

Assessment of excess N₂ for quantifying actual denitrification in New Zealand groundwater systems

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Abstract

Denitrification in groundwaters is a key nitrate (NO₃⁻) attenuation process, where leached NO₃⁻ can be reduced to gaseous forms of nitrogen (N₂). In this study, calculation of the concentration of excess N₂ is applied to the New Zealand context to quantify the extent of denitrification occurring in groundwater systems under natural flow conditions. The concentration of dissolved atmospheric N₂, according to the recharge conditions of the water, can be established by the measurement of two noble gases that are also part of the atmosphere. This enables differentiation of the excess N₂ gas produced via denitrification reactions from atmospherically derived dissolved N₂ gas. The excess N₂ method was applied to ten shallow piezometers in the Lake Taupo catchment, in combination with other denitrification proxies: δ¹⁸O and δ¹⁵N isotopes of NO₃⁻; identification of microbial denitrifying genes; and redox conditions. Eight of the ten sites had measurable excess N₂. Excess N₂ was detected at sites where δ¹⁸O and δ¹⁵N did not identify denitrification and at some sites

that were oxic or in a mixed redox state. This is because the redox classification only indicates the potential for denitrification to occur, unlike the excess N₂ method (which detects the actual accumulated denitrification product), and the dual nitrate isotope method is limited by the potential variation in source signatures and the nitrate itself being consumed.

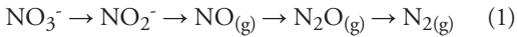
Keywords

water quality; nitrate attenuation; neon; argon; excess nitrogen; denitrification

Introduction

Nitrate (NO₃⁻) is the most pervasive contaminant in New Zealand groundwaters. Approximately 40% of long-term groundwater monitoring sites show above-natural concentrations of nitrate (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 (N₂) (Chapelle, 1993):



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 groundwater-fed receiving waters (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 include dissimilatory nitrate reduction to ammonia and anaerobic ammonia oxidation (anammox) (Tiedje, 1988; Smith *et al.*, 2017).

The extent of denitrification occurring in New Zealand's groundwater systems, according to Equation 1, is poorly known. Several approaches have been developed and applied to determine if denitrification has occurred or might occur, but these techniques have a variety of important limitations.

The aim of this study was to test the 'excess N₂' method for assessing groundwater denitrification in the New Zealand context. Groundwaters contain dissolved gases derived from the atmosphere during recharge, including N₂. In addition to the dissolved atmospheric N₂, groundwaters can also contain excess N₂ that has accumulated from denitrification reactions (i.e., the product of Eq. 1). The dissolved atmospheric N₂, according to the recharge conditions of the water, can be established by the measurement of two or more noble gases that are part of the atmosphere. Historically, this has usually been argon (Ar) and N₂ (e.g., Vogel *et al.*, 1981). However, as the concentration of N₂ can increase due to denitrification a third gas, neon (Ne), can be measured to more

precisely refine the derivation of the recharge temperature (e.g., Seltzer *et al.*, 2015). This enables differentiation of excess N₂ produced via denitrification reactions from the atmospherically derived dissolved N₂.

Despite its known potential (e.g., Stenger *et al.*, 2013; Wilson, 1990), the excess N₂ technique, as based on measurement of dissolved N₂, Ar and Ne, has not yet 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 Ne concentration in groundwater in New Zealand. In this paper we present an analytical method for determining excess N₂ based on the simultaneous measurement of Ne, Ar and N₂ via gas chromatography, and we validate this method against other methods that have been applied to evaluate denitrification in groundwater systems in the New Zealand context.

Existing approaches for evaluating denitrification in groundwater

Denitrification is dependent on abiotic and biotic factors. Important abiotic factors include the oxygen status of the groundwater and the availability of electron donors (Korom, 1992; Rivett *et al.*, 2008). Important biotic factors include the presence of an active denitrifier population that performs the denitrification steps in soil-water systems (Saggar *et al.*, 2013). Occurrences of partial or complete denitrification lead to a variable microbial community structure in the sub-soil or groundwater (Wakelin *et al.*, 2011; Philippot *et al.*, 2013). The following paragraphs describe the four main existing methods, and their limitations, for evaluating denitrification in groundwater: assessment of redox conditions; evaluation of the microbial community; measurement of the stable isotope ratios of nitrate ($\delta^{18}\text{O}$ and $\delta^{15}\text{N}$); and push-pull tests to quantify nitrate removal in situ.

For assessing denitrification in New Zealand groundwater, much emphasis has been placed on identifying where optimal redox conditions are present to allow for the facilitation of denitrification (Stenger *et al.*, 2008; Rivas *et al.*, 2017). The redox state of the groundwater is commonly determined by measuring the dissolved concentrations of redox-sensitive elements and compounds (McMahon and Chapelle, 2008). However, redox reactions may not reach thermodynamic equilibrium in groundwater systems and so comparisons of the concentrations of redox-sensitive elements must be undertaken with caution (Langmuir, 1997). Another method for evaluating the redox status involves the use of redox electrodes (e.g., platinum). However, these may not always provide meaningful measurements, for example where the dominant redox-sensitive elements are non-electroactive (e.g., C, O, H, N, S) (Langmuir, 1997). The Childs test can also be applied to visually detect reducing zones by applying a dye (α, α' -dipyridyl) to a soil or aquifer core, where the dye reacts with reduced iron (Fe^{2+}) in the soil or aquifer material indicating reducing conditions (Childs, 1981). However, 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).

An important limitation of the above-listed methods for evaluating the redox status of groundwater is that they only indicate potential occurrence of denitrification in an aquifer, but not whether it has actually occurred, or to what extent (Langmuir, 1997). For example, Daughney *et al.* (2010) reported that, in New Zealand (as elsewhere in the world), the oldest groundwaters are most likely to be reduced, but the study could not determine the actual amount of denitrification that had occurred. Furthermore, old groundwater tends not to be contaminated with nitrate in the

first place due to limited anthropological influence. Another study that compared groundwater age to redox status concluded that many reduced (anoxic) zones are relatively 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 as readily through these zones. Despite this, denitrification can have significant influence at the paddock scale whereby anoxic pools can form within a small area.

Microbial communities within aquifers have also been investigated to identify the potential for denitrification to occur. For example, Sirisena *et al.* (2018) collected groundwater samples at 35 selected sites from the New Zealand National Groundwater Monitoring Programme, sequencing the 16S rRNA gene. Metabolic inferences were made based on the taxonomic composition of the microbial community at each site to predict oxygen requirements, metabolic potential and dominant energy sources of the constituent bacteria. The study showed that the bacterial community structure was related to the redox condition of its groundwater.

Another molecular approach for assessing denitrification potential in groundwater systems involves analysis 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, like the redox approach, only indicate whether there is

potential for denitrification to occur and do not provide a quantitative measure of the amount of denitrification that has occurred or is currently occurring in a groundwater sample.

The stable isotope composition of nitrate, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$, can be used as direct measure of denitrification, under ideal conditions where the inputs are constant and the flow paths are well known. Isotopic compositions can vary as a result of numerous 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. The $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of residual nitrate can increase exponentially as nitrate concentrations decrease from denitrification, leading to a characteristic geochemical signature which enables identification of the occurrence of denitrification (Kendall, 1998). 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. Low nitrate concentrations from partial denitrification are also challenging, as they make the analysis of the $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ difficult (Clague *et al.*, 2015). The 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).

Since the 1970s, 'push-pull' or 'recirculating well' tests have been used to measure the amount of nitrate removed from injected, and subsequently extracted, groundwater samples (Yoshinari *et al.*, 1976). The tests are performed by adding acetylene gas to extracted water samples, reinjecting the prepared samples, then subsequently extracting groundwater samples at pre-determined time intervals.

Acetylene inhibits the reduction of N_2O to N_2 . The rate and production of N_2O during a push-pull test can allow estimation of denitrification rates.

Such push-pull 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 (Burbery *et al.*, 2013; Kim *et al.*, 2005). Furthermore, acetylene can inhibit the production of nitrate via nitrification (Mosier, 1980). It also has the ability to 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.

Study sites

Ten groundwater sampling sites were selected in the Lake Taupo catchment, Waikato (Table 1 and Fig. 1). 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 subsurface denitrification (Rissmann, 2011). However, the historic volcanic activity in the area has created a sequence of interbedded paleosols, which can provide a significant source of organic carbon (Hadfield, 2001; Clague *et al.*, 2013).

Eight of the ten selected sites were paired piezometers, with paired groups identified in Table 1. 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 readily infiltrates into the groundwater

Table 1 – Summary of well information and sampling details

Site Name	Grouping ¹	Screen depth (m)	Sampling date	Easting (NZTM)	Northing (NZTM)
72_4958	1	15.0–21.0	8/05/2018	1839515	5690839
72_1087	2a	0.6–6.6	8/05/2018	1834175	5698625
72_4095	2b	12.0–16.0	8/05/2018	1834189	5698630
72_1082	3a	1.9–7.9	8/05/2018	1832842	5694922
72_4093	3b	15.9–21.9	8/05/2018	1832835	5694923
72_4970	4a	20.0–23.0	9/05/2018	1851290	5722101
72_4971	4b	2.0–8.0	9/05/2018	1851285	5722099
72_1007	5a	1.4–7.4	9/05/2018	1860345	5713192
72_4085	5b	7.0–9.8	9/05/2018	1860350	5713178
Wastewater	6	6.77–11.67	9/05/2018	1864133	5712473

¹ Arbitrarily assigned number to identify piezometer pairs. Piezometers that have the same grouping number are located within metres of each other.

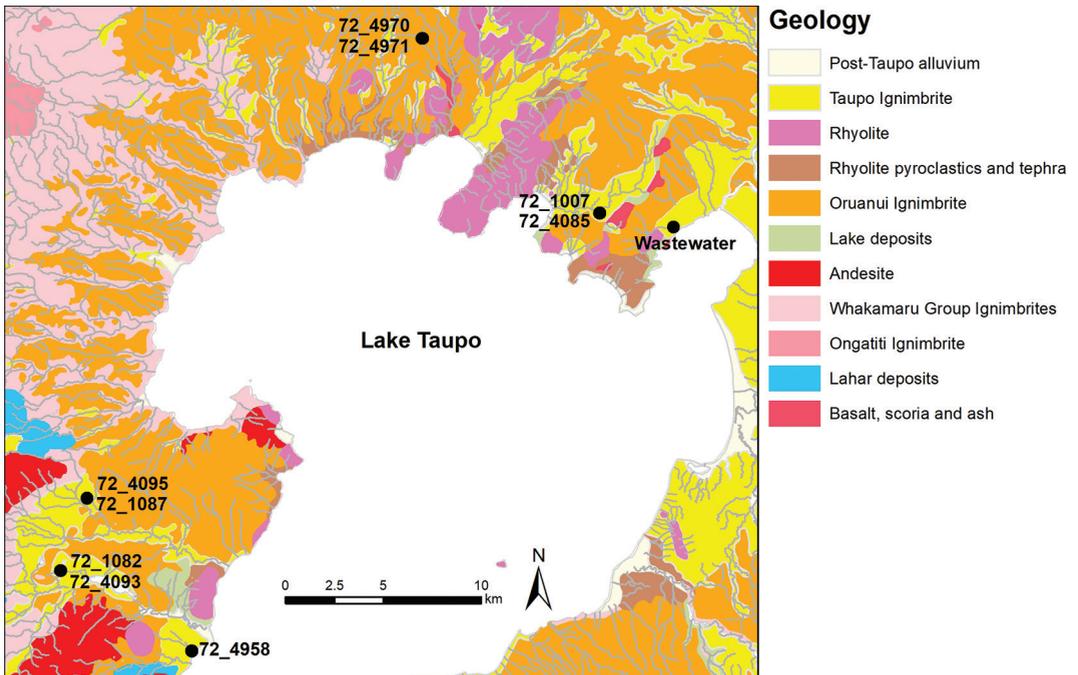


Figure 1 – Sampling site location and geology (Leonard *et al.*, 2010).

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 is through welded, fractured Whakamaru ignimbrite and andesite, and is mostly via much quicker near-surface runoff. Hydraulic properties can be a good initial indicator for subsurface denitrification potential. Groundwater that has a longer residence time is typically expected to show a greater extent of denitrification (Daughney *et al.*, 2010), provided the conditions are conducive for denitrification to occur (Rissmann, 2011).

Methods

Field sampling and analytical methods

Groundwater samples for Ar, N₂ and Ne were collected from each of the selected 10 piezometers on 8–9 May 2018. Before any samples were collected, the piezometers were purged at least three times the volume of the piezometer, using a continuous submersible pump, and sampling only commenced once the field dissolved oxygen measurement had become stable. Other proxies for measuring denitrification, or for demonstrating the potential for denitrification to occur, were sampled in conjunction with the Ne samples. These proxies included dissolved oxygen, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of nitrate, redox status assessed by hydrochemistry (McMahon and Chappelle, 2008), and DNA analysis for the abundance of the *nirS*, *nirK* and *nosZ* genes. Additionally, Childs' tests were previously carried out at the sites. For all sites sampled, the dissolved oxygen, temperature and pH were measured in the field at the time of sampling. Furthermore, samples for analysis of age tracers (tritium, chlorofluorocarbons (CFCs) and sulfur hexafluoride (SF₆)) were collected at each site.

Neon

Evacuated 1 L glass flasks were used for groundwater sample collection. When approximately 900 mL of sample had entered the flask the sample inlet valve was closed, leaving a headspace of approximately 100 mL.

The system developed for the measurement of Ne is shown in Figure 2. Two detectors, a PDHID (Valco Instruments D-4-I-SH14-R) and a thermal conductivity detector (TCD) (Shimadzu TCD-2014), are used, requiring two independent carrier gas flows (HF1 and HF2) of ultra-high purity helium (He) gas.

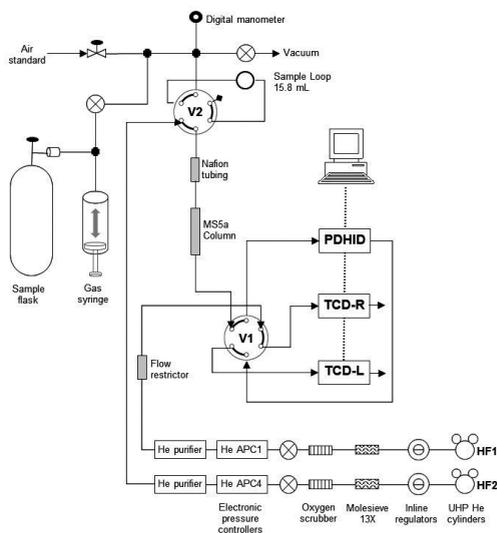


Figure 2 – Schematic of the analytical set up of the Ne measurement system.

A standard curve for Ne, Ar and N₂ 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 8 m 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 highly sensitive 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 12 minutes to allow for the separation of Ar and O₂, after which it is placed in a hot water bath, of approximately 90°C, to remove N₂ 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 the 1000 mL stainless steel syringe which is extended to its maximum volume. 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 and maximising the number of moles of the sample which can be injected and analysed. 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 N₂) in the headspace. The original sample concentration (C_i) of a particular gas can be calculated using Equation 2:

$$C_i = C_g (K+r) \quad (2)$$

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:

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

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 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.

The above-mentioned analytical set up was validated by sample comparisons with the CSIRO Environmental Tracer and Noble Gas Laboratory in Adelaide, Australia and "paleo" groundwater samples measured in 2013 by Seltzer *et al.* (2015) at the Lamont-Doherty Earth Observatory.

We point out that an important limitation of this method is that it cannot distinguish excess N₂ produced from denitrification and excess N₂ produced from the anaerobic oxidation of ammonia (annamox). However, both processes (denitrification and annamox) have the net effect of removing fixed nitrogen from the system (Smith *et al.*, 2017). A further limitation of this method is that large sample volumes are required for measurement. Furthermore, these samples are collected in

evacuated glass flasks, which are not ideal for transportation.

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

Nitrate samples for isotopic analysis were collected in 125 mL plastic vials and preserved by acidifying in the field with a solution of Sulfanilic acid in 10% HCl. $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of dissolved nitrate were measured at the Stable Isotope Laboratory at GNS Science using a method modified from McIlvin and Altabet (2005). The analytical precision for these measurements is 0.3% for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. The minimum N concentration for reliable analysis for this dataset was 0.1 mgL^{-1} .

DNA analysis

Groundwater samples for DNA analysis were collected in sterile plastic bags and subsequently chilled. Approximately 3 L of sample were collected for each site. Analysis for DNA was carried out by Massey University. To analyse the samples, 500 mL of sample water was filtered through $0.22 \mu\text{m}$ S-Pak® membrane filters. The filters were then subjected to DNA extraction using a DNA isolation kit Genomic DNA kit (Plant). The concentration of extracted DNA was quantified, and its quality assessed using a DS-11 spectrophotometer (DeNovix Inc. Wilmington, DE USA). The extracted DNA was then used for polymerase chain reaction analysis of *nosZ*, *nirS*, and *nirK* genes. Polymerase chain reactions 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).

Childs' Test

Childs' tests (Childs, 1981) 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.

Chemistry

Hydrochemistry samples were collected in sterile plastic bottles following standard collection protocols (Daughney *et al.*, 2006). Samples were analysed by Hill Laboratories using standard methods (APHA, 2012).

Mean residence time

Samples were collected for age tracers (tritium, CFCs and SF_6) following internationally reviewed sampling protocols (Daughney *et al.*, 2006; 2007). Care was taken to exclude air from the CFC and SF_6 samples. CFC samples were collected underwater in 125 mL glass bottles and sealed using foil-lined caps to prevent contact with the present-day atmosphere. SF_6 samples were collected in 1 L glass bottles with Polyseal caps, which displace the headspace. Tritium samples were collected in 1 L Nalgene plastic bottles. Localised tritium sources, such as luminous watches, were avoided.

CFCs and SF_6 were analysed by gas chromatograph (GC) using an electron capture detector (GC ECD) with detection limits of approximately $3 \times 10^{-15} \text{ mol.kg}^{-1}$ for CFCs and $1.5 \times 10^{-16} \text{ mol.kg}^{-1}$ for SF_6 . The analytical system for CFCs is similar to that of Busenberg and Plummer (1992). The analytical system for SF_6 is described in van der Raaij (2003). CFC samples were analysed in duplicate. Dissolved Ar, N_2 and CH_4 were measured simultaneously with CFCs by a GC/ TCD. CFCs and SF_6 concentrations were subsequently converted to atmospheric equivalents using Ar/ N_2 -derived temperatures and corrected for excess air.

Tritium was measured by electrolytic enrichment and liquid scintillation counting using Quantulus low-level counters (Morgenstern and Taylor, 2009). The detection limit is approximately 0.025 tritium units (1 TU is a $^3\text{H}/^1\text{H}$ ratio of $1:1 \times 10^{18}$).

Groundwater sampled from a groundwater outflow such as a well or spring is a mixture of water from various flow lines, and therefore of different residence times. This age distribution can be described by lumped parameter mixing models that provide a system response function (Maloszewski and Zuber, 1982). Convolution of known tracer inputs to the system using the chosen system response function and matching to the measured tracer concentrations allows calculation of the mean residence time (MRT) of the groundwater, along with the associated distribution of groundwater residence times (Zuber and Małoszewski, 2001). In this paper the exponential-piston flow model (EPM) was applied to calculate MRT.

Results and discussion

Measured excess N₂

Of the ten sites sampled, eight sites had measurable excess N₂. The measured concentrations of Ne, Ar and N₂ are provided in Table 2.

Comparison of excess N₂ to other indicators of denitrification

Table 3 gives a comparison of all the denitrification proxies and indicates which proxies identified or suggested denitrification was occurring at each site, and also lists the MRTs. Figure 3 shows the total nitrate at each site, divided into the proportion that has been denitrified and that which remains in the groundwater. Results from the isotopic fractions of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ and the DNA analysis are shown in Figures 4 and 5. The chemistry results are provided in Table 4.

All samples had measurable counts of both *nosZ* and *nirK+S* denitrifying genes, suggesting the potential for denitrification at all sites. For some sites, the gene abundances appeared to correlate to the measured concentrations of excess N₂. For example, the shallow piezometers 72_1087 and

72_1082 had relatively low abundances of *nosZ*, relatively high ratios of *nirK+S* to *nosZ*, and no measured excess N₂, whereas their paired deeper piezometers 72_4095 and 72_4093 showed essentially opposite characteristics. Therefore, qualitatively, when these two pairs of piezometers are compared, they support the denitrification findings shown by the excess N₂ method. Note, however, that gene populations from different sampling sites cannot be directly compared because environmental differences can influence populations (Groffman *et al.*, 2006). Moreover, when the results from all piezometers were considered together, there were very poor correlations between the concentration of excess N₂ and the abundance of *nosZ* or the ratio of *nirK+S* to *nosZ*. Therefore, we conclude that in this study the DNA method gave mixed results as a discriminator of potential denitrification, and in any case did not provide a quantitative estimate of the amount of denitrification that actually occurred. Future research could usefully focus on proteomic approaches to discriminate between denitrifier genes that are present but inactive versus those genes that are actively expressed (Larceda and Reardon, 2009).

No site showed an isotopic denitrification signal, despite the detection of excess N₂ and anoxic classification based on the hydrochemistry at some sites. At four sites (72_4095, 72_4093, 72_4958 and 72_4085) the concentrations of nitrate were too low for isotopic measurement, presumably because all of the nitrate had already been removed from the system by denitrification (all of these sites had high excess N₂, indicating that denitrification had occurred). For the other six sites, the signals from the $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ data were varied. Some sites had an isotopic signature indicative of normal N retention, suggesting that the soil organic matter appears to remain an effective reservoir for nitrate from urine and urea (i.e., isotopically the

Table 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	±
72_4958	361	0.000201	0.000003	0.366	0.010	15.174	0.244	11.1	1.4
72_1087	414	0.000209	0.000006	0.356	0.009	13.966	0.257	12.8	1.5
72_4095	414	0.000204	0.000003	0.370	0.011	16.764	0.330	10.5	1.6
72_1082	418	0.000222	0.000005	0.370	0.012	14.735	0.243	11.7	1.8
72_4093	418	0.000221	0.000004	0.390	0.013	17.844	0.587	9.0	1.7
72_4970	363	0.000206	0.000004	0.370	0.010	16.689	0.273	10.9	1.4
72_4971	363	0.000209	0.000007	0.370	0.014	16.419	0.319	11.1	2.1
72_1007	395	0.000218	0.000006	0.358	0.010	15.694	0.248	13.4	1.6
72_4085	395	0.000217	0.000005	0.365	0.009	17.904	0.266	12.2	1.4
Wastewater	369	0.000199	0.000007	0.332	0.009	14.380	0.241	16.0	1.7

Table 3 – Denitrification proxies for all sampled sites (shaded fields show which measurements indicate or suggest denitrification is occurring or has occurred).

Site Name	Excess N ₂ (mL(STP).kg ⁻¹)	Positive Childs' Test depths (m)	General Redox Category ³	Redox Process	CH ₄ μmol/kg	Denitrification indicated by δ ¹⁵ N δ ¹⁸ O	Denitrification indicated by presence of nosZ, nirK+S	Calculated MRT (years) ⁴
72_4958	1.11 ± 0.39	8.8–10.65 10.85–25.5	Mixed (anoxic)	NO ₃ ⁻ Fe(III)/SO ₄ ²⁻	0	Not measurable ²	Yes	16–20
72_1087	0.00 ± 0.40	No positive test ¹	Oxic	O ₂	0	No	Yes	3–5
72_4095	2.48 ± 0.46	10.5–16.0	Mixed (anoxic)	NO ₃ ⁻ CH ₄	118 ± 6	Not measurable ²	Yes	17–22
72_1082	0.00 ± 0.44	No positive test ¹	Oxic	O ₂	0	No	Yes	2–3
72_4093	2.51 ± 0.71	13.2–21.9 14.84–15.1	Mixed (anoxic)	NO ₃ ⁻ CH ₄	207 ± 188	Not measurable ²	Yes	11–12
72_4970	2.36 ± 0.41	18.6–18.8 21.1–21.5	Anoxic	NO ₃ ⁻	0	No	Yes	90–93
72_4971	2.01 ± 0.55	22–23 No positive test ¹	Oxic	O ₂	0	No	Yes	22–29
72_1007	1.36 ± 0.41	6.8–7.4 ¹	Mixed (oxic-anoxic)	O ₂ -Fe(III)/SO ₄ ²⁻	0	No	Yes	10–11
72_4085	3.40 ± 0.40	6.8–9.8	Mixed (anoxic)	NO ₃ ⁻ Fe(III)/SO ₄ ²⁻	0.6 ± 0.1	Not measurable ²	Yes	13–15
Wastewater	1.32 ± 0.40	n/a	Anoxic	NO ₃ ⁻	0	No	Yes	13–15

¹ Childs' test results are assumed based on the results from the deeper paired piezometer located several metres away.² Nitrate concentration was too low to allow for isotopic analysis.³ Redox threshold criteria used by Stenger et al. (2018) has been applied.⁴ Groundwater mean residence times (MRTs) calculated based on an assumed exponential mixed flow mixing of 50–70%.

Table 2 – continued

Re-constructed N_2 mL(STP).kg ⁻¹	±	ΔN_2 mL(STP).kg ⁻¹	±	moles N mmol.kg ⁻¹
14.680	0.481	1.11	0.39	0.10
14.699	0.482	-0.06	0.40	-0.01
14.985	0.535	2.48	0.46	0.22
15.483	0.598	-0.06	0.44	-0.01
16.072	0.631	2.51	0.71	0.22
14.946	0.493	2.36	0.41	0.21
15.019	0.718	2.01	0.55	0.18
14.989	0.534	1.36	0.41	0.12
15.167	0.472	3.40	0.40	0.30
13.634	0.510	1.32	0.40	0.12

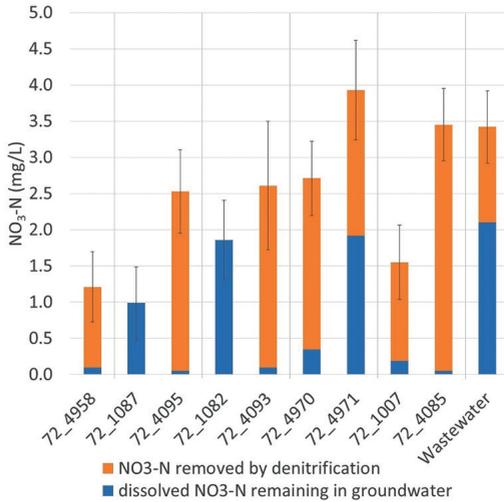
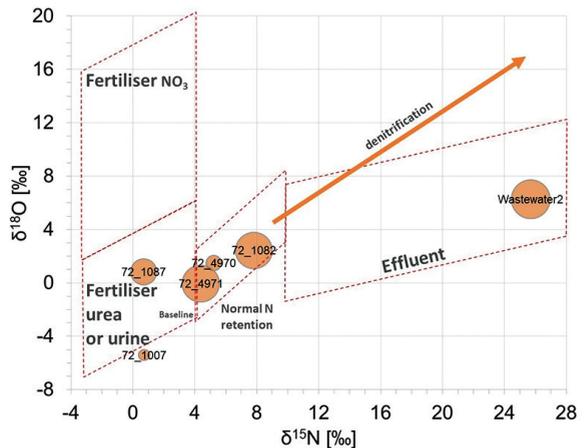


Figure 3 – Total nitrate in the groundwater at each site, divided into the proportion that has been denitrified and that which remains in the groundwater. The vertical lines identify and group the paired piezometers.

Figure 4 – Plot of $\delta^{18}O$ against $\delta^{15}N$ in nitrate samples. Symbol size is proportional to NO_3-N concentration. Plot areas that identify nitrate sources are taken from Morgenstern *et al.* (2018).



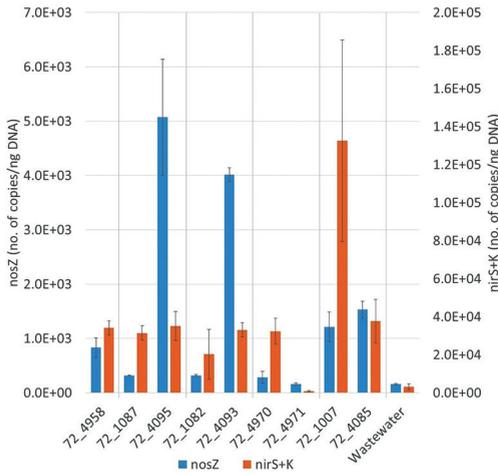


Figure 5 – Counts of denitrifier genes *nosZ*, *nirK+S* in different samples, normalised by the concentration of DNA extracted. The vertical lines identify and group the paired piezometers.

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). The other sites showed the influence of breakthrough of urine, urea or some other inorganic

nitrogenous fertiliser, without alteration by cycling or denitrification within the soil zone (Fig. 4). As nitrate concentrations decrease with increasing denitrification signal, any isotopic indication of denitrification is likely masked by the presence of predominantly oxic groundwater with baseline levels of nitrate.

That no site showed an isotopic signature of denitrification does not mean that the isotopic method provided no insight in this study. Disagreement or inconsistency between different but complementary methods can sometimes provide useful information. For example, Site 72_1007 does not show an isotopic signal of denitrification, but other types of measurements suggest that denitrification is occurring. The lack of isotopic signature of denitrification, combined with the mixed redox state implied by the hydrochemistry, could indicate complex groundwater flow conditions at this site, whereby water from both oxic and anoxic waters are represented in the sample. If this is the case, the exponential-piston flow model is overly simplistic for this site and thus the modelled MRT in Table 2 may not be accurate.

Table 4 – Chemistry parameter concentrations used for the redox state determination.

Site name	Dissolved oxygen (mgL ⁻¹)	NO ₃ -N (mgL ⁻¹)	Mn (dissolved) (mgL ⁻¹)	Fe (dissolved) (mgL ⁻¹)	SO ₄ (mgL ⁻¹)
72_4958	0.28	0.1	0.066	5.8	6.5
72_1087	8.68	0.99	<0.0005	<0.02	6.2
72_4095	0.15	0.05	0.44	7.8	0.6
72_1082	5.66	1.86	0.0006	<0.02	2.8
72_4093	0.3	0.1	0.166	5.8	0.5
72_4970	0.27	0.35	0.114	0.16	12.4
72_4971	2.63	1.92	<0.0005	<0.02	17.6
72_1007	2.82	0.19	0.22	0.81	16.2
72_4085	0.34	0.05	0.39	4.3	17.6
Wastewater	1.79	2.1	0.0008	<0.02	32

There was generally good agreement between the inferred redox condition and the measured excess N_2 . Where hydrochemistry and/or Childs' Test indicated reduced (anoxic) conditions, excess N_2 was generally detectable. Excess N_2 offers additional insight by providing a quantitative measure of the extent of denitrification that has occurred, whereas hydrochemistry and Childs' test can only indicate where denitrification might possibly occur.

However, there were some sites that had contradictory results between the hydrochemistry and excess N_2 . For example, Site 72_4971 is a shallow piezometer that had hydrochemistry indicative of oxic water, a negative Childs' test and 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 dissolved gas, is easily transported through the groundwater but the denitrifier microbes and their associated genes, being less mobile, have not been captured.

The combination of groundwater denitrification proxies with groundwater age determination as used in this study gives a useful inference of future nitrate reduction in the aquifer. The youngest water did not show any capacity for denitrification by almost all of denitrification proxies analysed, excluding the DNA analysis (Table 2). Flow of nitrate through the groundwater system to reducing or partial reducing zones is indicated to take more than 11 years. Such information should be taken into account when considering nitrate attenuation in future land use planning.

It is important to bear in mind that there is a distribution of groundwater ages at any site, and the MRT is one statistic that describes this distribution. For example, Site 72_4970 is of interest as it has excess N_2 , but the MRT of the groundwater is approximately 90 years. Land use intensification is assumed to have

started in 1955 (Vant and Smith, 2004) and so, at first impression, an excess N_2 signal of this size is unexpected. However, given that the sample contains a distribution of ages, the measured denitrification signal potentially relates to the fraction of groundwater younger than post-land use intensification.

Conclusions

The excess N_2 method has been demonstrated to be tractable and useful for understanding denitrification in New Zealand groundwaters. While groundwater redox characterisations (e.g., based on hydrochemistry or the presence of denitrifier genes) assess the potential for denitrification to occur, the excess N_2 method verifies its occurrence and quantifies the amount that has occurred. Furthermore, the excess N_2 method could identify denitrification at the study sites that had a mixed redox state, where redox state is less conclusive. Correlations between the excess N_2 method and the DNA method were mixed, indicating that further work is required to assess how the DNA molecular method can be more definitively used in the New Zealand environment as a denitrification proxy. The excess N_2 method also demonstrated its strength compared to the $\delta^{18}O$ and $\delta^{15}N$ measurements, as many of the study sites that exhibited excess N_2 did not have high enough nitrate concentrations for isotope measurement, presumably because of the nitrate removal via reduction.

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