Measurement of neon in groundwaters for quantification of denitrification in aquifers

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ABSTRACT

Nitrate is the most pervasive contaminant in New Zealand's groundwaters. Thus, understanding and managing nitrogen loads through New Zealand's aquifers is vital for maintaining the quality of groundwaters and connected surface waters.

Denitrification is a natural process that is mediated by the metabolism of aquifer microorganisms and by which dissolved nitrate is reduced eventually to nitrogen gas. However, the extent of denitrification occurring within New Zealand's groundwater system is largely unknown, because there has historically been no straightforward, reliable and accurate technique to measure it.

Calculation of the concentration of excess nitrogen in groundwaters is a promising technique to quantify the amounts of denitrification occurring in the groundwater system. The concentration of dissolved atmospheric nitrogen, according to the recharge conditions of the water, can be established by the measurement of two noble gases, such as neon and argon, which are part of the atmosphere. This enables differentiating the excess nitrogen gas produced via denitrification reactions from atmospherically derived dissolved nitrogen gas.

This report details the development, validation and application of an analytical method to simultaneously measure dissolved neon, argon and nitrogen in groundwater. The method is compared to other denitrification proxies across three different regions in New Zealand, so that the potential for applying the newly developed procedure for quantification of excess N_2 in groundwater can be assessed.

KEYWORDS

Noble gas, neon, argon, excess nitrogen, denitrification, groundwater

1.0 INTRODUCTION

In this report, the development and validation of a technique to measure dissolved neon (Ne) in New Zealand groundwaters is outlined. The simultaneous measurement of dissolved Ne and argon (Ar) allows us to determine the recharge temperature of groundwater, the concentration of excess air (Heaton 1981), and consequently the concentration of nitrogen from atmospheric sources. Simultaneous measurement of the dissolved N_2 concentration in groundwater will subsequently allow us to establish the concentration of excess N_2 that results from denitrification. For method validation, this report also compares the quantification of excess N_2 in New Zealand groundwaters via this Ne measurement to other proxies for measuring denitrification, including chemistry, Childs' tests, stable isotopes of the nitrate (15N, 18O), as well as microbial DNA analysis of groundwater.

1.1 Nitrate and Denitrification

Nitrate (NO_3) is the most pervasive contaminant in New Zealand groundwaters. Approximately 40% of long-term groundwater monitoring sites show above-natural concentrations of nitrate, with no conclusive evidence of improvements over the last decade (Daughney and Wall 2007; Moreau et al. 2016). Understanding and managing nitrogen loads through New Zealand's aquifers is, therefore, vital for maintaining and/or improving the quality of groundwater and connected surface waters.

Denitrification is a natural process that is mediated by the metabolism of microorganisms in the aquifers and by which dissolved nitrate is reduced eventually to nitrogen gas (Chapelle 1993):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO_{(g)} \rightarrow N_2O_{(g)} \rightarrow N_{2(g)} \qquad \textbf{Equation 1}$$

Denitrification can, therefore, remove nitrate from groundwater by conversion to gaseous forms. This process can potentially lead to a significant nitrate reduction in the aquifer and lessening of nitrogen loads into receiving waters such as groundwater-fed streams, springs, wetlands, and lakes (Woodward et al. 2013). Nitrate and other forms of fixed nitrogen can also be removed in natural systems by other processes which occur concurrently with denitrification. These processes included dissimilatory nitrate reduction to ammonia and anaerobic ammonia oxidation (anammox) (Tiedje 1988; Smith et al. 2015).

1.2 Measurement of Denitrification

Denitrification is primarily thought of as an anaerobic respiration process by which facultative heterotrophic denitrifying bacteria (e.g. *Pseudomonas* sp and *Bacillus* sp) simultaneously oxidise organic carbon compounds (as an electron donor) and utilise nitrogen oxides as the terminal electron acceptor (Delwiche, 1981), e.g.:

$$4NO_3^- + 5CH_2O \rightarrow 2N_{2\ (g)} + 5CO_{2\ (g)} + 3H_2O + 4OH^- \qquad \textbf{Equation 2}$$

Denitrification can also occur as a mixotrophic process utilising ferrous iron (such as in pyrite) as the electron donor (Korom et al. 2012; Robertson and Thramdrup 2017)

The extent of denitrification occurring in New Zealand's groundwater systems is largely unknown. However, there are many procedures that have been developed to assess qualitatively and quantitatively whether the denitrification process demonstrated in Equation 2

is occurring within an aquifer (e.g. Groffman et al 2010). The following sections briefly describe the existing methods for assessing denitrification in groundwater.

1.2.1 Redox state

Much emphasis has been placed on identifying where optimal redox conditions are present to allow for the facilitation of denitrification (Stenger et al. 2008). Denitrification primarily occurs under reducing (i.e., oxygen depleted) conditions, after the dissolved oxygen is consumed by microorganisms during the oxidation of organic matter. Once oxygen is depleted, other redox reactions occur in a sequence dictated by the most favourable terminal electron-accepting process (TEAP). The order of these processes is O_2 > NO_3^- > Mn(IV) > Fe(III) > SO_4^{2-} > methanogenesis (McMahon and Chappelle 2008).

One of the most common ways to infer the redox state of a groundwater sample is to measure the dissolved concentrations of redox-sensitive elements and compounds, notably oxygen, nitrate, ammonium, iron, manganese, and sulphate. McMahon and Chapelle (2008) show how the succession of TEAPs allows categorisation of groundwater into different redox states based on the concentrations of these relevant redox species (Table 1.1).

Table 1.1 Threshold criteria for identifying redox processes. From McMahon and Chapelle (2008).

Redox process	O_2 (mg/L)	NO_3-N (mg/L)	Mn^{2+} (mg/L)	Fe^{2+} (mg/L)	SO_4^{2-} (mg/L)	Comments
Oxic						
O_2 reduction	≥0.5	-	<0.05	<0.1	-	-
Suboxic	<0.5	<0.5	<0.05	<0.1	-	Further definition of redox processes not possible
Anoxic						
NO_3 reduction	<0.5	≥0.5	<0.5	<0.1	-	-
Mn(IV) reduction	<0.5	<0.5	≥0.5	<0.1	-	-
Fe(III)/SO_4 reduction	<0.5	<0.5		≥0.1	≥0.5	-
Methanogenesis	<0.5	<0.5		≥0.1	<0.5	
Mixed	-	-	-	-	-	Criteria for more than one redox process are met

A second approach for evaluation of redox state is to use a redox electrode, typically of platinum. Although such redox electrodes are in common use, there are numerous studies that illustrate that they may not always provide meaningful measurements of the redox state of natural waters, for example where the dominant redox sensitive elements are non-electroactive (e.g. C, O, H, N, S) (Langmuir 1997).

Childs' test is another method that can be used to assess the redox condition in an aquifer or soil. Childs' test is a chemical tracer experiment used to visually detect reducing zones (Childs 1981). The test involves applying a dye, such as α,α'-dipyridyl, to a soil or aquifer core. The dye reacts with only Fe^{2+}. The reaction induces a colour change and demonstrates that Fe^{3+} in the soil or aquifer material has been reduced, and thus anaerobic conditions are present. Lack of a colour change simply indicates that no Fe^{2+} is present. This means that either the soil is aerobic or that the soil is anaerobic but not Fe reduced (Vepraskas et al. 2016).

It must be stressed that the assessment of the redox status of the groundwater only suggests whether denitrification could be possible in an aquifer, but not whether it has actually occurred (Langmuir 1997). For example, a comparison of groundwater age versus redox status suggests that many reduced (anoxic) zones are, in effect, stagnant or very slow moving (Morgenstern et al. 2014), and hence, any potential for denitrification may have little effect on reducing nitrogen loads to receiving waters because the water does not flow through these zones. Furthermore, the above-listed methods for assessment of redox state each have their own limitations, such as the requirement to extract a core sample to apply Childs' test or the uncertain robustness of redox electrode measurements in some hydrochemical conditions.

1.2.2 Molecular microbiological approaches

Another proxy for identification of denitrification is by the examination of the microbial community within the aquifer. One approach is to conduct a high-throughput analysis of the microbial population as a whole. For example, Sirisena et al. (2018) collected groundwater samples from selected sites across the New Zealand National Groundwater Monitoring Programme and performed amplicon sequencing of the V5-V7 hypervariable region of 16S rRNA gene using the 454 pyrosequencing platform. Metabolic inferences were made based on the taxonomic composition of the microbial community at each site, for example, to predict the oxygen requirements, metabolic potential and dominant energy sources of the constituent bacteria. This showed that the bacterial community structure at a given site was related to the redox condition of its groundwater.

A second molecular approach is to analyse for specific denitrifier genes in the microbiological community within the aquifer (Bakken and Dorsch 2007; Chon et al. 2011). Analysis of the abundance and stoichiometry of the nitrite reductase genes, *nirS* and *nirK*, as well as the nitrous oxide reductase gene, *nosZ*, can identify which, if any, step in the denitrification reaction is dominant and whether it is likely that the denitrification reaction goes to completion (N_2) or is only partially completed to NO_2 or N_2O (Bakken and Dorsch 2007).

These molecular microbiological approaches are insightful but do not provide a quantitative measure of the amount of denitrification that has occurred in a groundwater sample. The whole-of-community DNA approach provides an indication of the *potential* for denitrification, but not whether it has actually occurred. The denitrifier gene method has the same limitation, in that it does not necessarily correlate to the extent of denitrification occurring in the aquifer system, as the population abundance does not shed any light on the rate at which the denitrification process is occurring. Furthermore, gene populations from different sampling sites with varying geology and lithology cannot be directly compared because environmental differences can influence populations (Groffman et al 2006).

1.2.3 $\delta^{18}O$ and $\delta^{15}N$ in dissolved nitrate

Nitrate in the environment can have a wide range of $\delta^{18}O$ and $\delta^{15}N$ compositions (Figure 1.1). These compositions are the result of various sources of nitrate and subsequent chemical transformations that occur in the soil and groundwater zones. These transformations include mineralisation, nitrification of ammonia, and nitrate attenuation processes such as denitrification.

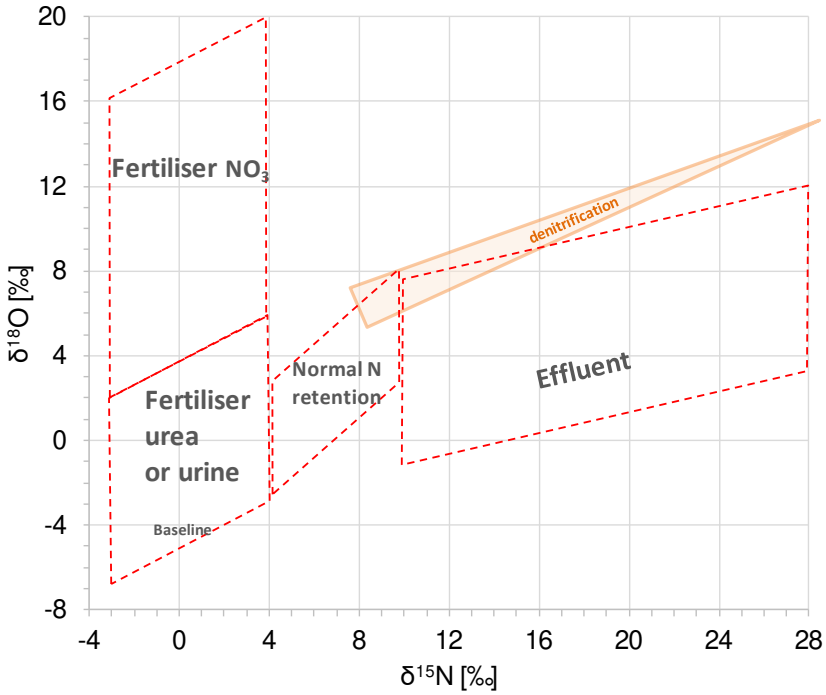

Figure 1.1 Typical ranges of $\delta^{18}O$ and $\delta^{15}N$ for different sources of nitrate (Morgenstern et al. 2018).

Based on previous isotope results from within New Zealand, sites have been assigned an indicator classification based on the relationship between $\delta^{15}N$, $\delta^{18}O$ and nitrate concentrations (Morgenstern et al. 2018). Characteristics of each class are given in Table 1.2. For example, low $\delta^{15}N$ and $\delta^{18}O$ with low nitrate concentrations are classed as background (baseline) results, while high nitrate concentrations imply breakthrough of urine or nitrogenous fertilisers such as urea (after conversion to nitrate).

In general, many New Zealand samples fall into the "Normal N retention" class. In this class $\delta^{15}N$ increases from around 4 to 9 ‰ with a general increase in nitrate concentrations, and $\delta^{15}N$ and $\delta^{18}O$ sit on a 1:1 line. We term this normal N retention because the soil organic matter appears to remain an effective sink for nitrate from urine and urea, and isotopically the nitrate looks like the soil organic matter N. The balance between competing processes such as nitrogen cycling in the soil, increasing nitrate inputs and nitrate attenuation lead to this characteristic signal (Wells 2015, 2016; Stevenson et al. 2010). In the “mixed UU/normal” category, nitrate concentrations are much higher than expected for the “normal N retention” category (> 4 mg/L) such that there appears to be a mixture of UU and normal N.

Table 1.2 Description of $\delta^{18}O$ against $\delta^{15}N$ indicator classifications.

Class		Description
Baseline		Low concentration background nitrate with low $\delta^{15}N$ and $\delta^{18}O$
UUF	Urine Urea Fert	Breakthrough of Urine, Urea or other inorganic nitrogenous fertiliser (such as ammonium compounds)
Denitrif	Denitrified	High $\delta^{15}N$, $\delta^{18}O$ but low NO_3 concentrations suggest denitrification has occurred
NormNRetn	Normal N Retention	Normal N Retention: Typical nitrate from pastoral soils matches soil $\delta^{15}N$, sits on 1:1 line with moderate $\delta^{15}N$; NO_3 concentration increases with $\delta^{15}N$
MixedUU	Mixed UU/Normal	high concentration but relatively low $\delta^{15}N$, $\delta^{18}O$ suggests a likely mixture of "ordinary" and "UUF"

The $\delta^{18}O$ and $\delta^{15}N$ values of residual nitrate increase exponentially as nitrate concentrations decrease from denitrification, leading to a characteristic geochemical signature which enables identification of the occurrence of denitrification (Kendall 1998) (Figure 1.1). For example, Murgulet and Tick (2013) used $\delta^{18}O$ and $\delta^{15}N$ values of nitrate in groundwater to investigate its source and fate in a highly developed aquifer system. Clague et al. (2015) applied a similar approach to evaluate the locations and rates of denitrification occurring in shallow groundwater in a small agricultural catchment in the Waikato region.

This technique is limited by the fact that the isotopic signature disappears once denitrification has progressed to completion, and all nitrate has been removed from the system. This creates a challenge because the nitrate concentrations are very low, making the analysis of the $\delta^{18}O$ and $\delta^{15}N$ difficult (Clague et al. 2015). This technique is also complicated by a lack of knowledge of flow paths, and/or by multiple sources of nitrate that have overlapping isotopic signatures (Böttcher et al. 1990; Clague et al. 2015). To illustrate, the above-mentioned study by Murgulet and Tick (2013) concluded that the dual isotope technique did not perform as well as simple mass ratios for inferring the impact of denitrification within the aquifer.

1.2.4 Acetylene inhibition

'Push-pull' or 'recirculating well' tests have also been used to measure the amount of nitrate that is removed from injected and subsequently extracted groundwater samples since the 1970's (Yoshinari et al. 1977). The tests are performed by adding acetylene gas to extracted water samples. Acetylene inhibits the reduction of N_2O to N_2. The rate and production of N_2O during a push pull test can allow for estimation of denitrification rates.

Such tests are complicated by local groundwater flow and, because the system is perturbed artificially, do not indicate the extent of denitrification that is likely to occur under natural conditions (Burberry et al. 2013; Kim et al. 2005). Furthermore, acetylene can inhibit the production of NO_3^- via nitrification (Mosier 1980). It also can scavenge NO, increasing the oxidation of NO to NO_2 thus reducing N_2O production (e.g. Nadeem et al. 2013). These factors lead to large uncertainty in the results produced from acetylene push pull tests.

1.2.5 Simultaneous measurement of dissolved N_2, Ar and Ne

Measurement of 'excess N_2', the product of the denitrification reaction ($N_{2(g)}$ in Equation 2), is a promising method for directly measuring denitrification that has occurred in an aquifer (Stenger et al. 2013; Wilson 1990). All groundwaters contain dissolved gases derived from the atmosphere during recharge, including N_2. In addition to the dissolved atmospheric N_2,

groundwaters can also contain excess N_2 that has accumulated from denitrification reactions. The dissolved atmospheric N_2, according to the recharge conditions of the water, can be established by the measurement of two noble gases that are part of the atmosphere, usually Ar and Ne. This enables differentiating the excess N_2 produced via denitrification reactions from atmospherically derived dissolved N_2.

A limitation of this method is that it cannot distinguish excess N_2 produced from denitrification and excess N_2 produced from annamox, the anaerobic oxidation of ammonia. However, both processes (denitrification and annamox) have the net effect of removing fixed nitrogen from the system (Smith et al. 2015).

Despite its potential, the excess N_2 technique, as based on measurement of dissolved N_2, Ar and Ne, has never previously been applied to quantify denitrification in New Zealand groundwater systems. This is because, historically, there has been no straightforward, reliable and accurate approach for measuring the concentration of Ne in groundwater in New Zealand.

2.0 METHODS

2.1 Field Sampling and Analytical Methods

2.1.1 Neon

Evacuated 1 L glass flasks are used for groundwater sample collection (Figure 2.1). Sample tubing from the sample source outlet is attached to one of the side-arms on the bottom of the flask. On the opposite side arm, exhaust tubing with a clamp is attached. The sample inlet valve is narrowed in the centre to allow water to pass from the sample source to the exhaust tubing when the sample inlet valve is closed. When it is visible that no air bubbles are passing through the tubing, the clamp is closed and the sample inlet valve is opened, allowing sample water to enter the flask. When approximately 900 mL of sample has entered the flask, the sample inlet valve is closed, leaving a headspace of approximately 100 mL.

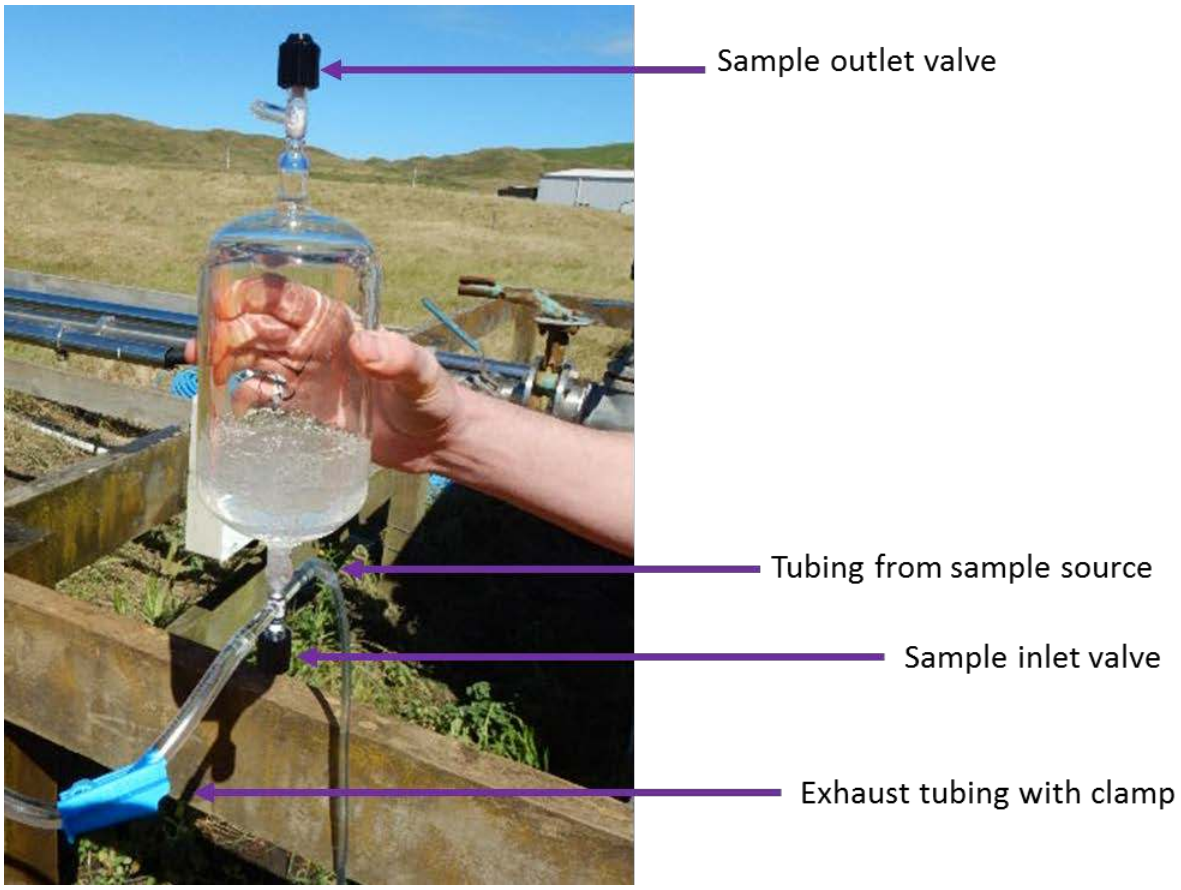

Figure 2.1 Annotated photograph of the Ne sample flask.

2.1.1.1 Neon analytical method, development and set up

Ne is a noble gas with a low solubility relative to Ar and N_2, and it is present in the atmosphere at a concentration of 0.001818%. The standard procedure for measuring dissolved gases (SF_6 CFCs, Ar, and N_2) at the GNS Science Water Dating Laboratory is by a "purge and trap" procedure (e.g., Swinnerton et al. 1962, van der Raaij 2003). However, this method is not suitable for Ne measurement because Ne is not condensable by commonly used trapping methods such as using liquid N_2 (-196 °C). Analysis of Ne using a thermal conductivity detector (TCD) on a gas chromatograph (GC) has been applied for measurement of Ne in gaseous mixtures (e.g., Sugisaki et al. 1982), but TCDs are generally not sensitive enough for measurement of low concentrations of dissolved Ne in groundwaters without pre-concentration

of Ne in the sample. To achieve adequate sensitivity, methods using gas chromatographs equipped with mass spectrometers (GC-MS) (e.g., Beyerle et al. 2000; Brennwald et al. 2013) have been developed. To minimise analytical costs, enable more widespread use and in order to encourage future uptake of the method by stakeholders, the GC-MS approach was not investigated in this study. Instead, a head space analysis approach using a TCD simultaneously with a pulse discharge helium ionisation detector (PDHID) has been used. The PDHID has been adapted for measurement of Ne following the methodology of Lasa et al. (2004). When used in this fashion, the PDHID has been shown to have a sensitivity to Ne an order of magnitude higher than that of the TCD (Lasa et al. 2004).

2.1.1.2 Description of the measurement system

The system developed for the measurement of Ne is shown in Figure 2.1. Two detectors, a PDHID (Valco Instruments D-4-I-SH14-R) and a TCD (Shimadzu TCD-2014), are used, requiring two independent carrier gas flows (HF1 and HF2) of ultra-high purity helium gas. The He is supplied from two gas cylinders, both of which flow through respective in-line regulators, set to 4790 kpa, and subsequent molesieve and oxygen scrubbers. HF1 flows through a pressure controller set to 414 kpa before being purified by a Valco Instuments He purifier. A flow restrictor, reducing the flow to 30 ml min^{-1}, is in place before HF1 reaches a 6 port valve, V1, and the PDHID. HF2 flows through a pressure controller set to 190 kpa. This flow controller is manually adjusted between sample measurements to ensure the flow is always 20.0 standard cubic centimetres per minute. HF2 flows through a 6 port valve, V2, to the standard loop.

A standard curve for Ne, Ar and N_2 is needed to measure groundwater samples. This is produced by evacuating the sample loop before allowing an air standard to enter the sample loop to the desired pressure. The air standard in the sample loop is then injected into the column via V2. Moisture is removed by Nafion tubing before the standard passes through an eight metre molesieve 5A column, which is cooled in an ethanol-dry ice bath to -30 °C. Ne is largely unrestricted through the column, taking approximately 4 minutes to pass through to the PDHID. After passing through the PDHID, HF2 then flows through the TCD resulting in the Ne being measured on two different detectors. Measurement of Ne on the TCD is necessary as the PDHID is highly susceptible to changes in flow and the TCD data are needed when a flow change interferes with the PDHID baseline when Ne flows through. After 5 min 30 sec, V2 is switched so that HF2 flows directly through the TCD and not through the PDHID. The column remains in the ethanol-dry ice bath for another twelve minutes to allow for the separation of Ar and O_2, after which it is placed in a hot water bath, of approximately 90 °C, to remove N_2 from the column.

The measurement of a sample uses the principles of head space analysis and Boyle's Law. The flask is attached to the inlet system via a Cajon fitting. The connection to the flask is then evacuated, as is stainless steel syringe which is extended to its maximum volume. Initially a 200mL stainless syringe was used but this was later increased to 1000 mL to increase the total Ne that was injected into the GC and thus increase the sensitivity of the Ne measurement. The outlet valve on the headspace sample is opened to allow the headspace to spread between the flask and the syringe. The outlet valve is then closed and the syringe is compressed, reducing the volume of the sample. The compressed sample is then injected from the sample loop and follows the same subsequent processes as the air standard for measurement. The area of the integrated peaks from the sample are used to calculate the concentration of each individual gas (Ne, Ar, and N_2) in the headspace.

The original sample concentration (C_i) of a particular gas can be calculated using equation 3:

$$C_i = C_g(K + r) \quad \textbf{Equation 3}$$

Where C_g is the measured concentration of the gas in the headspace, K is the partition coefficient between the gas phase and water, and r is the ratio of the headspace to the volume of water in the sample flask (Sliwka and Lasa 2000).

The uncertainty reported for each measurement of the original sample concentration is the standard measurement error (combined standard uncertainty) u_c of the measurements. This is the summation of all significant uncertainties involved in the analysis (Ellison and Williams 2012) such that the uncertainty for measurement x is given by:

$$u_c(x) = \sqrt{u(s)^2 + u(r)^2 + u(b)^2 + u(m)^2} \quad \textbf{Equation 3}$$

where:

- u(s) is the uncertainty from the calibration procedure arising through the use of least squares regression (Hibbert 2006);
- u(r) is the repeatability which is derived from the relative standard deviation (RSD) of multiple measured standards;
- u(b) is the uncertainty from the blank correction; and
- u(m) is the uncertainty from physical parameters such as standard loop and dead space volumes, pressures, temperatures, sample weights and sample volumes.

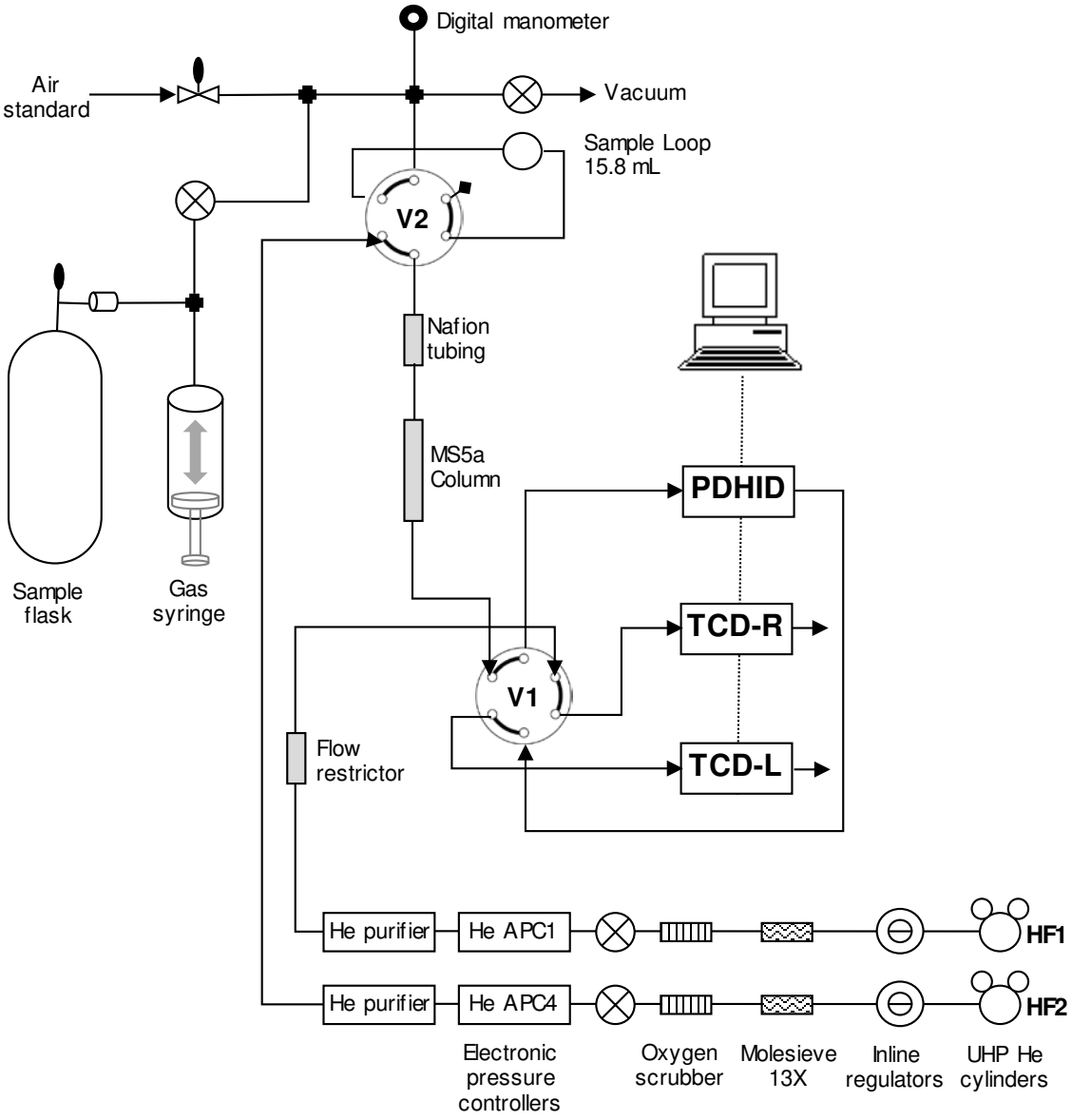

Figure 2.2 Schematic of the analytical set up of the Ne measurement system.

2.1.2 $\delta^{18}O$ and $\delta^{15}N$ in dissolved nitrate

Nitrate samples for isotopic analysis are collected in 125 mL plastic vials and preserved by acidifying in the field. $\delta^{18}O$ and $\delta^{15}N$ of dissolved nitrate are measured at the Stable Isotope Laboratory at GNS Science using a method modified from McIlvin and Altabet (2005). Nitrate (NO_3^-) is converted to nitrite (NO_2^-) using cadmium, then to nitrous oxide (N_2O) using sodium azide in an acetic acid buffer. The nitrous oxide is purged from the water sample and after going through a series of chemical traps to remove H_2O and CO_2 the N_2O is cryogenically trapped under liquid nitrogen. The N_2O passes through a GC column and into an Isoprime IRMS to determine its isotopic signature of nitrogen and oxygen. All results are reported as δ values with respect to AIR for $\delta^{15}N$ and VSMOW for $\delta^{18}O$ where, for example:

$$\delta^{15}N = \left[\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{AIR}} - 1\right] x1000\ ‰ \qquad \textbf{Equation 5}$$

$\delta^{18}O$ is calculated similarly. The analytical precision for these measurements is 0.3‰ for $\delta^{15}N$ and for $\delta^{18}O$, except for samples below 0.1 mg/L NO_3-N which may have lower precisions.

2.1.3 DNA analysis

Samples for DNA analysis were collected in sterile plastic bags. Approximately 3L of sample were collected for each site, and subsequently chilled. Analysis for DNA was carried out by Massey University. To analyse the samples, 500 mL of sample water was filtered through 0.22 µm S-Pak® membrane filters. The filters were then subject to DNA extraction using a DNA isolation kit Genomic DNA kit (Plant). The extracted DNA was quantified, and quality assessed using the DS-11 spectrophotometer (DeNovix Inc. Wilmington, DE USA). The extracted DNA was then used for polymerase chain reaction (PCR) analysis of *nos*Z, nirS, and *nir*K genes. PCRs were set up and conducted through Roche 480 lightcycler using the procedure and reaction setup described in Jha et al. (2017) and Morales et al. (2015).

2.1.4 Childs' Test

Childs' tests were carried out by Waikato Regional Council on soil cores from nine of the ten Waikato piezometer sites. The tests were undertaken at the time of each piezometer installation.

2.1.5 Chemistry

Hydrochemistry samples were collected in sterile plastic bottles following standard collection protocols (Daughney et al. 2006). Samples collected in the Waikato and Canterbury regions were analysed by Hill Laboratories and provided to us by Waikato Regional Council and ESR. Chemical analysis in the Manawatu region was undertaken, and provided to us, by staff at Massey University.

The redox category and dominant redox process were determined by following the methodology of McMahon and Chapelle (2008). This uses a spreadsheet method to identify redox states and dominant redox processes based on threshold concentrations of dissolved oxygen, nitrate, and dissolved manganese, iron and sulphate (Table 1.1).

2.2 Inter-Comparison and Validation of Sampling and Measurement Procedure

To validate the analytical method for simultaneous measurement of Ne, Ar, and N_2, groundwater samples were collected from three deep wells with known Ne concentrations from previous measurements. In 2013, Seltzer et al. (2015) measured noble gases, including Ne and Ar, in "paleo" groundwaters in Taranaki, Marlborough, and Tasman via noble gas mass spectrometry at the Lamont-Doherty Earth Observatory (LDEO). The mean age of these groundwaters ranged from 14,000 to 40,000 years (Seltzer et al. 2015). Due to the very old groundwater, it is unlikely that concentrations of Ar and Ne in these groundwaters would differ significantly between now and 2013. In addition to samples from the three paleo wells, samples from two shallower wells, also previously sampled by Seltzer et al. (2015), were collected as part of this study.

The CSIRO Environmental Tracer and Noble Gas Laboratory in Adelaide, Australia has recently developed a high precision mass spectrometry set up for measuring noble gases. Samples for analysis at the CSIRO lab were taken from the above wells as well as from an additional two wells situated in Lower Hutt New Zealand.

As a further verification of the gas measurement system (that includes Ne), the measured Ar and N_2 concentrations were cross-calibrated against the Ar and N_2 measured in the gas measurement system used for CFC analysis at GNS Science.

2.2.1 Neon inter-comparison sample collection and measurement

Samples were collected from two wells in Taranaki, three wells in Marlborough and the aforementioned two wells in Lower Hutt (Table 2.1). Wells in Taranaki and Marlborough were sampled in triplicate. Three of these wells were artesian and contained paleo groundwater. The two other wells were shallow, and a submersible piston pump (P28w/398) as well as a pre-installed pump were used to sample these wells. The two wells in Lower Hutt were also sampled from pre-installed pumps, where quintuplicate samples were taken instead of triplicate. All wells were purged for three well volumes before sampling. Ne samples were collected following the procedure outlined in section 3.1.1. CFC samples were also collected at the sampling sites following standard groundwater sampling procedure (Daughney et al. 2006). Samples for measurement of Ne, Ar, and N_2 were measured within 24 hours after collection via the procedure outlined in section 3.1.1. Ne samples were also collected in copper tubes in duplicate, following the procedure of Weiss (1968), and were sent to the Environment Tracer and Noble Gas Laboratory at CSIRO in Adelaide, Australia for analysis. No difference was expected to be observed in the dissolved gas concentration from the different sampling methods.

Table 2.1 Summary of well information and sampling details for wells used in Ne measurement inter-comparison.

Site Name	Location	Age (kyr BP)[1]	Screen depth [m BGL] [2]	Sampling date	Easting (NZTM)	Northing (NZTM)	Dissolved Oxygen (mg/L)	Temp-erature (°C)	pH
GND585	South Taranaki	17.5 – 22.4	122.8 – 140.5	15/11/17	1748265	5588832	0.17	17.9	8.17
GND524	South Taranaki	<1	64 – 76.2	15/11/17	1726436	5606436	5.13	15.2	5.70
P28w/0980	Marlborough	39.8 – 43.3	n/a	20/11/17	1678884	5401360	0.13	15.0	7.64
P28w/3278	Marlborough	23.1 – 27.3	102 –187	20/11/17	1673937	5402672	0.07	15.7	8.05
P28w/398	Marlborough	<1		20/11/17	1667689	5406335	8.65	12.0	6.10
R27/1183	Lower Hutt	n/a	25 [3]	24/10/2017	1762179	5437686	n/a	n/a	n/a
R27/1086	Lower Hutt	n/a	181.4 [3]	24/10/2017	1759813	5433246	n/a	n/a	n/a

[1] Ages as published in Seltzer et al. (2015) where ages greater than 17,000 years are considered as paleo-waters

[2] Screen depths in m below ground level (m BGL).

[3] Well depths. Screen depth unknown for these wells.

2.3 Use of Neon for Measuring Denitrification in New Zealand Groundwaters

To validate the application of the Ne technique for measuring denitrification in groundwater systems, groundwater samples for Ne were collected from 27 piezometers. Geology and lithology can have a large impact on the denitrification potential of an aquifer (Rissmann 2011, Devito et al. 2000). Therefore, to assess the effectiveness of the excess N_2 technique in the New Zealand groundwater environment, the piezometers were selected for sampling across three regions (Manawatu, Waikato and Canterbury), with varying geological settings. Other proxies for measuring denitrification, or for demonstrating the potential for denitrification to occur, were sampled in conjunction with the Ne samples. In the Canterbury Region these proxies included dissolved oxygen, $\delta^{18}O$ and $\delta^{15}N$ of nitrate, and hydrochemistry. In the Manawatu and Waikato Region samples were also collected for DNA analysis for the abundance of the *nirS*, *nirK* and *nosZ* genes. Additionally, Childs' tests had been previously carried out at the Waikato Region sampling sites. For all sites sampled, the dissolved oxygen, temperature and pH measurements for each sample were measured in the field at the time of sampling. Furthermore, the piezometers are grouped together in pairs or threes, as designated by the 'Grouping' in Table 2.2, Table 2.3 and Table 2.4, whereby the piezometers are in close proximity to one another but have varying screen depths.

2.3.1 Sampling Sites

2.3.1.1 Manawatu

Nine sampling sites were selected from across the Manawatu region (Table 2.2), five of which are on active dairy farms (SC and Massey Dairy Farm sites). The sampling sites are spread over the Rangitikei catchment, Lower Manawatu Valley and the Mangatainoka catchment. This area consists mainly of Tertiary and Quaternary marine sediments bisected by a central backbone of Triassic to early Cretaceous greywacke rocks that form the Ruahine ranges (Begg and Johnston, 2000). Marine sediments have a greater potential for denitrification than sedimentary or alluvial sediments as they are often rich in material such as glauconite, iron pyrite and oxidisable organic carbon (Rissmann 2011). These materials can act as electron donors to the electron accepting nitrate, allowing for denitrification to occur.

Table 2.2 Summary of well information and sampling details for the Manawatu Region.

Site Name	Grouping	Screen depth (m)	Sampling date	Easting (NZTM)	Northing (NZTM)	Dissolved Oxygen (mg/L)	Temp-erature (°C)	pH
SC1	1	0.8-5.2	19/03/2018	1787655	5558338	0.1	17.1	7.3
SC2	1	0.8-3.3	19/03/2018	1787653	5558337	0.5	17.3	6.8
Massey Dairy Farm 1	2	0.8-9.0	19/03/2018	1820849	5526346	0.1	15.4	6.4
Massey Dairy Farm 2	2	0.8-7.75	19/03/2018	1820848	5526346	0.1	15.7	6.4
Massey Dairy Farm 3	2	0.8-5.9	19/03/2018	1820848	5526346	2.5	18.4	6.3
Burmeister 1	3	5.5-6.1	20/03/2018	1843647	5522245	36	12.2	6.0
Burmeister 2	3	3.3-4.3	20/03/2018	1843871	5522506	5.8	17.8	6.0
Armistead 1	4	Unknown	20/03/2018	1850545	5528738	0.1	10.4	6.3
Armistead 3	4	Unknown	20/03/2018	1850545	5528738	0.1	14.5	7.0

2.3.1.2 Waikato

The ten sampling sites in the Waikato region all fall within the Lake Taupo catchment (Table 2.3). Rhyolitic volcanics dominate the catchment (Leonard et al. 2010). Relatively thick unwelded Oruanui Ignimbrite, with areas of overlying Taupo Ignimbrite, overlies much older welded Whakamaru Group ignimbrites in the northern part of the catchment. The western part of the catchment is dominated by the older welded Whakamaru Group ignimbrites overlain by thinner Oruanui Ignimbrite (Hadfield 2001; Morgenstern 2007a). The south-western part of the catchment is dominated by andesitic and basaltic lava, partially overlain by the Oruanui and Taupo ignimbrites (Morgenstern 2008). In general, consolidated volcanic rocks have low levels of organic carbon, which reduces the potential for denitrification (Rissmann 2011). However, the historical volcanic activity in the area has created a sequence of interbedded paleosols which can provide a significant source of organic carbon (Hadfield 2001). Eight of the ten selected sites are paired piezometers, with paired groups identified in Table 2.3. These paired piezometers allow for screening below and above various paleosols.

Groundwater age-tracer data indicate that these geological units have very different hydraulic properties (Morgenstern 2008). Rain infiltrates readily into the groundwater system through the unwelded Taupo and Oruanui ignimbrites. Groundwater flow into the lake in the northern catchment through these unwelded ignimbrites is mostly via lake bed seepage with long time delay in the groundwater system. Flow into the lake in the western catchment with welded fractured Whakamaru ignimbrite and andesite is mostly via much quicker near-surface runoff. Hydraulic properties can be a good initial indicator for denitrification potential. Groundwater

that has a longer residence time is typically expected to show a greater extent of denitrification, provided the conditions are conducive for denitrification to occur (Rissmann 2011).

Table 2.3 Summary of well information and sampling details for the Waikato region.

Site Name	Grouping	Screen depth (m)	Sampling date	Easting (NZTM)	Northing (NZTM)	Dissolved Oxygen (mg/L)	Temp-erature (°C)	pH
72_4958	9	15.0-21.0	8/05/2018	1839515	5690839	0.3	13.1	6.6
72_1087	10	0.6-6.6	8/05/2018	1834175	5698625	8.7	13.1	6.4
72_4095	10	12.0-16.0	8/05/2018	1834189	5698630	0.2	12.5	6.8
72_1082	11	1.9-7.9	8/05/2018	1832842	5694922	5.7	13.6	6.0
72_4093	11	15.9-21.9	8/05/2018	1832835	5694923	0.3	13.4	6.5
72_4970	12	20.0-23.0	9/05/2018	1851290	5722101	0.3	12.8	7.4
72_4971	12	2.0-8.0	9/05/2018	1851285	5722099	2.6	13.5	6.6
72_1007	13	1.4-7.4	9/05/2018	1860345	5713192	2.8	13.8	6.3
72_4085	13	7.0-9.8	9/05/2018	1860350	5713178	0.3	13.4	6.6
Wastewater2	14	6.77-11.67	9/05/2018	1864133	5712473	1.8	17.6	6.4

2.3.1.3 Canterbury

Eight piezometers were sampled in the central Canterbury plains (Table 2.4). These plains cover some 2700 km^2 between the Waimakariri River to the north and the Rakaia River to the south. The basement of Mesozoic age greywacke is overlain by extensive Quaternary fluvial and glacial deposits consisting mainly of greywacke gravels. Two thirds of the plains are covered by Waimakariri River fan deposits (Brown & Weeber 2001) Aquifers are present in glacial outwash deposits, interglacial and postglacial deposits derived from reworked older deposits, forming ypical heterogeneous fluvial aquifers with both lateral and vertical variation in aquifer yields (Brown & Weeber 2001). The denitrification potential of such materials is generally low as they contain low concentrations of oxidisable organic carbon. However, towards the coast, the aquifers are overlain by and interbedded with confining layers of silt, sand and peat forming the confined aquifers of the Christchurch artesian system (Taylor et al. 1989). Peat has high concentrations of organic carbon and, therefore, a higher denitrification potential.

Groundwater flows in a general south-eastward direction from the foothills to the coast (Hanson & Abraham 2009). Flow patterns are controlled by the degree of sorting of deposits with old buried river channels and other preferential flow paths forming zones of high transmissivity (Brown & Weeber 2001). Recharge to the groundwater system comes primarily from the alpine rivers and land surface recharge consisting of rainfall and irrigation water (Taylor et al. 1989; Stewart et al. 2002).

Table 2.4 Summary of well information and sampling details for the Canterbury region.

Site Name	Grouping	Screen depth (m)	Sampling Date	Easting (NZTM)	Northing (NZTM)	Dissolved Oxygen (mg/L)	Temp-erature (°C)	pH
RF2	5	3.5 - 6.5	17/04/2018	1545323	5164043	4.3		6.9
RF3	5	4.26 - 7.26	17/04/2018	1545391	5164068	4.4		6.1
E1	6	0.0-4.5	17/04/2018	1567825	5193437	0.3		6.2
N3	6	0.0-0.45	17/04/2018	1567733	5193350	<0.1		6.2
SR2	7	0.6 – 9.6	18/04/2018	1517836	5182964	3.7	13.6	6.8
SR1	7	0.75-6.75	18/04/2018	1517849	5182991	4.1	13.9	6.7
BW19	8	12-18	18/04/2018	1544260	5170061	7.6	13.7	6.2
BW8	8	12-18	18/04/2018	1544248	5170101	6.8	13.2	6.2

2.3.2 Sample collection and measurement for denitrification validation

Samples were collected for Ne in triplicate at the nine Manawatu sites, the eight Canterbury sites and the ten Waikato sites (Table 2.3) following the procedure described in Section 3.2.1. Most of the piezometers sampled were grouped, where multiple piezometers were located next to one another but had different screen depths. The groupings are defined in Table 2.2 and Table 2.3. Seven of the nine Manawatu sites were sampled using a peristaltic pump as the piezometer diameter did not allow for a submersible piston pump to be lowered. The other two Manawatu sites, at Burmeister, were sampled using the submersible piston pump. A difference may be expected between the two sampling methods as a peristaltic pump can induce degassing during sampling due to the pressure differential. All wells in the Waikato and Canterbury regions were sampled with a submersible piston or centrifugal pump. In all three regions, wells were purged for three well volumes before sampling. CFC samples were also collected at the sampling sites following standard groundwater sampling procedure (Daughney et al. 2006).

3.0 RESULTS AND DISCUSSION

3.1 Neon inter-comparison

Validation of the newly developed Ne measurement system is demonstrated by the results of the inter-comparison (Table 3.1). At ten of the twelve sites used for the laboratory inter-comparison, the GNS Science results were equivalent, within analytical uncertainty, to Ne concentrations determine by CSIRO or Seltzer et al. (2015).

There were two of the twelve sites at which the GNS Science results were not equivalent to the Ne concentrations determined by other laboratories. The CSIRO samples measured at the two paleo sites in Marlborough, P28w/3278 and P28w/0980, did not fall within the range of uncertainty of both the LDEO and GNS measurements. Furthermore, while the GNS Science measurements at well P28w/0980 were equivalent to results from Seltzer (2015), the duplicate samples measured by CSIRO did not correlate (Figure 3.1). A possible reason for the discrepancy is due to the 2016 Kaikoura earthquake which caused the hydraulic head of both these Marlborough wells to increase by 6m. Sampling was difficult under such high-pressure. Visible degassing was also observed in the sampling tube, which may have resulted in extra gas being collected in CSIROs copper tubes.

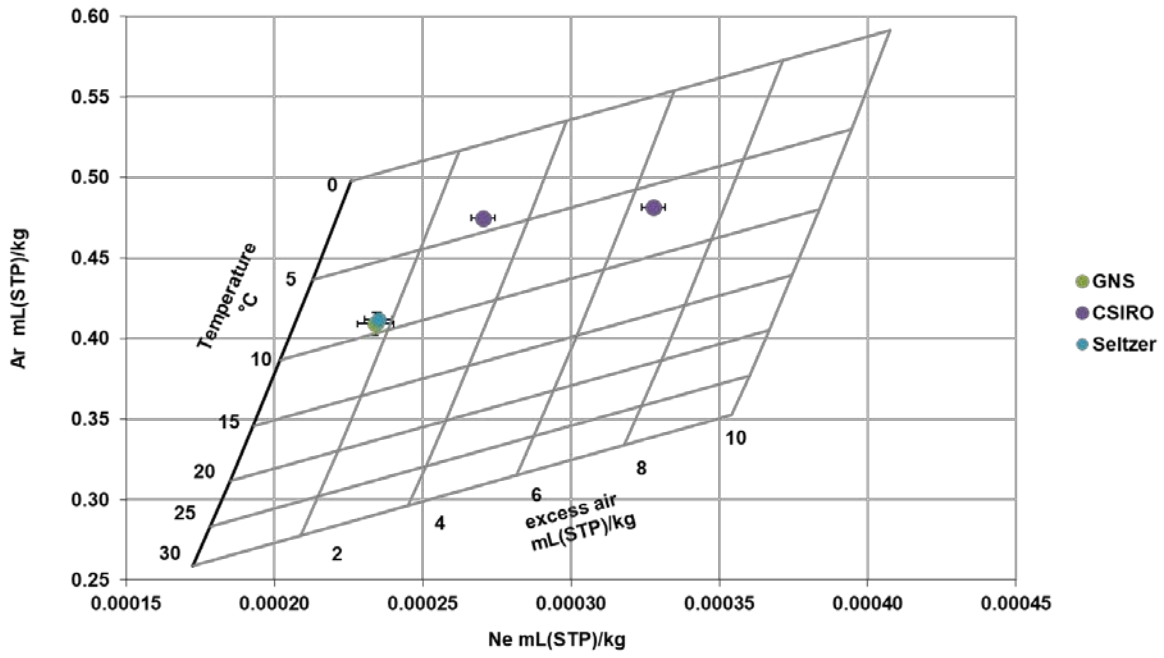

Figure 3.1 Comparison of the measured Ne and Ar concentrations measured by GNS Science (green), Seltzer et al. (2015) (blue) and CSIRO (purple) at well P28w/0980 in Marlborough. The GNS triplicate measurements have been averaged, whereas the duplicate CSIRO measurements are plotted separately as the duplicates did not correlate.

At one of the shallow, non-paleo, wells sampled, GND524, measurements from Seltzer's research group and GNS Science correlated well. However, at P28w/380 the measurements between Seltzer's research group and GNS Science did not correlate. Because these shallow wells have younger waters, they are subject to seasonal and short term climatic changes, so it cannot be expected that the concentrations of Ne and Ar would remain constant over time. However, at P28w/380, the Ne and Ar concentrations between CSIRO and GNS Science correlated well (Figure 3.2).

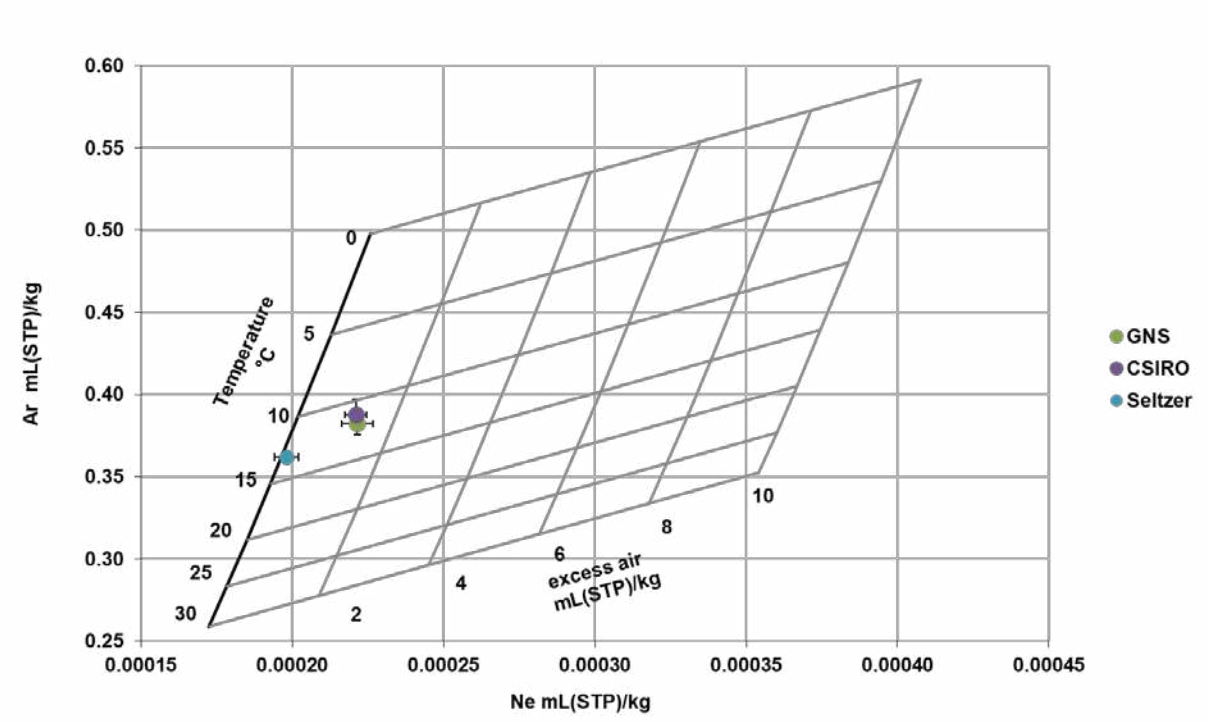

Figure 3.2 Comparison of the measured Ne and Ar concentrations measured by GNS Science (green), Seltzer et al (2015) (blue) and CSIRO (purple) at well P28w/380 in Marlborough

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Table 3.1 Measured Ne, Ar and N2 concentrations at sites used for neon laboratory inter-comparison[1].

Site name	GNS Ne (x 10^{-4})	± (x 10^{-4})	CSIRO Ne (x 10^{-4})	±(x 10^{-4})	Seltzer et al. (2015) Ne (x 10^{-4})	± (x 10^{-4})	GNS Ar	±	CSIRO Ar	±	Seltzer et al. (2015) Ar	±	GNS N_2	±	CSIRO N_2	±	GNS CFC Method Ar	±	GNS CFC Method N_2	±
GND585	2.34	0.06	2.43	0.03	2.30	0.05	0.409	0.005	0.411	0.002	0.412	0.004	16.709	0.387	16.823	0.160	0.409	0.012	16.718	0.267
GND524	1.93	0.05			1.99	0.04	0.367	0.004			0.359	0.010	14.620	0.254			0.366	0.012	14.15	0.24
P28w/0980	2.34	0.06	3.28[2] 4.75[2]		2.35	0.05	0.410	0.010			0.410	0.00	17.910	0.331			0.435	0.016	18.931	0.897
P28w/3278	2.28	0.06	2.54	0.02	2.35	0.05	0.416	0.007	0.420	0.003	0.410	0.009	18.190	0.317	17.790	0.434	0.411	0.012	17.273	0.280
P28w/398	2.21	0.05	2.21	0.04	1.98	0.04	0.382	0.007	0.388	0.004	0.362	0.004	15.160	0.291	14.918	0.139	0.375	0.012	14.391	0.279
R27/1183	2.12	0.04	2.24	0.04			0.384	0.004	0.399	0.005			14.965	0.258	15.366	0.501	0.390	0.009	14.779	0.231
R27/1086	2.23	0.05	2.24	0.04			0.393	0.004	0.394	0.001			15.946	0.297	15.644	0.163	0.384	0.008	15.325	0.243

[1] All units are in mL(STP).kg[1]

[2] The duplicate results did not correlate so both measurement results are reported.

3.2 Using neon to measure denitrification

3.2.1 Overview

The results for the simultaneous measurement of Ne, Ar and N_2 for the 27 sites in the Waikato, Manawatu and Canterbury regions are presented in Table 3.2. Recharge temperatures and excess air concentrations derived from the Ar and Ne concentrations allow calculation of the equivalent concentrations of N_2 unaffected by denitrification, i.e. N_2 derived from equilibration with the atmosphere and from excess air. These reconstructed N_2 concentrations can then be used to calculate the contribution of excess N_2 resulting from denitrification. The concentration of excess N_2 produced from denitrification assumes that all the excess N_2 measured is derived from the denitrification process described in equations 1 and 2. The calculated uncertainties in Table 3.2 are derived following the method described by Equation 4 in section 4.1. Of the twenty-seven sites sampled, fifteen had N_2 in excess above the range of uncertainty, and four sites were on the bounds of the uncertainty limits (Table 3.2). Sections 5.2.2. to 5.2.4 below discuss the results of the excess N_2 measured from the groups of the piezometers sampled, in the context of the other proxies measured for identifying denitrification.

Table 3.2 Measured Ne, Ar and N2 concentrations used to derive calculated excess N2 concentrations.

Site name	Altitude (m)	Measured Ne mL(STP).kg^{-1}	±	Measured Ar mL(STP).kg^{-1}	±	Measured N_2 mL(STP).kg^{-1}	±	Temp. °C	±	Re-constructed N_2 mL(STP).kg^{-1}	±	ΔN2 mL(STP).kg^{-1}	±	moles N mmol.kg^{-1}
Burmeister 1	50	0.000229	0.000004	0.361	0.012	14.602	0.299	15.3	1.9	14.718	0.551	-0.05	0.46	0.00
Burmeister 2	50	0.000195	0.000004	0.346	0.01	13.828	0.402	14.9	1.6	13.325	0.470	0.57	0.50	0.05
Armistead 1	50	0.000189	0.000004	0.364	0.011	16.106	0.736	11.5	1.5	13.688	0.510	2.51	0.81	0.22
Armistead 3	50	0.000142	0.000005	0.309	0.01	12.728	0.527	16.0	1.5	10.797	0.481	2.02	0.61	0.18
Massey Dairy Farm 1	10	0.000220	0.000004	0.379	0.005	15.992	0.653	12.2	0.8	14.910	0.284	1.10	0.68	0.10
Massey Dairy Farm 2	10	0.000223	0.000004	0.392	0.005	16.884	0.689	10.6	0.8	15.346	0.292	1.54	0.71	0.14
Massey Dairy Farm 3	10	0.000183	0.000003	0.356	0.005	14.492	0.592	12.7	0.7	13.208	0.256	1.31	0.61	0.12
SC1	3	0.000312	0.000008	0.442	0.012	23.671	0.995	10.1	1.7	19.279	0.625	4.39	1.08	0.39
SC2	3	0.000202	0.000004	0.408	0.006	19.806	0.808	7.4	0.7	15.21	0.292	4.60	0.83	0.41
RF2	70	0.000235	0.000003	0.380	0.010	15.743	0.309	12.9	1.5	15.478	0.462	0.39	0.43	0.03
RF3	70	0.000225	0.000003	0.371	0.009	15.616	0.255	13.4	1.4	14.922	0.422	0.80	0.37	0.07
N3	5	0.000215	0.000004	0.394	0.011	18.950	0.310	9.9	1.5	15.180	0.521	3.78	0.46	0.34
E1	6	0.000216	0.000003	0.388	0.010	16.637	0.287	10.7	1.4	15.042	0.456	1.61	0.41	0.14
BW8	60	0.000272	0.000004	0.433	0.013	18.913	0.546	8.5	1.6	18.014	0.608	1.00	0.67	0.09
BW19	60	0.000227	0.000003	0.394	0.011	15.955	0.295	10.5	1.5	15.642	0.509	0.43	0.44	0.04
SR2	211	0.000217	0.000005	0.384	0.010	15.446	0.246	10.4	1.4	15.369	0.500	0.45	0.40	0.04
SR1	211	0.000213	0.000003	0.382	0.010	15.203	0.238	10.4	1.4	15.207	0.472	0.38	0.38	0.03
72_1087	414	0.000209	0.000006	0.356	0.009	13.966	0.257	12.8	1.5	14.699	0.482	-0.06	0.40	-0.01
72_4095	414	0.000204	0.000003	0.370	0.011	16.764	0.330	10.5	1.6	14.985	0.535	2.48	0.46	0.22
72_1082	418	0.000222	0.000005	0.370	0.012	14.735	0.243	11.7	1.8	15.483	0.598	-0.06	0.44	-0.01
72_4093	418	0.000221	0.000004	0.390	0.013	17.844	0.587	9.0	1.7	16.072	0.631	2.51	0.71	0.22
72_4958	361	0.000201	0.000003	0.366	0.010	15.174	0.244	11.1	1.4	14.680	0.481	1.11	0.39	0.10
72_1007	395	0.000218	0.000006	0.358	0.010	15.694	0.248	13.4	1.6	14.989	0.534	1.36	0.41	0.12
72_4085	395	0.000217	0.000005	0.365	0.009	17.904	0.266	12.2	1.4	15.167	0.472	3.40	0.40	0.30
Wastewater	369	0.000199	0.000007	0.332	0.009	14.380	0.241	16.0	1.7	13.634	0.510	1.32	0.40	0.12
72_4970	363	0.000206	0.000004	0.370	0.010	16.689	0.273	10.9	1.4	14.946	0.493	2.36	0.41	0.21
72_4971	363	0.000209	0.000007	0.370	0.014	16.419	0.319	11.1	2.1	15.019	0.718	2.01	0.55	0.18

Thirteen sites were classed as anoxic (including DO< 0.5mg/L, and mixed anoxic). Three sites were classed as mixed oxic-anoxic, and 12 sites were classed as oxic (Table 3.3). For the anoxic sites, the predominant redox process was Fe(III) reduction (Figure 3.3). All Waikato sites that had positive Childs' tests are classed as anoxic, except for one site with mixed oxic-anoxic status.

Table 3.3 Chemistry data and redox classification for sampled piezometers in the Waikato, Manawatu and Canterbury regions.

Site name	DO	NO_3-N	Mn (dissolved)	Fe (dissolved)[1]	SO_4	General Redox Category	Redox Process
Burmeister 1	3.58	0.74	0.00	0.01	3.3	Oxic	O2
Burmeister 2	5.85	2.40	0.01	0.01	6.0	Oxic	O2
Armistead 1	0.08	0.01	0.09	3.59	5.52	Anoxic	Fe(III)/SO4
Armistead 3	0.09	0.02	0.05	0.72	0.04	Anoxic	CH4gen
Massey Dairy Farm 1	0.10	0.00	0.19	3.86	2.14	Anoxic	Fe(III)/SO4
Massey Dairy Farm 2	0.06	0.01	0.18	3.67	2.22	Anoxic	Fe(III)/SO4
Massey Dairy Farm 3	2.53	0.16	0.01	0.03	7.35	Oxic	O2
SC1	0.59	0.05	0.29	0.67	11.6	Mixed(oxic-anoxic)	O2-Fe(III)/SO4
SC2	0.13	0.01				O2 < 0.5 mg/L	Unknown
RF2	4.4	4.3		0.081	9.8	Oxic	O2
RF3	4.3	6.5		<0.021	12.1	Oxic	O2
N3	0.02	3.7		0.69	12.5	Mixed(anoxic)	NO3-Fe(III)/SO4
E1	0.25	6.5		0.104	12.9	Mixed(anoxic)	NO3-Fe(III)/SO4
BW8	6.82	3.9		0.082	6.3	Oxic	O2
BW19	7.64	2.9		0.103	6.0	Mixed(oxic-anoxic)	O2-Fe(III)/SO4
SR2	3.7	0.22		<0.021	4.2	Oxic	O2
SR1	4.1	0.34		<0.021	4.3	Oxic	O2
72_1087	8.68	0.99	<0.0005	<0.02	6.2	Oxic	O2
72_4095	0.15	0.05	0.44	7.8	0.6	Anoxic	Fe(III)/SO4
72_1082	5.66	1.86	0.0006	<0.02	2.8	Oxic	O2
72_4093	0.3	0.1	0.166	5.8	0.5	Anoxic	Fe(III)/SO4
72_4958	0.28	0.1	0.066	5.8	6.5	Anoxic	Fe(III)/SO4
72_1007	2.82	0.19	0.22	0.81	16.2	Mixed(oxic-anoxic)	O2-Fe(III)/SO4
72_4085	0.34	0.05	0.39	4.3	17.6	Anoxic	Fe(III)/SO4
Wastewater	1.79	2.1	0.0008	<0.02	32	Oxic	O2
72_4970	0.27	0.35	0.114	0.16	12.4	Anoxic	Fe(III)/SO4
72_4971	2.63	1.92	<0.0005	<0.02	17.6	Oxic	O2

[4] Iron concentrations for Canterbury sites are Total Fe.

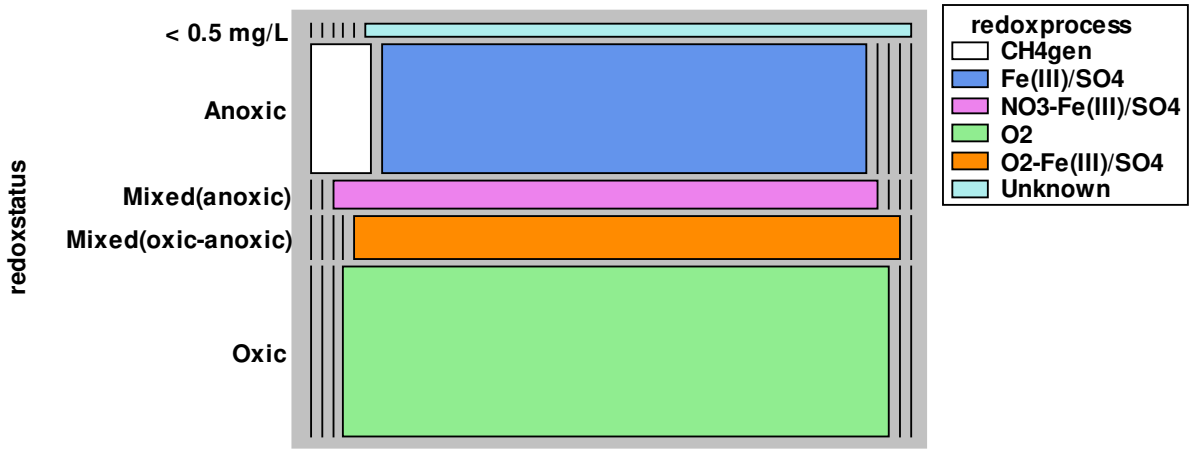

Figure 3.3 Cross-tabulation plot showing redox categories by dominant redox processes.

Sites have been assigned an indicator classification based on the relationship between $\delta^{15}N$, $\delta^{18}O$ and nitrate concentrations, as described in Section 3.1.2 (Table 3.4). Most samples fall into the "Normal N retention" class, with evidence of breakthrough of urine, or fertiliser N in some samples. Only a few samples show evidence suggestive of denitrification. Many of the anoxic sites had insufficient nitrate for isotopic analysis (where no result is reported in Table 3.4), and therefore, any isotopic denitrification signal that may have been apparent at these sites has been lost.

Table 3.4 $\delta^{18}O$ and $\delta^{15}N$ isotopic ratios from sampled piezometers in the Waikato, Manawatu and Canterbury regions.

Site name	Nitrate-N (mgL^{-1})	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	Indicator classification
Burmeister 1	0.735	10.1	6.3	Denitrif
Burmeister 2	2.404	7.6	3.2	NormNRetn
Armistead 1	0.007			
Armistead 3	0.015			
Massey Dairy Farm 1	0.006			
Massey Dairy Farm 2	0.025			
Massey Dairy Farm 3	0.157	6.1	1.8	NormNRetn
SC1	0.053	12.2	4.7	Denitrif
SC2	0.004			
RF2	4.3	5.5	0.7	MixedUU
RF3	6.5	5.4	0.9	MixedUU
N3	3.7	19.6	11.6	Denitrif/ Effluent
E1	6.5	7.5	3.0	NormNRetn
BW8	3.9	3.1	-0.3	UUF
BW19	2.9	3.2	-0.5	UUF
SR2	0.22	4.1	-1.4	NormNRetn
SR1	0.34	3.5	-1.6	Baseline
72_1087	0.99	0.7	0.8	UUF
72_4095	< 0.05			
72_1082	1.86	7.8	2.4	NormNRetn
72_4093	0.1			
72_4958	0.1			
72_1007	0.19	0.7	-5.4	Baseline
72_4085	< 0.05			
Wastewater	2.1	25.7	6.3	Effluent
72_4970	0.35	5.2	1.5	NormNRetn
72_4971	1.92	4.4	-0.1	MixedUU

Most piezometers in both the Waikato and Manawatu regions identified populations of both the *nosZ* and *nirK+S* denitrifier genes (Figure 3.4 and Figure 3.5), indicating the potential for denitrification to occur. Results for gene counts were normalised by the concentration of DNA in each sample to consider that every sample has a different bacterial biomass.

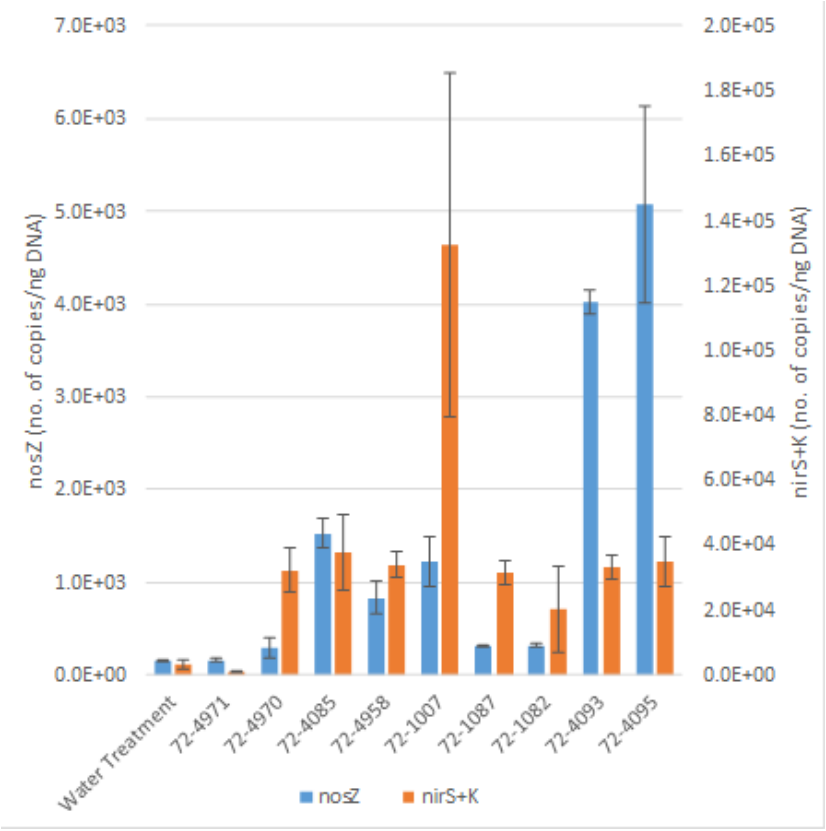

Figure 3.4 Normalised counts of denitrifier genes nosZ, nirK+S at the Waikato sites.

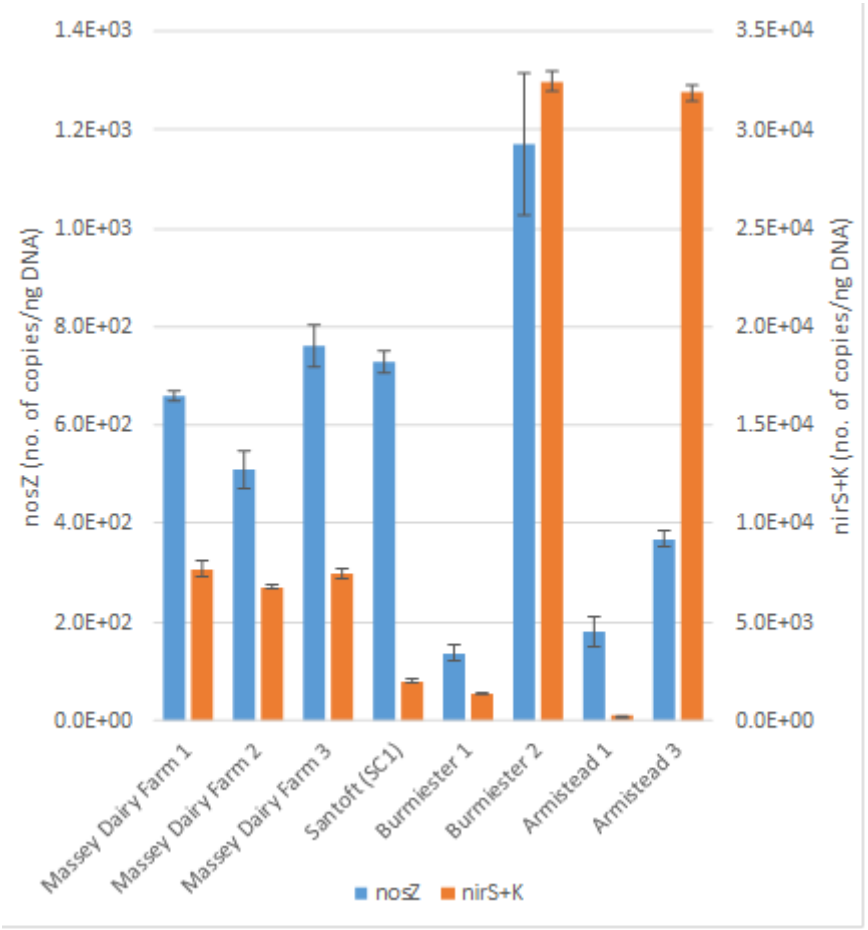

Figure 3.5 Normalised counts of denitrifier genes nosZ, nirK+S at the Manawatu sites.

Correlations between excess N_2, gene counts and redox parameters such as DO, dissolved Fe and dissolved methane were assessed using the non-parametric Spearman correlation test (Table 3.5). Excess N_2 is significantly correlated ($p<0.05$) to redox status indicators such as DO or Fe, but not to the reductase genes *nos*Z or *nir*S+K. Normalised counts of the reductase genes show negative relationships to NO_3, and positive relationships to Fe and excess N_2 (Figure 4-6). Of these relationships, only the correlation to Fe is significant ($P<0.05$). NO_3-N concentrations show a negative relationship and significant correlations ($p<0.05$) to excess N_2, methane and N_2O, and a significant positive correlation to DO. The negative correlation between excess N_2 and the normalised reductase gene counts is consistent with the literature (e.g. Hernandez-del et al. (2018)).

Table 3.5 Spearman Rank correlations with sample size (in brackets) and significance (P-values). P values <0.05 (red coloured text) show statistically significant correlations.

	DO	NO_3-N	Fe	excessN2	nirS+K norm	nosZ norm	N_2O
DO	-						
	-						
	-						
NO3-N	0.5983*	-					
	(27)**	-					
	0.0023***	-					
Fe	-0.3911	-0.2993	-				
	(26)	(26)	-				
	0.0505	0.1345	-				
excessN2	-0.7603	-0.4703	0.6388	-			
	(27)	(27)	(26)	-			
	0.0001	0.0165	0.0014	-			
nirS+K_norm	0.0361	-0.0506	0.5089	0.0692	-		
	(18)	(18)	(18)	(18)	-		
	0.8816	0.8347	0.0359	0.7753	-		
nosZ_norm	-0.1126	-0.303	0.5479	0.304	0.782	-	
	(18)	(18)	(18)	(18)	(18)	-	
	0.6424	0.2116	0.0239	0.2100	0.0013	-	
N_2O	-0.7857	-0.8571	0.4392	0.5476	0.0952	-0.2619	-
	(8)	(8)	(8)	(8)	(8)	(8)	-
	0.0376	0.0233	0.2453	0.1474	0.8011	0.4883	-
CH_4	-0.6788	-0.6172	0.4616	0.6046	0.055	0.3319	0.8286
	(24)	(24)	(24)	(24)	(16)	(16)	(6)
	0.0011	0.0031	0.0268	0.0037	0.8314	0.1986	0.0639

*Spearman Rank correlations
**(Sample Size)
***P-Value

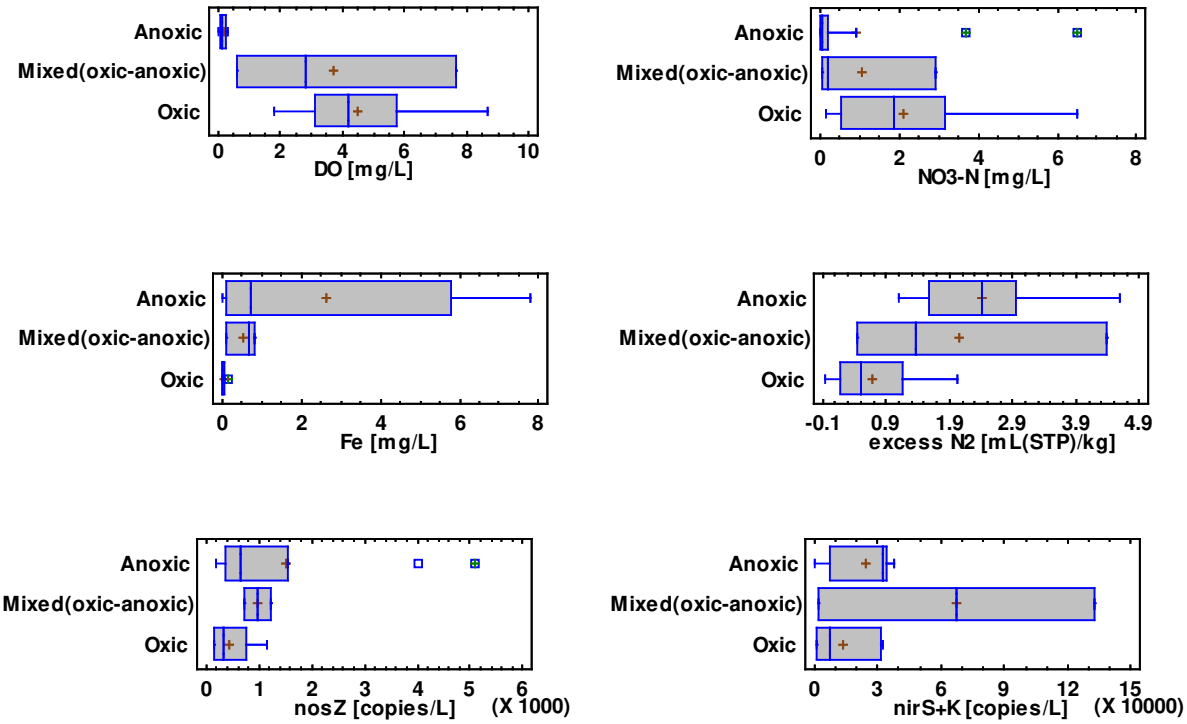

Figure 3.6 Boxplots of redox parameters (DO, NO3-N, Fe), excess N2 and denitrifier gene copies sorted by redox status.

Relationships of excess N_2 and gene counts to redox categories were assessed using a one-way analysis of variance (ANOVA) test and non-parametric tests such as the Mann-Whitney and Kruskal-Wallis tests. As expected, excess N_2 shows an association with redox status, with a significant difference ($p<0.05$) between anoxic and oxic groundwater (Figure 3.6). For normalised *nos*Z or *nir*S+K counts, differences between oxic and anoxic sites are not significant ($p<0.05$).

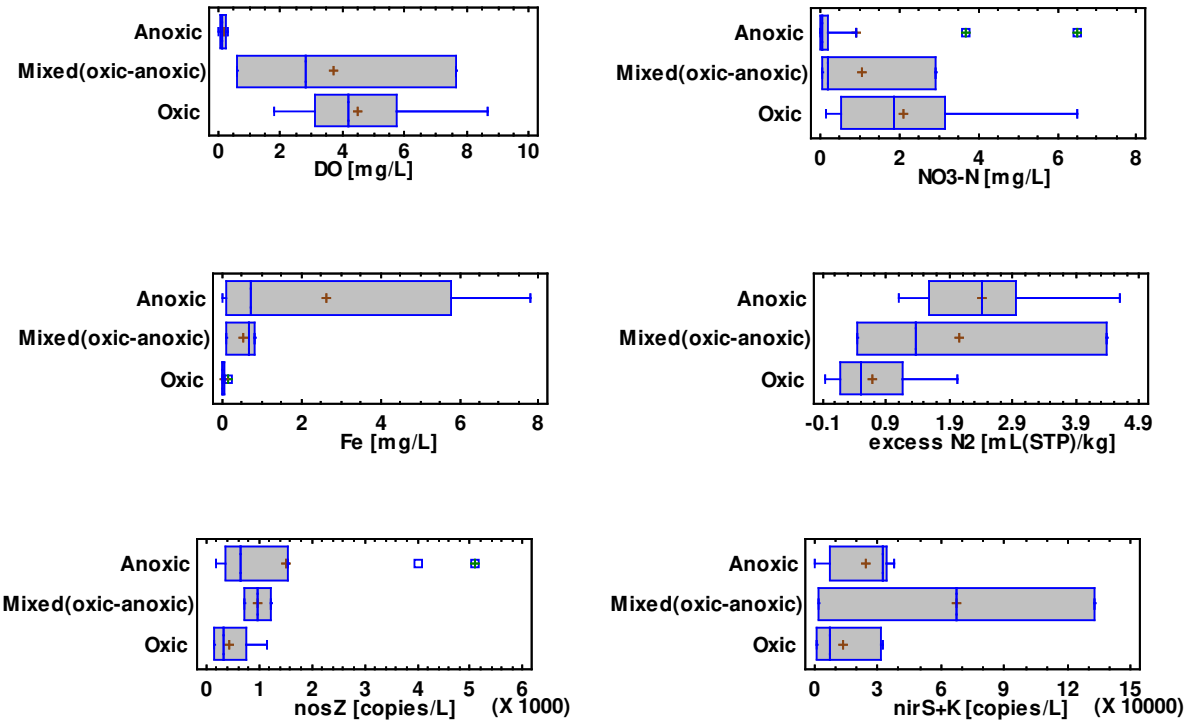

Figure 3.7 Boxplots of redox parameters (DO, NO3-N, Fe), excess N2 and denitrifier gene copies sorted by redox status.

3.2.2 Manawatu

Difficulties during sampling were encountered at the Manawatu region sites. The majority of these sites, excluding the Burmeister sites, had a non-standard piezometer diameter which was too narrow for the piston flow pump. Instead, at these seven sites a peristaltic pump was used. Because a peristaltic pump uses suction to draw up water from the ground, it can induce degassing. Heavy degassing was observed at all sites except the Burmeister sites (e.g. Figure 3.8). At Burmeister 1, samples were taken using both the peristaltic pump and the submersible piston pump. The sample taken via the peristaltic pump is degassed in comparison to the one taken with the submersible piston pump (Figure 3.9). Due to this, and the observed degassing in the sampling tube, all seven other piezometers are assumed to have undergone some degassing during sampling. The effect of this is that the results in Table 3.2 (except for the Burmeister sites) are most likely not representative of the actual excess nitrogen in the groundwater. Therefore, the excess N_2 concentrations described in Table 3.2 are considered qualitatively.

Figure 3.8 Observed degassing in the sampling tube while sampling for dissolved gasses using a peristaltic pump at the Armistead sites.

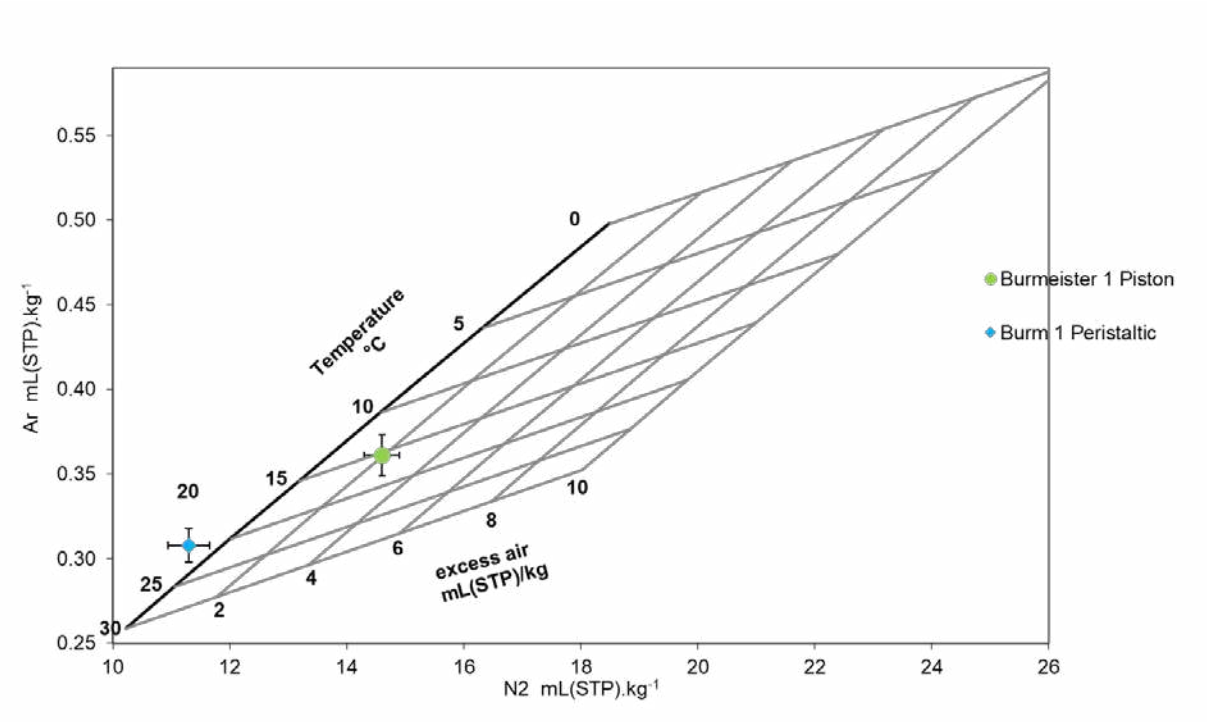

Figure 3.9 Measured N_2 and Ar concentrations using a submersible piston flow pump and a peristaltic pump at Burmeister 1.

All of the sites sampled in the Manawatu region, except for Burmeister 1 and 2, showed excess N_2 concentrations, indicating occurrence of denitrification Table 3.2). However, because of degassing while sampling, the actual amount of denitrification occurring in the aquifer system is probably greater than that measured. For example, when looking at the Ne-Ar ratios for the SC sites (Figure 3.10) you can see that SC2 falls to the left of the water equilibrated with

atmosphere (WEA) line, indicating the occurrence of degassing. The same was observed at Massey Dairy Farm 3 (Figure 3.10) and both Armistead sites.

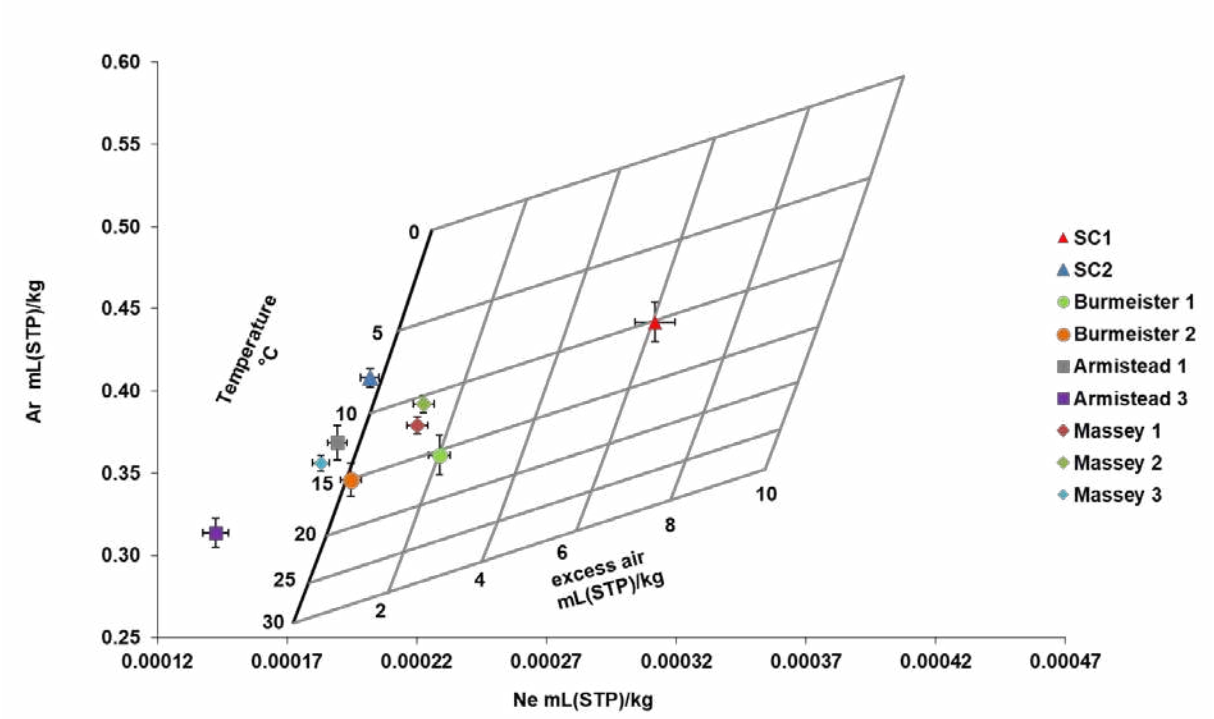

Figure 3.10 Measured Ne and Ar concentrations at the Manawatu sites where the bolded line represents the WEA line.

All Manawatu sites with significant excess N_2 concentrations are classed as anoxic using the redox classification system of McMahon and Chapelle (2008), with the exception of Dairy Farm 3, which is classed as oxic (having a DO concentration of 2.54 mg/L) and SC1 which is classed as mixed oxic/anoxic (Table 3.4). Interestingly, historical push-pull tests carried out at SC1 found the site to be highly anoxic with denitrification occurring slowly (Collins 2015). The redox status of groundwater can indicate that the potential for denitrification occurs (Thayalakumaran et al. 2008). In this respect, the DO and other chemistry data for these wells support the excess N_2 findings that denitrification is occurring.

Both the *nirK+S* and *norZ* genes were found in all samples from the Manawatu. Piezometers at Massey Dairy Farm all showed excess N_2 and all have high population counts of both denitrifying genes. There appears to be no difference between the redox state of these three piezometers and the normalised denitrifier gene counts. The two Armistead sites show interesting results. While both piezometers were anoxic and showed excess N_2, Armistead 3 showed the highest counts for the *NirS+K* gene, the gene responsible for the reduction of nitrous oxide. Potentially this could indicate the production of annamox, the reduction of which also terminates at N_2 gas. However, as gene counts were high for piezometers in which no denitrification was occurring, conclusions based on the gene counts cannot be made with any certainty.

Samples from four sites (Burmeister 1 and 2, Dairy Farm 3 and SC1) from the Manawatu region had sufficient nitrate for isotopic analysis (Table 3.4). Of these four sites, Burmeister 1 and SC1 show a possible denitrification signal (Figure 3.11). The other two sites fall into the range expected for normal N retention. The result for Burmeister 1 is unexpected, as this site is oxic, has no excess N_2, and relatively low *nosZ* gene copy abundance, therefore we do not expect significant denitrification to be occurring at this site. However, the isotopic denitrification

signal at this site is marginal, and may be an artefact of nitrogen cycling and retention in the soil.

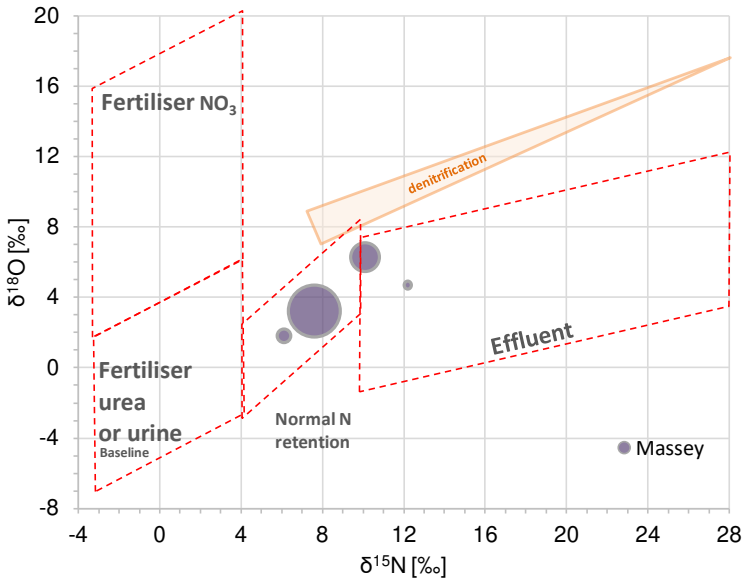

Figure 3.11 Plot of $\delta^{18}O$ against $\delta^{15}N$ in nitrate samples from the Manawatu Region sites. Symbol size is proportional to NO_3-N concentration.

The results in Figure 3.12 depict dissolved N_2O and excess N_2 in the Manawatu groundwaters. N_2O concentrations are low and are near or below the expected concentration for dissolution of atmospheric N_2O in recharge waters (Hiscock et al. 2003). Excess N_2 concentrations are significantly higher than N_2O concentrations, indicating that when denitrification is occurring within the aquifer, the process is essentially proceeding to completion with the end product N_2.

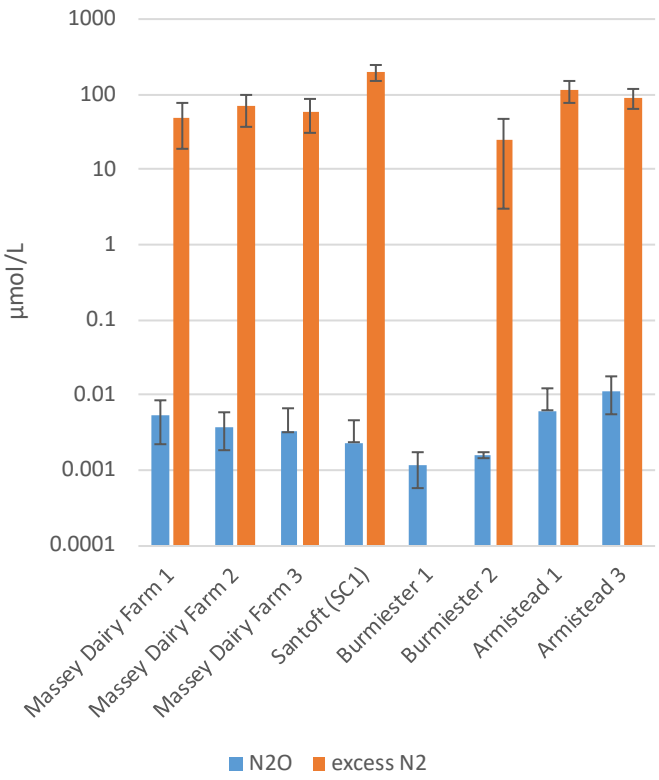

Figure 3.12 Dissolved N_2O and excess N_2 concentrations at Manawatu region sites.

3.2.3 Canterbury

Only two of the eight sites sampled in the Canterbury region had measured excess N_2 well outside the bounds of uncertainty. Of these two sites, E1 and N3, N3 showed the greatest excess N_2.

The assessment of denitrification by the Ne method is supported by the other proxies measured. Dissolved oxygen was low at sites E1 and N3 (<0.5mg/L). However, the chemistry data indicated a mixed anoxic redox status due to the high concentrations of nitrate (still) present. Despite this, site N3 appears to be highly reduced, with the presence of dissolved methane. $\delta^{18}O$ and $\delta^{15}N$ data from dissolved nitrate show that denitrification is occurring at site N3, whereas at site E1, the denitrification signal is most likely swamped by the high concentration of nitrate still present.

Most of the other Canterbury sites are classed as oxic by the McMahon and Chapelle (2008) redox classification scheme. This supports the Ne measurements as no excess N2 outside the bounds of uncertainty were found here. $\delta^{18}O$ and $\delta^{15}N$ data for these other sites is mixed, with some sites falling into the normal N retention class, and others showing the influence of the breakthrough of urine, urea or some other inorganic nitrogenous fertiliser (Figure 3.13).

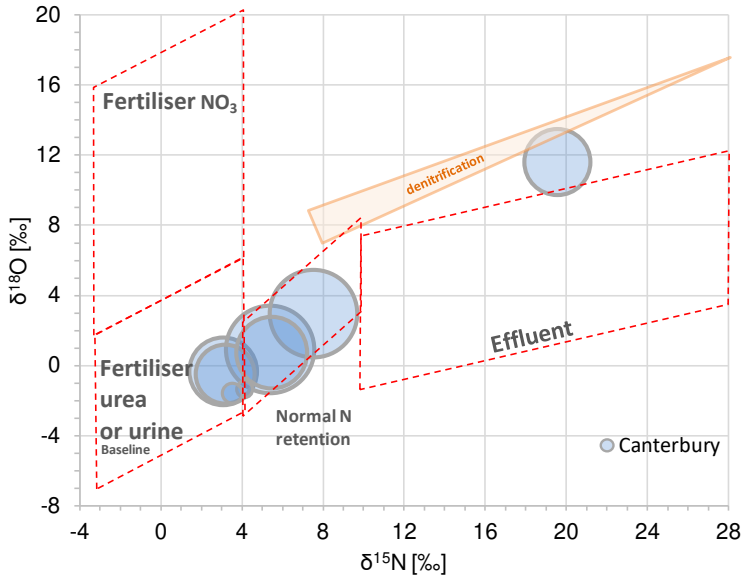

Figure 3.13 Plot of $\delta^{18}O$ against $\delta^{15}N$ in nitrate samples from the Canterbury sites. Symbol size is proportional to NO_3-N concentration.

3.2.4 Waikato

Of the ten sites sampled in the Waikato Region, eight had measurable concentrations of excess N_2. Five of these eight sites had measured excess N_2 well above the bounds of uncertainty.

The two sites with no detectable excess N_2 were shallow wells 72_1087 and 71_1082. Childs' tests done on sediment cores of these wells gave negative results Table 3.6). Under the redox classification of McMahon and Chappelle (2008), these two sites are classed as oxic (Table 3.4), as were two other sites (72_4971 and Wastewater). Thus, these two proxies for determining whether there is denitrification potential support the observed lack of excess N_2.

Both of 72_1087 and 71_1082 were within a few metres of deeper piezometers that crossed a redox zone (72_4095 and 72_4093). These deeper piezometers had excess N_2 well above the bounds of uncertainty and had positive Child's tests on their sediment cores. Under the redox classification of McMahon and Chappelle (2008), sites 72_4095 and 72_4093, were classed as anoxic (Table 3.4), which is consistent with the observance of excess N_2 at these sites.

Another pair of deep and shallow piezometers, 72_1007 and 72_4085, located metres apart from each other, also gave varying results. While both had positive Child's tests, the deeper, piezometer showed excess N_2 well above the bounds of uncertainty, whereas the shallower piezometer had excess N_2 concentrations on the bounds of uncertainty. This shallow piezometer had varying DO concentrations depending on the depth the pump was lowered to, likely indicating that the piezometer is drawing from both oxic and anoxic waters. With simultaneously high concentrations of DO and dissolved Fe, this site is classed as mixed oxic/anoxic (Table 3.4) under the redox classification of McMahon and Chappelle (2008), while site 72_4085 is classed as anoxic.

Of the four sites with higher excess N_2, two of the sites (72-4093 and 72-4095) have a significantly higher abundance of *nosZ* gene copies (Figure 3.14). The high abundance of the *nosZ* gene could indicate the occurrence of complete denitrification and supports the observance of excess N_2 at these sites. However, site 72_4970 has high excess N_2

concentrations, but was found to be not as abundant in *nos*Z gene copies. It is unclear why this is so.

One piezometer sampled in the Waikato region gave quite contradictory results. Piezometer 72_4971 was shallow, oxic, had a negative Childs' Test, had very low denitrifying gene counts, yet it showed high excess N_2. A possible explanation is that the denitrification occurs within the aquifer matrix but not close to the well screen. The excess N_2, as it is a gas, is easily transported through the groundwater but the denitrifying genes, being less mobile, have not been captured.

Samples from six sites in the Waikato region had sufficient nitrate for isotopic analysis (Table 3.3). Despite the detection of excess N_2 and anoxic classification of some sites, no site shows an isotopic denitrification signal (Figure 3.14). $\delta^{18}O$ and $\delta^{15}N$ data for the sites is mixed, with some sites falling into a normal N retention, and others showing the influence of the breakthrough of urine, urea or some other inorganic nitrogenous fertiliser. Site 72_1007 shows a baseline isotopic signal, even though there is some likelihood of denitrification occurring at this site due to the mixed oxic/anoxic state. As nitrate concentrations decrease with increasing denitrification signal, any such signal is likely swamped by the presence of oxic groundwater with baseline levels of nitrate. As might be expected, the Wastewater site has an isotopic signal for wastewater effluent.

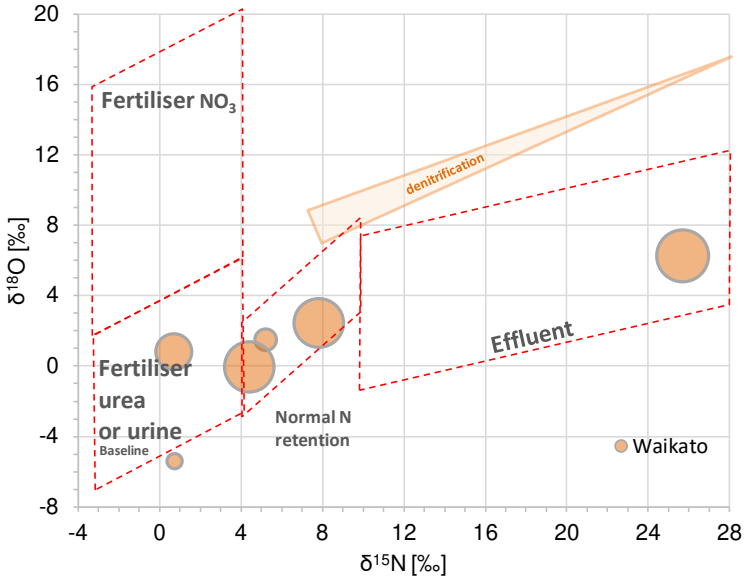

Figure 3.14 Plot of $\delta^{18}O$ against $\delta^{15}N$ in nitrate samples from the Waikato sites. Symbol size is proportional to NO_3-N concentration.

Table 3.6 Childs' Test results for soil cores from Waikato piezometer sites.

Site name	Grouping	Screen depth (m)	Positive Childs' Test depths (m)
72_5948	9	15.0-21.0	8.8-10.65 10.85-25.5
72_1087	10	0.6-6.6	No positive test*
72_4095	10	12.0-16.0	10.5-16.0
72_1082	11	1.9-7.9	No positive test*
72_4093	11	15.9-21.9	13.2-21.9
72_4970	12	20.0-23.0	14.84-15.1 18.6-18.8 21.1-21.5 22-23
72_4971	12	2.0-8.0	No positive test
72_1007	13	1.4-7.4	6.8-7.4*
72_4085	13	7.0-9.8	6.8-9.8
Wastewater	14	6.77-11.67	

* Child's test results are assumed based on the results from the deeper paired piezometer located several metres away.

4.0 CONCLUSIONS AND RECOMMENDATIONS

An analytical system to measure Ne in groundwater has been developed to accurately calculate groundwater recharge temperature and concentration of excess nitrogen. This system set up was successfully validated against noble gas measurements by GCMS from two other reputable institutions, CSIRO and LDEO.

The application of the method to the New Zealand groundwater environment also proved to be successful. When compared to the denitrification proxies of chemistry/redox status, and $\delta^{18}O$ and $\delta^{15}N$ ratios the excess N_2 method predominantly correlated well. The excess N_2 method was shown to be particularly useful at sites which had a mixed redox state, where denitrification proxies such as chemistry and redox state were less conclusive in identifying the potential for denitrification to occur. However, the results between the excess N_2 method and the molecular method did not correlate. The excess N_2 method also demonstrated its strength compared to the $\delta^{18}O$ and $\delta^{15}N$ measurements, as many of the sites which exhibited excess N_2 did not have high enough nitrate concentrations for isotope measurement, presumably because the nitrate had been reduced.

While results presented in this report show that the Ne measurement method for quantifying excess N_2 in groundwaters is successful, there are further improvements which can be made to increase the robustness of the study. Groundwater flow paths are complex and not in a steady state. To further clarify the denitrification processes occurring within groundwater systems the following future work is proposed:

- To understand any seasonal changes in the denitrification processes it is recommended that a sampling round is undertaken during the end of the wet season, because the samples in this study were collected at the end of summer.
- Another sampling round in the Manawatu Region would also be of benefit if the degassing issues while sampling could be resolved. This would allow for quantitative excess N_2 data to be derived.
- Only a small number of piezometers were sampled in the study. To better understand denitrification at a national level it would be beneficial to expand the sampling to a national level, such as sampling for denitrification at National Groundwater Monitoring Programme Wells (NGMP).
- A limitation of one of the proxies sampled, $\delta^{18}O$ and $\delta^{15}N$ ratios, was that often if denitrification is occurring the concentration of nitrate in the water is too low for measurement. Development of a procedure to measure the ^{15}N isotopic ratio in the dissolved N_2 would provide another proxy for denitrification detection.

5.0 ACKNOWLEDGMENTS

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