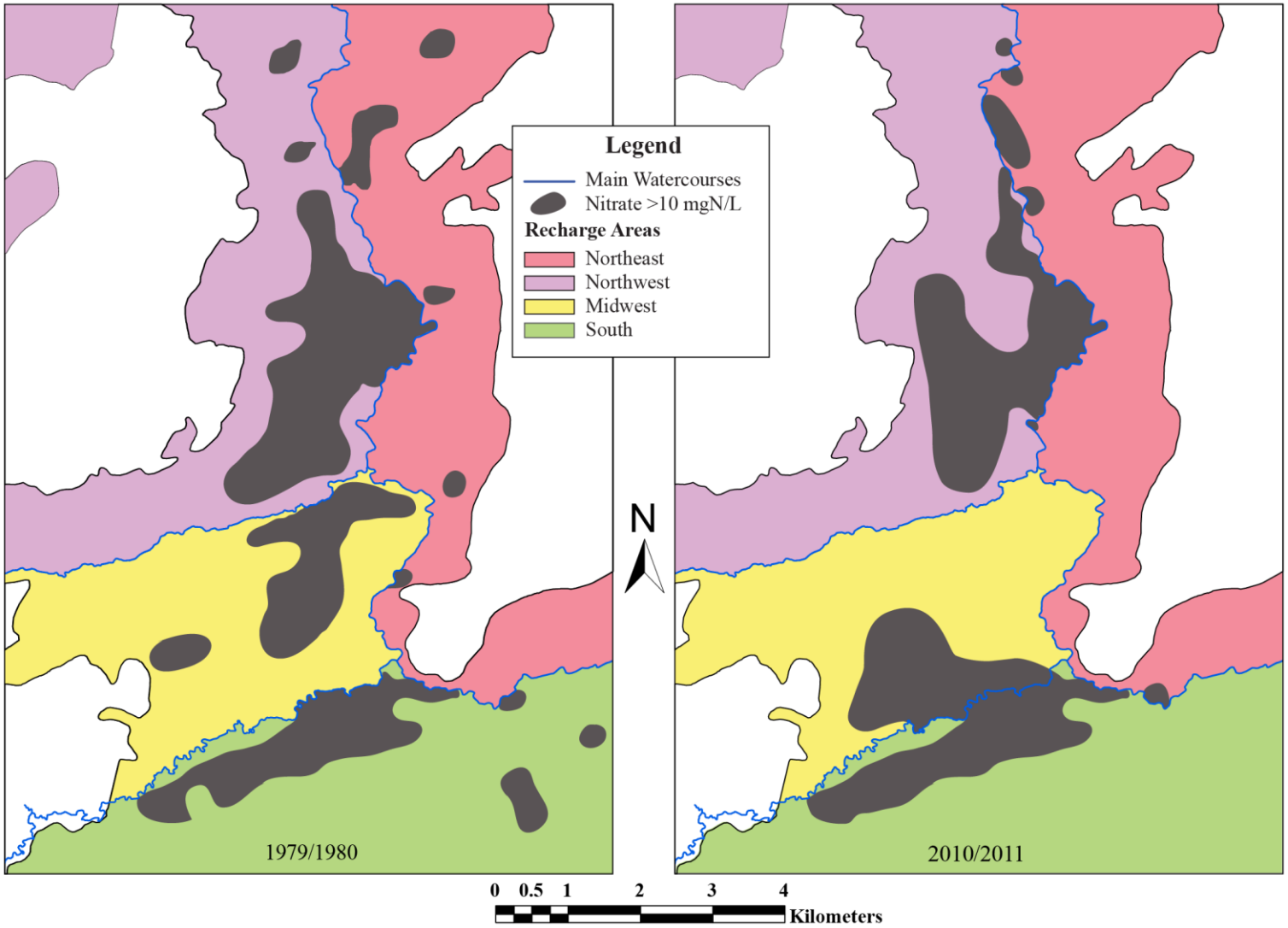
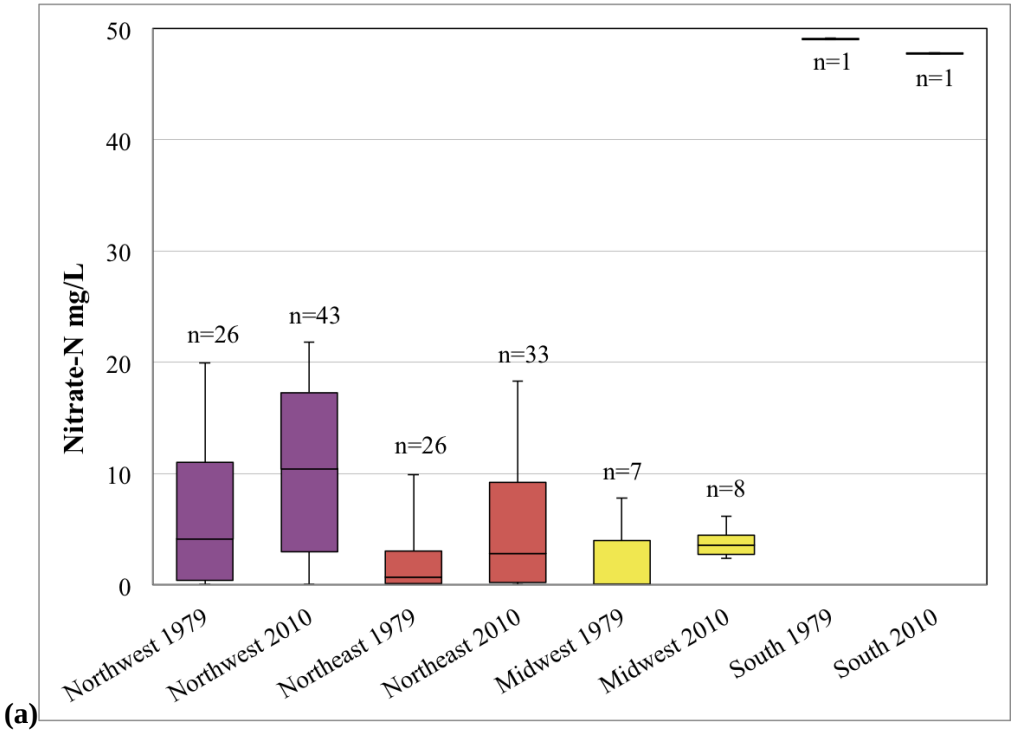
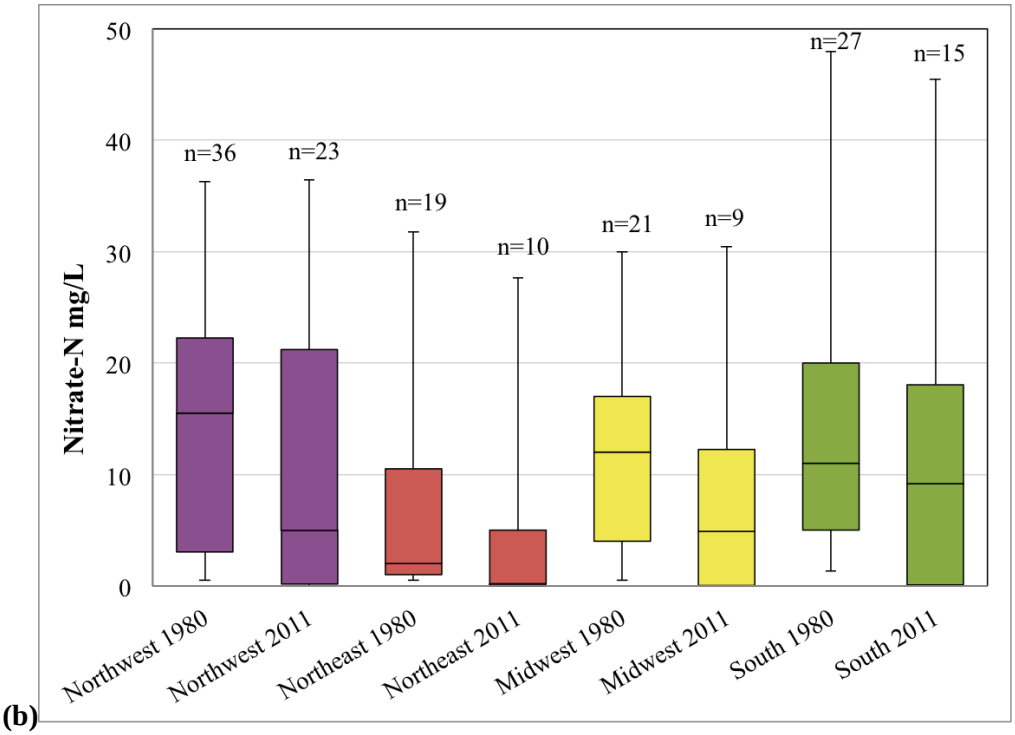



**Figure 3.2** Delineation of areas containing nitrate above the drinking water limit (>10 mgN/L) measured in domestic wells and groundwater seeps in the LASA in 2010/2011 (right) and 1979/1980 (left).




**(a)**




**(b)**

**Figure 3.3** Box and whisker plots for nitrate in (a) groundwater seeps, and (b) domestic wells, partitioned by recharge area; n=sample population for the given category.

*3.3.1.2 Multi-level wells*

MC2

Box and whisker plots (Figure 3.4) compare the nitrate concentration for the historical and current study for the various multi-level wells. Samples collected in the summer show an increase in all percentile points in 2010. The spring data show an increase in the 95$^{th}$, 75$^{th}$ and 50$^{th}$ percentiles of the 2011 nitrate, whereas the 5$^{th}$ and 25$^{th}$ percentiles are slightly less than the 1983 data. The spring 2011 data has a greater range in concentrations than the summer 2010 data, which is due to the measurement of a sample at 3.5 mbgs that could not be measured in the summer due to a lower water table. A substantial increase in nitrate in the current study's data, compared to the 1982/83 data, is noted in the upper portion of the aquifer, distinctly between 4.3 mbgs and 6.7 mbgs (Figure 3.5). At 8.5 mbgs, 2010/11 nitrate is within the range of concentrations measured in 1982/83. Below 8.5 mbgs, 2010/11 nitrate is almost negligible with concentrations measuring barely above zero. These data are lower than 1982/83 nitrate which were measured at ~3 mgN/L.

MC4

Sample points between 6.3 mbgs and 8.3 mbgs have lower nitrate concentrations in 2010/11 than the 1982/83 samples, for all seasons (Figure 3.5). The concentrations for 2010/11 are highest at 9.1 mbgs, with a measurement of 51.0 mgN/L. The seasonal concentration ranges for 2010/11 are consistently less than 1982/83 samples. Furthermore, at 12.2 mbgs, 2010/11 nitrate concentrations are consistently measured below that of the drinking water limit. At 13.7 mbgs, all samples show an agreement of having very low nitrate concentrations, and measured slightly above zero values. Box and whisker plots show a decrease for all percentile points for 2010/11 data for both the summer and spring sample sets in comparison to the 1982/83 data (Figure 3.4).

MC6

Insufficient recharge for sample collection within the MC6 piezometer bundle prohibited sampling of all but one sample, that at a depth of 8.5 mbgs. The concentration of nitrate at this depth measured 94.5 mgN/L, which was much greater than the highest concentration measured in 1982/83 at the same depth, 30.8 mgN/L (Figure 3.6).

MC7

In 2010/11, nitrate concentrations at piezometer MC7 were within the range of those measured in 1982/83 (Figure 3.6). At 6.8 mbgs, samples taken in the summers of 1983 and 2010 both had extremely high values, and measured 103.0 mgN/L. At 8.1 mbgs, 2010/2011 samples had a lower maximum

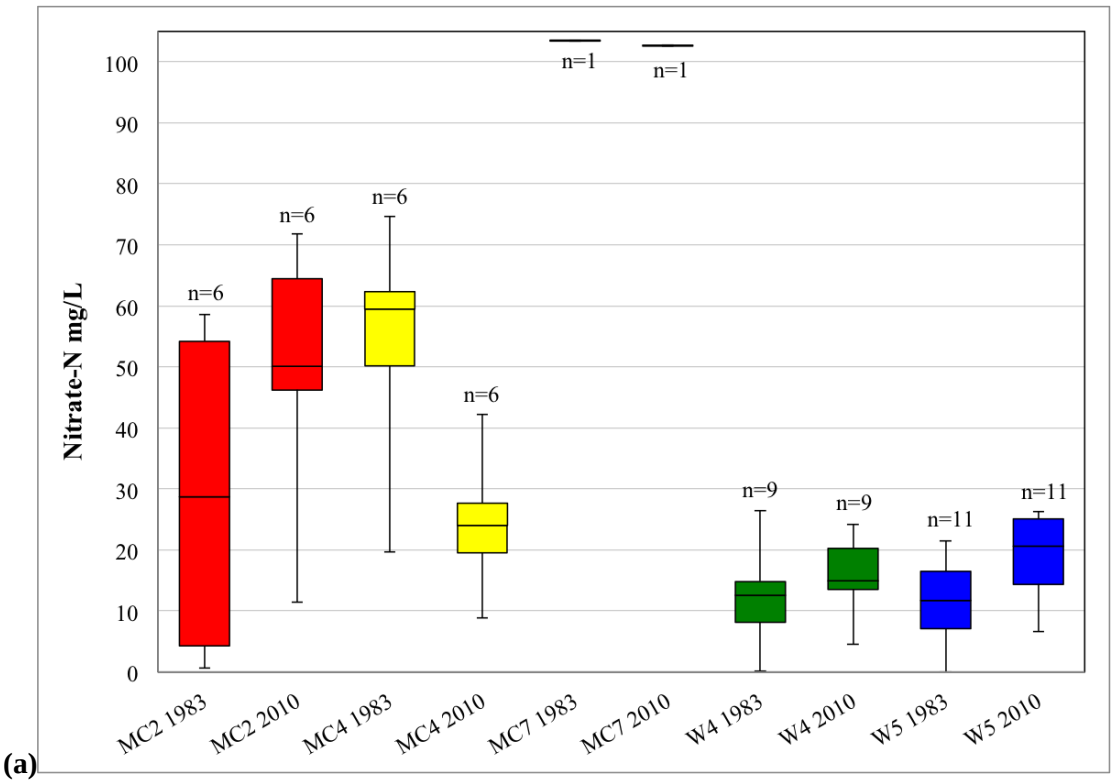
concentration than the 1982/83 samples, but it should be noted that a sample for August 2010 could not be obtained due to insufficient recharge within the piezometer owing to a low water table. The box and whisker plot for summer samples are higher in all percentile points for the 2010, compared to the 1982 study.

W4

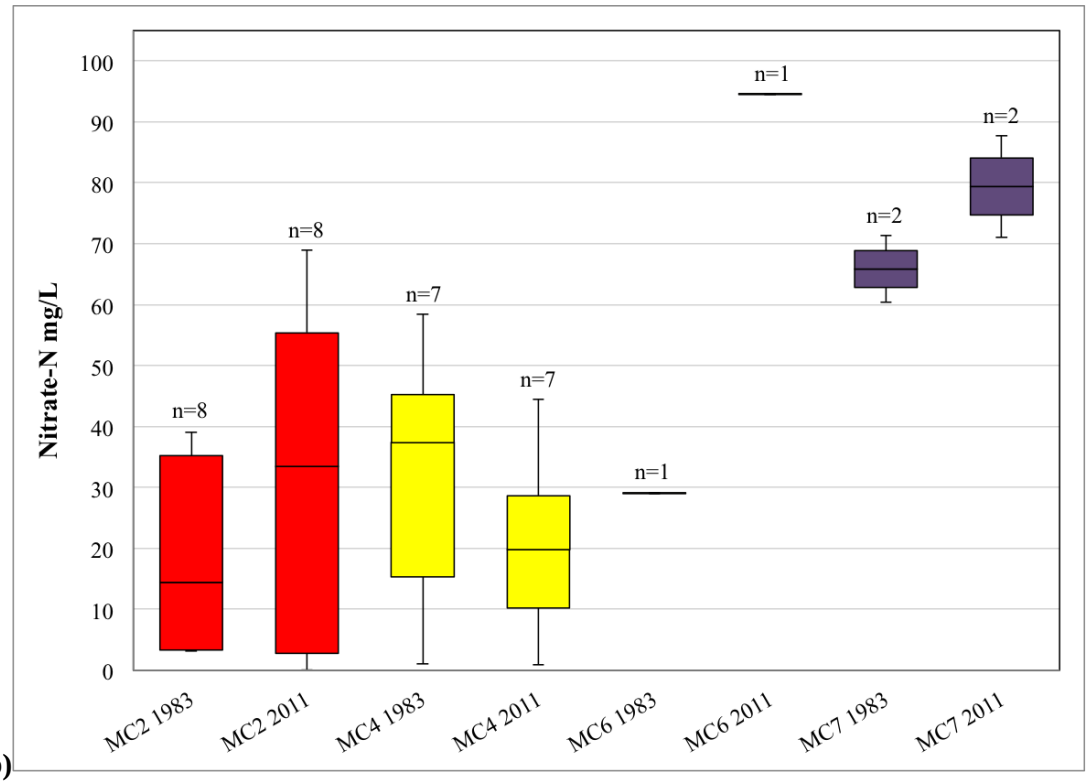
Between 6.3 mbgs and 10.9 mbgs, trends in nitrate concentration vary when compared to the 1982/83 data (Figure 3.4). However, between 13.0 mbgs and 18.9 mbgs, sample concentrations for 2010/11 consistently exceed that of the 1982/83 data, with concentrations of nitrate exceeding the drinking water limit concentrations down to a depth of 18.9 mbgs. From 21.4 mbgs and beyond, both datasets show that nitrate concentrations were negligible, and hardly present above the detection limit. The box and whisker comparison for summer samples shows an increase in the $5^{th}$, $25^{th}$, $50^{th}$ and $75^{th}$ percentiles, but a decrease in the $95^{th}$ percentile. Although the median ($50^{th}$ percentile) has increased, the increase is small, having a value of 12.6 mgN/L in 1983 and 14.6 mgN/L in 2010, of nitrate. A spring comparison could not be computed because no samples were taken in the spring of 1983.

W5

For 2010/11 data, nitrate concentrations show a somewhat consistent, increasing trend, to a depth of 17.4 mbgs (Figure 3.6). Between 9.2 mbgs and 15.9 mbgs, nitrate concentrations vary between being higher or lower than those measured in 1982/83. Between 15.9 mbgs and 20.5 mbgs, nitrate concentrations are consistently greater than those measured in 1982/83 and exceed the drinking water limit. Beyond 20.5 mbgs both historical and recent nitrate concentrations are measured between 0 and 0.5 mgN/L. The box and whisker plot for the summer samples shows increased values for all points of the 2010 data compared to the 1982 data (Figure 3.7). A spring comparison could not be generated because no samples were collected in the spring of 1983.




**(a)**




**(b)**

**Figure 3.4** Box and whisker plots for multi-level piezometers; (a) samples collected in the summer of 1983 and 2010, and (b) samples taken in the spring of 1983 and 2010.

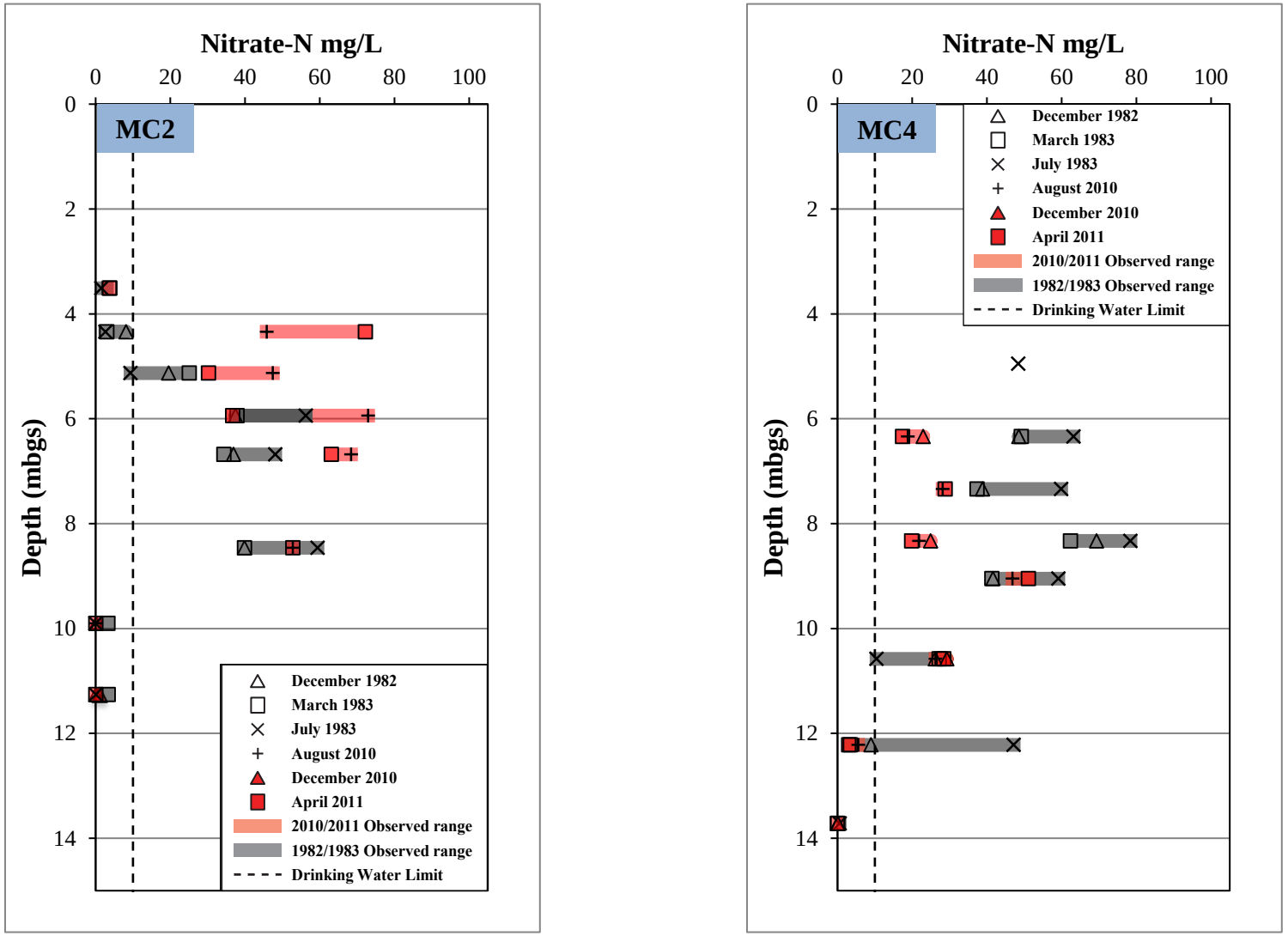



**Figure 3.5** MC2 and MC4 multi-level nitrate concentrations for 1982/1983 and 2010/2011.

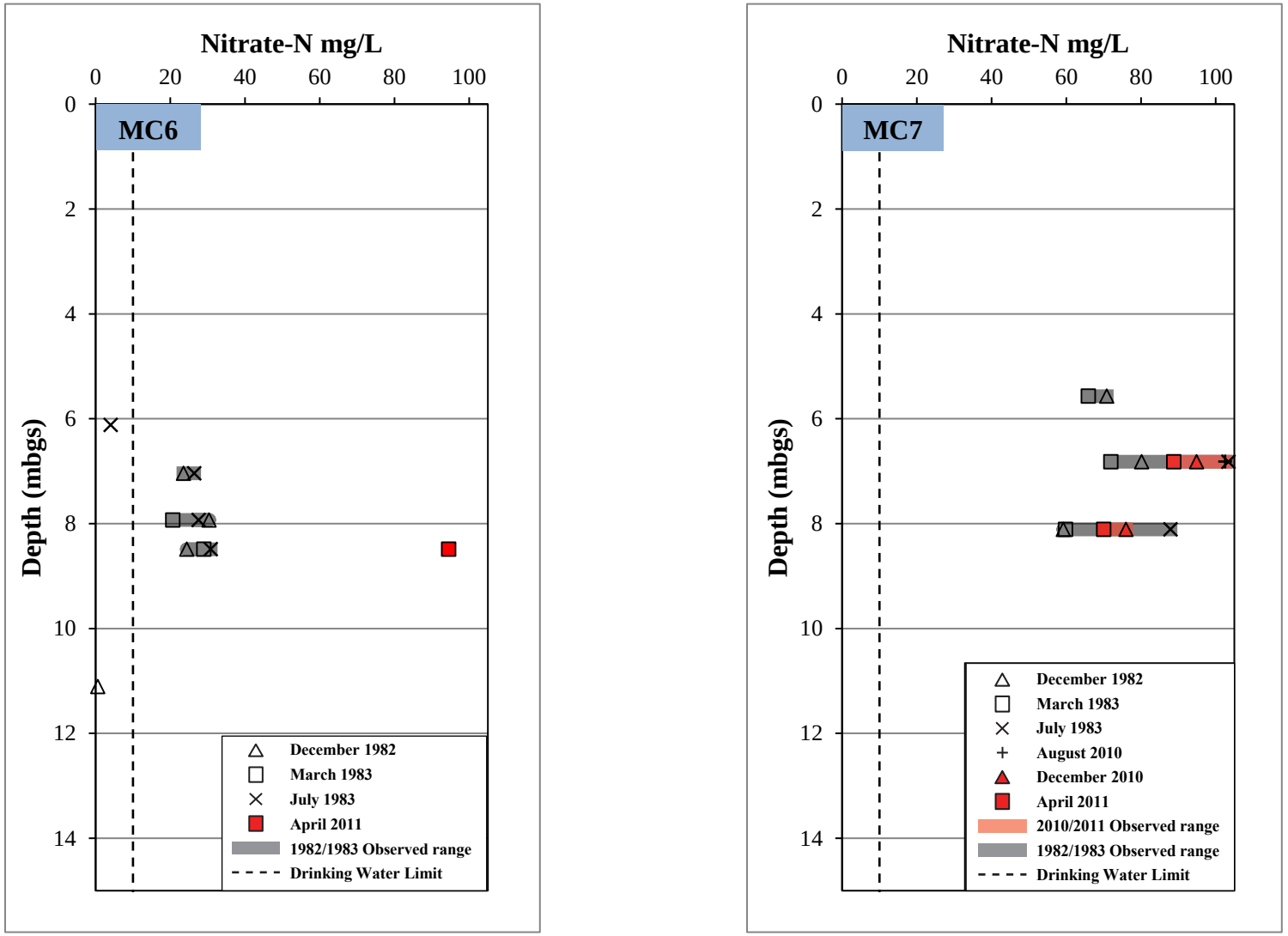



**Figure 3.6** MC6 and MC7 multi-level nitrate concentrations for 1982/1983 and 2010/2011.

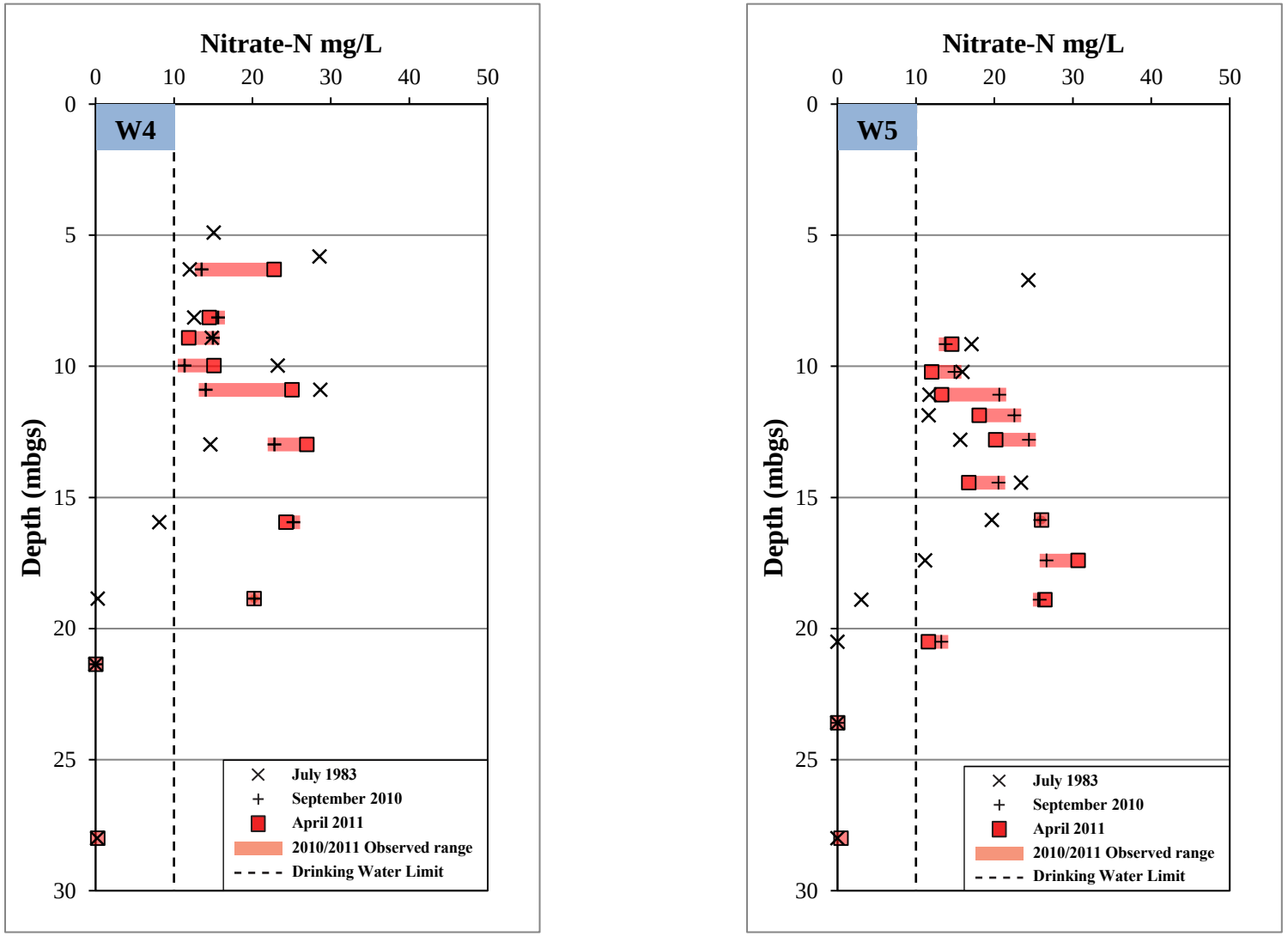



**Figure 3.7** W4 and W5 multi-level nitrate concentrations for 1982/1983 and 2010/2011.

## 3.4 Discussion

### 3.4.1 Domestic wells and groundwater seeps

#### *3.4.1.1 Mann-Whitney rank-sum test for nitrate comparison*

The expected result was a reduction in groundwater nitrate concentrations relative to values measured in the study area 30 years prior, relating to increased awareness and application of nutrient management practices. Domestic well nitrate concentrations for 2011 within each recharge area are not statistically different from data collected in 1980, when separate recharge area datasets are compared (ie. Northeast, Northwest, Midwest, South). However, when grouped together and compared as one large dataset, the two sample sets were found to be statistically different, with the wells having lower nitrate concentrations in 2011 (mean = 11.5 mgN/L) than in 1980 (mean = 13.7 mgN/L). Domestic wells are likely to have a high responsiveness to the changes in local land management and farming practices. Wells are drilled at shallow depths to provide adequate water supply for a single dwelling home, and deeper drilling than necessary is discouraged because of the high cost. Therefore lateral flow into the well is assumed to be from shorter distances (i.e. up to few hundred meters; see section 3.4.2) and reflective of nearby overlying land types and use. Increased efficiency in nutrient applications and changes in crop diversification are thought to be the driver for lower overall nitrate in 2011, however this cannot be quantified. The percentage of wells with nitrate above 10 mgN/L was 36% in the current study, compared to 51% in 1980.

Overall, nitrate in groundwater seeps was higher in 2010 (mean = 8.5 mgN/L) than in 1979 (mean = 5.0 mgN/L). This is the opposite of what was expected and what was found for the domestic wells. This may be related to an overall decrease in the depth of the water table in the LASA. This was noted in the multi-level wells (section 3.4.2), and could result in younger waters existing at deeper depths, as well as bringing more oxygenated water deeper into the aquifer. This would decrease the change of nitrate removal via denitrification or other dissimilatory processes that function under anoxic conditions, and increase the likelihood of having nitrate. This, however, is assuming that the trend of the depressed water table, south of the Upper Nottawasaga River, is prevalent in the rest of the study area.

#### *3.4.1.2 Effects of land use and nutrient management*

A survey of land use, with a focus on crop type identification, was completed in the study area in the summer of 2011 (Post, 2011). Sample locations with nitrate above 10 mgN/L have been superimposed upon the resulting land use map (Figure 3.8), in order to observe correlations. Nitrate and chloride

concentrations of domestic well samples were categorized based on the crop type in the immediate vicinity of the wells. Averages of both parameters were calculated for each land use type (Table 3.2) and the box and whisker plots for each are illustrated in Figure 3.8. For the purpose of this comparison, it is assumed that the land use survey in 2011 is representative of the farming practices for that year, and likely correlates relatively well to other years, since crops are commonly rotated on a seasonal and sometimes annual basis. Also, as previously mentioned, domestic wells are shallow and therefore influenced by local land use and farming practices.

Nitrate concentrations of domestic well samples were generally lower in 2011 than measured in 1980, and 36% of samples still had nitrate above 10 mgN/L in 2011, compared to 51% in 1980. The data show that the one well in the vicinity of corn crops had the highest mean nitrate and chloride concentrations at 57.0 mgN/L and 108.0 mg/L, respectively; however, this is only based on one sample. Wells under potato crops had the second highest mean values for both nitrate and chloride (18 and 63 mgN/L, respectively, averaged over 22 samples), and makes up the largest fraction of the sample population at 38%. Half of those samples, 19% of the total sample population, had nitrate concentrations above 10 mgN/L. Land use types having successively lower mean nitrate and chloride concentrations were 'sod', 'other', 'forest', and 'pasture'. The ranking of chloride concentration with respect to land type follows that of nitrate (Table 3.2 and Figure 3.8). Chloride is analyzed with nitrate because both are soluble and present in fertilizer application; chloride is applied in the form of KCl. If chloride and nitrate are positively correlated, it would be reasonable to conclude that fertilizer application is the source responsible for elevated nitrate (Hill, 1986; Starr, et al., 1987).

In previous research, Hill (1982) found that nitrate and chloride in groundwater underlying potato fields were strongly correlated to, and derived from, synthetic fertilizers applied to potato crops. Additionally, he pointed out that concentrations of nitrate less than 1 mgN/L were characteristic of forest and pasture areas. The current study shows that the correlations to land use and nitrate in shallow groundwater are in good agreement with the results of Hill (1982). However, the amount of potato farming in the LASA has decreased since the 1980s, due to the diversification to other crops such as sod and soy. The category labeled 'other', in Table 3.2 and Figure 3.8, encompasses many of these different types of crops, which make up slightly less of the total sample population than potato crops, at 36%. The percentage of total samples categorized as 'other' and having nitrate above 10 mgN/L make up 10% of the total sample population. This suggests that diversification away from potato crops may help reduce high concentrations of groundwater nitrate.

The relationship between nearby land use and nitrate concentration for groundwater seeps is quite similar to that of domestic wells. Figure 3.9 shows nitrate concentration grouped by nearby land use

type that was provided by the 2011 land use survey (Post, 2011). Overall, results show 'forest' and 'pasture' as having the lowest groundwater nitrate whereas 'potato' has the highest, followed by 'sod', 'corn', and 'other'.

Although the reduction of nutrient loading in agricultural practices in Ontario is encouraged through the NMA, data regarding the extent and details of the actual nutrient applications and crop rotation practices within the southern Nottawasaga Watershed have not been documented in the current study. It has been assumed, for the purposes of this study, that farming practices have evolved since the 1980s to improved nutrient management and increased adoption of practices that decrease nutrient leaching to the LASA.

Similar studies regarding nitrate leaching into shallow groundwater and the effectiveness of BMPs are being tested globally. In France, 'Good Agricultural Practices' (GAP) at the small catchment scale were monitored, between 1991-1999, to assess the usefulness for reducing N leaching. Detailed analysis of fertilizer application rates, soil type, crop type, use of catch crops (i.e. cover crops), and precipitation were used to delineate the efficacy of GAP and the limits of their application. Fluctuations of N leaching, which varied between a three to five-fold factor, were dependent on the year, crop type, and soil type. A correlation between drained water (that is water which was not used by the crop and would recharge into the groundwater system) and nitrate concentration was not evident. Nitrate concentrations were highly influenced by soil type, with the highest values correlating to shallow sandy soils. Nitrate leaching was not significantly lowered with the reduction of fertilization rates, especially in sandy rocky soils. It was concluded that N fertilizer optimization in use with catch crops and straw incorporation is the most efficient method for limiting nitrate pollution to the aquifer (Beaudoin, et al., 2005). In British Columbia, Canada, Wassenaar et al. (2006) examined the impact of voluntary BMPs with respect to groundwater nitrate contamination. A distinction was made that young groundwater (<5 years) had increases in nitrate concentration compared to older groundwater, and stable isotopic analysis revealed a slow shift away from the use of animal manure and toward synthetic fertilizers. A conclusion stated that voluntary BMPs were not useful in achieving groundwater targets and that BMPs should be used in conjunction with nutrient monitoring programs to more quickly address BMP deficiencies. This advice should be used within the LASA study area to identify how BMPs such as fertilizer application types and rates, and crop type and rotation affect the leaching of nitrate to groundwater.

**Table 3.2** Mean values of nitrate and chloride, and samples containing nitrate above 10 mgN/L for domestic wells.

| | | | Category populations | | $NO_3^-$ >10 mgN/L | | |
|---|---|---|---|---|---|---|---|
| **Land Type Category** | **Mean $NO_3^-$ (mgN/L)** | **Mean $Cl^-$ (mg/L)** | **# of samples** | **% of total** | **# of samples** | **% of category** | **% of total population** |
| Corn | 57.2 | 108 | 1 | 1.7 | 1 | 100 | 1.7 |
| Forest | 3.4 | 32.1 | 8 | 13.8 | 1 | 12.5 | 1.7 |
| Other | 7.3 | 42.9 | 21 | 36.2 | 6 | 28.6 | 10.3 |
| Pasture | 0.0 | 1.2 | 3 | 5.2 | 0 | 0 | 0 |
| Potato | 17.6 | 63.5 | 22 | 37.9 | 11 | 50.0 | 10.0 |
| Sod | 9.6 | 49.7 | 3 | 5.7 | 2 | 66.7 | 3.5 |

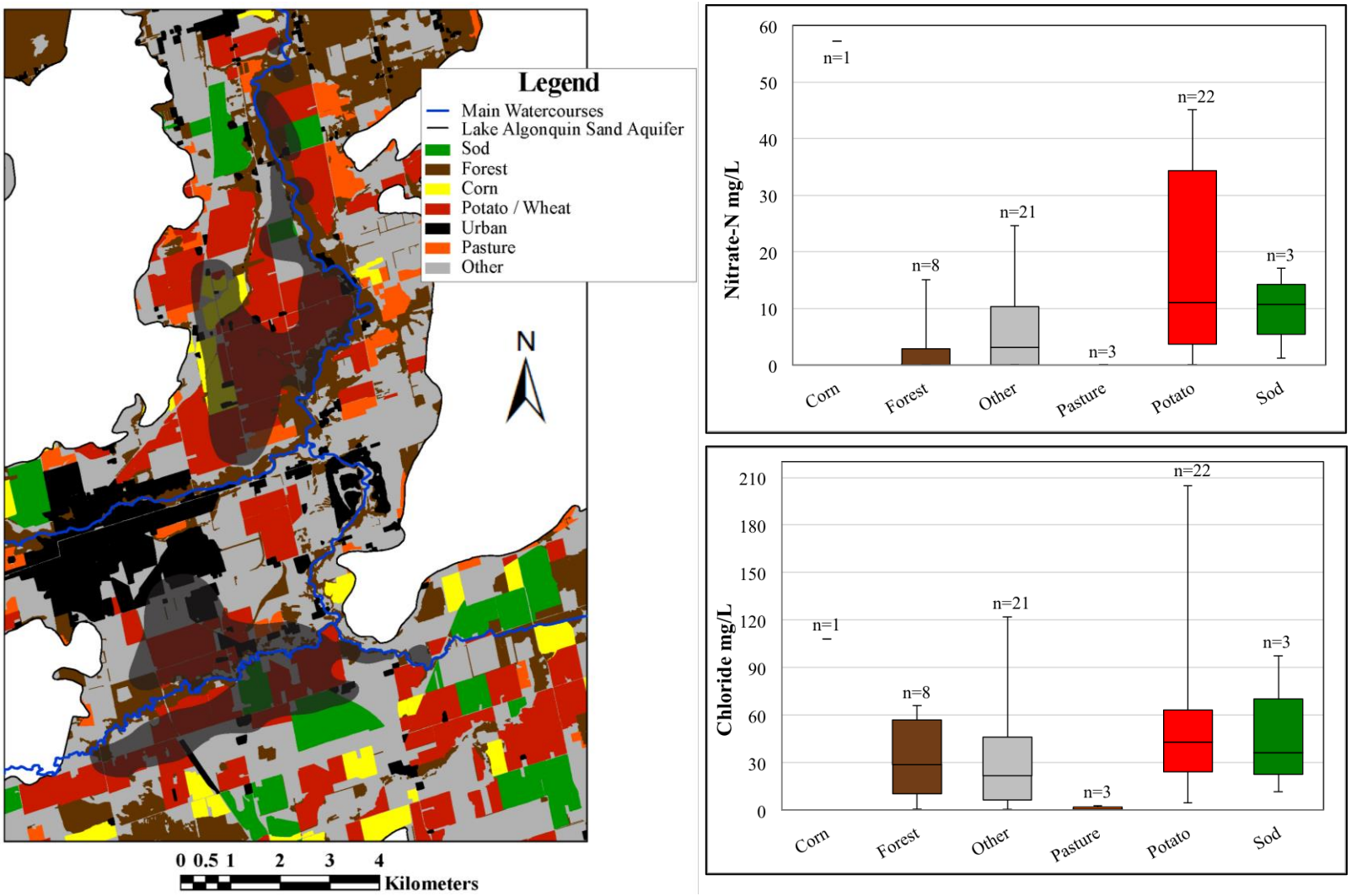



**Figure 3.8** Left: 2011 land use map (Post, 2011) with shaded areas representing areas with nitrate >10 mgN/L. Right: Box and whisker plots for nitrate (top) and chloride (bottom) of the 2010 domestic well samples, categorized by land use type.

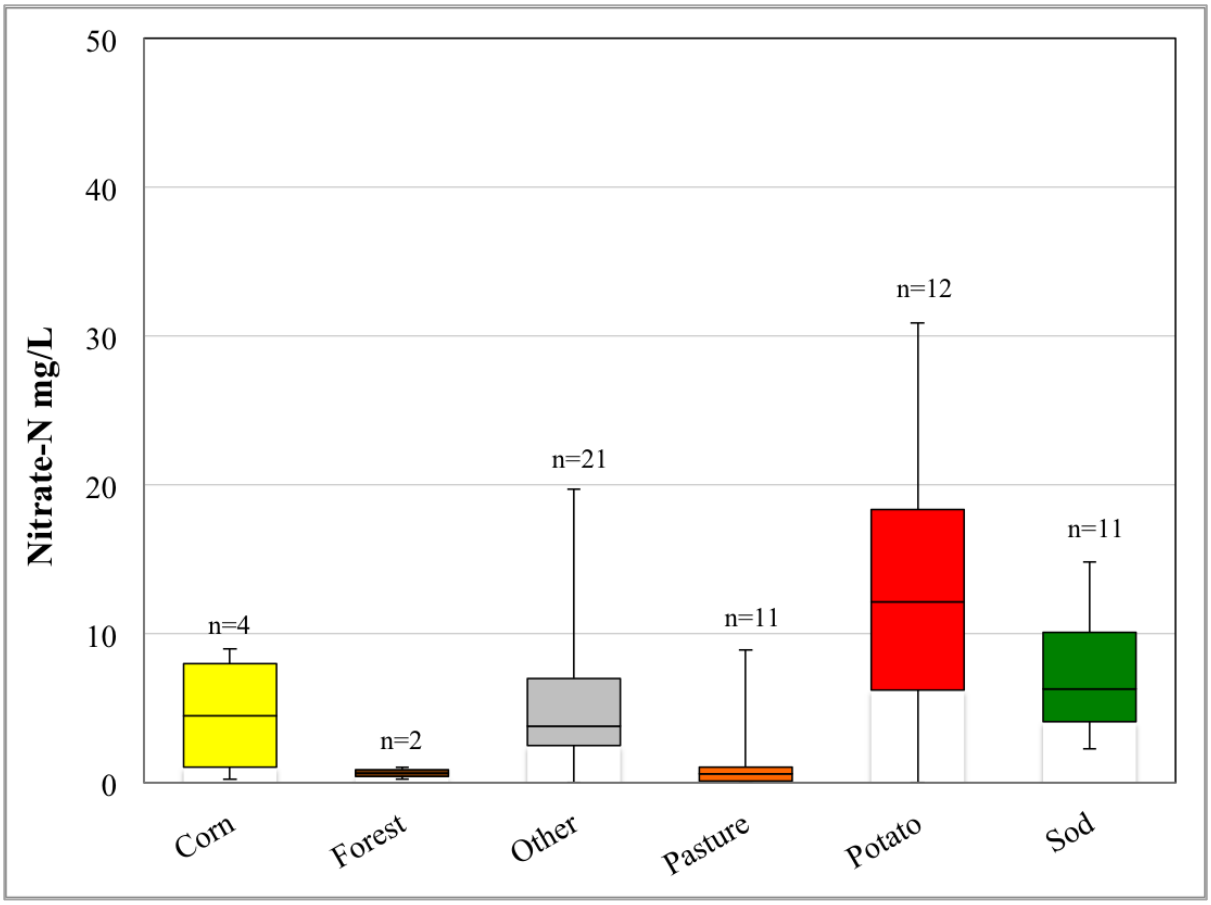



**Figure 3.9** Nitrate concentrations from groundwater seeps collected in 2010. The land use type for each sample has been determined from the land use map in Figure 3.8, according to the closest land package to the sample location. Areas with small riparian zones use the land type immediately adjacent to these areas.

### 3.4.2 Multi-level wells

Estimations of groundwater velocity, both horizontal and vertical components, are necessary for interpreting changes in nitrate in the multi-level wells. Recharge into the LASA has been measured to range between 26-36 cm/year (Hill, 1986), and can be used to estimate the vertical velocity of groundwater flow. For the purpose of estimating vertical groundwater flow rates in the unsaturated zone, a recharge rate (R) of 30 cm/year will be used. A general sense of the vertical velocity ($V_{unsat}$) in the unsaturated zone can be approximated:

$$V_{unsat} = R/\phi \qquad \text{(Eq. 3.1)}$$

The porosity ($\phi$) estimated to be approximately 0.35, which gives a $V_{unsat}$ of 86 cm/year. However, the isotopic compositions of water collected at MC2 (Chapter 4, section 4.3.3.2) give support for winter recharge waters entering the saturated zone (~3m) over a time period of less than a year. Therefore, the calculated $V_{unsat}$ is interpreted as a baseline estimate of vertical flow in the unsaturated zone and the actual velocity may range from ~80 cm/year to several hundred cm/year.

For the saturated zone, groundwater age can be modelled using the 'Vogel' model, and can be characterized as being exponentially slower with depth, or, with respect to groundwater age, groundwater age increases exponentially with depth (Sebol, et al., 2007). The model uses recharge rate (R), the aquifer saturated thickness (H), depth below the water table (z), and the aquifer saturated porosity ($\xi$):

$$t_z = (H\xi)/R*\ln(H/H-z) \qquad \text{(Eq. 3.2)}$$

Approximations for groundwater age for MC2, MC4, W4, and W5 are summarized in table 3.3, and are helpful for interpreting changes in nitrate in the multilevel wells.

Horizontal velocity and transit time in the saturated zone for groundwater flow between multi-level wells that are sub-parallel to groundwater flow are calculated in Chapter 4 (Section 4.3.3.1). Flow between MC2 and MC4 is interpreted as being parallel to the flow direction and has a calculated maximum value of 90 m/year. Between W4 and W5, horizontal velocity has a maximum 72 m/year and is interpreted as subparallel to the flow direction. Actual groundwater velocities are more likely closer to the maximum calculated values than the minimum values, as velocities of tens of meters per year is normal among unconfined aquifers with similar sandy soils (ex. Strathroy, ON) (Sebol, et al., 2007).

In the multi-level nitrate profiles, exceptionally low nitrate at the top of multi-level well MC2 (3.5 mbgs, April) is attributed to recharge from melted snow that covered the area during the winter months.

This is similar to the trend noted in the historical dataset (Starr, et al., 1987). Between 4.3 and 6.7 mbgs, nitrate was higher than historical measurements. Using the 'Vogel' model (Table 3.3) samples from 4.3 and 6.7 mbgs would have ages of approximately 2 and 5 years, respectively (water table at ~3 mbgs). The maximum flow velocity at MC2 would be less than that calculated between MC2 and MC4, as gradient increases towards the main tributaries (Figure 1.3). The source of high nitrate in MC2 is therefore estimated to be 2-300m away. Therefore, the increase in nitrate here is likely a result of the removal of the woodlot which occupied the space south of MC2 (Figure 2.5) until 2005, after which it was removed and the area was cropped. Below 6.7 mbgs, nitrate concentrations for both sets of data were within range of one another and do not indicate any major changes from the previous study. The source of groundwater exists south of the old woodlot; a section of land that was under cultivation during the 1982/1983 study (Starr, et al., 1987) and during 2010/2011 (Figure 2.4), and likely was under constant cultivation between the two study dates.

Nitrate in MC4 was notably lower between 6.3 and 8.3 mbgs, for all samples taken in the current study compared to the historical dataset, although values still exceeded the drinking water limit. Again, 'Vogel' model dates (Table 3.3) correspond to groundwater ages of roughly 2 and 5 years (water table at ~5 mbgs). The source location would be as much as 450m away, and therefore be impacted by the old woodlot area. However, the transit time of groundwater in the unsaturated zone would increase the actual groundwater age by a few years, and therefore low nitrate here might be attributed to the woodlot area before its removal. The reason for higher nitrate in the 1982/1983 dataset could be explained by a pervasively lower water table during the 2010/2011 study. Samples at 6.3 and 8.3 mbgs in 1982/1983 would be older in age, and therefore be sourced farther away, possibly south of the woodlot area where crops were present.

Only one sample could be obtained from MC6 throughout all sampling dates; the resulting nitrate at 8.5 mbgs was much higher than the historical data. MC7 was consistently sampled at two of the depths and had concentrations within range of those measured in 1982/1983. The current data shows similar nitrate concentrations for samples in MC6 and MC7, all having concentrations within range of 70 to 103 mgN/L. The field south of both sites (upgradient) was rotationally cropped with potatoes, and was still under similar rotation during the current study. It is unlikely that the adjacent field underwent unequal fertilizer application for the same crop type, or had vastly different recharge patterns. The cause of the high nitrate in MC6 is not clearly understood, but it could result from intense precipitation such as storm events, which increases nitrate leaching into the aquifer (Hill, 1986).

For both W4 and W5 multi-level wells, a pattern of increased nitrate existed in the lower portion of the aquifer. Similar to MC4, this can be explained by the depression of the water table, which was more

extreme at these sites, and was 2-3m lower than 1982/1983 data. This has a more profound effect on groundwater at depth (15-20 mbgs), as groundwater age exponentially increases with depth. Therefore, at each sample depth, groundwater age is 3-6 years younger in the current study. Oxygenated water now exists deeper below the ground surface and subsequent redox boundaries are deeper. Therefore, nitrate removal occurs at a lower depth. The source would then be a few hundred meters closer, however the area was intensively cropped in both studies and is not expected to impact groundwater nitrate. Besides this, the top five sample points for W4 and W5 are within close range of the historical data. In both wells, the water table was lower, which was consistent through all the sample dates.

The comparison of nitrate in multi-level wells to that measured in 1982/1983 shows that groundwater concentrations vary widely, and can be broadly linked to changes in land use. The distances at which recharge occurs, relating to the various well depths, is approximately within a few hundred meters for the first ~5m below the water table. The best method for measuring changes in nitrate with respect to land use or BMPs would be to measure groundwater chemistry as close to the water table as possible, at locations where water table fluctuations does not change the flow source. The range of nitrate in groundwater may be more closely linked with recharge events such as considerable storm related precipitation. Hill (1986) found that such events reduced transit time of waters moving from the unsaturated zone to the saturated zone by elevating the water table, and had a direct influence on increased nitrate in groundwaters down gradient of cropped fields. Where uniform and regular crop production practices occurred, nitrate could be more closely related to the occurrence of recharge events that increase nitrate leaching and transit time to the aquifer.

**Table 3.3** Approximations of groundwater age ($t_z$) with respect to transit in the saturated zone in the multi-level wells using the 'Vogel' model for distance intervals (meters) below the water table (z). Recharge rate is estimated at 30cm/year and the aquifer saturated porosity is estimated at 0.35.

| | **MC2** (H=9) | **MC4** (H=10) | **MC6** (H=5) | **MC7** (H=6) | **W4** (H=23) | **W5** (H=21) |
|---|---|---|---|---|---|---|
| z (mbwt) | $t_z$ (years) | $t_z$ (years) | $t_z$ (years) | $t_z$ (years) | $t_z$ (years) | $t_z$ (years) |
| 1 | 1.2 | 1.2 | 1.3 | 1.3 | 1.2 | 1.2 |
| 2 | 2.6 | 2.6 | 3.0 | 2.8 | 2.4 | 2.5 |
| 3 | 4.3 | 4.2 | 5.3 | 4.9 | 3.8 | 3.8 |
| 4 | 6.2 | 6.0 | 9.4 | 7.7 | 5.1 | 5.2 |
| 5 | 8.5 | 8.1 | - | 12.5 | 6.6 | 6.7 |
| 6 | 11.5 | 10.7 | - | - | 8.1 | 8.2 |
| 7 | 15.8 | 14.0 | - | - | 9.7 | 9.9 |
| 8 | 23.1 | 18.8 | - | - | 11.5 | 11.7 |
| 9 | - | 26.9 | - | - | 13.3 | 13.7 |
| 10 | - | - | - | - | 15.3 | 15.8 |
| 11 | - | - | - | - | 17.5 | 18.2 |
| 12 | - | - | - | - | 19.8 | 20.8 |
| 13 | - | - | - | - | 22.3 | 23.6 |
| 14 | - | - | - | - | 25.2 | 26.9 |
| 15 | - | - | - | - | 28.3 | 30.7 |
| 16 | - | - | - | - | 31.9 | 35.2 |
| 17 | - | - | - | - | 36.1 | 40.6 |
| 18 | - | - | - | - | 40.9 | 47.7 |
| 19 | - | - | - | - | 46.9 | 57.6 |
| 20 | - | - | - | - | 54.7 | 74.6 |
| 21 | - | - | - | - | 65.5 | - |
| 22 | - | - | - | - | 84.1 | - |

### 3.5 Conclusion

Changes in groundwater nitrate over the past ~30 years were varied among the domestic wells, groundwater seeps, and multi-level wells, in the LASA. Domestic wells and multi-level wells had lower mean nitrate, and groundwater seeps had higher mean nitrate when compared to the respective historical datasets (Figure 3.10). When whole datasets were compared, nitrate in both the groundwater seeps and domestic wells of the current study showed statistical differences from the historical data; groundwater seeps were higher and domestic wells were lower. When the domestic well and groundwater seep datasets were compared by recharge area, a statistical difference of nitrate between the 2010/2011 and 1979/1980 datasets was only true for the northwest recharge area of groundwater seeps dataset.

The spatial distribution of high nitrate (>10 mgN/L) from domestic well and groundwater seep data shows similar patterns to that compiled by Hill (1982). High concentrations of nitrate were most

prevalent in the northwest and south portions of the aquifer. High nitrate existed in the midwest area for both studies, but was spatially different in the current study. The northeast was consistently lower in nitrate, in both studies, and had few areas with high nitrate.

Although nitrate in domestic wells is lower in the current study compared to the 1980 dataset, the concentration of nitrate in domestic well samples was still high, with 36% of samples having nitrate above 10 mgN/L. High concentrations were correlated to 'potato', 'other', and 'corn' land types with less linked to 'sod', 'corn', and 'forest', and no correlation to 'pasture'. This agrees with relationships found by Hill (1982). Groundwater seeps show a similar trend of high nitrate correlating to 'corn' and 'potato' land use, and low nitrate to 'forest' and 'pasture'.

While the mean nitrate of the multi-level wells for the current study was less than that in 1982/1983, nitrate was not consistently lower at each site. At MC2 nitrate increased substantially for the first few meters below the water table, and was attributed to the removal and subsequent cultivation of the old woodlot area that is located directly upgradient. Lower nitrate for the first few meters of sampling in MC4 could be linked to the woodlot area and reflects the groundwater geochemistry before the woodlot removal. Also, a decrease in the water table, according to the 'Vogel' model, would decrease the age of the groundwater for the current study, compared to the same depths sampled in 1982/1983. Therefore, older ages in the historical dataset would be related to a source located more upgradient, possibly south of the old woodlot area where cultivated fields have existed throughout both studies. Depression of the water table is also evident for the multi-level profiles of W4 and W5. Nitrate at both sites exists at deeper depths and is attributed to a 2-3m drop in the water table, bringing younger, more oxygenated water deeper into the aquifer. Lastly, distinct increases in nitrate, like that in MC6, are likely related to intense precipitation events which drastically increase the leaching of nitrate into the aquifer.

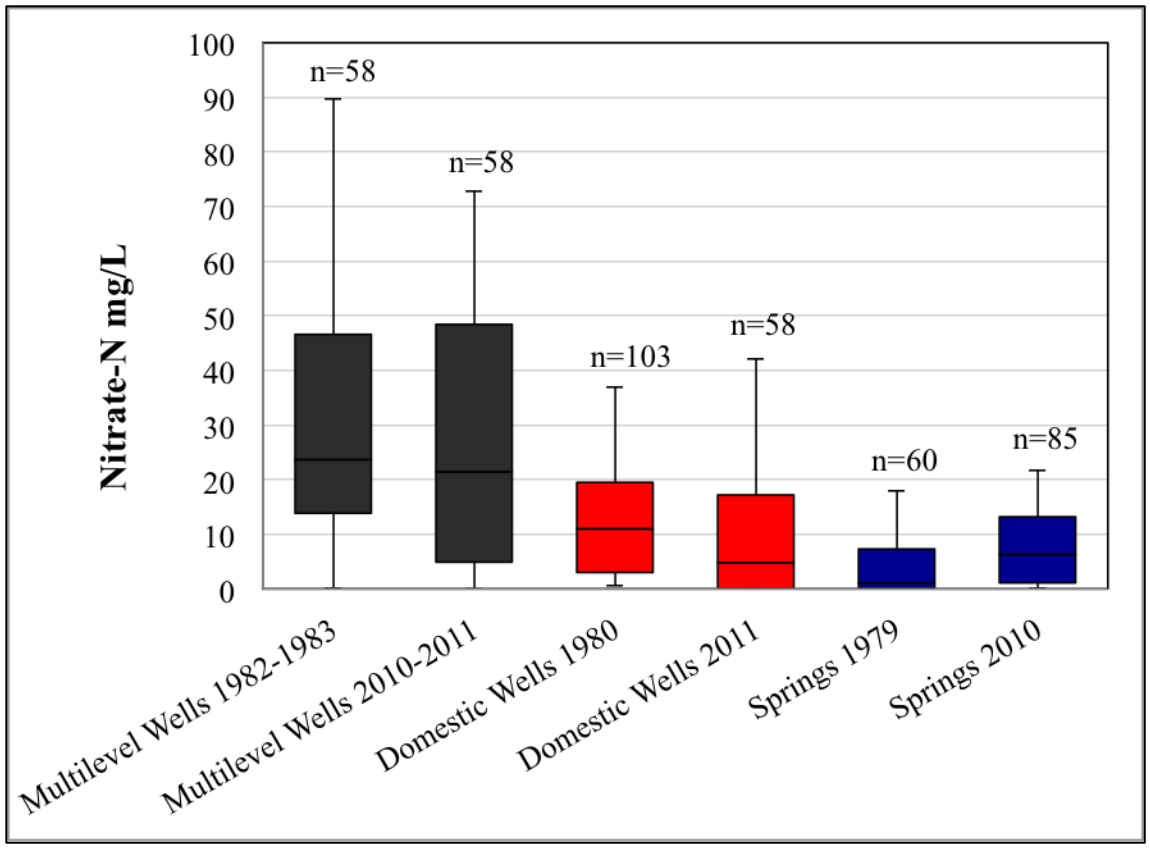



**Figure 3.10** Complete comparison of nitrate concentrations as grouped by sample type, with domestic well and groundwater seep data ('Springs') from Hill (1982) and multi-level well data from Starr et al. (1987).

# 4. NITRATE SOURCES AND REMOVAL PROCESSES IN THE LAKE ALGONQUIN SAND AQUIFER

## 4.1 Introduction

In regions where nitrate contamination exists and often exceeds the drinking water limit of 10 mgN/L, identifying the source of contamination is important for reducing further contamination. Measurement of the isotopic composition of nitrate is often useful for distinguishing sources of nitrate. The ranges in $\delta^{18}O$ and $\delta^{15}N$ values of nitrate are well defined for sources including atmospheric deposition, nitrate fertilizer, or that produced from nitrification, otherwise known as microbial nitrate (Kendall, et al., 2007). Furthermore, microbial nitrate can be categorized as being nitrified from $NH_4^+$ in fertilizer, from soil organic N, or from human or animal waste. The source of high groundwater nitrate concentrations in the LASA has previously been attributed to fertilizer application (Hill, 1982). Furthermore, it was proposed that high nitrate in the LASA persists as a result of low labile organic carbon, which otherwise acts as an electron donor for the process of denitrification, a mechanism that naturally occurs and converts nitrate to $N_2$, thereby removing it from the groundwater (Starr & Gillham, 1993; Starr, et al., 1987). Isotopic compositions of nitrate can also be used for detecting denitrification activity in groundwater; the increase of $\delta^{18}O$ and $\delta^{15}N$ values as nitrate concentration decreases commonly follows a ratio of 1:2 ($\delta^{18}O$:$\delta^{15}N$). Therefore, stable isotopes of nitrate can be used to identify sources of nitrate in the LASA and whether denitrification occurs to remove nitrate.

In addition to nitrate stable isotopes, artificial sweeteners can be measured in groundwater to identify sources of wastewater such as septic systems and wastewater treatment plant effluent (Scheurer, et al., 2009; Van Stempvoort, et al., 2011a; Buerge, et al., 2009; Van Stempvoort, et al., 2011b). Artificial sweeteners are added to many processed food and beverage products and resist digestion, therefore accumulating in human sewage. The artificial sweeteners that are increasingly being used to trace wastewater in the environment are saccharin, cyclamate, acesulfame, and sucralose. In Canada, both acesulfame and sucralose have been introduced fairly recently into food and beverages; in 1988 and 1992, respectively, and therefore can be good indicators relative water ages (Gougeon, et al., 2004; Van Stempvoort, et al., 2011a; Robertson, et al., 2016). In the LASA, septic beds located in the rural neighborhoods may influence the presence of nitrate in the wells and the groundwater seeps via nitrification of human waste (Denver, 1989; Robertson, et al., 1991; Aravena, et al., 1993; Aravena & Robertson, 1998). In sand aquifers with low transverse dispersion, septic plumes have been found to exist at more than 130 m beyond the septic bed (Robertson, et al., 1991). Both stable isotopes of nitrate

and artificial sweeteners are novel tools that could be important for defining sources and nitrate cycling responsible for nitrate contamination in the LASA.

## 4.2 Methodology

Methodology for field procedures and laboratory analysis are outlined in sections 2.3.2 and 2.3.3, respectively.

## 4.3 Results

### 4.3.1 Domestic wells

#### *4.3.1.1 Stable isotopes*

Of the domestic wells samples measured for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ (Figure 4.1) many samples plot in a source region that corresponds to 'microbial nitrate from soil N', 'ammonium in fertilizer', or 'nitrified septic/manure N'. No samples plot within the 'nitrate fertilizer' or 'atmospheric nitrate' source boxes. Several samples also plot above the 'nitrification' source boxes and suggest increases in nitrate isotope compositions are a result of denitrification. The $\delta^{15}N\text{-}NO_3^-$ values ranged from +1.0‰ to +29.5‰ and the $\delta^{18}O\text{-}NO_3^-$ values ranged from -2.7‰ to +10.6‰. Samples with the highest nitrate isotope ratios had nitrate <10 mgN/L (Figure 4.1 and Figure 4.2), however, samples with more average $\delta^{15}N$ values also had low nitrate. Overall, nitrate concentrations ranged from 0 to 57.2 mgN/L. Using the denitrification trend line (Figure 4.1), original isotope ratios of nitrate (pre-denitrification) can be interpreted to lie within the 'ammonium in fertilizer' source box', as well as the 'microbial nitrate from soil N' and with few samples crossing into the 'nitrified septic/manure N'.

Artificial sweeteners (acesulfame, saccharin, cyclamate, sucralose), used to detect contamination associated with septic systems and subsequent plumes. One or more of the sweeteners was detected in 18 domestic well samples (31% of the dataset) and summed sweetener concentrations ranged from 0.012 to 69.43 µg/L (Figure 4.3). This suggests that 31% of the domestic well samples originate from septic wastewaters or are to some degree mixed with septic wastewaters, and nitrate in these samples could be derived from wastewater or a mixture of wastewater and other nitrate sources. A better approximation of wastewater impact is calculated using a ratio of acesulfame (ACE) to dissolved inorganic nitrogen (DIN), to give a semi-quantitative estimate of wastewater proportion (Robertson, et al., 2016). Table 4.1 outlines the proportion of nitrate attributed to wastewater in domestic wells and categorizes four samples as consisting of >50% nitrate from wastewater. However, three of these

samples have low DO, and therefore low nitrate concentration may be impacted and artificially lowered by denitrification, which affects the TIN/ACE ratio. One sample, DW-52, with a DO value of 3.6 mg/L, can be said to have significant contribution to nitrate from wastewater (septic system). This sample also has the highest sum of sweeteners 69.43 μg/L, and nitrate is over the drinking water limit, 34.8 mgN/L. Overall, wastewater is a minor contributor to groundwater nitrate in domestic wells.

Nitrous oxide concentrations range from 0 to 43000%sat (with respect to atmospheric $N_2O$ concentration) in domestic well samples (Figure 4.4). $N_2O$ was above 100%sat in a total of 40 out of 59 samples (68%). In these samples, the $N_2O$ excess is likely the result of N cycling mechanisms. Samples with nitrous oxide higher than 100 %sat had nitrate that ranged from 0 to 57.2 mgN/L, and 80% were oxic (DO >2mg/L). Where nitrous oxide was lower than 100 %sat, nitrate ranged from 0 to 10.7 mgN/L, and 16% of samples were oxic. Therefore, high $N_2O$ produced from N cycling was more commonly associated with oxic conditions in the domestic wells, and with a high range of nitrate concentrations.

Few samples were measured for $\delta^{15}N\text{-}NH_4^+$ (Table 4.2) and have isotopic compositions that are characteristic of synthetic fertilizer. One sample (DW-48) had a $\delta^{15}N\text{-}NH_4^+$ value of +11.3‰, and $NH_4^+$ was 10.1 mgN/L. All samples tested for $\delta^{15}N\text{-}NH_4^+$ composition had low nitrate (>0.2 mgN/L) and only DW-48 had sufficient nitrate to test for nitrate isotope values, which were +29.5‰ and +7.3‰ for $\delta^{18}O$ and $\delta^{15}N$, respectively. This is consistent with the elevated $\delta^{15}N\text{-}NH_4^+$ which is characteristic of manure/septic N.

Isotopic compositions of nitrous oxide ranged from -49.3‰ to +9.4‰, and +7.5‰ to +61.5‰, for $\delta^{15}N$ and $\delta^{18}O$, respectively (Table 4.3). Differences in isotopic composition for nitrous oxide and nitrate ($\Delta_{N20\text{-}NO3}$) were calculated, where stable isotopes of nitrate and nitrous oxide were both measured. For $\delta^{18}O$, the $\Delta_{N20\text{-}NO3}$ ranged from +0.2‰ to +58.7‰, with 71% ranging between +32‰ and +60‰, $\varepsilon^{18}O$ outlined by Snider et al. (2013). For $\delta^{15}N$, the $\Delta_{N20\text{-}NO3}$ ranged from -60.9‰ to -13.9‰ and is largely consistent with $\varepsilon^{15}N$ summarized by Remple (2008). A summary of isotope compositions of nitrate and nitrous oxide relating to nitrification and denitrification are presented in Table 4.4 and used to outline expected nitrous oxide isotope ratios (Figure 4.5). Domestic well samples dominantly fall within the denitrification box, with one sample plotting at the edge of the nitrification box (DW-11). Therefore, nitrous oxide is present as a product or sub-product of denitrification which can exists in the LASA in aerobic groundwaters.

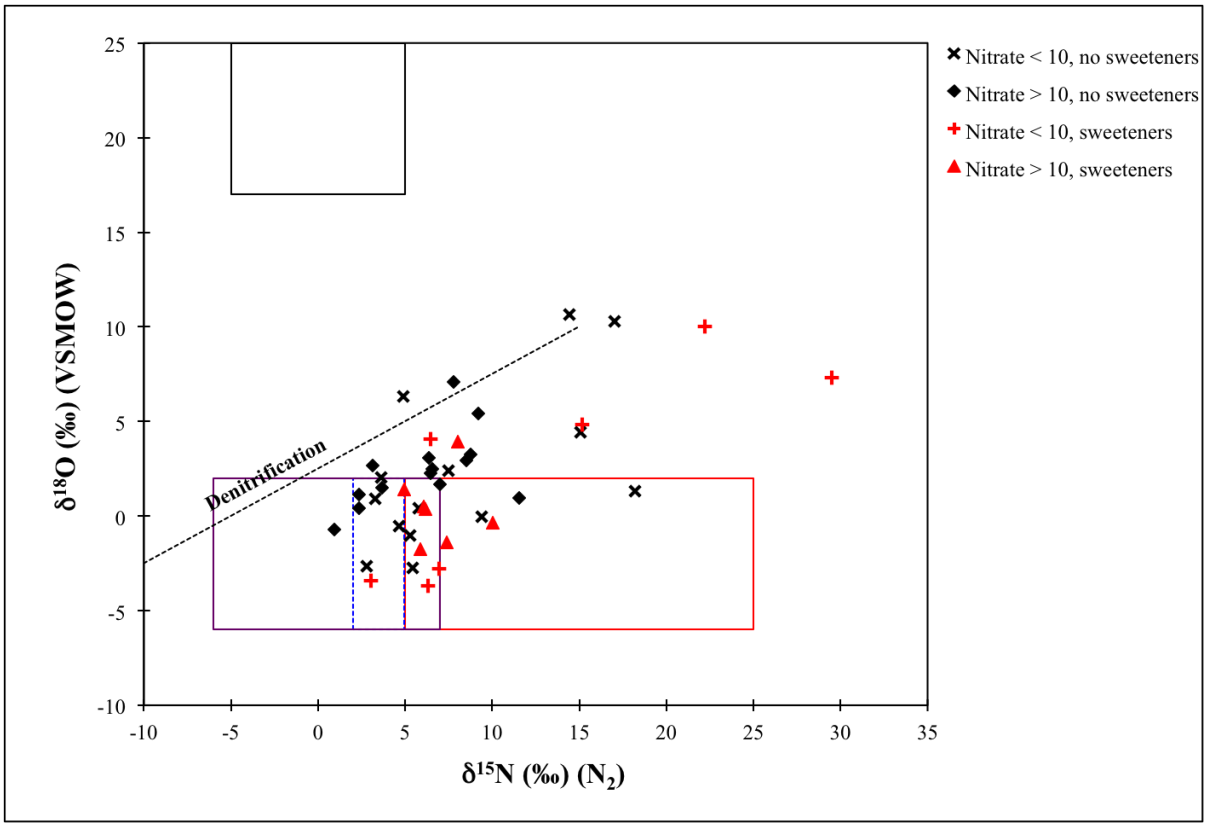



**Figure 4.1** The dual nitrate isotope plot for all domestic well samples with sufficient nitrate for analysis (>0.5 mg/L). Samples are categorized based on the presence of at least one sweetener (acesulfame, saccharin, sucralose, cyclamate) and concentrations of nitrate.

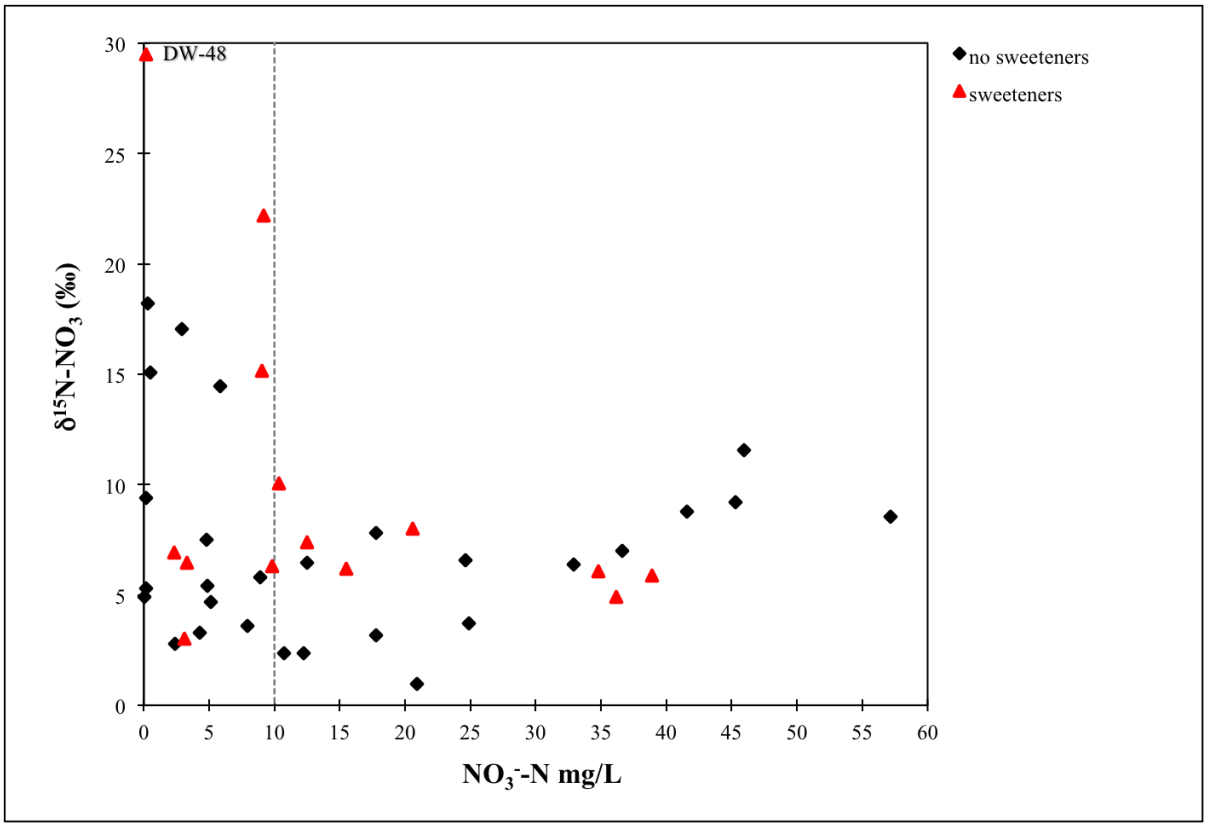



**Figure 4.2** $\delta^{15}N\text{-}NO_3^-$ composition and nitrate concentrations for all domestic well samples with sufficient nitrate for isotopic analysis (>0.5 mg/L). Samples are categorized based on the presence of at least one artificial sweetener (acesulfame, saccharin, sucralose, cyclamate).

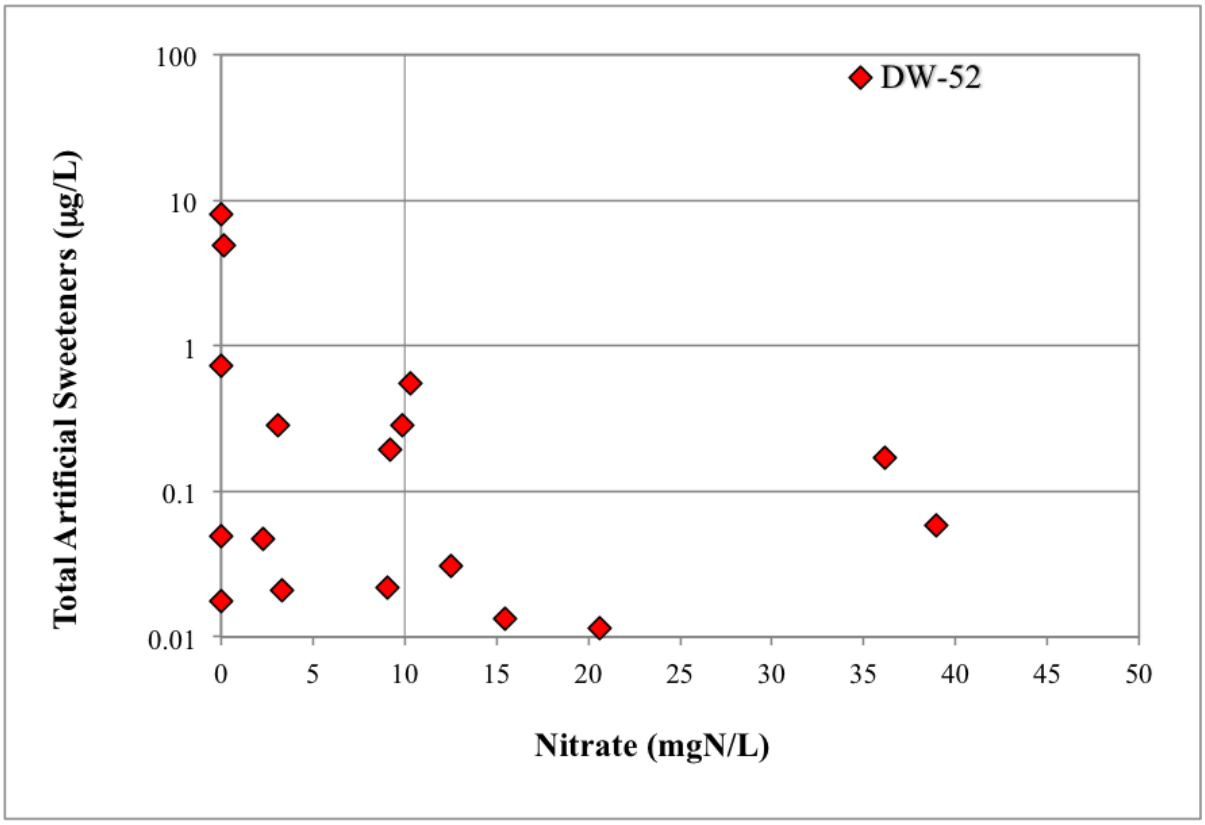



**Figure 4.3** Artificial sweeteners and nitrate for domestic well samples collected in 2011. Only samples with one or more artificial sweeteners are plotted here, which accounts for a total of 18 samples out of 58 samples. Sweetener concentrations are summed to give a total concentration of cyclamate, saccharin, acesulfame, and sucralose. Ranges of nitrate and presence of artificial sweeteners are outlined in Figure 4.2.

**Table 4.1** Estimates of septic wastewater proportion in domestic well samples collected in 2011, based on concentrations of acesulfame (ACE) and dissolved inorganic nitrogen (DIN). Contribution to nitrate is expressed as a percentage and assumes nitrate removal processes are not prevalent (Robertson, et al., 2016). Italicized values represent estimates that are exaggerated due to decreased DIN via denitrification.

| Sample ID | $NO_3^-$ (mgN/L) | DO (mg/L) | TIN (mg/L) | ACE (µg/L) | TIN/ACE | Waste water $NO_3^-$-N (%) |
|---|---|---|---|---|---|---|
| DW-03 | 9.9 | 5.2 | 9.9 | 0.28 | 35000 | 2-10 |
| *DW-04* | *0* | *0.1* | *0.9* | *5.4* | *200* | *100* |
| DW-06 | 9.1 | 5.9 | 9.1 | 0.02 | 455000 | 0-1 |
| DW-08 | 15.5 | 7.3 | 15.5 | 0.01 | 1550000 | 0 |
| DW-11 | 20.6 | 4.9 | 20.6 | 0.01 | 2060000 | 0 |
| *DW-14* | *0* | *1.6* | *0.02* | *0.05* | *300* | *100* |
| DW-18 | 2.3 | 1.2 | 2.3 | 0.05 | 46000 | 1-8 |
| DW-22 | 10.3 | 6.4 | 10.3 | 0.55 | 19000 | 4-18 |
| DW-24 | 36.2 | 5.0 | 36.3 | 0.17 | 214000 | 0-2 |
| *DW-26* | *0.01* | *0.1* | *0.04* | *0.29* | *200* | *100* |
| DW-29 | 39 | 7.1 | 39 | 0.06 | 650000 | 0-1 |
| DW-31 | 3.1 | 5.9 | 3.1 | 0.28 | 11000 | 6-32 |
| DW-35 | 12.5 | 4.1 | 12.5 | 0.03 | 417000 | 0-1 |
| DW-42 | 3.3 | 6.0 | 3.4 | 0.02 | 170000 | 0-2 |
| DW-48 | 0.2 | 0.8 | 10.3 | 0.67 | 15000 | 5-23 |
| DW-52 | 34.8 | 3.6 | 34.9 | 26.4 | 1300 | 52-100 |

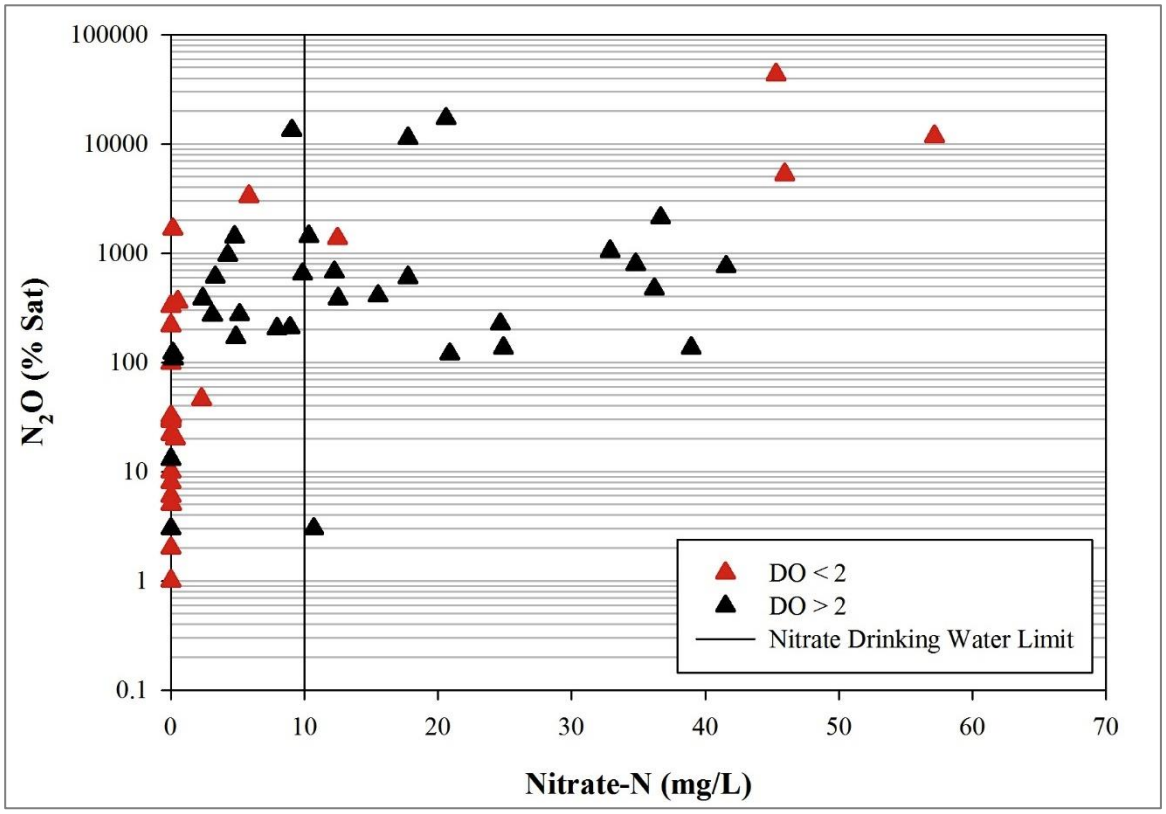



**Figure 4.4** $N_2O$, nitrate, and DO (mg/L) for domestic well samples from 2011. $N_2O$ concentrations are expressed as a percentage of the concentration expected if the groundwater $N_2O$ concentration was in equilibrium with the atmospheric $N_2O$ concentration. Therefore, samples with dissolved $N_2O$ >100%sat are influenced by a $N_2O$ production process in the sub-surface.

**Table 4.2** Domestic well sample concentrations and stable isotopes of nitrate and ammonium, for samples with ammonium concentrations >0.5 mgN/L. Samples with insufficient nitrate concentrations to measure $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ (<0.5 mgN/L) are labeled '-'.

| Sample ID | $NO_3^-$ (mgN/L) | DO (mg/L) | $NH_4^+$ (mgN/L) | $\delta^{15}N\text{-}NO_3^-$ (‰) | $\delta^{18}O\text{-}NO_3^-$ (‰) | $\delta^{15}N\text{-}NH_4^+$ (‰) |
|---|---|---|---|---|---|---|
| DW-04 | 0.0 | 0.1 | 0.9 | - | - | 5.5 |
| DW-15 | 0.0 | 0.2 | 0.5 | - | - | 4.0 |
| DW-16 | 0.0 | 0.2 | 0.8 | - | - | 2.0 |
| DW-23 | 0.0 | 0.5 | 5.4 | - | - | 1.9 |
| DW-28 | 0.0 | 0.1 | 3.3 | - | - | 2.0 |
| DW-46 | 0.0 | 0.1 | 0.5 | - | - | 0.9 |
| DW-48 | 0.2 | 0.8 | 10.1 | 29.5 | 7.3 | 11.3 |
| DW-51 | 0.0 | 0.2 | 0.7 | - | - | 1.9 |

**Table 4.3** Domestic well sample concentrations, stable isotopes, and calculated $N_2O$ isotope effects of denitrification.

| Sample ID | $NO_3^-$ (mgN/L) | DO (mg/L) | $\delta^{15}N\text{-}NO_3^-$ (‰) | $\delta^{18}O\text{-}NO_3^-$ (‰) | $N_2O$ (% sat) | $\delta^{15}N\text{-}N_2O$ (‰) | $\delta^{18}O\text{-}N_2O$ (‰) | $^{15}N$: $\Delta_{N20\text{-}NO3}$ (‰) | $^{18}O$: $\Delta_{N20\text{-}NO3}$ (‰) |
|---|---|---|---|---|---|---|---|---|---|
| DW-05 | 41.6 | 6.2 | 8.8 | 3.6 | 760 | -19.1 | 52.3 | -27.8 | 49.1 |
| DW-06 | 9.1 | 5.9 | 15.2 | 4.8 | 13290 | -10.0 | 57.3 | -25.2 | 52.4 |
| DW-07 | 9.2 | - | 22.2 | 10.0 | 800 | -17.4 | 61.5 | -39.5 | 51.5 |
| DW-09 | 12.5 | 0.2 | 6.4 | 2.3 | 1370 | -14.3 | 61.0 | -20.7 | 58.7 |
| DW-10 | 46.0 | 1.7 | 11.6 | 1.0 | 5260 | -49.3 | 35.2 | -60.9 | 34.2 |
| DW-11 | 20.6 | 4.9 | 8.0 | 3.9 | 17050 | -19.2 | 48.8 | -27.2 | 44.8 |
| DW-19 | 4.8 | 2.1 | 7.5 | 2.4 | 1410 | -15.2 | 44.6 | -22.7 | 42.2 |
| DW-22 | 10.3 | 6.4 | 10.0 | -0.3 | 1430 | -13.0 | 38.5 | -23.0 | 38.8 |
| DW-34 | 36.7 | 5.8 | 7.0 | 1.7 | 2100 | -12.4 | 28.1 | -19.4 | 26.4 |
| DW-37 | 4.3 | 6.5 | 3.3 | 0.9 | 950 | -10.6 | 37.8 | -13.9 | 36.9 |
| DW-41 | 32.9 | 5.4 | 6.4 | 3.1 | 1050 | -19.5 | 24.6 | -25.9 | 21.5 |
| DW-48 | 0.2 | 0.8 | 29.5 | 7.3 | 1670 | 9.4 | 7.5 | -20.1 | 0.2 |
| DW-50 | 17.8 | 2.3 | 7.8 | 7.1 | 11320 | -28.6 | 34.6 | -36.4 | 27.5 |
| DW-52 | 34.8 | 3.6 | 6.1 | 0.5 | 790 | -17.6 | 26.7 | -23.7 | 26.2 |
| DW-53 | 5.9 | 1.9 | 14.5 | 10.6 | 3300 | -7.0 | 47.1 | -21.4 | 36.4 |
| DW-55 | 57.2 | 1.7 | 8.5 | 2.9 | 11740 | -23.3 | 40.8 | -31.9 | 37.8 |
| DW-59 | 45.3 | 0.1 | 9.2 | 5.4 | 42980 | -16.7 | 59.5 | -25.9 | 54.1 |

**Table 4.4** Parameters used to calculate the expected ranges of $\delta^{15}N$ and $\delta^{18}O$ values of $N_2O$ produced from nitrification and denitrification in the domestic well samples.

| | **Nitrification** | **Denitrification** |
|---|---|---|
| Substrate | $NH_4^+$ | $NO_3^-$ |
| Product | $N_2O$ | $N_2O$ |
| $\varepsilon^{15}N$ (‰) | -45 to -68[a] | -10 to -45[b] |
| $\varepsilon^{18}O$ (‰) | NA | +32 to +60[c] |
| $\delta^{15}N$ (‰) (substrate) | +5 | +3 to +30 |
| $\delta^{18}O$ (‰) substrate | NA | 0 to +11 |
| $\delta^{15}N\text{-}N_2O$ (‰) (product) | -63 to -40 | -42 to +20 |
| $\delta^{18}O\text{-}N_2O$ (‰) (product) | +13 to +35[d] | +32 to +71 |

[a] Shearer and Kohl, (1986); Yoshida, (1988); Kendall, (1998); Perez et al., (2001); Stein and Yung, (2003); Sutka et al., (2006); Snider (2011).

[b] Remple (2008) and references therein.

[c] Snider et al. (2013).

[d] Snider et al. (2012).

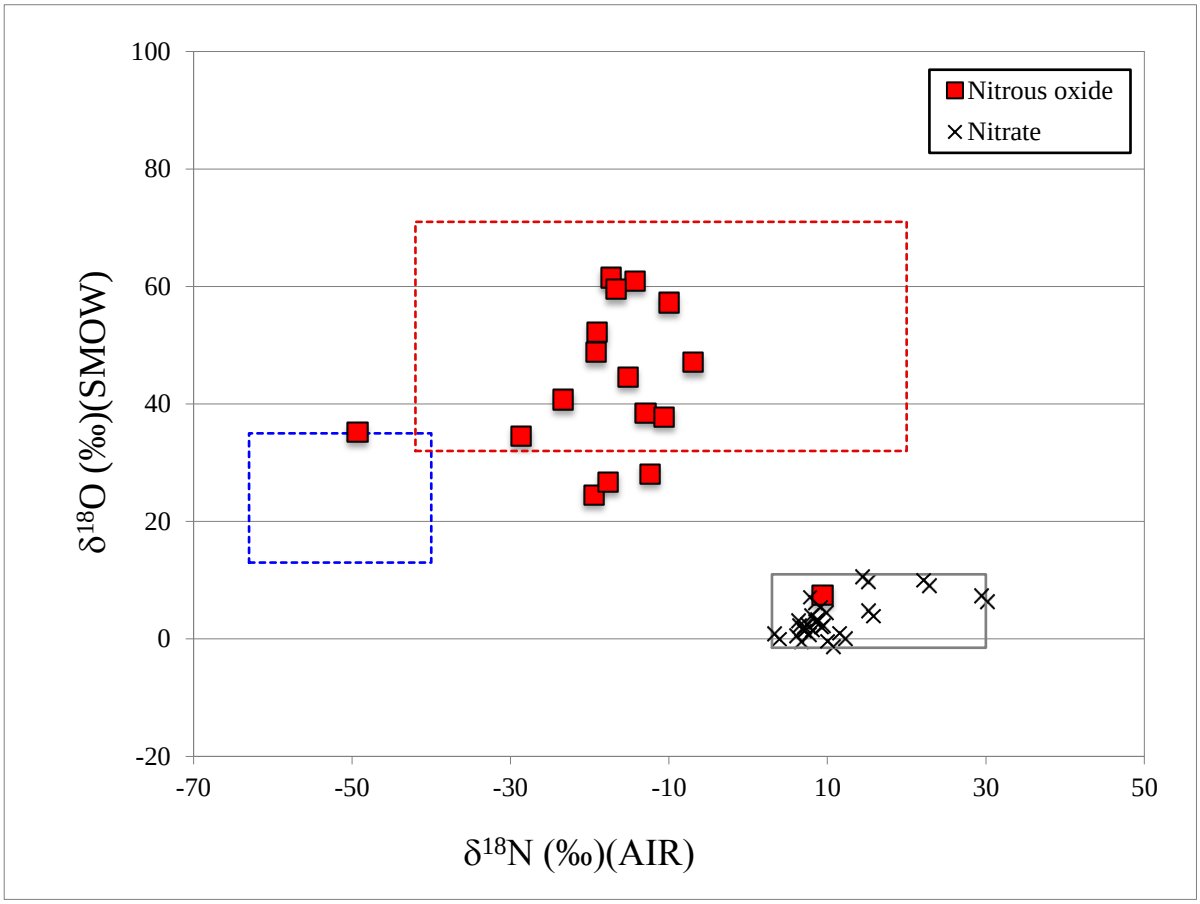



**Figure 4.5** Isotopic compositions of nitrous oxide and nitrate in domestic wells sampled in 2011. Black box encompasses the range of nitrate isotope compositions, red and blue line outlines expected ranges in subsequent nitrous oxide composition resulting from denitrification and nitrification, respectively (see Table 4.4).

*4.3.1.2 Geochemistry*

Samples on a ternary cation plot of $Ca^{2+}$, $Mg^{2+}$, and $Na^{+}$ + $K^{+}$ show trends with respect to nitrate concentration, but no distinct trend for artificial sweeteners (Figure 4.6). Groundwater with higher relative Calcium and magnesium are interpreted as being younger, and sodium and potassium generally increase with age. Therefore, high nitrate samples correspond to younger groundwater, and lower nitrate corresponds to older groundwater, relatively. Few samples have very high sodium and potassium, and may be impacted by water softeners.

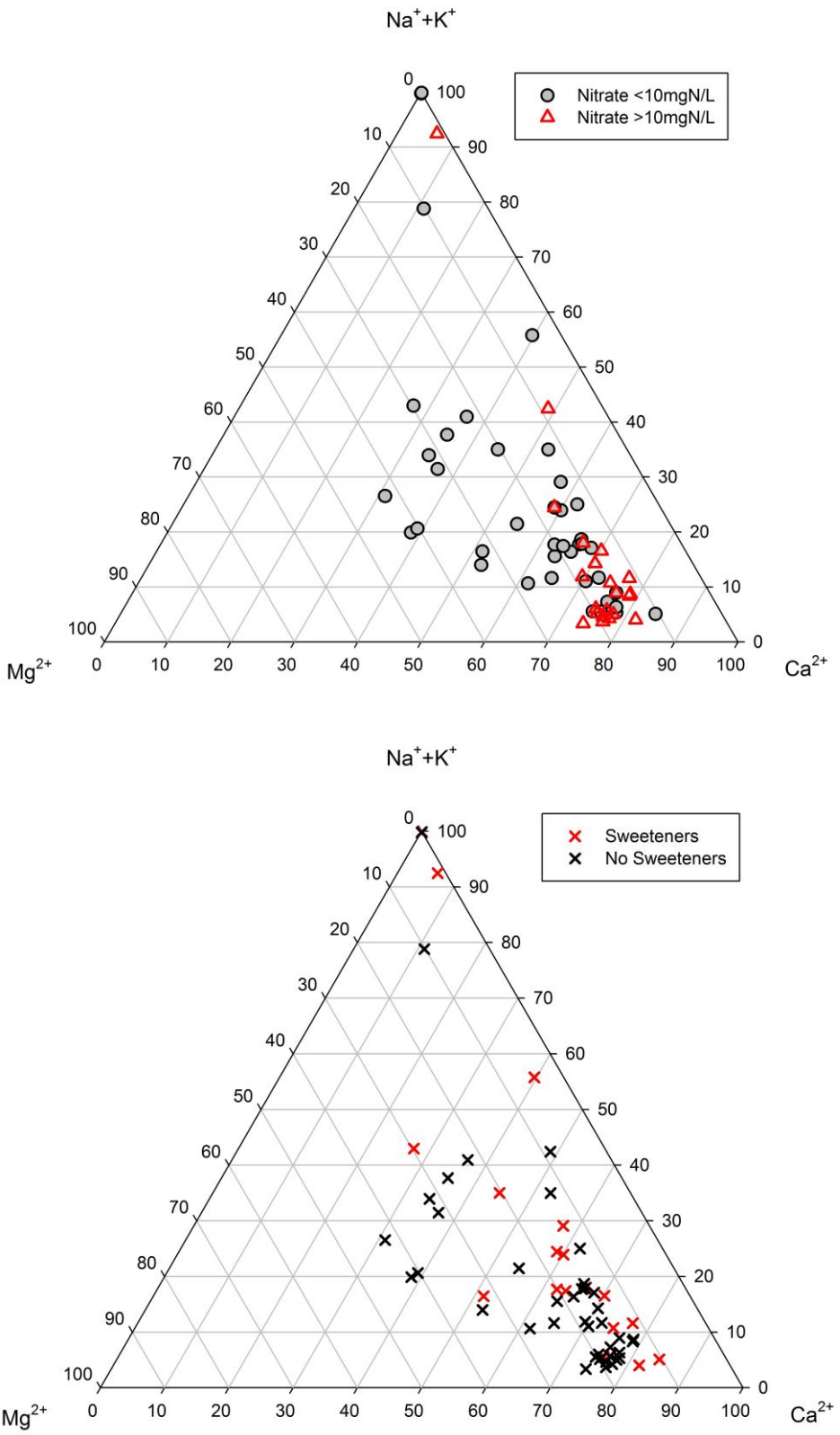



**Figure 4.6** Ternary cation plots for domestic well samples from 2011.

Several parameters were used to investigate the nature of the domestic well groundwater redox conditions in relation to nitrate concentration and potential nitrogen cycling mechanisms, namely $SO_4^{2+}$, DO, Fe, SRP, DOC and Cl. When DO of domestic well samples was >3.6 mg/L, Fe was not present (Figure 4.7). When DO was <3.6 mg/L, Fe ranged from 0 to 6.2 mg/L. Overall, 33 samples (56%) were oxic (DO >2 mg/L). Fe was highest when DO was <1, with 2 samples >6 mg/L Fe, and otherwise was below 2 mg/L. Under anoxic conditions (DO <2 mg/L) nitrate was high (>10 mgN/L) in 4 samples, however Fe was not present. Sulfate and DO did not correlate or show clear relationships (Figure 4.8), however a small cluster of 8 samples (14%) with low DO (<1 mg/L), sulfate (<5 mg/L), and nitrate (<0.1) existed and represent highly reduced conditions. These samples ranged in DOC from 0.4 to 7.2 mgC/L (Figures 4.9 and 4.10). Further relationships between DOC, sulfate, and nitrate were not evident. A plot of SRP to DO shows an inverse relationship, with SRP increasing as DO decreased (Figure 4.11), and so SRP is generally correlated to reducing conditions.

Since chloride is largely non-reactive and does not undergo removal via redox processes, it can be used as a tracer to better indicate if reduction has lowered or removed nitrate. Knowing that SRP is increased as DO decreases (Figure 4.11), SRP can be used as a proxy for reduced conditions. Samples with high SPR (>30 μg/L) have chloride that ranges from 0.5 to 31.2 mg/L (Figure 4.12), and no detectable nitrate. Four of the five samples have chloride below 6 mg/L and were less likely to have high nitrate before undergoing reduction. The one sample with higher chloride, 31.2 mg/L may have had higher nitrate prior to being reduced.

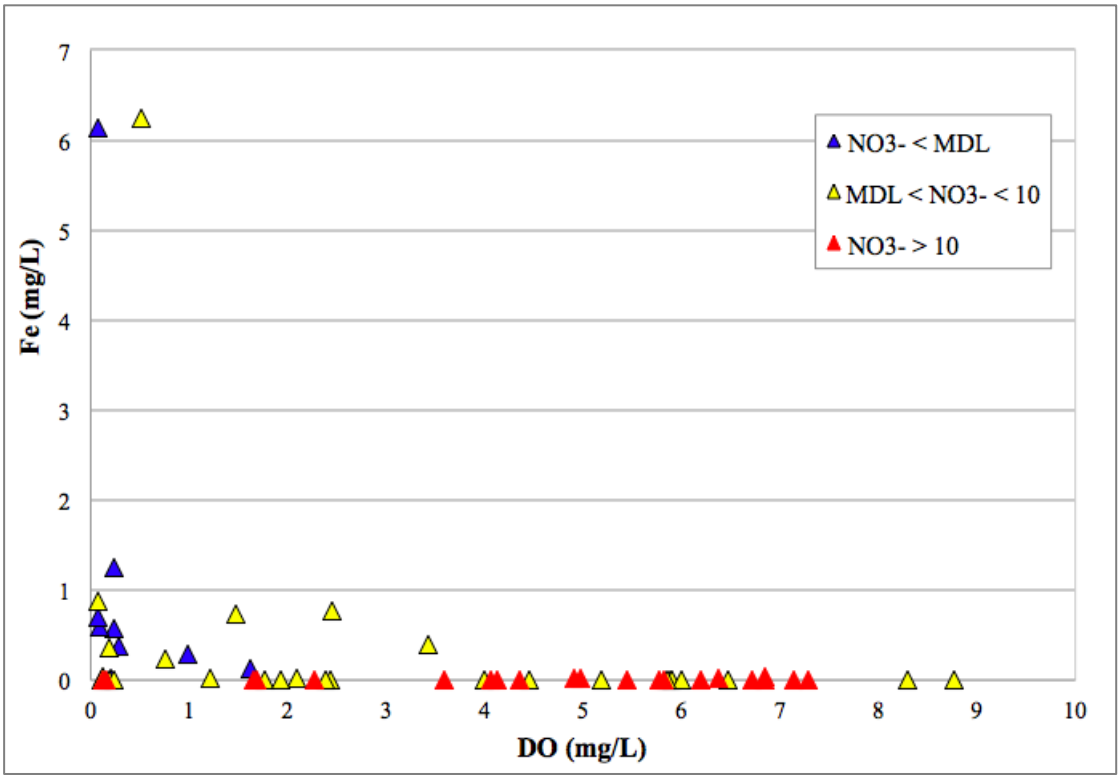



**Figure 4.7** Fe, DO, and nitrate (mgN/L) for domestic well samples from 2011.

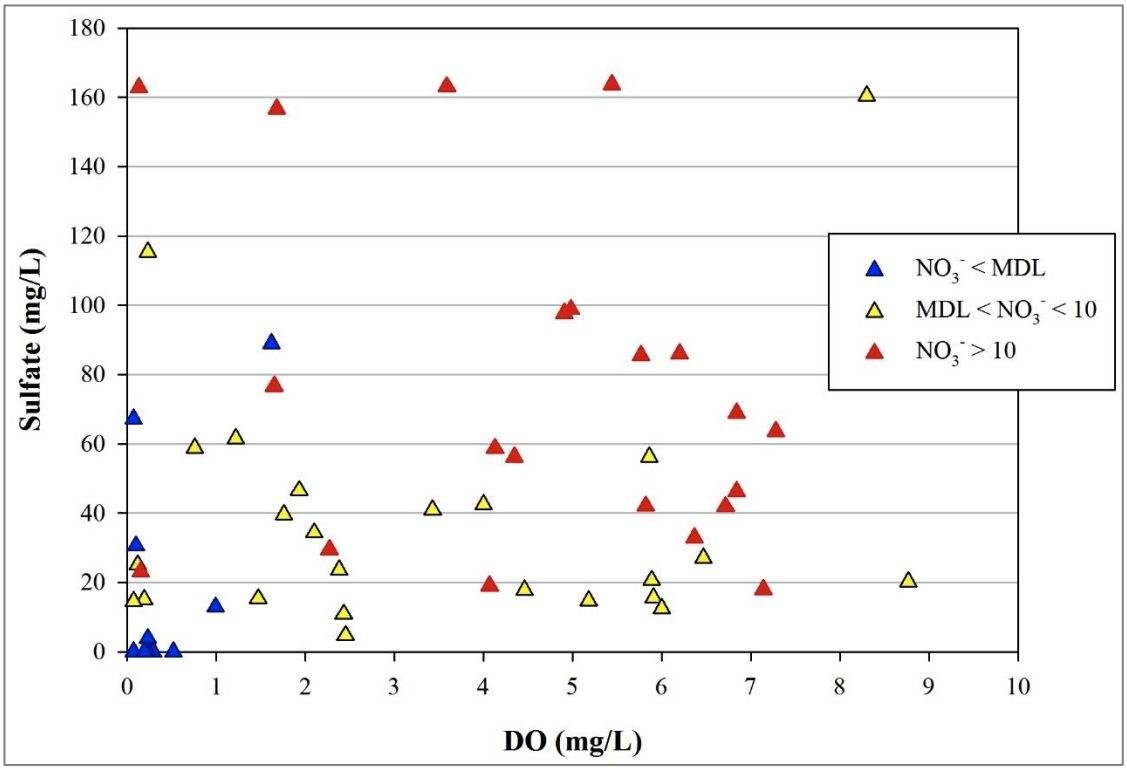



**Figure 4.8** Sulfate, DO, and nitrate (mgN/L) for domestic well samples from 2011.

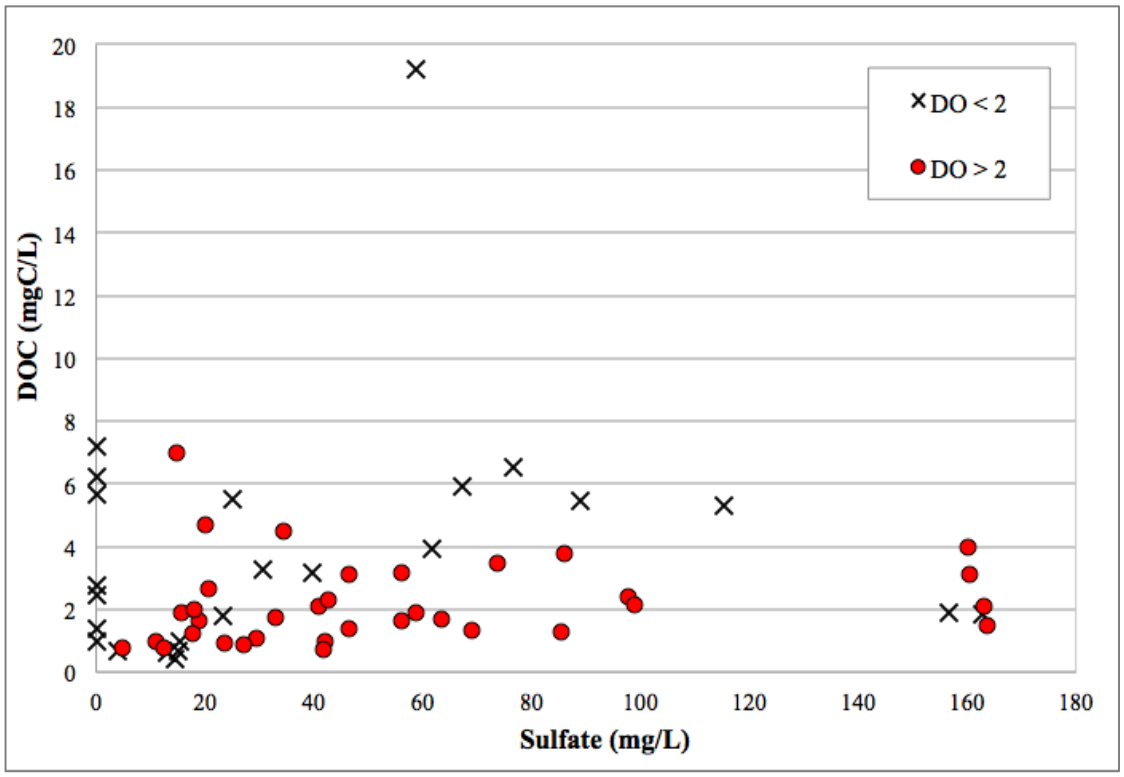



**Figure 4.9** Sulfate, DOC, and DO (mg/L) for domestic well samples from 2011.

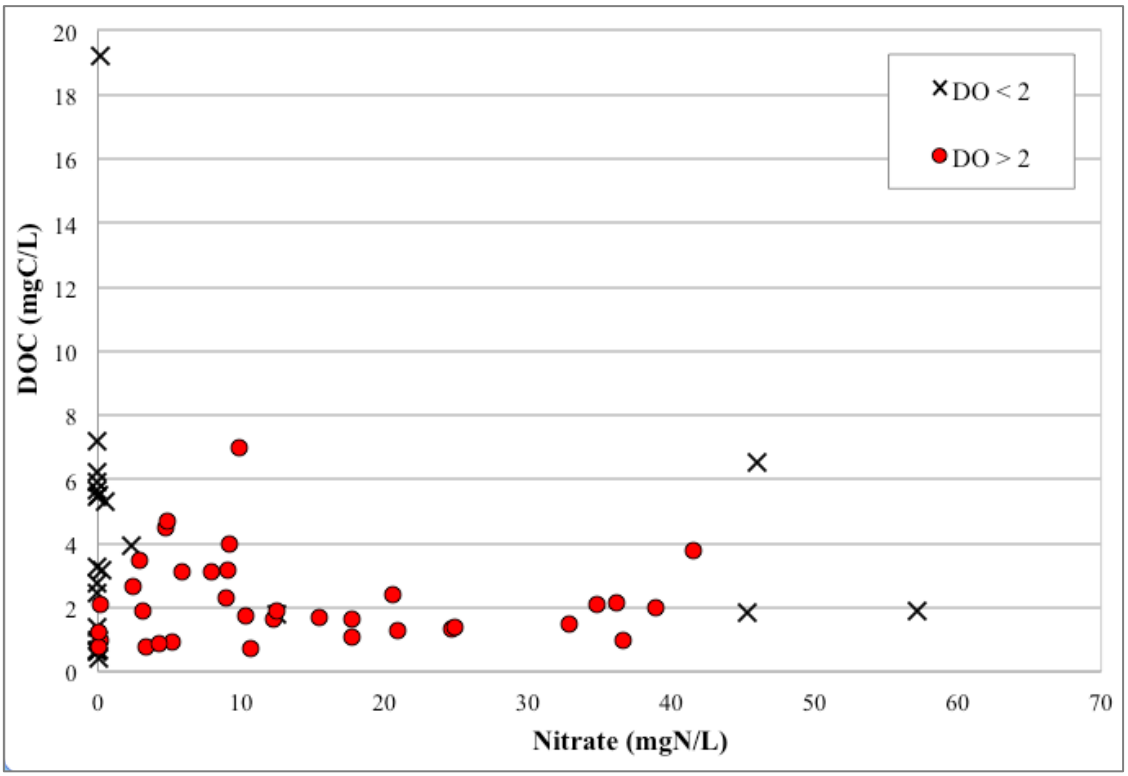



**Figure 4.10** DOC, nitrate, and DO (mg/L) for domestic well samples from 2011.

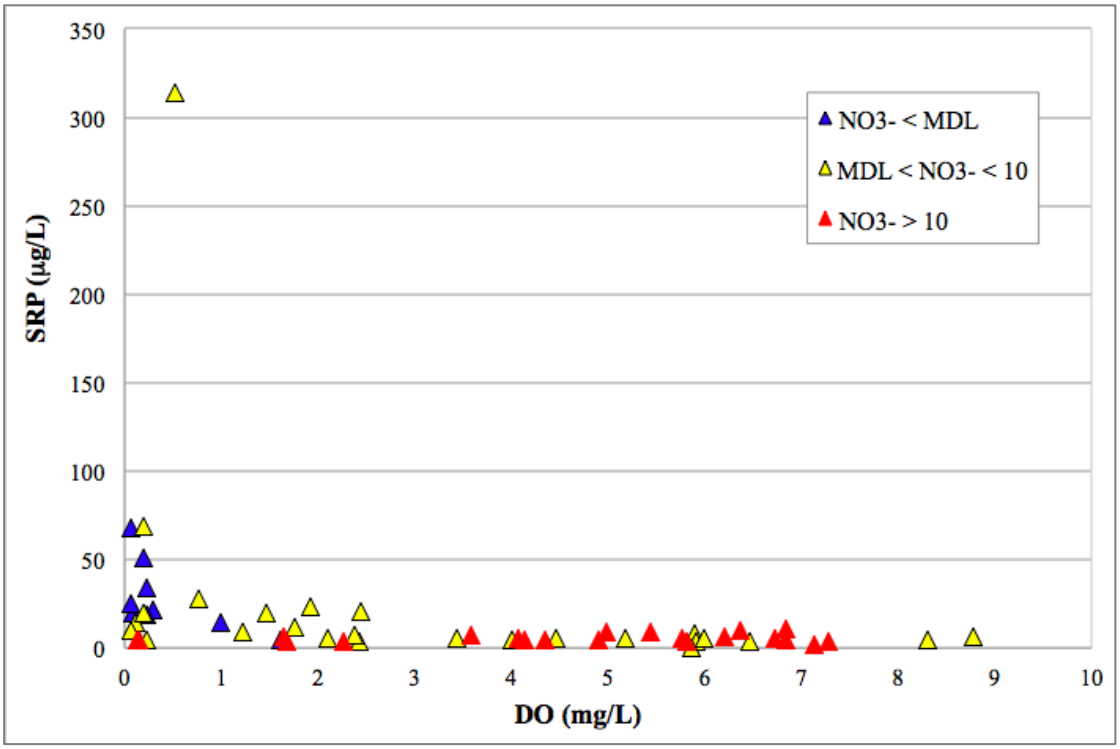



**Figure 4.11** SRP, DO, and nitrate (mgN/L) for domestic well samples from 2011.

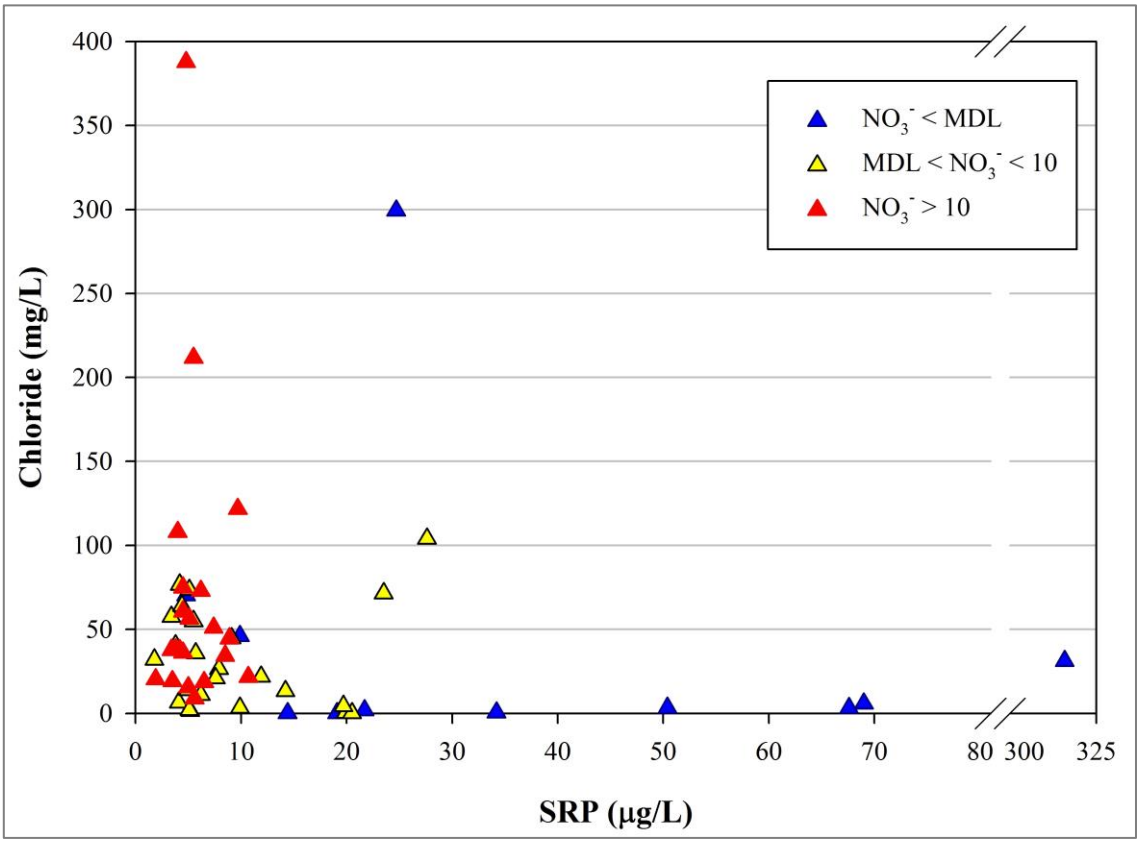



**Figure 4.12** Chloride, SRP, and nitrate (mgN/L) for domestic well samples from 2011.

### 4.3.2 Groundwater seeps

#### *4.3.2.1 Stable isotopes*

Isotopic compositions of nitrate largely plot above the signature boxes (ie. higher $\delta^{18}O$) outlined for nitrification of various sources (Figure 4.13). Similar to the domestic well samples, no samples plot within the 'nitrate fertilizer' or 'atmospheric nitrate' signature categories. Assuming $\delta^{18}O$ values have been enriched as a result of denitrification, the initial $\delta^{15}N$ values would trace back to the 'ammonium in fertilizer' source box, with few samples overlapping in 'microbial nitrate from soil N' or 'nitrified septic/manure N'. Few samples plot within these isotope composition ranges and are less likely to have undergone substantial denitrification. For all groundwater seeps and river samples, isotopic compositions of nitrate ranged from -4.8‰ to +26.7‰ for $\delta^{15}N$, and from -8.1‰ to +15.3‰ for $\delta^{18}O$. Samples with higher $\delta^{15}N$ and $\delta^{18}O$ ratios of nitrate were correlated to lower concentrations of nitrate (Figures 4.14 and 4.15). For example, when $\delta^{15}N$ >10‰, nitrate ranged from 0 to 19.2 mgN/L. In addition to this, samples with $\delta^{15}N$ <10‰, have nitrate that ranges from 0 to 47.7 mgN/L. This shows that nitrate does naturally exists at concentrations below the drinking water limit without nitrate reducing processes such as denitrification. The $\delta^{15}N$ and $\delta^{18}O$ of nitrate for samples with nitrate <10 mgN/L ranged from -4.8‰ to +26.7‰, and -8.1‰ to +15.1‰, respectively. The $\delta^{15}N$ and $\delta^{18}O$ values of nitrate for samples with nitrate >10 mgN/L ranged from +4.0‰ to +17.3‰, and from +5.7‰ to +15.3‰, respectively. No trend in nitrate and $\delta^{15}N$ of nitrate was noted with respect to the various recharge areas (Figure 4.15). River samples were well constrained for nitrate isotopes and range in $\delta^{15}N$ between +7.2‰ and +8.9‰, and $\delta^{18}O$ between +4.0‰ and +5.6‰.

A total of 35% of the 2011 groundwater seep samples (30 out of 85 samples) contained one or more artificial sweeteners (acesulfame, saccharin, cyclamate, sucralose). Concentrations of sweeteners ranged between 0.001 and 1.3µg/L (Figure 4.16), and did not correlate to nitrate, which ranged between 0 and 30 mgN/L where artificial sweeteners were present. This, however, still suggests that 35% of the groundwater seep samples have some influence of septic wastewater, whether or not this correlates to high nitrate. The TIN/ACE ratio and estimated proportions of wastewater contributing to nitrate in groundwater are summarized in Table 4.5. Samples NRS-64, NRS-85, NRS-88, and NRS-89 are estimated to have as much as 80-100% nitrate sourced from wastewater, however, DO could not be collected for groundwater seeps and so TIN/ACE ratios can only be used as a general guideline for wastewater source impact. Nitrate in the aforementioned samples is 0.2 to 4.4 mgN/L, so even if samples have not been denitrified and do dominantly consist of wastewater as a source of nitrate, the

concentration of nitrate is still below the drinking water limit and far lower than many samples in all datasets.

All river samples had the artificial sweetener acesulfame present due to the upstream presence of municipal wastewater effluent discharges. Only one sample had both nitrate >10 mgN/L and at least one artificial sweetener; the $\delta^{15}N$ and $\delta^{18}O$ values of nitrate were +6.2‰ and +7.9‰, respectively. When nitrate was <10 mgN/L and sweeteners were present, the $\delta^{15}N$ value ranged from +3.0‰ to +26.7‰, and the $\delta^{18}O$ value ranged from -8.0‰ to +15.1‰.

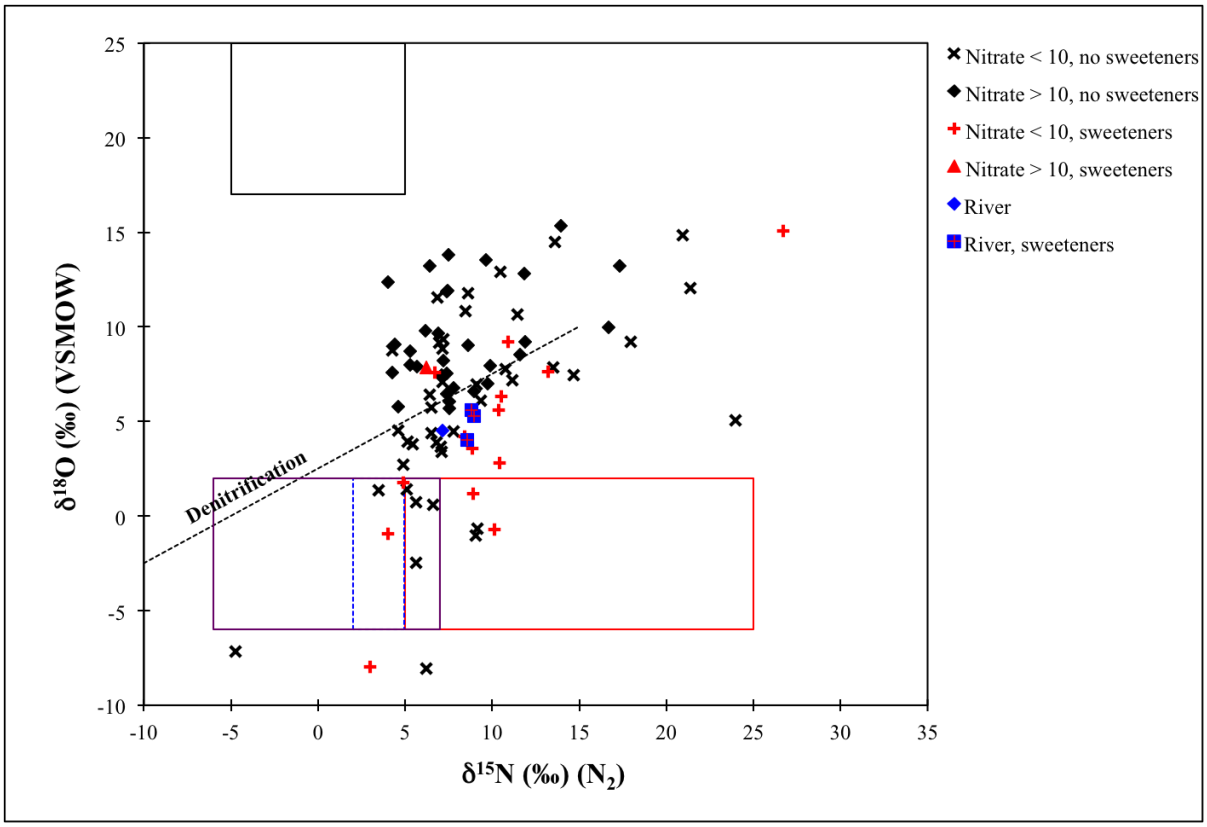



**Figure 4.13** Dual nitrate isotope plot for all groundwater seep samples collected in 2010 and 2011 with sufficient nitrate for analysis (>0.5 mgN/L). Samples are categorized based on the presence of at least one sweetener (acesulfame, saccharin, sucralose, cyclamate) and concentrations of nitrate.

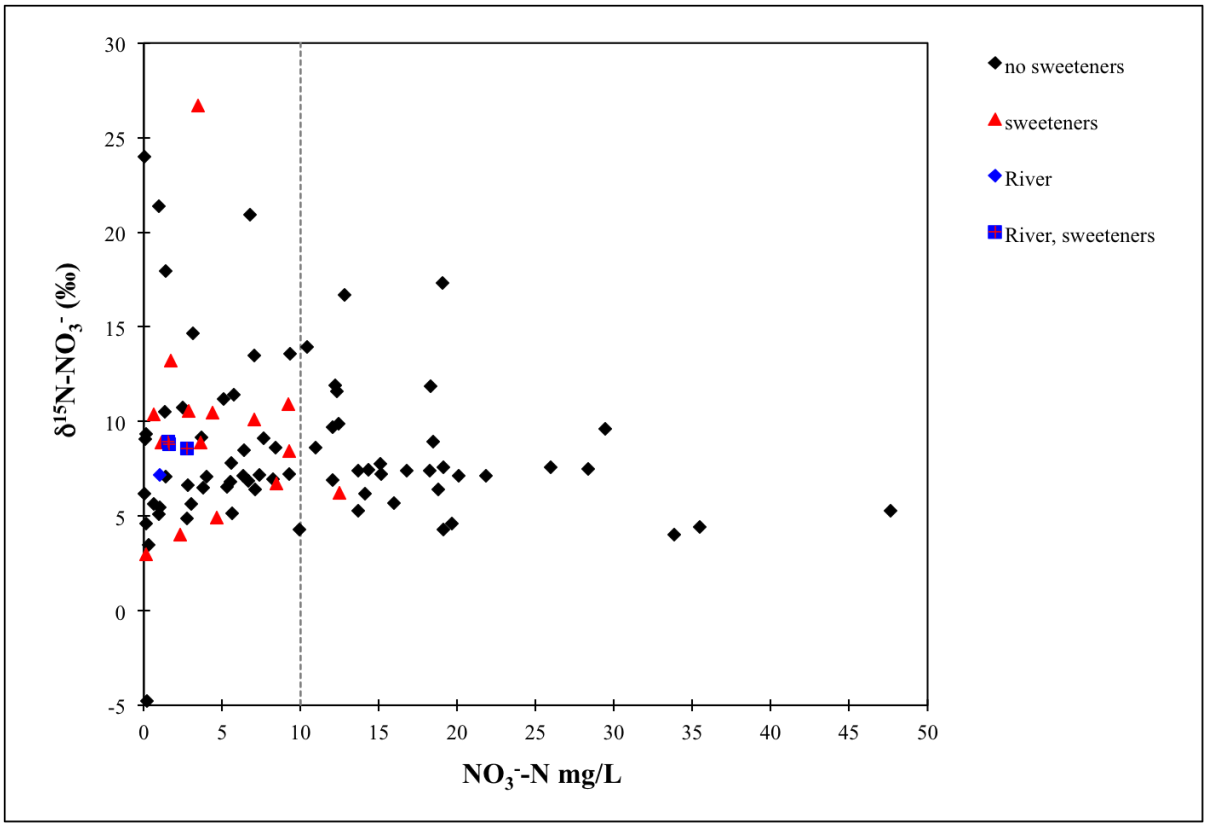



**Figure 4.14** $\delta^{15}N\text{-}NO_3^-$ and nitrate concentrations for all groundwater seep samples collected in 2010 and 2011 with sufficient nitrate for analysis (>0.5 mgN/L). Samples are categorized based on the presence of at least one sweetener (acesulfame, saccharin, sucralose, cyclamate).

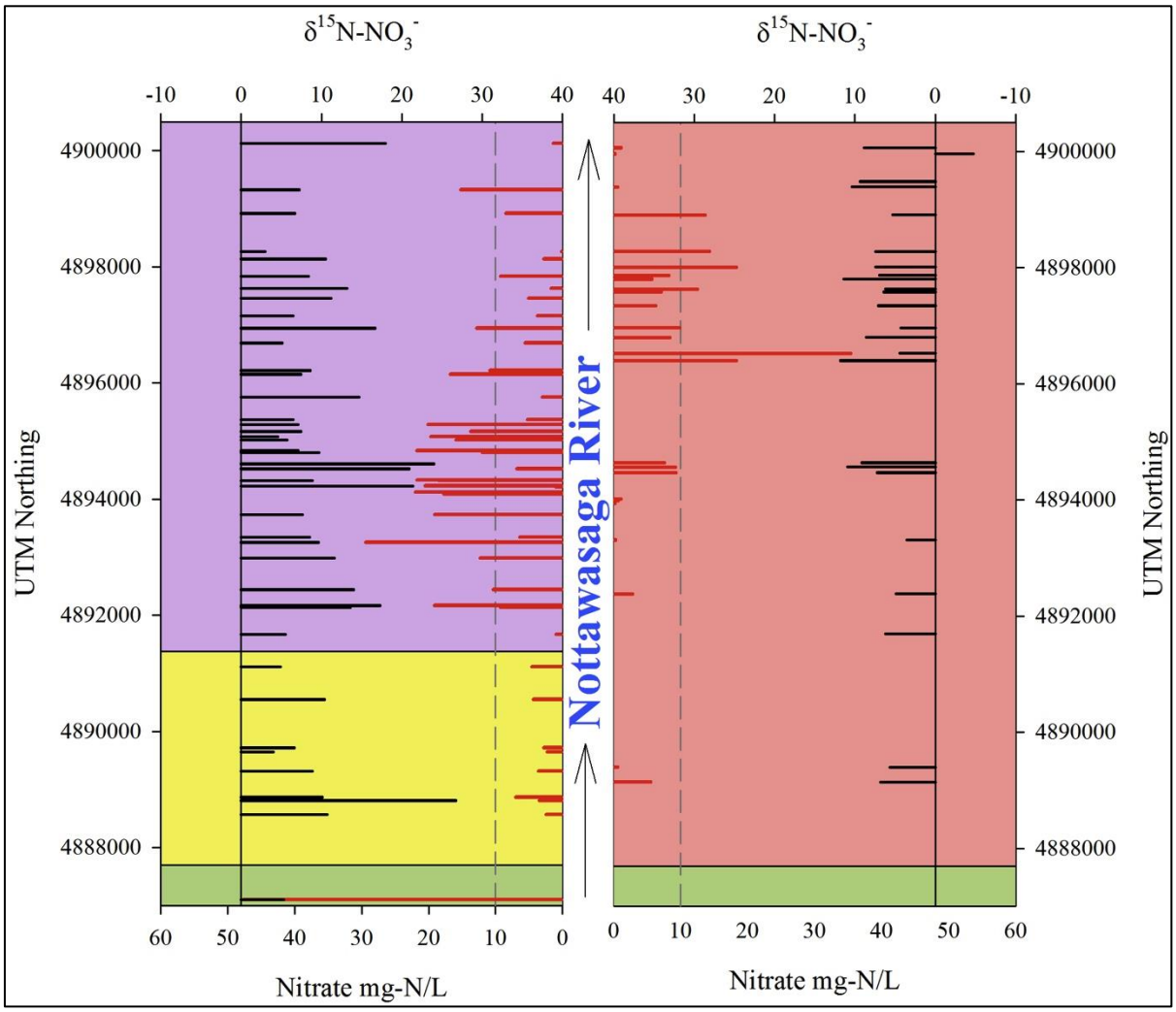



**Figure 4.15** Spatial representation of nitrate (**red**) and $\delta^{15}N\text{-}NO_3^-$ (**black**) along the Nottawasaga River for samples collected in September 2010. The various recharge areas are colored as outlined in Figure 2.3.

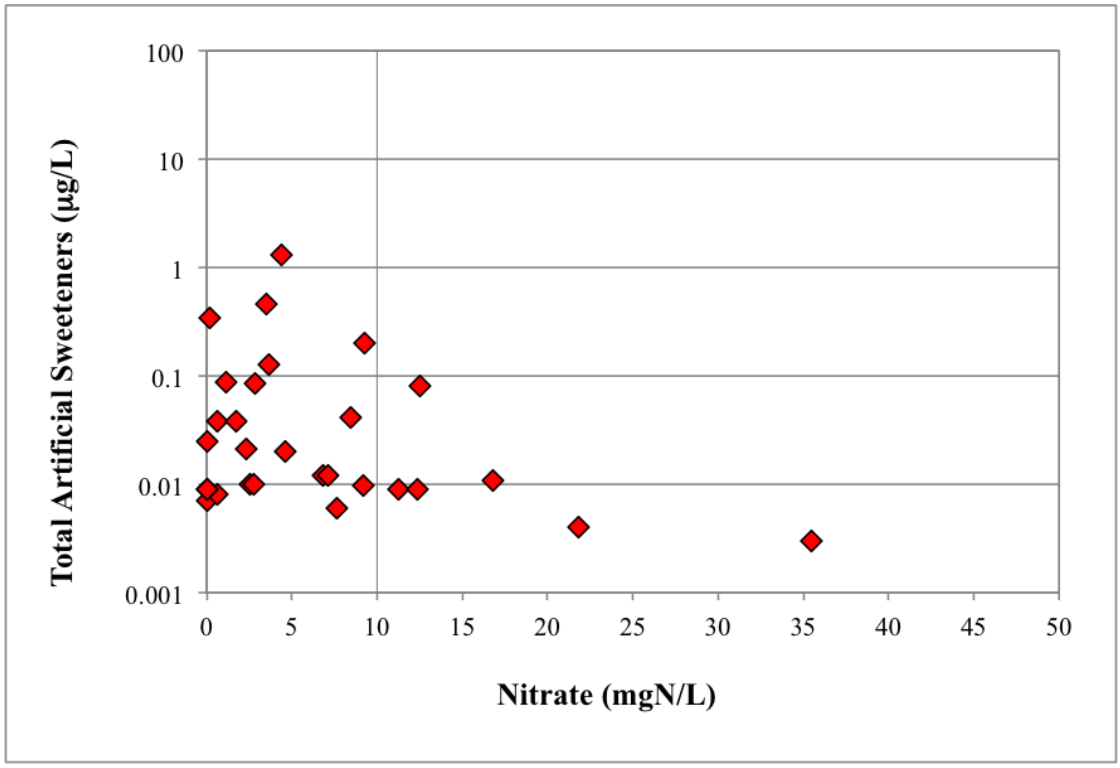



**Figure 4.16** Concentration plot of artificial sweeteners and nitrate for all measured values in 2011 groundwater seep samples. Only samples with measurable concentrations of one or more artificial sweeteners are plotted here, which accounts for a total of 30 samples out of 85 samples. Sweetener concentrations are summed to give a total concentration of cyclamate, saccharin, acesulfame, and sucralose. Ranges of nitrate and presence of artificial sweeteners are outlined in Figure 4.14.

**Table 4.5** Estimates of septic wastewater proportion in domestic well samples collected in 2011, based on concentrations of acesulfame (ACE) and dissolved inorganic nitrogen (DIN). Contribution to nitrate is expressed as a percentage and assumes nitrate removal processes are not prevalent (Robertson, et al., 2016). Note: DO was not measured in groundwater seeps.

| Sample ID | $NO_3^-$ mgN/L | TIN mg/L | Acesulfame µg/L | TIN/ACE | Waste water $NO_3^-$-N (%) |
|---|---|---|---|---|---|
| NRS-13 | 4.6 | 4.8 | 0.02 | 240000 | 0-1 |
| NRS-57 | 1.7 | 1.7 | 0.039 | 44000 | 2-8 |
| NRS-59 | 9.3 | 9.3 | 0.203 | 45800 | 1-8 |
| NRS-62 | 2.8 | 2.9 | 0.027 | 107000 | 1-3 |
| NRS-64 | 0.2 | 0.3 | 0.068 | 4400 | 15-80 |
| NRS-67 | 8.5 | 8.5 | 0.042 | 202000 | 0-2 |
| NRS-70 | 0.6 | 0.6 | 0.038 | 16000 | 4-22 |
| NRS-73 | 1.1 | 1.1 | 0.087 | 13000 | 5-27 |
| NRS-76 | 0 | 1.1 | 0.025 | 44000 | 2-8 |
| NRS-78 | 3.5 | 3.5 | 0.456 | 7700 | 9-45 |
| NRS-81 | 3.6 | 3.6 | 0.128 | 28000 | 2-13 |
| NRS-83 | 2.3 | 2.4 | 0.021 | 114000 | 1-3 |
| NRS-85 | 4.4 | 4.5 | 1.32 | 3400 | 20-100 |
| NRS-88 | 3.0 | 3.1 | 1.215 | 2600 | 26-100 |
| NRS-89 | 3.7 | 3.7 | 0.967 | 3800 | 18-92 |
| NRS-109 | 5.6 | 5.6 | 0.081 | 69000 | 1-5 |

*4.3.2.2 Geochemistry*

Ternary cation molarity diagrams for groundwater seeps (Figure 4.17) had similar results to the domestic wells; samples with high nitrate samples having high calcium and magnesium relative to sodium and potassium. Samples with low nitrate have a larger range of magnesium and generally have more sodium and potassium. This similarly suggests that groundwater that has high nitrate is younger, based on the geochemical evolution expected with residence time in the aquifer. Artificial sweeteners do no show a valuable trend in their geochemical trend and are not distinctly related to groundwater age.

To assess the redox conditions of groundwater seep samples, Fe, sulfate, SRP, DOC, and chloride were used. As DO was not collected in this dataset, inferences regarding redox geochemistry from the domestic wells is used to guide interpretations. The relationship of sulfate and Fe was unclear; when Fe was above zero, corresponding sulfate concentrations ranged considerably, showing no clear trend (Figure 4.18). However, nitrate showed a general linear relationship with sulfate. Sulfate and SRP had a broad inverse relationship (Figure 4.19) showing that when sulfate is low and SRP is high, reducing conditions exist. However, this was only true for one sample out of the whole dataset. Samples with Fe that did not have elevated SRP had considerable nitrate and are not categorized as being of reducing conditions. When SRP was high, Fe was present, and nitrate was not, conditions are interpreted as being highly reducing (Figure 4.20). However, measurement of high SRP and Fe alone cannot guarantee reducing conditions exist. These results are consistent with conditions outlined in the Boyne River riparian zone where increased SRP and elevated iron exist where buried channel deposits act as reducing sites for groundwater (Carlyle & Hill, 2001). Chloride and SRP were not correlated and the one sample with high SRP, interpreted as being reduced, had considerable chloride and potentially had higher nitrate before reduction (Figure 4.21). Finally, DOC and nitrate do not have a clear relationship, and low nitrate correlates to a range of DOC concentrations (Figure 4.22).

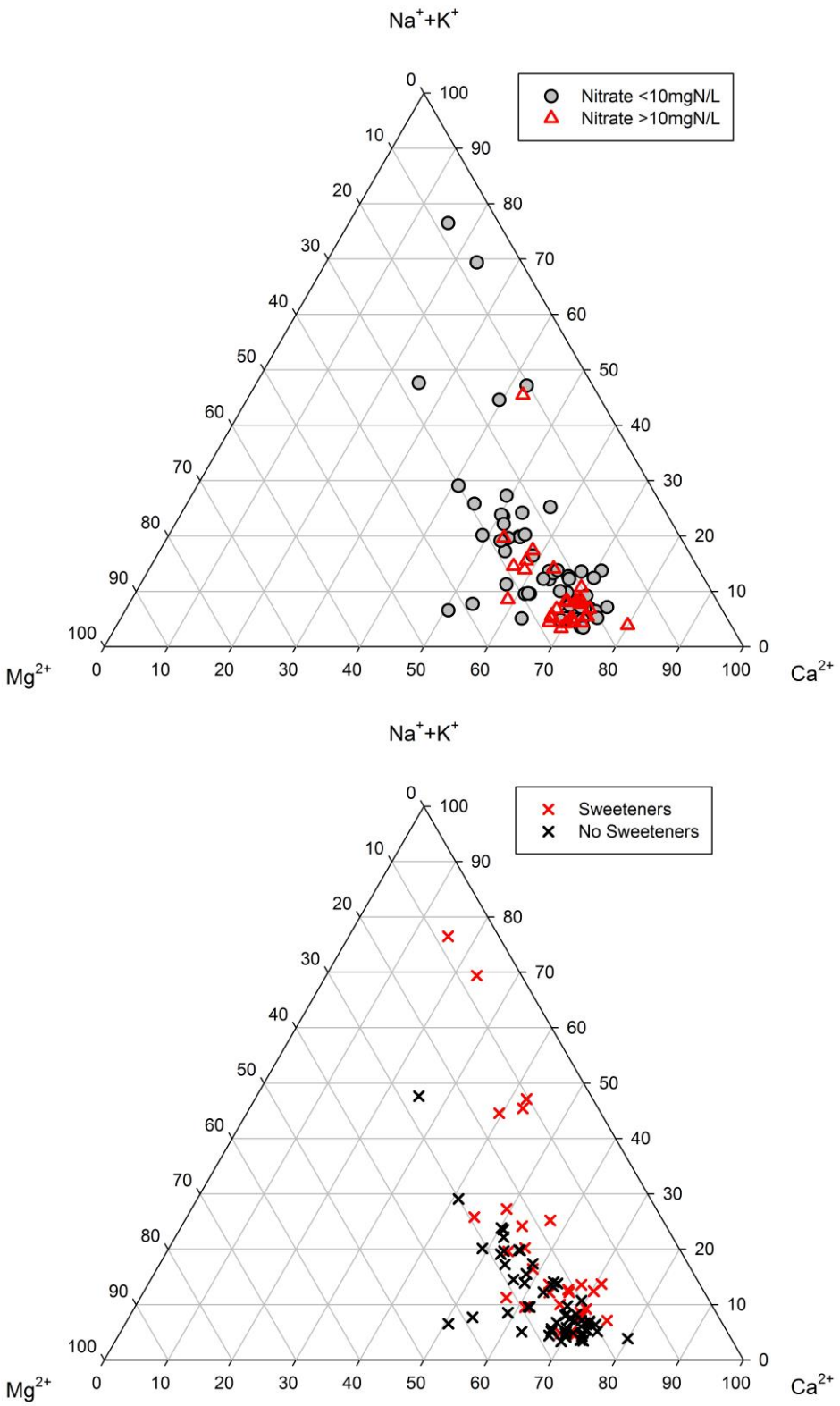



**Figure 4.17** Ternary cation plots for groundwater seeps from 2010.

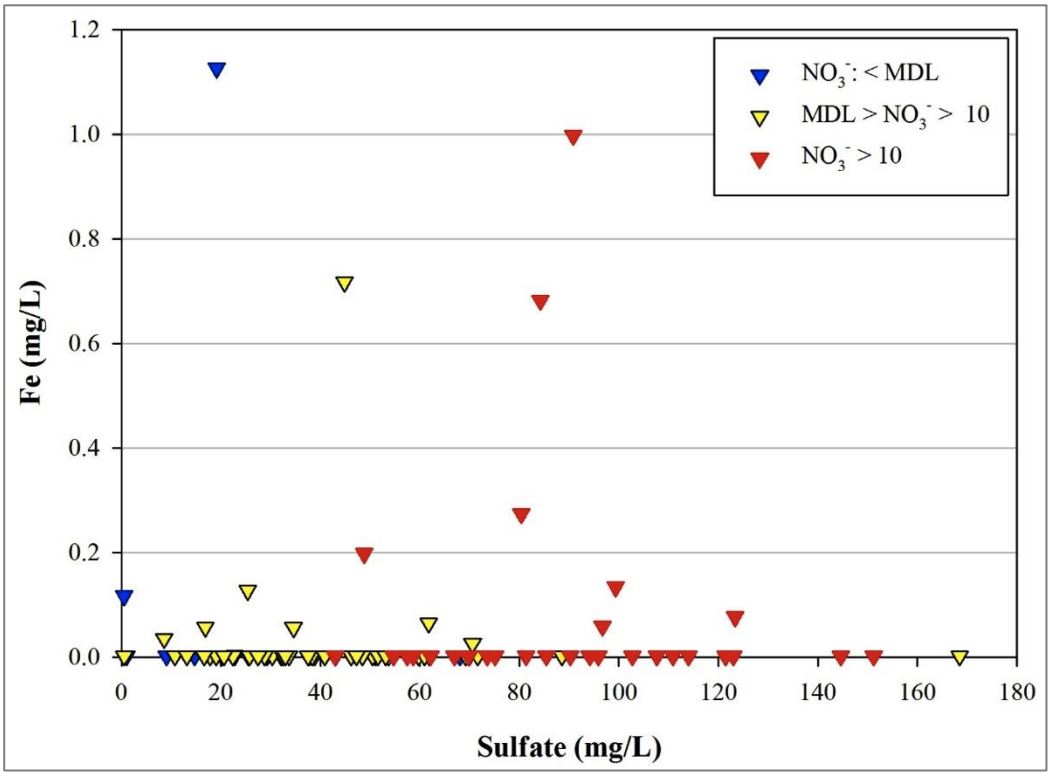



**Figure 4.18** Iron, sulfate, and nitrate (mgN/L) for groundwater seeps collected in 2010.

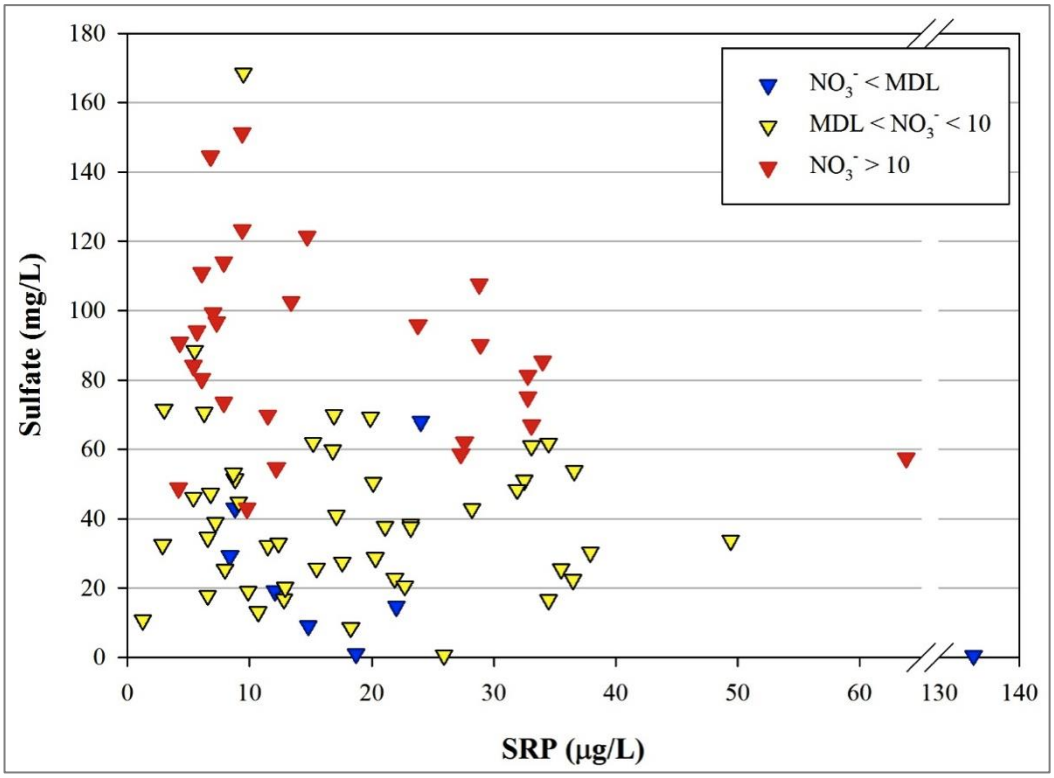



**Figure 4.19** Sulfate SRP, and nitrate (mgN/L) for groundwater seep samples collected in 2010.

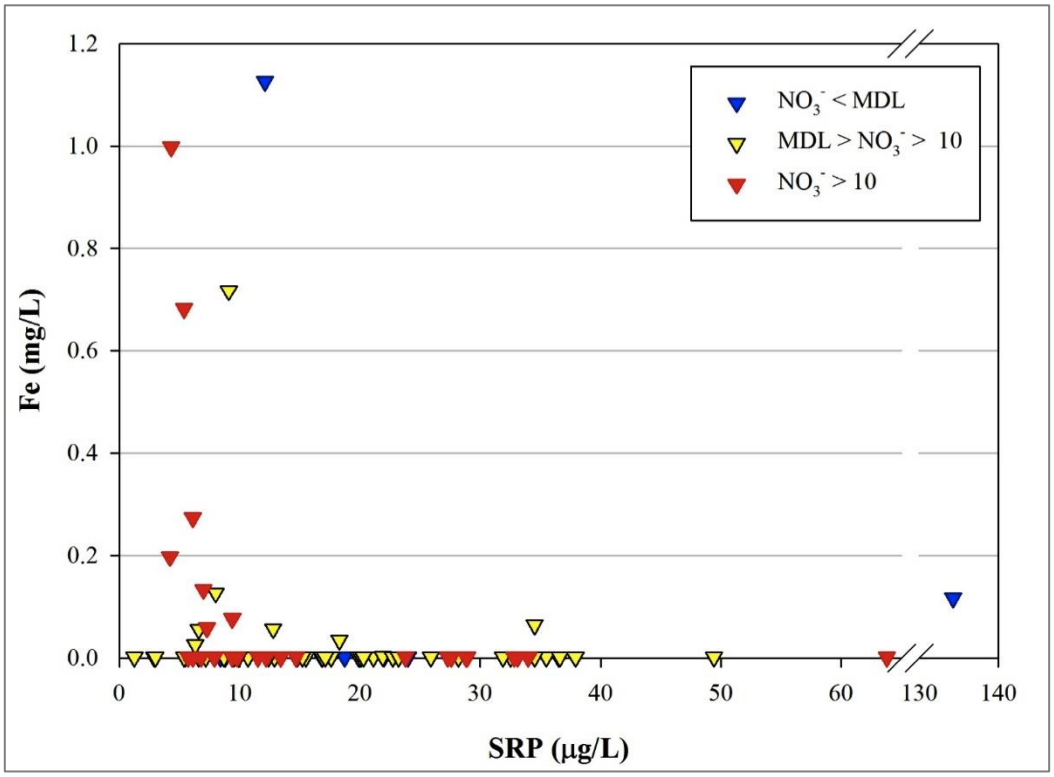



**Figure 4.20** Iron, SRP, and nitrate (mgN/L) for groundwater seep samples collected in 2010.

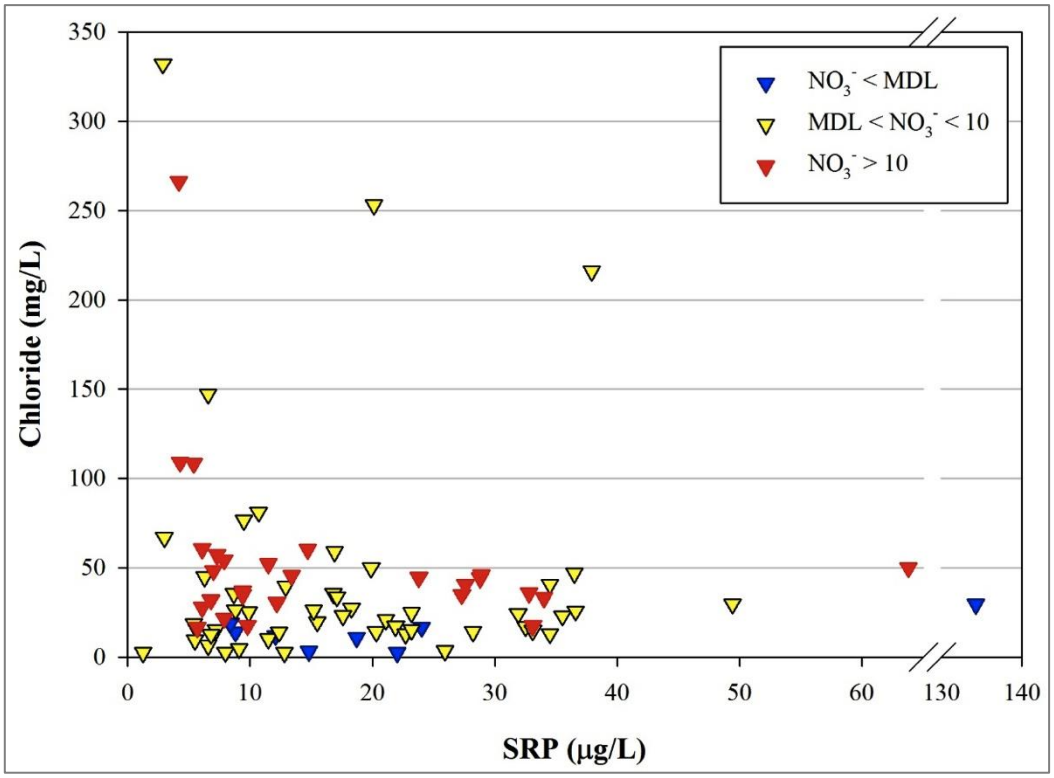



**Figure 4.21** Chloride, SRP, and nitrate (mgN/L) for groundwater seeps collected in 2010.

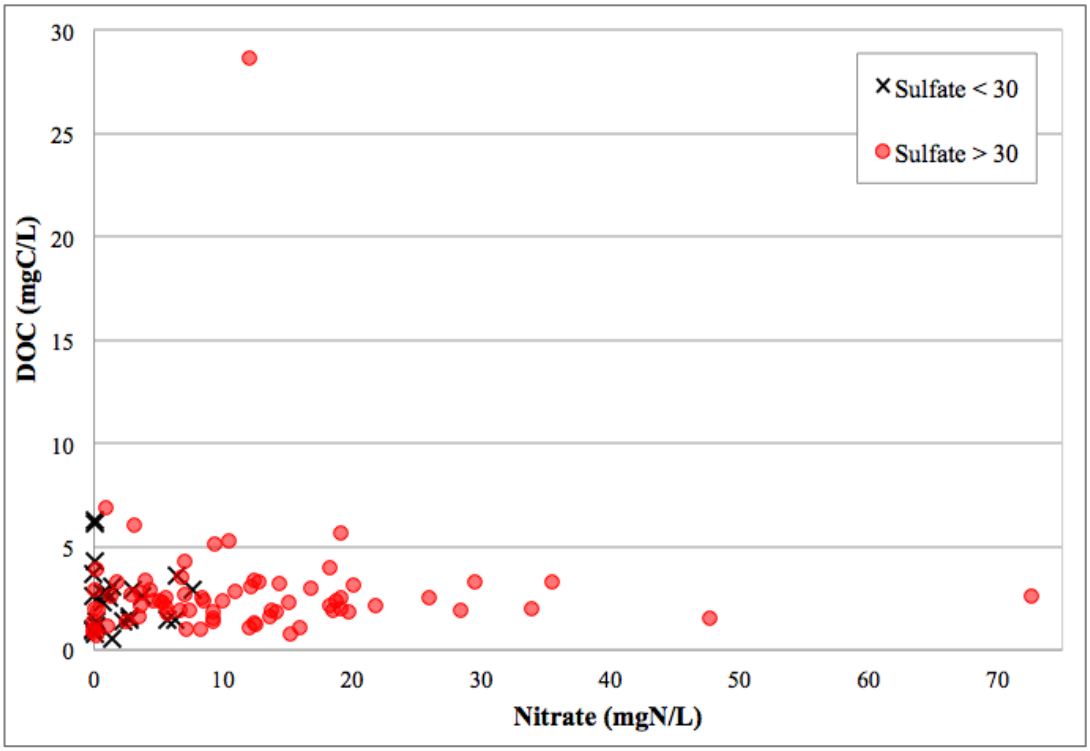



**Figure 4.22** DOC, nitrate, and sulfate (mg/L) for groundwater seeps collected in 2010.

4.3.3 Multi-level profiles

*4.3.3.1 Hydrogeology*

Head measurements (Figure 4.23) have been used to construct velocity and time estimates of groundwater flow subparallel to the flow direction (Table 4.6). Vertical hydraulic gradients for samples collected in April 2011 for MC site are also summarized (Table 4.7). Within each piezometer bundle, head measurements showed minor fluctuations, likely caused by the heterogeneity of aquifer material or the undulating aquitard in the LASA, such as that summarized by Sibul and Choo-Ying (1971). The range of vertical hydraulic gradients was minimal, but identified a common downward gradient nearing the base of the aquifer. Vertical gradients were greatest at MC6, which is located closest to the Upper Nottawasaga River, consistent with the groundwater contour map provided by Hill (1982) that showed the groundwater table decreasing with closer proximity to the Nottawasaga River and its main tributaries. Sample tubes with lower head than the surrounding points may highlight horizons with greater permeability, possibly consisting of slightly coarser sands than what is present in the adjacent horizons.

Minimum and maximum groundwater velocities, (Table 4.6), have been calculated using hydraulic conductivities that have been measured by previous researchers in the LASA (Devito et al., 2000; MacFarlane et al., 1983; Sibul and Choo-Ying, 1971). The time for groundwater to travel between multi-level wells stations sub-parallel to groundwater flow (MC2 to MC4, and W4 to W5) are calculated. Groundwater flow from MC2 to MC4 was calculated as being the fastest, with a maximum velocity of 89.7 m/yr. Flow between MC2 and MC4 are parallel to the actual groundwater flow direction (Figure 2.5), whereas Flow between W4 and W5 also provided a fairly high velocity, 71.8 m/year, and the trajectory of flow likely was sub-parallel to actual groundwater flow.

Groundwater flow in this part of the LASA, south of the Upper Nottawasaga River, likely has characteristics that reflect velocities closer to the $v_{max}$ in Table 4.6. High banks along the river expose profiles of the aquifer material, which was commonly fine to medium grains sands, with minor horizons of finer loamy sand. However, heterogeneities did occur as lenses of both more and less permeable materials, such as gravels and silt/clay lenses, and therefore flow would be altered accordingly. Overall, the velocities of groundwater flow summarized in Table 4.6, are a guide for estimating groundwater travel in this area.

**Table 4.6** Groundwater velocity and transit time calculations between multi-level wells stationed sub-parallel to the direction of groundwater flow.

| **From** | **To** | **Δh (mbgs)** | **Δl (mbgs)** | **i (Δh/Δl)** | **$v_{min}$** (m/year) | **$v_{max}$** (m/year) | **Minimum time (years)[1]** | **Maximum time (years)[2]** |
|---|---|---|---|---|---|---|---|---|
| MC2 | MC4 | 2.66 | 265 | 0.010 | 0.45 | 90 | 591 | 3 |
| W4 | W5 | 2.2 | 264 | 0.008 | 0.36 | 72 | 736 | 4 |

[1]: Minimum time was calculated using a hydraulic conductivity of 0.043 and an effective porosity of 0.35, based on measurements from MacFarlane et al. (1983) in silty fine-grained sand in the LASA.
[2]: Maximum time was calculated using a hydraulic conductivity of 8.6 and an effective porosity of 0.35, based on measurements from MacFarlane et al. (1983) in fine and medium grained sand in the LASA.

**Table 4.7** Vertical hydraulic gradients ($i_v$) for MC site piezometer bundles sampled in the spring of 2011, where $i_v$ is calculated from the change in hydraulic head ($\Delta h$) divided by the difference in elevation head ($\Delta z$); ($i_v = \Delta h/\Delta z$) (Fetter, 1994).

| From | To | Δh (m) | Δz (m) | $i_v$ |
|---|---|---|---|---|
| MC2 3.51 | MC2 4.34 | -0.040 | 0.890 | -0.045 |
| MC2 4.34 | MC2 5.13 | 0.090 | 0.740 | 0.122 |
| MC2 5.13 | MC2 5.94 | 0.000 | 0.890 | 0.000 |
| MC2 5.94 | MC2 6.68 | -0.110 | 1.000 | -0.110 |
| MC2 6.68 | MC2 8.46 | 0.360 | 1.450 | 0.248 |
| MC2 8.46 | MC2 9.90 | -0.240 | 1.520 | -0.158 |
| MC2 9.90 | MC2 11.26 | 0.840 | 1.490 | 0.564 |
| | | | | |
| MC4 6.34 | MC4 7.34 | 0.060 | 1.030 | 0.058 |
| MC4 7.34 | MC4 8.33 | -0.050 | 0.990 | -0.051 |
| MC4 8.33 | MC4 9.05 | -0.030 | 1.000 | -0.030 |
| MC4 9.05 | MC4 10.58 | 0.210 | 1.420 | 0.148 |
| MC4 10.58 | MC4 12.22 | 0.390 | 1.490 | 0.262 |
| MC4 12.22 | MC4 13.72 | -0.370 | 1.590 | -0.233 |
| | | | | |
| MC6 7.28 | MC6 8.49 | 0.350 | 0.890 | 0.393 |
| MC6 8.49 | MC6 9.25 | 0.230 | 1.120 | 0.205 |
| MC6 9.25 | MC6 11.68 | 1.550 | 2.060 | 0.752 |
| | | | | |
| MC7 6.82 | MC7 8.11 | 0.150 | 1.210 | 0.124 |

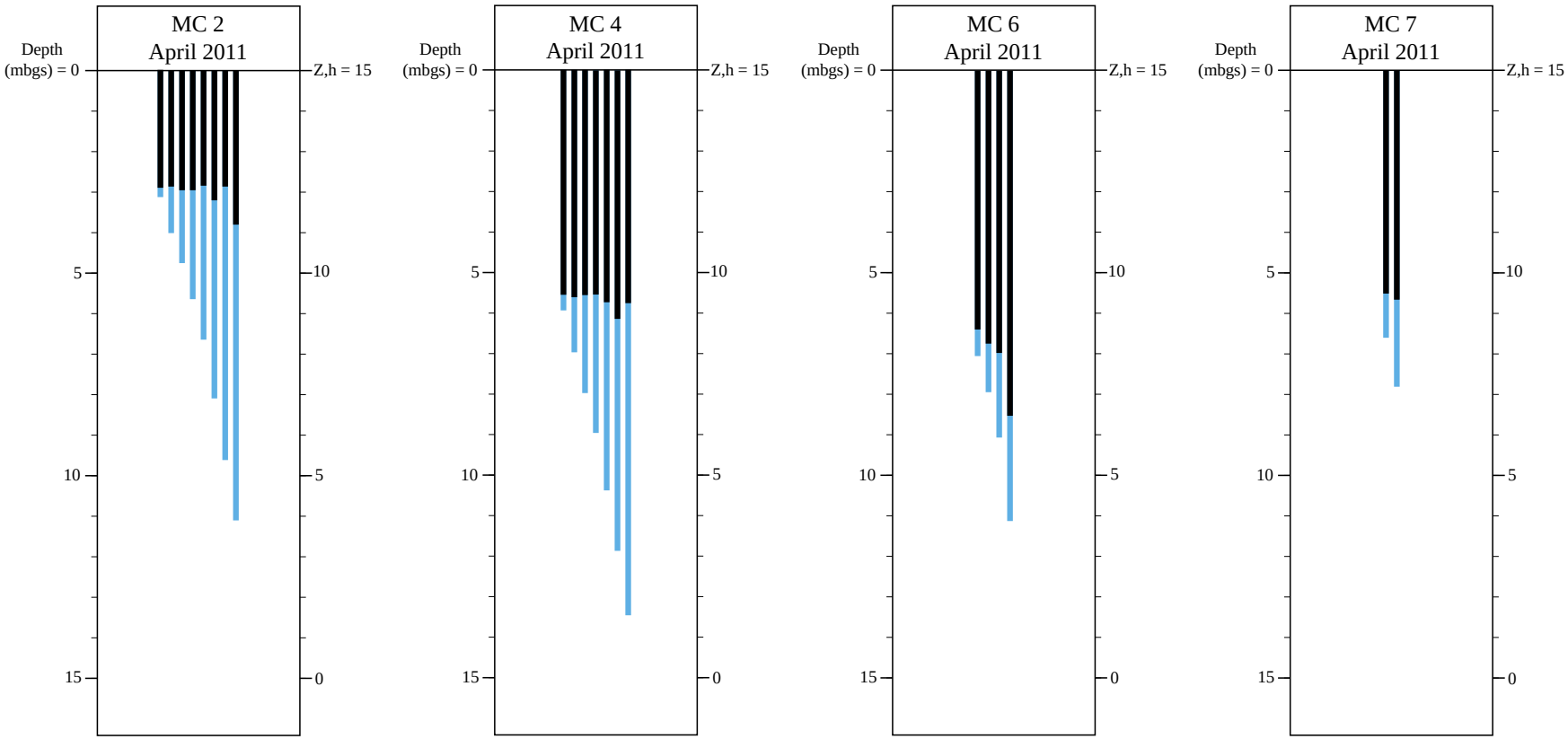



**Figure 4.23** Detailed measurements of hydraulic head (h) and elevation head (Z) for MC site piezometers, in April 2011. Blue represents the hydraulic head. Depth of the saturated zone and piezometer screen intake depth (bottom of blue line) can be measured using the left hand scale of each figure, whereas the elevation head and the hydraulic head are indicated with the scale along the right hand side. Topography of the LASA in this area is extremely flat and well locations are plotted as having the same elevation.

*4.3.3.2 Stable isotopes*

4.3.3.2.1 MC Site

Nitrate in MC2 was high and commonly well above 10 mgN/L in samples collected down to a depth of 8.5 mbgs (Figure 4.24). Below this, nitrate drastically decreased to near negligible concentrations. Stable isotope ratios of nitrate gradually increased with depth, where nitrate was high, but could not be measured for the bottom two samples due to insufficient nitrate. At the top of the profile, 3.5 mbgs, nitrate was lower (3.7 mgN/L, April) compared to the rest of the samples in the top of the profile. Nitrate isotope ratios were also lower than the rest of the profile, $\delta^{15}N$ and $\delta^{18}O$ of nitrate of +1.5‰ and -5.9‰, respectively. The $\delta^{2}H$ and $\delta^{18}O$ values of water were distinctly low for this depth, -100.2‰ and -14.5‰, respectively. Between 4.3 mbgs and 8.5 mbgs, nitrate ranged between 30.2 and 72.9 mgN/L, and samples at 4.3 mbgs, 5.1 mbgs, and 5.9 mbgs had a greater range in concentration than the deeper samples at 6.7 mbgs and 8.5 mbgs. Throughout this section of the profile, isotopic ratios of nitrate gradually increase with depth. Stable isotopes of water are consistent between 4.3 mbgs and 5.9 mbgs, and increase at 6.7 mbgs. This trend is consistent with both sample dates. From 6.7 to 9.9 mbgs, nitrate decreases substantially, and isotope ratios of water are constant. At 11.3 mbgs, water isotope ratios decreased, minimally but consistently for both sample dates.

Trends in nitrous oxide isotopic compositions are not correlated to nitrate or isotopic ratios of either nitrate or water. The pattern of $\delta^{18}O$-$N_2O$ values correlate to that of $\delta^{15}N$-$N_2O$ values between 5.9 mbgs and 8.5 mbgs, but is distinctly different at 4.3 mbgs. At this depth, the $\delta^{18}O$-$N_2O$ value is higher than the next sample, but the $\delta^{18}O$-$N_2O$ value is considerably lower. Samples at 5.9 mbgs, 6.7 mbgs, and 8.5 mbgs were well constrained for $\delta^{15}N$ and $\delta^{18}O$ of nitrous oxide, and ranged between -20.6‰ and -+26.3‰ for $\delta^{15}N$, and +30.3‰ and +39.6‰ for $\delta^{18}O$.

The first sample in the profile (3.5 mbgs, April) is likely derived from spring recharge derived from snow from the overlying fields. Stable isotopes of water support this with distinctly lower values than the rest of the profile, which is consistent with snowmelt water. Samples between 4.3 mbgs and 9.9 mbgs have very high nitrate and have isotopic signatures consistent with soil ammonium and nitrified septic/manure N, with a few samples that overly the in the 'ammonium in fertilizer' source area. Stable isotopes of water suggest two separate packages of source waters exist between 4.3 mbgs and 9.9 mbgs, which are divided between the 5.9 mbgs and 6.7 mbgs sample points. If this is accurate, the drastic decrease in nitrate at 9.9 mbgs is more likely to be a result of nitrate removal rather than a change in source. The general increase in nitrate isotope ratios down profile is not attributed to denitrification of the same package of water but perhaps of a different source area that has a different nitrate isotope

composition. The isotopic ratio of water at 11.3 mbgs is consistent for both sample dates, it is thought that this sample again is derived from a different source than the overlying water column.

In MC4, nitrate was high (>10 mgN/L) to a depth of 10.6 mbgs, and coincided nitrate isotope ratios that vary only slightly in their signature (Figure 4.25). Below 10.6 mbgs, nitrate was much lower (0 to 5.5 mgN/L) and has isotopic ratios that much higher than the sample points higher in the profile. Isotopic ratios of water gradually decreased with depth, then abruptly increased between 8.3 mbgs and 9.1 mbgs before decreasing again with depth. This change in isotopic composition could be indicative of a seasonal change in recharge water, similar to that outlined in MC2. The dual nitrate isotope plot for MC4 samples had a ratio of $\delta^{18}O$ to $\delta^{15}N$ of 0.35. Although this ratio is slightly lower than what is expected for denitrification, it is likely due to the signature of the overlying sample points, which likely aren't from the same source as those at depth, therefore they would have a slightly different isotopic signature. The $\delta^{18}O$ values of nitrate were almost all higher than what was expected and either suggest that some denitrification has increased the isotopic composition, even at the top of the profile, or that $\delta^{18}O$ values of nitrate are higher than what was expected. Nitrous oxide isotope ratios could only be measured at 9.1 mbgs and 10.6 mbgs. The $\delta^{15}N$ value of nitrous oxide ranged between -20.6‰ and -22.8‰, and $\delta^{18}O$ range between +30.4‰ and +32.7‰. Isotope effects of denitrification ($\boldsymbol{\varepsilon_{N20\text{-}NO3}}$) are well constrained and range from -24.3‰ to -28.3‰ for $\delta^{15}N$ and from +26.2‰ to +28.0‰ for $\delta^{18}O$ (Table 4.8). For $\delta^{15}N$, these values are within range for isotope effects of denitrification calculated by Snider et al., (2012) (-30‰ to -9‰). The isotope effects of $\delta^{18}O$ of denitrification are slightly lower than those calculated by Snider et al. (32‰ to 60‰).

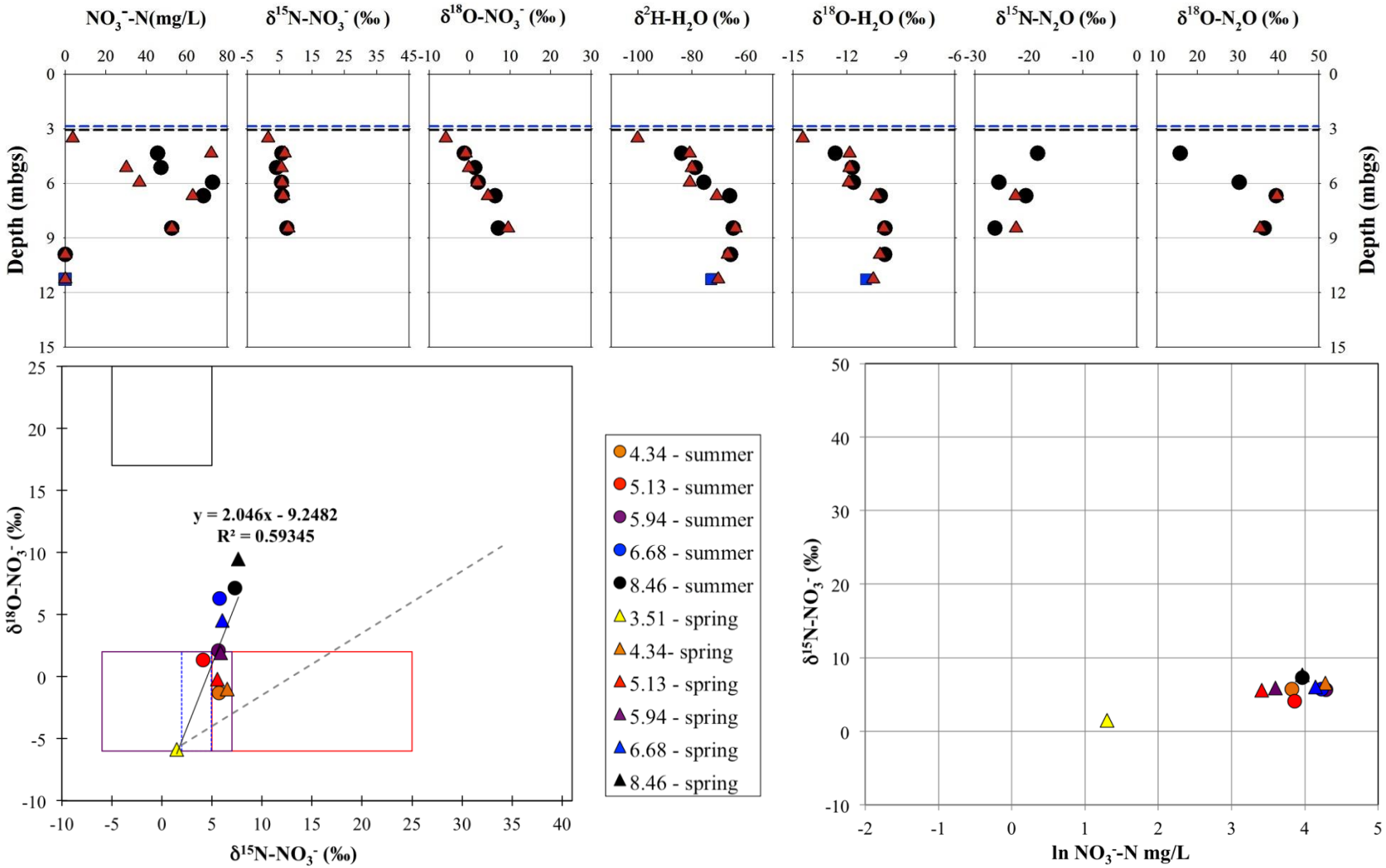



**Figure 4.24** MC2 nitrate and stable isotope behaviour. Top: Black circles are from summer 2010, blue squares from the late fall 2010, and red triangles from spring 2011. Bottom left: Dual nitrate isotope plot, boxes indicate expected ranges for source types as inidicated by previous studies, as oulined in Figure 2.4, and the grey dashed line outlines the increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ at a ratio of 2:1 for denitrification. Bottom right: $\delta^{15}N\text{-}NO_3^-$ vs. ln nitrate plot.

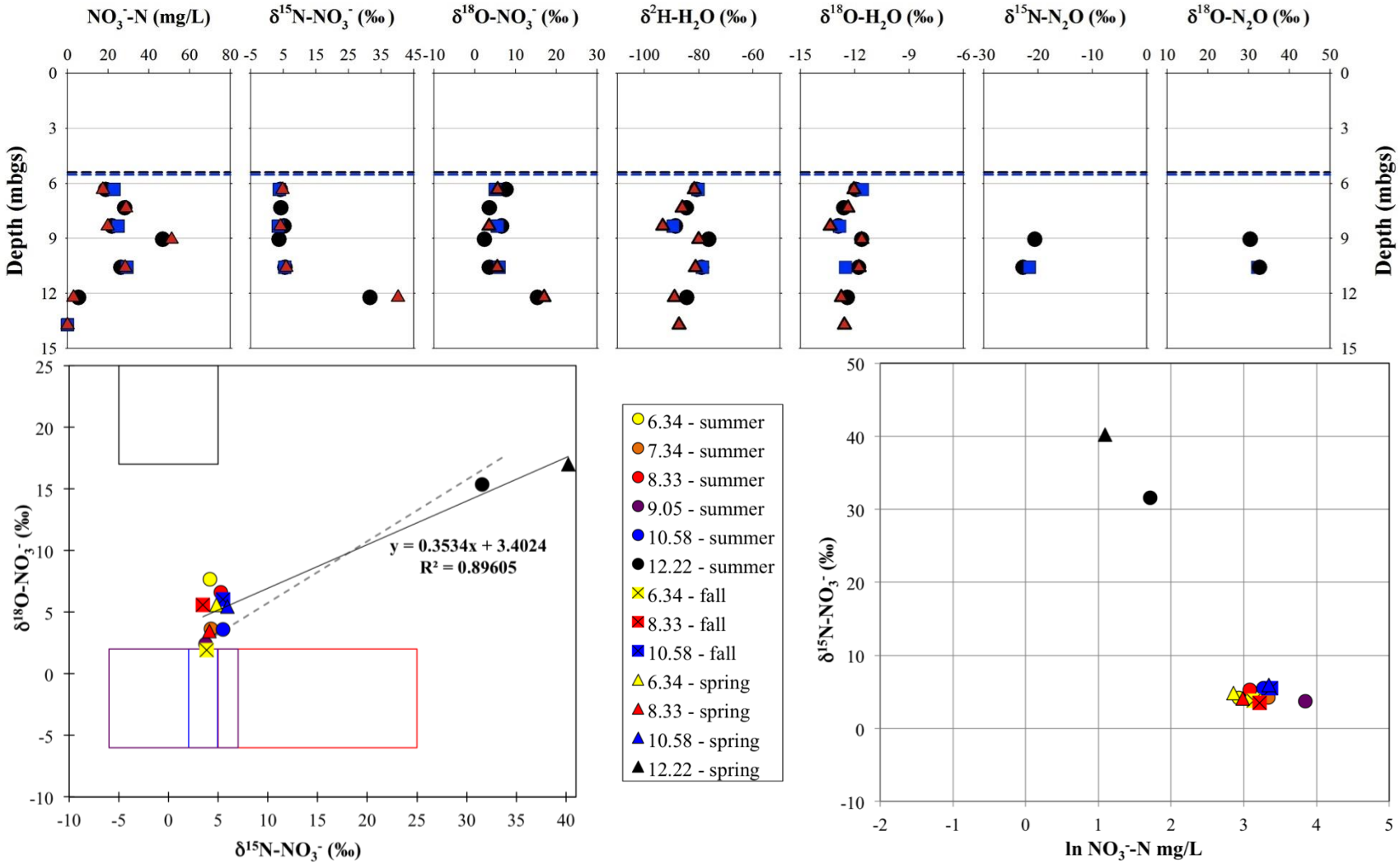



**Figure 4.25** MC4 nitrate and stable isotope behaviour. Top: Black circles are from summer 2010, blue squares from the late fall 2010, and red triangles from spring 2011. Bottom left: Dual nitrate isotope plot, boxes indicate expected ranges for source types as inidicated by previous studies, as oulined in Figure 2.4, and the grey dashed line outlines the increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ at a ratio of 2:1 for denitrification. Bottom right: $\delta^{15}N\text{-}NO_3^-$ vs. ln nitrate plot.

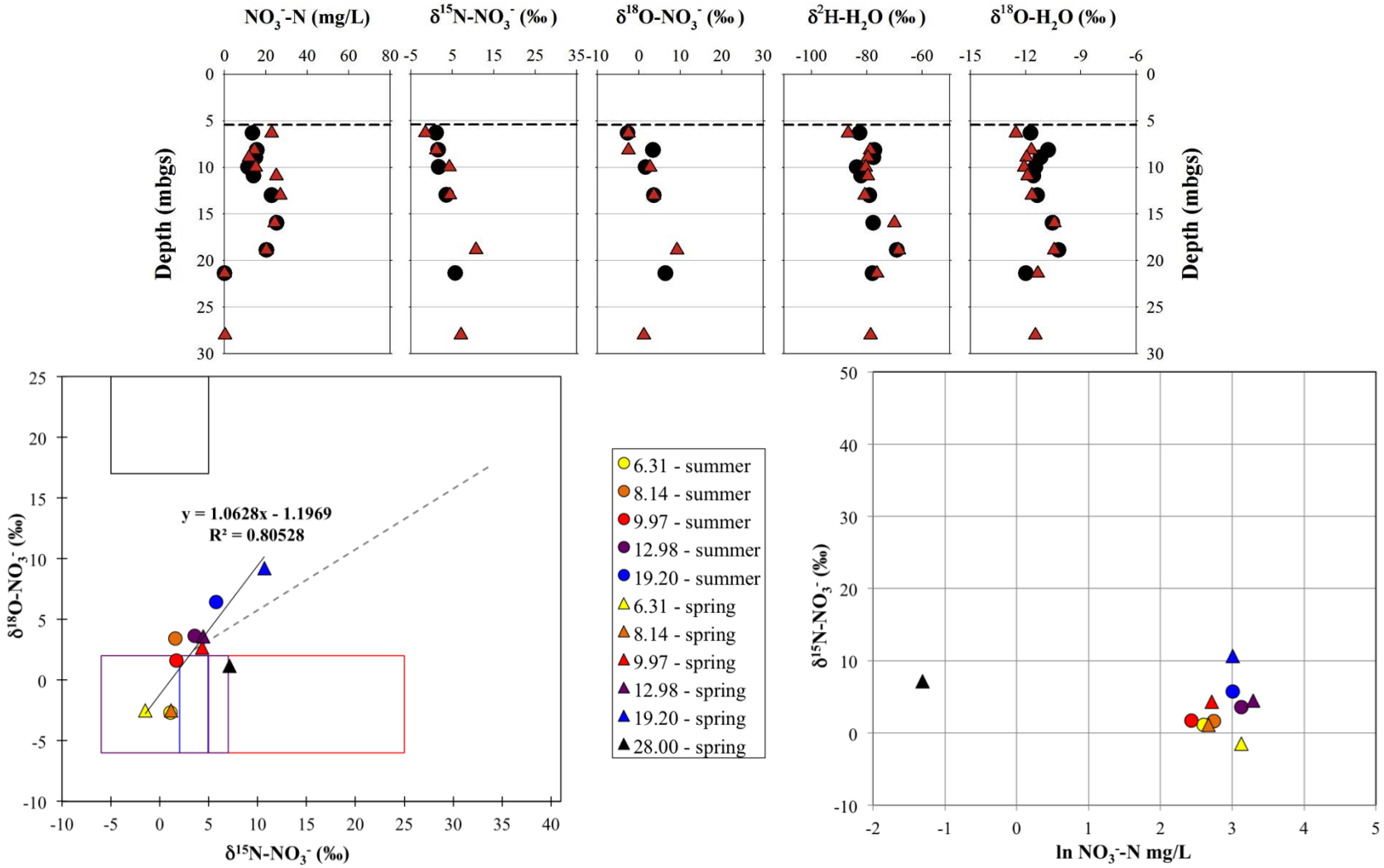



**Figure 4.26** W4 nitrate and stable isotope behaviour. Top: Black circles are from summer 2010, blue squares from the late fall 2010, and red triangles from spring 2011. Bottom left: Dual nitrate isotope plot, boxes indicate expected ranges for source types as inidicated by previous studies, as oulined in Figure 2.4, and the grey dashed line outlines the increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ at a ratio of 2:1 for denitrification. Bottom right: $\delta^{15}N\text{-}NO_3^-$ vs. ln nitrate plot.

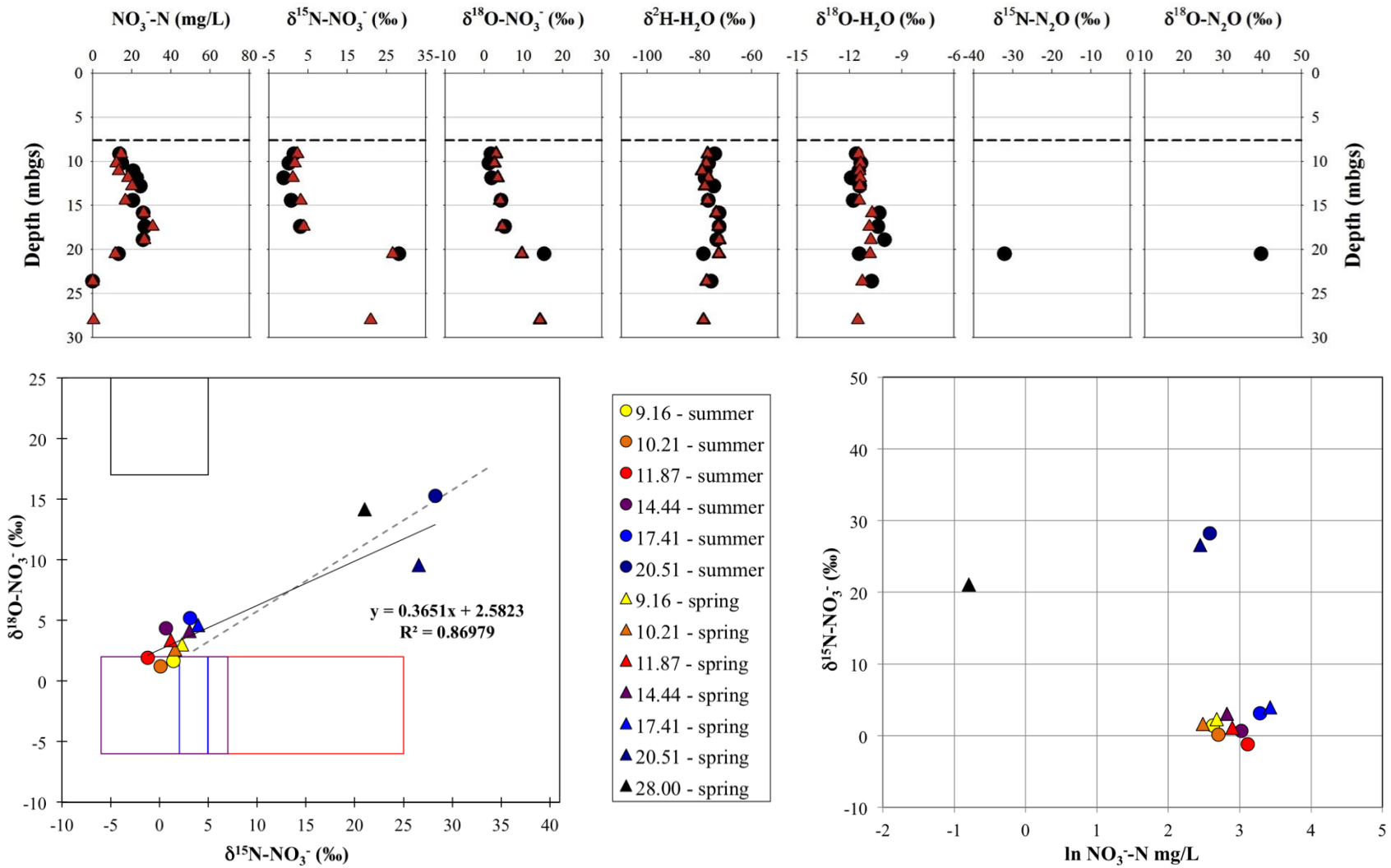



**Figure 4.27** W5 nitrate and stable isotope behaviour. Top: Black circles are from summer 2010, blue squares from the late fall 2010, and red triangles from spring 2011. Bottom left: Dual nitrate isotope plot, boxes indicate expected ranges for source types as inidicated by previous studies, as oulined in Figure 2.4, and the grey dashed line outlines the increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ at a ratio of 2:1 for denitrification. Bottom right: $\delta^{15}N\text{-}NO_3^-$ vs. ln nitrate plot.

4.3.3.2.2 W Site

Nitrate in W4 was high (11.3 to 26.9 mgN/L) from the top of the profile, to a depth of 19.2 mbgs (Figure 4.26). Isotopic ratios of nitrate generally increased with depth in this section of the profile. Below 19.2 mbgs, nitrate was low (0 to 0.3 mgN/L) and isotopic compositions of nitrate were within range of those up profile. Isotopic ratios of water varied with depth, however they showed consistency between the spring and summer datasets. The dual nitrate isotope plot shows samples plotting in the 'ammonium in fertilizer' box, at the lower end of the 'manure/septic nitrate' box, and above the three soil nitrification source boxes. The isotope-concentration plot reiterates the lack of increase in $\delta^{15}N$ of nitrate with respect to the decrease in nitrate concentration at depth. A linear relationship is expected if denitrification is responsible for the decrease in nitrate. Therefore a change in source is expected to be responsible for low nitrate, rather than by nitrate removal processes.

The trend in nitrate in W5 is similar to that of W4; nitrate is high (11.6 to 30.7 mgN/L) from the top of the profile to a depth of 18.9m (Figure 4.27). Isotope ratios of nitrate range from -1.2‰ to +3.9‰ for $\delta^{15}N$ and +1.2‰ to +5.2‰ for $\delta^{18}O$ in this part of the profile. Below 19.8m nitrate decreased, and ranged from 0 to 13.2 mgN/L. Corresponding $\delta^{15}N$ and $\delta^{18}O$ values of nitrate both increase at 20.5m, but decreased at the lowest sample point, 28.0 mbgs.

A distinct break in the isotopic signature of water existed between 14.4 mbgs and 15.9 mbgs, where the samples down profile have slightly higher $\delta^{18}O$ and $\delta^{2}H$ values compared to samples higher in the water column. For nitrate, samples between 9.4 mbgs and 11.9 mbgs actually decreased slightly in the $\delta^{15}N$ value with depth, while $\delta^{18}O$ values was fairly stable, and nitrate concentrations gradually increased. Between 15.9 mbgs and 18.9 mbgs nitrate concentrations were fairly consistent, and isotope compositions increased slightly with depth. The last sample point in the profile had very low nitrate, and highly enriched isotope signatures compared to the dataset in general. Taking into account all the data points in the profile, the ratio of $\delta^{18}O$ to $\delta^{15}N$ on the dual nitrate isotope plot was 0.36. The isotope-concentration plot clearly illustrates the lack of relationship between groundwater at 20.5 mbgs and 28.0 mbgs and suggests that low nitrate in the last sample is from a different source than that at 20.5 mbgs. Nitrous oxide was high enough to measure isotope compositions at a single depth, 20.5 mbgs. The $\delta^{18}O$ value was +39.7‰ and was similar to data from MC2 at 6.9 mbgs. However, the $\delta^{15}N$ value, was lower than those comparable data for MC2. The $\varepsilon_{N2O\text{-}NO3^-}$ for $\delta^{15}N$ at this point is -60.4‰, and for $\delta^{18}O$ is +24.5‰; these data are fairly different from the rest of the dataset, where $N_2O$ stable isotopes were measured (Table 4.8).

**Table 4.8** Dual isotopes for $NO_3^-$ and $N_2O$ in multi-level wells where $N_2O$ was present in sufficient quantities to measure isotopic signatures. Enrichment factors are calculated for denitrification.

| Sample ID | $NO_3^-$ (mgN/L) | $\delta^{15}N\text{-}NO_3^-$ (‰) | $\delta^{18}O\text{-}NO_3^-$ (‰) | $N_2O$ (% sat) | $\delta^{15}N\text{-}N_2O$ (‰) | $\delta^{18}O\text{-}N_2O$ (‰) | $^{15}N$: $\Delta_{N20\text{-}NO3}$ (‰) | $^{18}O$: $\Delta_{N20\text{-}NO3}$ (‰) |
|---|---|---|---|---|---|---|---|---|
| [1]MC2 4.34 | 45.8 | 5.7 | -1.3 | 7220 | -18.4 | 15.7 | -24.1 | 17.0 |
| [1]MC2 5.94 | 72.9 | 5.6 | 2.1 | 9040 | -25.6 | 30.3 | -31.2 | 28.2 |
| [1]MC2 6.68 | 68.4 | 5.8 | 6.3 | 79580 | -20.6 | 39.4 | -26.3 | 33.1 |
| [1]MC2 8.46 | 52.8 | 7.3 | 7.1 | 69070 | -26.3 | 36.5 | -33.6 | 29.3 |
| [2]MC2 6.68 | 63.1 | 6.1 | 4.5 | 58120 | -22.5 | 39.6 | -28.6 | 35.1 |
| [2]MC2 8.46 | 52.8 | 7.7 | 9.5 | 6600 | -22.4 | 35.4 | -30.0 | 26.0 |
| [1]MC4 9.05 | 68.4 | 3.7 | 2.4 | 5320 | -20.6 | 30.4 | -24.3 | 28.0 |
| [1]MC4 10.58 | 52.8 | 5.5 | 3.6 | 6760 | -22.8 | 32.7 | -28.3 | 29.1 |
| [3]MC4 10.58 | 29.3 | 5.5 | 6.0 | 6920 | -21.6 | 32.2 | -27.1 | 26.2 |
| [1]MC7 6.82 | 102.6 | 7.4 | 4.1 | 9980 | -21.5 | 35.1 | -28.9 | 31.0 |
| [4]W5 20.51 | 13.2 | 28.2 | 15.3 | 63420 | -32.1 | 39.7 | -60.4 | 24.5 |

[1]Sample collected August 16, 2010.
[2]Sample collected April 12, 2011.
[3]Sample collected December 1, 2010.
[4]Sample collected September 16, 2010.

*4.3.3.3 Geochemistry*

4.3.3.3.1 MC Site

Results of geochemical parameters measured at MC2 are summarized for all sample dates in Figure 4.28. For all sample dates nitrate concentrations were well above the drinking limit between 4.3 mbgs and 8.5 mbgs, but were almost negligible at 9.9 mbgs and 11.3 mbgs. In April 2011 the water table was higher than in August 2010 and a sample at 3.5 mbgs had a relatively low nitrate value of 3.6 mgN/L. Nitrite concentrations were mostly negligible for all sampling periods and depths, except for in August at a depth of 5.9 mbgs, where 0.1 mgN/L was detected. Nitrous oxide was the highest at 6.7 mbgs and 8.5 mbgs, for both August and April. In December, at 11.3m, nitrous oxide was high, but this value may be falsely elevated as it was obtained by forcing pressurized air into the piezometer via an air compressor, in order to retrieve the sample. The high DO value at the same depth supports this reasoning. $NH_4^+$ and SRP were high at 11.3m, much more so than the sample taken in April 2011, and so it is suggested that these values were also affected by the method of sample removal. Sulfate, chloride and magnesium quite closely mimic the trend in nitrate; very low concentrations were measured at 3.5 mbgs in April, concentrations peaked between 5.9 mbgs and 8.5 mbgs, and were detectable at 9.9 mbgs and 11.3 mbgs. Calcium, sodium and DOC were lowest at 3.5 mbgs but did not directly mimic the concentration trend of nitrate. At all other depths, sodium was moderately constant, between 4 mg/L and 8 mg/L. DOC and calcium were more variable with DOC having the highest

concentration at 11.3 mbgs, and calcium was not constant between sampling dates and depths. A peak in potassium at 9.9 mbgs was consistent through both August and April, and may indicate a more coarse-grained horizon within the aquifer where adsorption may be even less, due to a potential decrease in clay sorption sites. In comparison to domestic wells and groundwater seeps, geochemical maturation with depth (ie. age) is evident in the profiles of at least calcium and magnesium, and possibly potassium, and is consistent with the relationship between age and nitrate concentration for domestic wells and groundwater seeps.

The geochemistry of MC4 is outlined in Figure 4.29. The concentration of DO was consistent for each sampling date, with oxic conditions existing between 6.3m and 8.3m, suboxic to anoxic conditions occurring between 9.1 mbgs and 10.6 mbgs, and anoxic conditions occurring down profile. Nitrate was also consistent for all dates and was moderately high within the top three sample points, and then peaked at 9.1 mbgs. Beyond 9.1 mbgs nitrate decreased, reaching low and near negligible concentrations at 12.2 mbgs and 13.7 mbgs, respectively. Nitrite was very low within the profile, but showed a small but consistent increase at 12.2 mbgs, for both the August and April sampling dates. At 6.3 mbgs, nitrous oxide was much higher in December than in April and August. Besides this one sample point, nitrous oxide seemed to be low between 6.3 mbgs and 8.33 mbgs and then increased to reach a maximum that ranged between 22.5 μg/L and 34.8 μg/L. At 12.2 mbgs and 13.7 mbgs nitrous oxide had diminished to below 2 μg/L. Concentration profiles for ammonium and iron were consistently low for all sample depths. SRP was moderately low (<30 μg/L) for all depths and sample dates with minor variance throughout. DOC generally decreased from the top to the bottom of the profile with few exceptions including, 1) an elevated concentration at 13.7 mbgs in the December sample, compared to the April sample, and 2) an elevated concentration at 10.6 mbgs in the August sample, compared to the December and April samples. The elevated DOC at 13.72 mbgs may be a result of first time sampling (this depth was not sampled in August), which could give a high DOC value if there was insufficient pumping of the static water in the well. An explanation for the slightly higher DOC concentration in the sample for August at 10.6 mbgs is unknown; the only slight correlation exists between it and SRP, which had a profile maximum value at the same depth in August. Calcium and Magnesium had noticeably strong trends between all sample dates and may suggest leaching of lime application. The decrease in calcium and magnesium at depth correspond to an increase and potassium and sodium, which again indicates geochemical maturation of groundwater and supports the cation ternary data provided by the domestic well and groundwater seep datasets. It is also possible to distinguish three water parcels with clear geochemical trends from these two profiles. However, the pattern of magnesium and calcium did not agree well with that of nitrate, like the geochemistry of MC2. Also, sulfate was poorly correlated with

nitrate and was very different from that of MC2. In MC4 sulfate was extremely high at 6.3 mbgs, with a maximum concentration of 453.6 mg/L. At 7.3 mbgs sulfate was still slightly high but below that was <100 mg/L and fairly constant with depth. Sulfate existed even in the last sample point and therefore redox conditions were not sulfate reducing. Chloride behaved vaguely like that of magnesium and calcium, and may be related to fertilizer application, however potassium and chloride had drastically different behaviors.

4.3.3.3.2 W Site

The geochemistry of W4 is illustrated in Figure 4.30, for summer 2010 and spring 2011 dates. At both dates, the aquifer was oxic between 6.3 mbgs and 13.0 mbgs, suboxic at 16.0 mbgs, and anoxic between 19.2 mbgs and 28.0 mbgs. The concentration of nitrate was also consistent at both dates, with fairly consistent concentrations ranging between 11.4 mgN/L and 26.9 mgN/L, between 6.3 mbgs and 19.2 mbgs. At 21.4 mbgs and 28.0 mbgs nitrate was low, and had a maximum value of 0.3 mg/L. At 19.2 mbgs, nitrite was present at 0.5 mgN/L and 1.1 mgN/L and was not measured above the detection limit for all other sample points. Nitrous oxide had a similar behavior but peaked in concentration before that of nitrite. The maximum concentrations of nitrous oxide were 8.5 μgN/L and 8.1 μgN/L at 16.0m and 13.0m, respectively. Below 16.0 mbgs, nitrous oxide was below 1 μg/L. Ammonium was present below the detection limit for all depths down to and including 19.2 mbgs. A maximum value of 0.5 mgN/L was measured at 28.0 mbgs in April. The profiles of sulfate were fairly consistent for both sample dates, and ranged from 16.5 mg/L to 56.1 mg/L, except for at 28.0 mbgs where it was below the detection limit of 0.1 mg/L. This trend is indicative of sulfate reductive redox conditions. The trend of iron was similar to that of ammonium with concentrations near 0 measured down to 19.2 mbgs and increases occurring at 21.4 mbgs and 28.0 mbgs. The highest value was 1.8 mgN/L, measured at 21.4 mbgs. SRP is somewhat similar down to 21.4 mbgs and then increased at 28.0m to 82.4 μg/L. The magnitude of both calcium and magnesium were fairly steady at both sample dates and all sample points and show a similar trend in the variance of each concentration between depths. Potassium was generally measured close to 1 mg/L, except for at 8.1 mbgs where it spiked to 2.8 mg/L and 3.1 mg/L for the summer and spring dates, respectively. The consistency of this trend may indicate a horizon containing coarser sediments where adsorption occurs than within the surrounding material. Sodium and chloride showed fairly similar trends with depth, and seasonal variance was minimal for both datasets. The trend consists of slightly higher concentrations between 8.1 mbgs and 13.0 mbgs, with slightly lower concentrations below 13.0 mbgs, and a very strong increase at 28.0 mbgs. The profile of DOC had concentrations ranging between 0 mg/L and 1.7 mg/L between 6.3 mbgs and 19.2 mbgs, with increasing concentrations at 21.4 mbgs, reaching a maximum at 28.0 mbgs.

W5 geochemistry is outlined in Figure 4.31, showing the summer 2010 and spring 2011 data. For both dates, oxic conditions existed between 9.2 mbgs and 14.4 mbgs, suboxic conditions at 15.6 mbgs, and anoxic conditions from 17.4 mbgs to 18.0 mbgs. The concentrations of nitrate were very consistent for the two sample dates. Nitrate, for the most part, gradually increased with depth, reaching a maximum value of 26.6 mgN/L and 30.7 mgN/L at 18.9 mbgs, for the summer and spring dates, respectively. Below 19.8 mbgs nitrate decreased, and was less than 0.5 mgN/L at 23.6 mbgs and 28.0 mbgs. The trends in both nitrite and nitrous oxide were similar, having relatively high concentrations at 20.5 mbgs. At 23.6 mbgs and 28.0 mbgs nitrite diminished, whereas nitrous oxide was low at 23.6 mbgs, but was present at 6.2 μgN/L. Similarly, ammonium increased significantly at 28.0 mbgs to 0.8 mgN/L but was generally below the detection limit for all other data points. Iron was similar to ammonium, but increased above the detectable limit at 23.6 mbgs and then was highest at 28.0 mbgs with a concentration of 2.3 mg/L. Sulfate was very consistent at both dates, and concentrations were lowest between 9.2 mbgs and 12.8 mbgs, then, gradually increased with depth to a maximum value of 71.5 mg/L at 23.6 mbgs. At 28.0 mbgs, sulfate decreased substantially to 0.5 mg/L, again, likely indicating sulfate reducing redox conditions. The trend in DOC was consistent for both sample dates, except for at 17.4 mbgs where the spring sample was substantially higher than the summer sample. The reason for this is unknown and the data does not seem to correlate to the other geochemical parameters. For the rest of the profile, DOC gradually decreased from about 2 mg/L at 9.2 mbgs, to about 0.5 mg/L at 18.9 mbgs. Below 18.9 mbgs, DOC increased and reached a maximum of 7.6 mg/L at 28.0 mbgs. The profiles of calcium and magnesium were very consistent for the two sample dates, and did not vary drastically with depth. Calcium ranged between 78.8 mg/L and 121.3 mg/L between both sample dates and all sample depths. Magnesium generally increased with depth, with values of less than 10 mg/L at 9.2 mbgs and just above 18 mg/L at 20.2 mbgs. The profiles of potassium and chloride were fairly well twinned; between 9.2 mbgs and 12.8 mbgs both gradually increased, although here potassium reached a local maximum and chloride continued to increase further at 14.4 mbgs. It is likely that these top 5 or 6 sample depths are derived from a parcel of land fertilized with KCl. From 15.9 mbgs to 23.6 mbgs both were relatively low and stable, and may indicate a separate source horizon. One other discordant value existed at 20.5 mbgs where potassium was higher than its surrounding values, and chloride did not trend likewise. The consistency of this increase for both sample dates suggests a coarser sediment horizon, which can decrease the amount of potassium adsorption to particle surfaces. Sodium was low and fairly constant with a mild decrease with depth; however at 28.0 mbgs, sodium was abnormally high, with a concentration of 27.3 mg/L. Lastly, the agreement in geochemistry for W4 and W5, at each 28.0 mbgs sample point, suggests that a common source impacted both of these depths.

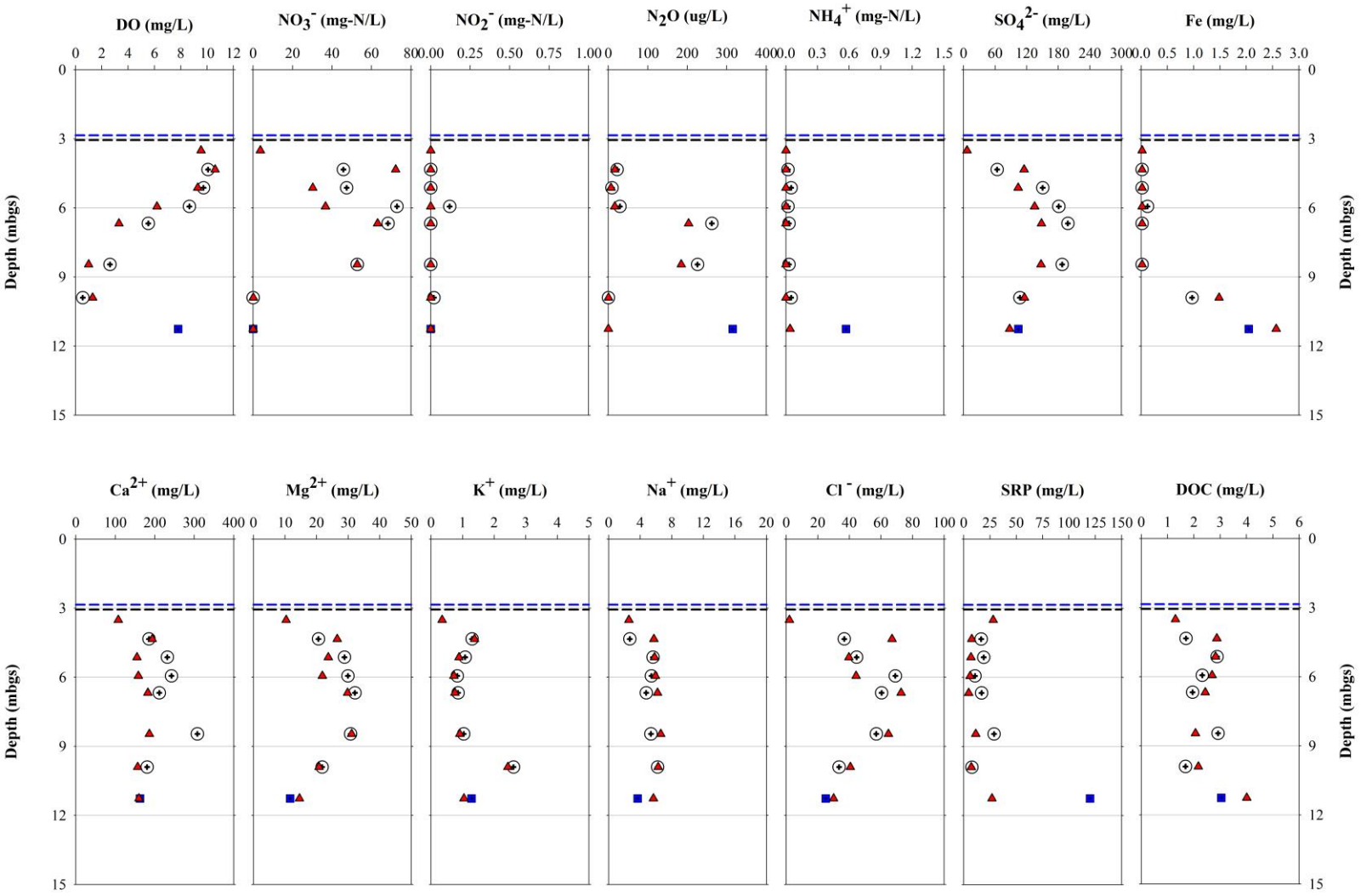



**Figure 4.28** Groundwater geochemical data for multi-level well MC2. Open circles with crosshairs represent samples collected in September 2010, red triangles in April 2011, and blue squares in December 2010.

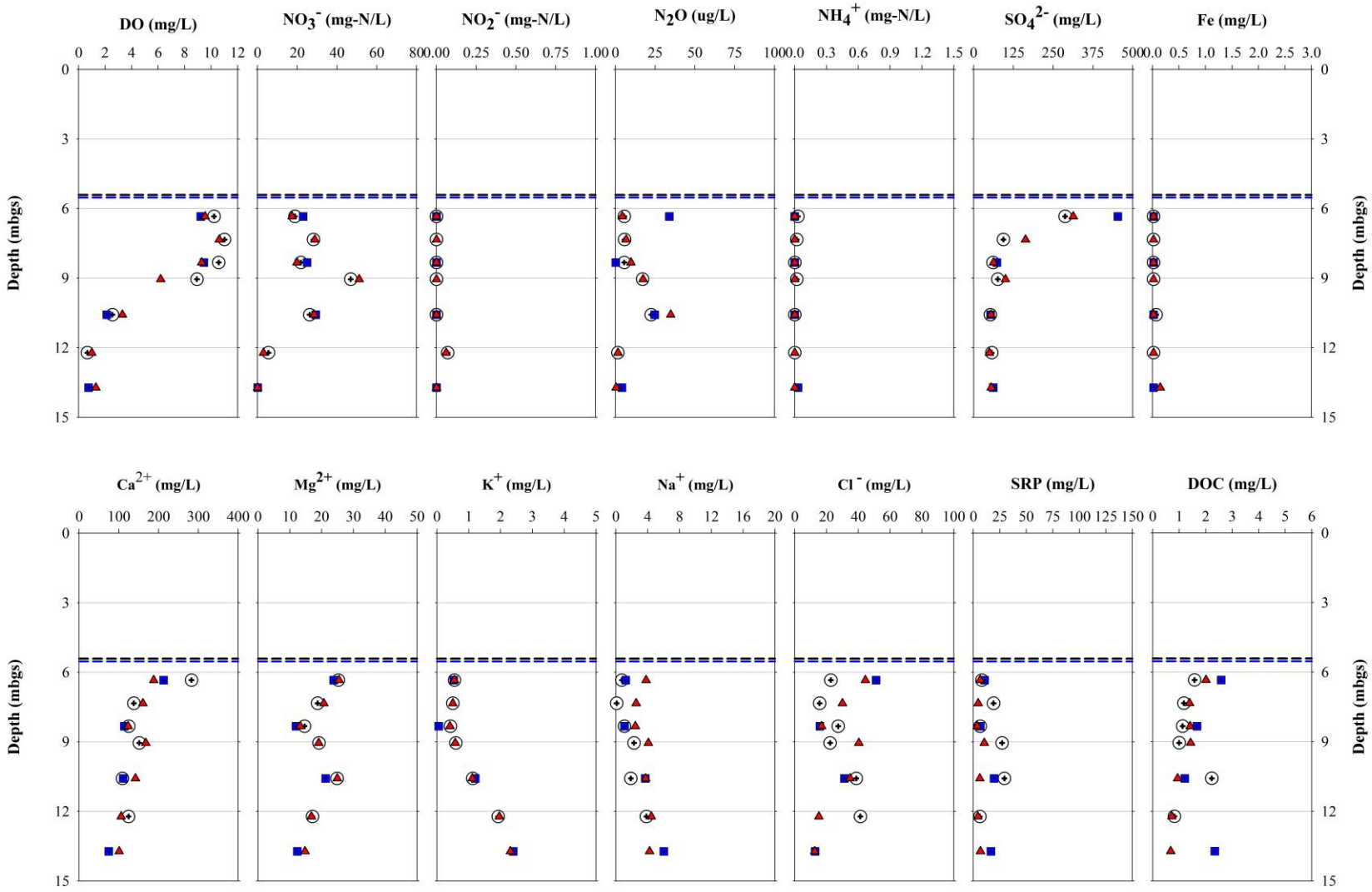



**Figure 4.29** Groundwater geochemical data for multi-level well MC4. Open circles with crosshairs represent samples collected in September 2010, red triangles in April 2011, and blue squares in December 2010.

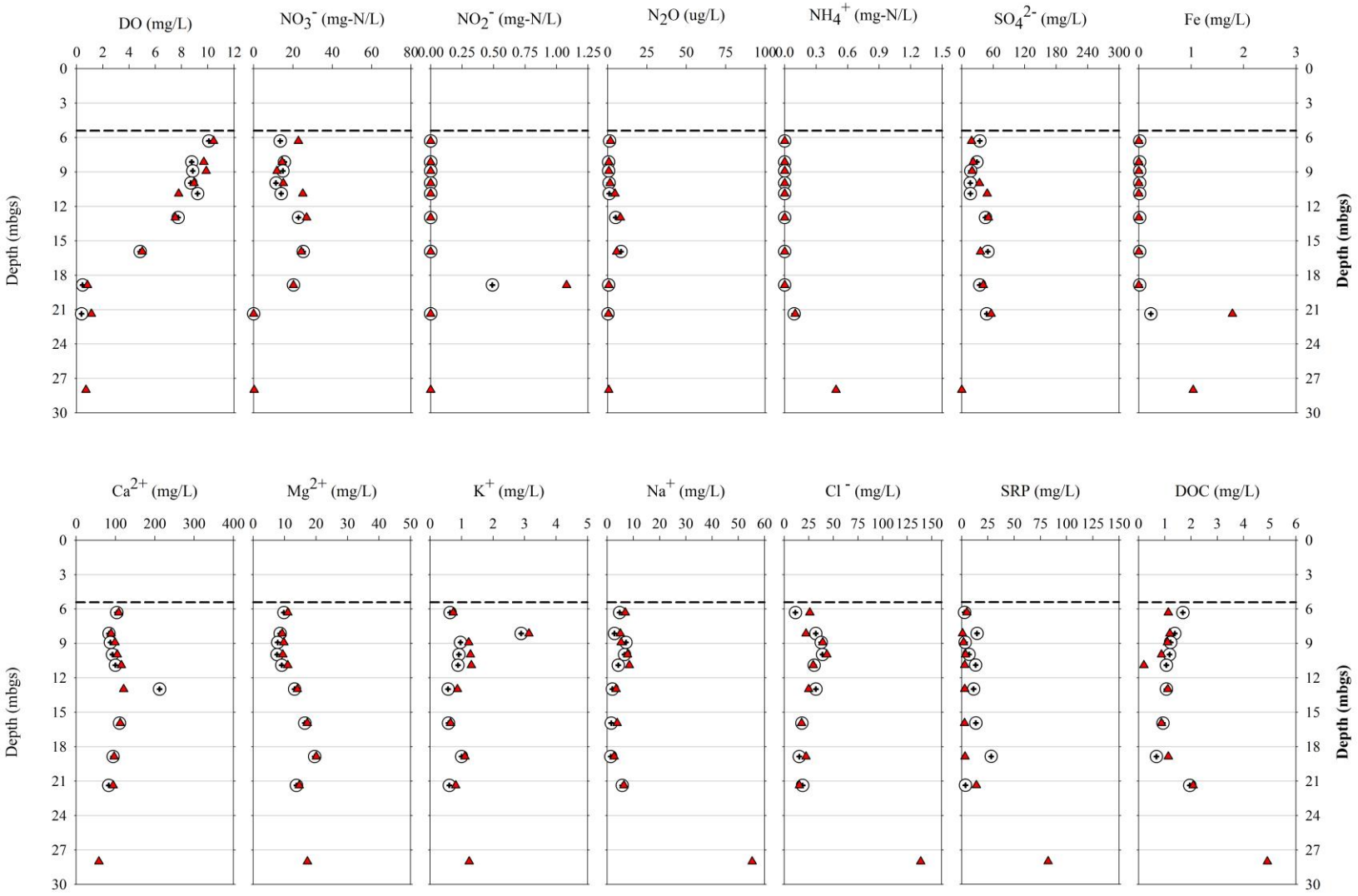



**Figure 4.30** Groundwater geochemical data for multi-level well W4. Open circles with crosshairs represent samples collected in August 2010 and red triangles in April 2011.

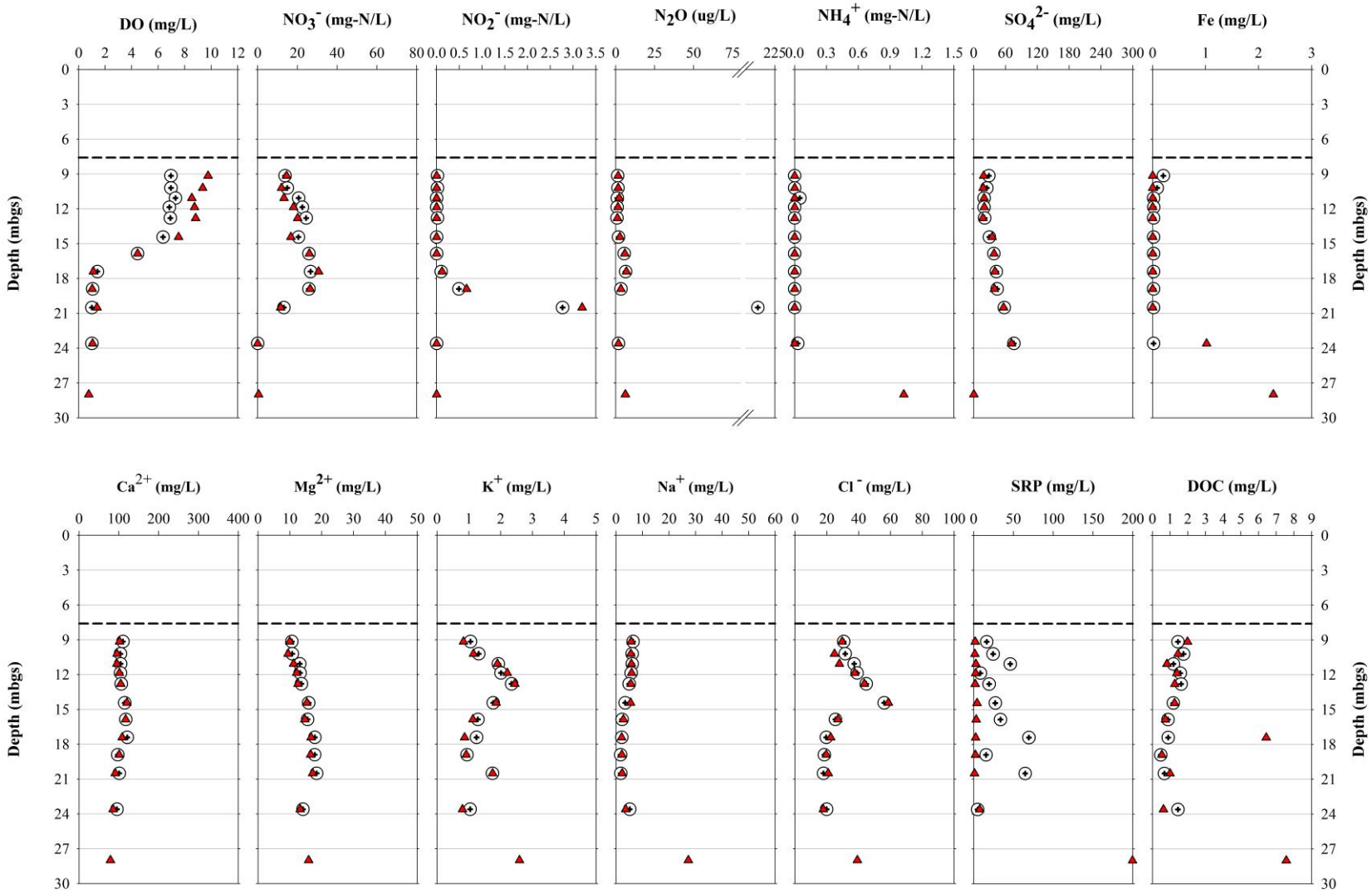



**Figure 4.31** Groundwater source geochemical data for multi-level well W5. Open circles with crosshairs represent samples collected in August, 2010 and red triangles in April 2011.

## 4.4 Discussion

### 4.4.1 Nitrate sources in the LASA

Based on previously defined source boxes for dual isotopes of nitrate, the dominant sources of nitrate in the LASA are identified as dominantly ammonium based synthetic fertilizer. This is inferred by assuming that samples with $\delta^{18}O$ values of nitrate that are greater than the expected upper limit (+2‰) have undergone denitrification and therefore increased in isotopic composition. To lower the $\delta^{18}O$ at a ratio of 1:2 for $\delta^{18}O$:$\delta^{15}N$ would therefore place most samples for all datasets in the source box 'ammonium in fertilizer'. Few samples are not elevated beyond the expected limits of $\delta^{18}O$ and plot within the intersection of 'ammonium in fertilizer', 'soil N' and 'manure/septic' boxes. Agricultural fields within the LASA are often are cropped in rotation and use different fertilizer types depending on the crop. For potato farming, which is commonly cropped in rotation with wheat and sometimes corn, chemical fertilizers and manure are used (Personal communication, local farmer, 2011). However, isotope ratios of nitrate indicate that chemical fertilizers are the source of high nitrate in the groundwater.

Artificial sweeteners were used to better interpret manure vs. septic sources that were categorized as such by stable nitrate isotopes. For domestic wells, 31% of the total sample population had one or more sweetener. The results are similar for groundwater seep samples, with 35% of the samples containing one or more sweetener. Samples with sweeteners are interpreted as being influenced by septic wastewaters. An interpretation of the proportion of wastewater from septic systems contributing to nitrate in the domestic wells and groundwater seeps shows that only few samples in each dataset are substantially impacted by septic waters. Only one sample had substantial nitrate (>10mgN/L) that was characterized as being sources almost fully from septic waters. Therefore the impact of septic wastewaters on groundwater nitrate in the LASA is minimal, and only of minor concern for well owners who could have the well source located too close to their septic system.

### 4.4.2 Nitrate cycling processes in the LASA

In domestic wells and groundwater seeps nitrate was low (<0.1 mgN/L) in 29% and 7% of the respective sample populations. Stable isotopes of nitrate show trends of increasing $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NO_3^-$ in several samples of each dataset, as well as at depth in multi-level wells. Generally, samples with $\delta^{18}O\text{-}NO_3^-$ >2‰ are interpreted as having undergone some amount of denitrification

In domestic wells, oxic waters had higher $N_2O$ and nitrate, and anoxic waters had lower $N_2O$ and nitrate, generally. Stable isotopes of nitrous oxide and isotope effects of denitrification and nitrification were

used to clarify sources of $N_2O$ and N cycling processes. Denitrification is interpreted to be the dominant process producing $N_2O$, even in oxic groundwaters. Since domestic wells must be able to produce enough water for household use, domestic wells are screened over a large enough interval that the infiltrating waters are sourced from a mix of redox horizons, since redox boundaries often occur at fairly abrupt horizons, or depths, within the aquifer (Aravena & Robertson, 1998). However, comparing the domestic wells and seeps to the multi-level piezometer samples, there is a common trend of high $N_2O$ where waters are oxic, and nitrate is present. This horizon seems to exist more or less directly above where nitrate abruptly decreases. Therefore it is likely that the increase in $N_2O$ in domestic wells is generally related to denitrification and is evidence thereof. As mentioned in previous sections, large pools of nitrate may inhibit the further reduction of $N_2O$, and therefore large $N_2O$ concentrations are likely to exist when a nitrate pool is present (Stein & Yung, 2003). The presence of some DO also inhibits $N_2O$ reduction to $N_2$ during denitrification (Snider, 2011). Data here are consistent with the findings of Hollingham (2011), who found that municipal well samples from Norfolk and Oxford counties, Ontario, were characterized by high $N_2O$ as a result of denitrification in the oxic zone, as opposed to nitrification.

The relationship of SRP, sulfate, nitrate, Fe, DOC, and chloride were useful for interpreting redox conditions in the LASA. Groundwaters were oxic in 56% of the domestic wells, and were not measured for DO in the groundwater seeps. For samples with no nitrate, redox conditions were anoxic, however high nitrate (>10 mgN/L) was present in few samples that were anoxic. Samples with no nitrate are categorized as having undergone nitrate, sulfate, and possibly iron reduction, as sulfate is also not present and iron is detected. SRP also increases where reducing conditions exist, and can be used as an indicator of reduced conditions along with sulfate and iron.

In the groundwater seep dataset, groundwater seep samples with no nitrate have varying amounts of sulfate, iron, and SRP, and therefore represent nitrate reducing conditions, but not sulfate or iron reducing conditions. Therefore, the reducing capacity in groundwater seeps is weaker than in the domestic wells. This trend could also indicate that groundwater collected in seeps is younger, relative to the domestic wells, and less geochemically mature.

The non-reactivity of chloride assists in outlining redox conditions, by comparison with SRP and nitrate, typically, the concentration of chloride should be relatively consistent along the flow path, and should represent source water conditions. If nitrate is low or not present and chloride remains, nitrate removal can be implied. The great range in chloride concentrations throughout the study area permits only a general interpretation of chloride in relation to SRP and nitrate. For the domestic well dataset, samples with elevated SRP and very low nitrate also had low chloride. This implies reduction occurred

to the point as to increase concentrations of SRP. Furthermore, samples with low chloride may indicate non-agricultural or non-septic sources, and nitrate may not have been initially high. Groundwater seeps, however, had chloride that was not as low as domestic wells, when nitrate was very low. Therefore samples that had low nitrate could have been sourced from agricultural or septic groundwaters.

## 4.5 Conclusion

Source classification of nitrate in the LASA was determined with the use dual nitrate isotope plots and supported by measurement of artificial sweeteners. Nitrate isotope ratios were commonly higher in $\delta^{18}O$ than expected and were interpreted as having undergone at least partial denitrification. Assuming that source $\delta^{18}O$ and corresponding $\delta^{15}N$ would have been lower, groundwater source signatures can be back calculated and would result in a dominance in the 'ammonium in fertilizer' source box, and suggests chemical fertilizers are the dominating source of nitrate in the LASA.

Artificial sweeteners were measured in 31% and 35% amongst the domestic well and groundwater seeps, respectively, however nitrate contribution from wastewater was only considered to be substantial in one domestic well sample. Therefore septic systems are not a main contributor to elevated nitrate in the LASA.

Ternary cation plots and multi-level profile geochemistry was useful for interpreting the relationship of nitrate contamination with respect to groundwater age and maturity. All three sample sets show geochemical evidence that nitrate is prevalent in geochemically immature, ie younger groundwaters. Although this data is used as a relative marker for contamination, it could be helpful when prospecting for uncontaminated groundwaters.

The presence of oxygen in the LASA at several meters below the water table provided conditions where nitrate could exist at high concentrations. Stable isotopes of nitrate displayed evidence for nitrate removal via denitrification for many samples where nitrate was still present, for all datasets. The production of $N_2O$ in domestic wells and multi-level wells was generally attributed to denitrification within the oxic zone. In domestic wells, where nitrate was not present, and at the deepest sample points in the multi-level wells, redox conditions were classified having undergone nitrate, sulfate, and iron reduction. However, multi-level well data outlines that this occurs at depths of at least 15m below the water table, and therefore the majority of the LASA has a limited reducing capacity. Groundwater seeps were less reduced and did not show evidence for sulfate or iron reduction. Although denitrification is evident to some degree in each of the datasets, nitrate was largely, incompletely removed in the LASA. Therefore the capacity of the aquifer to remove nitrate is not sufficient to cope with the amount of nitrate from fertilizers and local septic systems.

# CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

### 5.1.1 Changes in groundwater nitrate

Changes in groundwater nitrate over the past ~30 years were varied among the domestic wells, groundwater seeps, and multi-level wells, in the LASA. Overall, domestic wells had lower mean nitrate, groundwater seeps had higher mean nitrate, and multi-level wells had lower mean nitrate when compared the respective historical datasets. The Mann-Whitney rank-sum test confirmed that the domestic well data were statistically lower in nitrate, and groundwater seeps were statistically higher in nitrate in the current study. However, when the domestic well and groundwater seep datasets are compared by recharge area, a statistical difference of nitrate between the 2010/2011 and 1979/1980 datasets was only true for the northwest recharge area of groundwater seeps dataset.

The spatial distribution of nitrate >10 mgN/L for the combined domestic well and groundwater seep data were similar to those of Hill (1982). Nitrate plumes were prevalent in the northwest and south parts of the aquifer with less nitrate in the northeast. Changes in nitrate outlined in this fashion are also a factor of sample distribution, which did not perfectly match that of Hill (1982).

Between the different datasets, only the domestic wells exhibited an overall decrease in groundwater nitrate concentrations over time, which was hypothesized to result from the current and/or recent improvements in nutrient management. Changes in nitrate profiles at the MC site were attributed to the removal of a woodlot in 2005 that was positioned upgradient of both wells. Depression of the water table since 1982/1983 proved to move nitrate rich groundwaters deeper below the surface than historically measured, and also changed the source and age of groundwaters being sampled at the set depths.

Although nitrate in domestic wells was relatively lower than the historical data, 36% of samples had nitrate above 10 mgN/L. Elevated nitrate correlated to ‘potato’ and ‘other’ land types with less linked to ‘sod’, ‘corn’, and ‘forest’, and no correlation to ‘pasture’ and agreed with correlations provided by Hill (1982). Results of groundwater seeps were similar, and high nitrate correlated to ‘corn’ and ‘potato’ land use, and low nitrate to ‘forest’ and ‘pasture’.

### 5.1.2 Nitrate sources in the LASA

Source identification for nitrate was constrained with the use of stable isotopes of nitrate, artificial sweeteners, and ternary cation plots. Domestic wells, groundwater seeps, and multi-level wells were

all dominantly identified as nitrified ammonium from fertilizer, after having been interpreted as having undergone isotopic enrichment via denitrification. Artificial sweeteners were detected in 31% and 35% of the domestic well and groundwater seep datasets, respectively, and proved to be an effective tracer for septic sources. The estimation of wastewater proportion for those samples with acesulfame showed that septic systems are a minor contributor to groundwater nitrate. However, domestic wells with any amount of artificial sweetener present should still understand that septic wastewater is entering their drinking water source, and should have their water tested for coliform.

### 5.1.3 Nitrate cycling processes in the LASA

Oxic conditions extended several meters below the water table in the LASA and provided conditions where nitrate could exist at high concentrations. In the current study, this was exacerbated by a depressed water table which introduced oxic waters to greater depths. Denitrification was prevalent in all datasets, however, not always to the extent needed to remove nitrate or decrease the concentration below the drinking water limit. Denitrification in oxic groundwaters was revealed with isotopic compositions of nitrous oxide. In domestic wells, where nitrate was not present, and at the deepest sample points in the multi-level wells, redox conditions were classified having undergone nitrate, sulfate, and iron reduction. However, multi-level well data outlines that this occurs at depths of at least 15m below the water table, and therefore the majority of the LASA has a limited reducing capacity. Groundwater seeps were less reduced and did not show evidence for sulfate or iron reduction. Although denitrification is evident to some degree in each of the datasets, nitrate was largely, incompletely removed in the LASA. Therefore the capacity of the aquifer to remove nitrate is not sufficient to cope with the amount of nitrate from fertilizers and local septic systems.

### 5.1.4 Riparian zones and nitrate removal

A lack of denitrification in riparian zones is attributed to groundwater flow that does not interact with DOC rich sites in the riparian zone. Although the Nottawasaga River has a distinct riparian zone for the majority of the span of the river, the flow is too fast and avoids interaction with any organic carbon that can act to aid in denitrification. Widening the riparian zone and reducing the amount of area to be farmed beside the river is unlikely to help nitrate removal or sufficiently lower nitrate entering the aquifer.

## 5.2 Recommendations

1) A detailed, continuous monitoring of multi-level wells, either at the MC or W site, or installed elsewhere, where potato crops are used in rotation, to assess the changes in groundwater nitrate over the full crop rotation cycle. A tight spaced sample interval network (0.5m intervals), down to a maximum of 6m below the minimum water table depth would suffice to best capture the effect of crop type and fertilization application. This would also capture water table fluctuations and the effects of storm events on the transport of nitrate to the aquifer. The results would be compared to better understand where fertilizer application and management practices need improvement. Multi-level wells would ideally be installed at the farthest point along the groundwater path for a specific farm so that changes in the water table does not change the groundwater source. This could be performed in conjunction with other crop monitoring programs such as sod and soy.

2) More specific groundwater age dating to better interpret the residence time of nitrate in the LASA.

3) To better understand the input of nitrate into the Nottawasaga River and its tributaries, shallow monitoring wells could be installed at the edges of farms that boarder the rivers. Wells screened no more than 5m below the water table, or multi-level wells with several sampling depths would best reveal changes in groundwater nitrate with respect to recent and relevant changes in land use and crop management. Ideally, potato farms, as well as sod and soy should be considered.

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