

# Nitrate reactivity in groundwater: a brief review of the science, practical methods of assessment, and collation of results from New Zealand field investigations

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## Abstract

Nitrate attenuation factors are becoming an important and routine consideration in applied water resource management within New Zealand, as nitrogen load limits are being set for hydrological catchments. The few nitrate attenuation factors so far reported in the New Zealand scientific literature have been calculated from comparing landscape nitrogen yield with catchment nitrogen yield. An important limitation of the nutrient budgeting approaches so far employed is that they do not consider the fate and transport of nitrate in the vadose zone-groundwater continuum that links the base of the soil zone with surface waters. There have been few objective scientific studies targeting measurement of nitrate reactivity below the root zone in New Zealand. The few *in situ* measurements that have been made have mainly been focussed in the Waikato and Manawatu-Wanganui regions. Owing to the paucity of available data on nitrate reactivity below the root zone, much discussion pertaining to nitrate attenuation in New Zealand groundwater remains speculative.

The theme of this paper is a review of the topic of denitrification in groundwater, written from a New Zealand perspective, although anammox and dissimilatory nitrate reduction to ammonium reactions are covered also, for they represent alternative

potential natural attenuation processes for nitrate in groundwater. The review describes the various practical methods by which nitrate reactivity can be determined through *in situ* field measurement, to inform design of future applied research in New Zealand. Molecular bioassay and stable isotope chemical analytical techniques for diagnosing denitrification are highlighted. Examples of where the practical methods have been applied in New Zealand are described.

Potential for nitrate attenuation via heterotrophic and autotrophic denitrification pathways has so far been demonstrated in anoxic groundwater systems of the Waikato region, including on the Volcanic Plateau where the surficial geology comprises sandy volcanic deposits interbedded with palaeosols that formed as a consequence of episodic volcanic eruption events. Active nitrate reduction has also been confirmed in anoxic portions of the alluvial aquifer systems distributed across the Manawatu-Wanganui region. There is very low potential, however, for any effective nitrate attenuation in aerobic alluvial gravel aquifer systems, such as constitute the most significant groundwater systems in New Zealand.

Whilst the method itself has yet to be applied in New Zealand, excess  $N_2$  measurement from analysis of Ar/Ne/ $N_2$  ratios carried out in conjunction with groundwater

dating conceivably represents a useful method for determining nitrate attenuation rates at a scale commensurate with New Zealand resource management applications. To be effective, nitrate reaction rates ideally need to be determined on a site-specific basis and reconciled with local groundwater flow information, from which nitrogen fluxes can be quantified. The problems of up-scaling point observational data, constraining groundwater ages, and determining flow paths and historic nitrogen loadings are identified as continuing technical challenges in this field.

## Keywords

nitrate; attenuation processes; reaction rates; groundwater; New Zealand

## Introduction

Nitrate from agriculture has been described by the United Nations (2011) as the world's most common chemical contaminant of groundwater. In New Zealand it has been referred to as the 'elusive pollutant' (Ministry for the Environment [MfE], 2013). Nitrate is a contaminant of concern in groundwater for two reasons. Firstly, it poses a human health hazard in drinking water, owing to its ability to cause infant methaemoglobinaemia (blue-baby syndrome) and potentially other diseases (World Health Organization [WHO], 2016; Schullehner *et al.*, 2018; Ward *et al.*, 2018). For context, approximately 47% of New Zealand's drinking water is sourced from groundwater (MfE and Stats NZ, 2017). To mitigate health risks, a maximum acceptable value (MAV) of 50 mg/L nitrate ( $\text{NO}_3$ ) — equivalent to 11.3 mg/L nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) — is defined in the New Zealand Drinking-Water Standards (Ministry of Health, 2018). This value is consistent with the World Health Organization threshold (WHO, 2017).

Secondly, many streams and rivers are groundwater fed and nitrate contamination

can degrade water quality in these aquatic systems, leading to eutrophication, which has been referred to as the most prevalent water quality problem in the world (United Nations, 2014). Guideline values for protecting New Zealand freshwater ecosystems from the toxic effects of nitrate are much lower than the nitrate MAV for human health (e.g., ANZECC and ARMCANZ, 2000), as are concentrations at which nitrate can affect the trophic state of an aquatic system. Nutrient enrichment of surface waters with nitrate-nitrogen can lead to growth of nuisance periphyton and algal blooms that impact on the value of freshwater as a recreational resource, and in cases such as toxic cyanobacteria, can threaten human and animal health. Nitrate is an attribute listed in the National Objectives Framework, provided in the National Policy Statement for Freshwater Management (NPS-FM) (MfE, 2017). Therein, the New Zealand Government has set a median value of 6.9 mg/L  $\text{NO}_3\text{-N}$  as the National Bottom Line for New Zealand river systems.

There are no known geological sources of nitrate in New Zealand, where the natural baseline concentration in oxic groundwater is approximately 0.25 mg/L  $\text{NO}_3\text{-N}$  (Morgenstern and Daughney, 2012). By relating groundwater chemistry with water age, Morgenstern and Daughney (2012) demonstrated nitrate impacts in New Zealand groundwater have experienced two historical step-changes, caused by major land-use intensification events. Around 1880, at the time of European settlement, groundwater nitrate concentrations elevated from the natural baseline condition in response to development of the land for low-intensity agriculture. Then, following the expansion of high-intensity agriculture in the second half of the 20th century, maximum concentrations have increased above a threshold of 2.5 mg/L  $\text{NO}_3\text{-N}$  (Morgenstern and Daughney, 2012). The primary source of nitrate in New Zealand

has long been diffuse pollution from pastoral farming (Howard-Williams *et al.*, 2010). Industrial and human waste discharges, along with fertilizer, contribute to some of the nitrate load, but it is animal waste, and in particular urine patches from grazed animals, that is recognised as the main culprit (Di and Cameron, 2002).

It is 17 years since a scientific chapter dedicated to the topic of groundwater nitrate in New Zealand was published (Close *et al.*, 2001). At that time, practical examination of nitrate reactivity in New Zealand groundwater was effectively limited to the study published by Wang *et al.* (2003), which modelled the fate and transport of the nitrogen plume in ignimbrite deposits at Waitahanui wastewater treatment plant, Waikato. Since then, agricultural land-use intensification within New Zealand has proceeded at one of the highest rates in the world (OECD, 2017). The national resident population has increased by almost a million people<sup>1</sup>; the national dairy herd by 1.3 million cows<sup>2</sup>; and nitrogenous fertilizer usage is up 70%, to 425,000 tonnes per annum<sup>3</sup>. MfE (2014) estimate that between 1990 and 2012 there was an incremental increase of 29% in the amount of nitrogen leached from agricultural soils, the largest increases of which occurred within the Canterbury region (Dymond *et al.*, 2013) where some of the nation's most important groundwater resources are located (Brown, 2001; White *et al.*, 2004). Coincidentally, throughout the country there has been a measurable decline in water quality over the same period. Whilst in 2001 Close *et al.* could not determine any groundwater nitrate trends at the national scale, owing to a paucity of observational data, latest accounts from the National Groundwater Monitoring Programme (NGMP) confirm a worsening trend in groundwater nitrate

levels at 26% of the monitored sites when assessed over the period 2005–2014 (MfE and Stats NZ, 2017). It is worth noting that the NGMP shows that nitrate impacts are most apparent in, but not limited to, groundwater sampled from shallow wells that screen unconfined aquifers. Similarly, nitrate levels are increasing at over half of the sites that make up the national river water quality monitoring programme (Larned *et al.*, 2016; MfE and Stats NZ, 2017).

Much of the study into the fate of nitrate in the New Zealand environment has been made within the soil zone (e.g., Clough, 1996; Cameron *et al.*, 2002; Owens *et al.*, 2016). This is understandable, considering the pedosphere forms a strategic management unit in farming systems, being where nitrogen inputs are controlled and where nitrogen cycling activity is most concentrated. A lot of effort has also been put into measuring nitrate reactivity in New Zealand's rivers, lakes, riparian systems and wetlands (e.g., Burns and Nguyen, 2002; Tanner *et al.*, 2005; Matheson and Sukias, 2010; Wilcock *et al.*, 2012; Cox and Rutherford, 2012). Knowledge from these scientific works continues to be incorporated into tools like Overseer® and CLUES that are applied to water resource management decision support (e.g., Selbie *et al.*, 2013; Semadeni-Davies *et al.*, 2016).

Using such tools to analyse nitrogen budgets from land-use models and surface water quality monitoring data, Elliott *et al.* (2005) estimated that, at the national scale, just 45% of the total nitrogen mass lost from the land makes it to the sea, implying 55% is attenuated during landscape and aquatic transport. Similar nutrient budgeting approaches comparing landscape yield with catchment yield have been taken at more localised scales. Examples include

1 <https://www.stats.govt.nz/topics/population>.

2 <https://www.stats.govt.nz/topics/agriculture>.

3 Statistics provided by Fertiliser Association of New Zealand

the Waikato river system (Alexander *et al.*, 2002; Clothier *et al.*, 2007) and an upper part of the Manawatu catchment (Clothier *et al.*, 2007; Elwan *et al.*, 2015). The range of nitrate attenuation factors calculated for these cases were 0.39–0.75<sup>1</sup>, and 0.29–0.75, respectively. Such nitrate attenuation factors are becoming an important and routine consideration in applied water resource management, as nitrogen load limits are applied to New Zealand catchments, in alignment with the NPS-FM (MfE, 2017).

A significant limitation of the aforementioned works, however, is their failure to explicitly consider the fate and transport of nitrate in the vadose zone-groundwater continuum that links the base of the soil zone with surface waters. Incorporated in the reported nitrate attenuation factors is the effective sum of lag effects associated with storage of nitrate below the root zone plus any nitrate losses through natural biogeochemical reactions such as denitrification, occurring anywhere between the bottom of the root zone and the surface water monitoring site. It is widely recognised that the vadose zone-groundwater continuum can act as a significant store for reactive nitrogen, creating a legacy source of nitrate (Van Meter *et al.*, 2016; Ascott *et al.*, 2017). Since very little work has been undertaken in New Zealand to directly examine denitrification below the root zone it remains uncertain whether denitrification is active in that zone, providing a true sink for nitrogen leached from the land.

Being an oxidation-reduction process, it is possible to identify where nitrate reduction will occur considering soil and geological conditions together with observed water chemistry. Rissmann *et al.* (2018) refer to this as development of physiographic models. Recognising this potential, there is

an on-going drive to compile groundwater redox or nitrate vulnerability maps for New Zealand groundwater systems. Worked case examples include all of the Southland region (Rissmann, 2011; Rissmann *et al.*, 2016; Wilson *et al.*, 2018); Canterbury and Waikato regions (Close *et al.*, 2016), and parts of the Manawatu River catchment in the Manawatu-Wanganui region (Rivas *et al.*, 2017). While redox maps provide an indication of where nitrate reduction might occur, for practical management of catchment loads this indication needs to be reconciled with information on whether it is occurring and the capacity of the groundwater system to assimilate nitrate loads. Practical field assessments are required to ascertain these properties.

This paper provides a brief review of the topic of natural attenuation of nitrate in groundwater. In some regards it can be viewed as an addendum to the nitrate chapter (Chapter 8) in the Groundwaters of New Zealand book (Rosen and White, 2001). A focus of the paper is to offer insight to the practical methods by which nitrate reactivity can be examined in groundwater systems and so provide an informative guide for those practising applied contaminant hydrogeology and water resource management. The knowledge that can be gained from using the methods described herein can be applied to constrain groundwater vulnerability maps and assessments of nitrate attenuation factors in general. In particular, measured reaction rates conceivably might be used to parameterise mechanistic groundwater models, which have an integral role to play in landscape-catchment yield modelling. The paper is structured around three key objectives:

- i) to review the scientific concepts of denitrification as well as alternative nitrate

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<sup>1</sup> Clothier *et al.* (2007) actually calculated a transmission coefficient  $R$  for the Waikato region of  $0.25 < R < 0.61$ , where  $R = 1 - \text{attenuation factor}$ .

reduction pathways, and explain how these likely apply to New Zealand's hydrogeological conditions,

- ii) describe the field-investigative methods that can be applied to examine nitrate reactivity in groundwater, and
- iii) catalogue New Zealand case studies where such methods have been applied and collate the nitrate reaction rates that have been estimated from them.

Perspectives on future research on the topic of natural attenuation of nitrate in New Zealand groundwater are discussed.

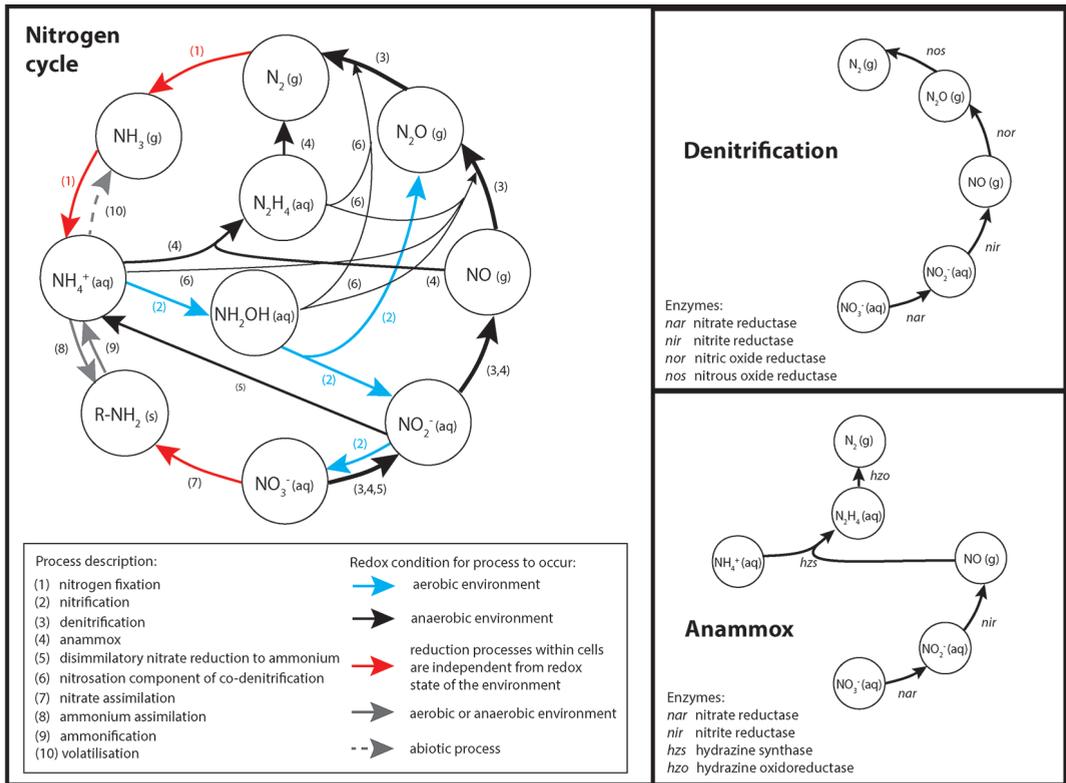
## Denitrification and other nitrate reactions

Detailed explanations of denitrification processes are provided in a number of published scientific articles, and a comprehensive review of the subject is not the aim of this paper. For that, readers are referred to the much-cited review articles by Korom (1992) and Rivett *et al.* (2008), or the more recent text on nitrogen transformation processes provided by Robertson and Groffman (2015). Here, discussion is limited to a brief summary of denitrification, highlighting key technical points applicable to investigations of the fate of nitrate in groundwater. Whilst denitrification has for a long time been considered the main process for nitrate loss in natural ecosystems, there is growing recognition that alternative nitrate reduction processes, such as dissimilatory nitrate reduction to ammonium and, more importantly, anammox, potentially play a more active role in groundwater systems than had previously been thought (Burgin and Hamilton, 2007; Smith *et al.*, 2015; Wang *et al.*, 2017). These processes are reviewed here. Furthermore, whilst there is no evidence to demonstrate it is important in groundwater systems, or expectation it should be, for completeness the recently discovered co-denitrification process is described also.

An important point to be mindful of when considering nitrate reactivity in groundwater is reaction kinetics. Denitrification, anammox and dissimilatory nitrate reduction to ammonium are microbially-mediated reactions involving enzymes. Their kinetics are thus best simulated following a Michaelis-Menten model and as a function of multiple reagents (Chapelle, 2000). Nonetheless, due to difficulties in parameterising such complex reaction models it is common practice to simply assume a pseudo-steady state condition and use a lumped model with a zero-order rate constant, or first-order nitrate reaction rate, to simulate denitrification reactions (e.g., Smith *et al.*, 2004; Buss *et al.*, 2005).

### Denitrification

Denitrification is a bacterial respiratory process that involves reduction of nitrate or nitrite to gaseous nitrogen, either as molecular N<sub>2</sub> or as an oxide of nitrogen (Payne, 1973). It is a true sink for aqueous nitrogen in the nitrogen cycle (Fig. 1). Denitrification occurs as a sequential chain of step-wise reduction reactions, generally involving consortia of micro-organisms that collectively possess the reductase enzymes necessary to catalyse reaction. Denitrifying bacteria are of diverse genera, most of which are facultative anaerobes, i.e., organisms that generally respire oxygen but can switch to anaerobic respiration if oxygen is absent. Denitrifying bacteria are considered to be ubiquitous in the natural environment (Martens, 2005). Whereas a wide variety of organisms express the nitrate reductase enzyme (*Nar*) and can reduce nitrate to nitrite (Philippot and Germon, 2005), a less diverse range of bacterial species possess the enzyme nitrite reductase (*Nir*), required to catalyse the reaction that reduces nitrite to nitrous oxide (Lycus *et al.*, 2017). This critical reduction step, in which aqueous nitrogen is transformed to a gaseous form,



**Figure 1** – Schematic representation of the nitrogen cycle, as conceivably relates to groundwater environments. Note: Any of the various pathways drawn for nitrification reactions that might contribute to co-denitrification are theoretically feasible. In co-denitrification an N atom produced from nitrosation reaction ultimately combines with an N atom produced from conventional denitrification, which proceeds as an independent process. Nitrous oxide production during nitrification conceivably occurs under low oxygen conditions. Inset figures show details of denitrification and anammox processes that are perceived to be the most significant potential nitrate attenuation processes in groundwater. The enzymes responsible for each biologically-mediated N-reduction step are labelled and can be examined through molecular genotyping methods, albeit the number of potential genes involved is numerous and to list them is beyond the scope of this review.

is irreversible and often regarded as true denitrification (Lycus *et al.*, 2017). *Nir* is occasionally referred to as the ‘denitrifying enzyme’ (Knowles, 1982). Nitric oxide (NO) is an intermediary compound and reaction tends to proceed to nitrous oxide (N<sub>2</sub>O) production and subsequently N<sub>2</sub>, if the denitrification process is completed. It is noteworthy that N<sub>2</sub>O is a greenhouse gas with a global warming potential approximately 300 times that of CO<sub>2</sub> (IPCC, 2007). Interest

in global N<sub>2</sub>O fluxes has prompted much of the recent research on nitrogen cycling and denitrification.

Denitrification in groundwater is limited to conditions where there is either very low, or no, dissolved oxygen (DO), under which nitrite reductase and nitric oxide reductase (*Nor*) gene expression is possible (Baek and Shapleigh, 2005). There is little consensus on the DO threshold below which denitrification can proceed in natural

groundwater systems, although Knowles (1982) and Seitzinger *et al.* (2006) state that denitrification is definitely favourable at DO concentrations below 0.2 mg/L. Positive indicators of denitrification have been detected in situations where groundwater DO concentrations have been as high as 4 mg/L (e.g., McMahon *et al.*, 2004; Tesoriero and Puckett, 2011), although as Tesoriero and Puckett (2011) note, this DO value is likely an artefact of scaling effects and not a threshold *per se*. They, along with others (e.g., Rivett *et al.*, 2008; Stenger *et al.*, 2018), suggest 2 mg/L O<sub>2</sub> is a more likely threshold, below which denitrification might be expected in field-settings. Whilst some facultative anaerobes are capable of denitrification under aerobic conditions above these thresholds, it is assumed aerobic denitrification is limited to situations where redox conditions fluctuate (Ferguson, 1994; Ji *et al.*, 2015). Only periplasmic nitrate reductase enzyme is active in aerobic denitrification and energy yields are only sufficient for cell maintenance purposes. As such, it is hypothesised aerobic denitrification is a temporary energy conservation measure exercised by facultative anaerobes transitioning between aerobic respiration with oxygen or anaerobic respiration with nitrate (Chen and Strous, 2013). Whilst genomic information is a powerful tool for verifying the presence of denitrifying organisms, there are as many as 50 genes involved in the denitrification process (Zumft, 1997) and genetic potential cannot be relied upon as a predictor of phenotype (Lycus *et al.*, 2017). Despite its limitations, quantification of the nitrous oxide reductase (*nosZ*) gene transcript is a commonly-applied method for verifying presence of active denitrifiers in field studies.

It is important to note that there are limitations with using nitrate concentrations as the sole basis for evaluating denitrification rates and conclusive proof of denitrification requires more than simply evidence of

nitrate losses. This is because nitrite, which is produced in the first reductive step of the denitrification process, can also be consumed by anammox and nitrification reactions, as can be seen in the nitrogen cycle shown in Figure 1. Whilst nitrification depends on oxygenated conditions, if it is active it has potential to cycle any nitrite-nitrogen produced from denitrification back to nitrate. The anammox process is described later in this paper.

Heterotrophic denitrification that is fuelled by organic carbon tends to be the most common form of denitrification in groundwater, although autotrophic denitrification is also viable. In autotrophic denitrification, reduced species of iron, sulphur, manganese and/or arsenic serve as electron donors for denitrifying organisms (autotrophs) that obtain the carbon necessary for cell synthesis from an inorganic form, usually carbon dioxide (Matějů *et al.*, 1992; Korom, 1992; Sun *et al.*, 2008). Sometimes, when it is known that substrates are derived from a geological source, autotrophic denitrification may be described as lithotrophic or lithoautotrophic denitrification. Autotrophic denitrification was first identified as a major nitrate reduction pathway in pyritic sandy aquifers distributed across Northern Europe, where it occurs together with heterotrophic denitrification (Frind *et al.*, 1990; Postma *et al.*, 1991). It has subsequently been demonstrated to be an important pathway by which nitrate is attenuated in many groundwater systems comprising fluvio-glacial sediments (e.g., Böhlke *et al.*, 2002; Rodvang and Simpkins, 2001; Smith *et al.* 2017). Iron sulphide minerals (pyrite) are recognised as a common source of both reduced iron and sulphide electron donors that can fuel autotrophic denitrification. Dissolution of silicate minerals like biotite, pyroxenes and amphiboles represent alternative sources of dissolved ferrous iron in aquifers (Rivett

*et al.*, 2008). In sand and gravel aquifers, ferrous iron may occur sorbed to reactive coatings on sediment grain surfaces (e.g., Smith *et al.*, 2017). Whilst pyritic aquifers do not represent any known important aquifer system in New Zealand, shallow aquifer systems in Southland that contact lignite deposits are perceivably closest to the systems described in Northern Europe. Just recently, active autotrophic denitrification has been demonstrated in New Zealand groundwater in unconsolidated sediments within the Taupo Volcanic Zone (Korom *et al.*, 2016).

A third documented denitrification process that deserves a mention in a review such as this is co-denitrification (Spott *et al.*, 2011). Co-denitrification involves denitrification (as described above) coupled with biotic N-nitrosation reactions. N<sub>2</sub>O and/or N<sub>2</sub> gas are the end-products of co-denitrification with the defining factor being they are formed with a hybrid N-N bond. That is, one nitrogen atom originates from inorganic-N (i.e., an N-species part of the true denitrification reaction) and the other nitrogen atom represents a nucleophilic N atom from a co-substrate (usually N<sub>3</sub>, NH<sub>3</sub> or a monomeric organic N source such as an amine) (Spott *et al.*, 2011; Clough *et al.*, 2017). Although only a few objective studies have been made, evidence is amassing that co-denitrification likely represents an important process and route for N<sub>2</sub> production in the terrestrial environment (Laughlin and Stevens, 2002; Selbie *et al.*, 2015). Its function in either aquatic or groundwater environments has yet to be evaluated, although at this stage, considering the small potential pools of organic nitrogen in most groundwater systems, one can speculate co-denitrification likely plays an insignificant role in groundwater nitrate attenuation (Spott *et al.*, 2011).

Nitrate reduction via abiotic chemical reactions involving reduced metal species like Fe<sup>2+</sup> and Mn<sup>2+</sup> is thermodynamically viable

(Fanning, 2000; Kappler and Straub, 2005; Doane, 2017), although it has been shown such reactions are very slow to proceed unless metal elements like copper, silver, cadmium, nickel, mercury or lead are available in solid phase to catalyse the reaction (Buresh and Moraghan, 1974; Ottley *et al.*, 1997). From an applied hydrogeology and environmental management perspective, it is reasonable to assume abiotic reaction processes are an insignificant control of nitrate in the New Zealand environment.

In addition to oxygen, there are a variety of compounds that can inhibit denitrification by blocking function of different reductase enzymes. Knowles (1982) lists sulphide, certain pesticides, acetylene, azide, cyanide and 2,4-dinitrophenol as known denitrification inhibitors. Acetylene, in particular, blocks the nitrous oxide reductase enzyme and this phenomenon provides the basis of the acetylene block method that is widely used as a practical denitrification assay (Tiedje, 1982; Sánchez-Pérez *et al.*, 2003). The limitations of the acetylene block method are discussed by Seitzinger *et al.* (1993) and Groffman *et al.* (2006). Most recently, it has been demonstrated that the polyphenolic compound procyanidin is an effective inhibitor of biological denitrification, as it interferes with expression of the membrane-bound nitrate reductase enzyme (Bardon *et al.*, 2016).

### **Anammox**

Anammox is similar to denitrification in that it is a microbially-mediated reaction that occurs under sub- or an-oxic conditions and involves reduction of nitrite nitrogen to dinitrogen gas, albeit directly in one step (e.g., Fig. 1). Unlike denitrification, however, anammox does not utilise organic carbon, reduced iron or mineral phase electron donors; rather, nitrite reduction is coupled to oxidation of ammonium.

Since the initial discovery of anammox in a wastewater treatment plant (Mulder *et al.*,

1995), it has become apparent anammox bacteria are broadly distributed in the natural environment (Penton *et al.*, 2006) and that the process itself probably plays a significant role in removal of fixed N in the natural environment, including groundwater systems.

Owing to dependency on a supply of ammonium, anammox reactions are conceivably limited to settings where oxic groundwater containing nitrate migrates to an anoxic zone where there has been some prior mineralisation of either extraneous or autochthonous nitrogen, whether it be a plume of contaminated groundwater or relict organic matter. At the Cape Cod (Massachusetts, USA) groundwater research site, where the sandy alluvial aquifer is contaminated with an aged plume of wastewater, Smith *et al.* (2017) recently estimated that 39–90% of the N<sub>2</sub> produced in the aquifer is attributed to anammox, not denitrification as had previously been assumed. Moore *et al.* (2011) similarly reported that anammox accounted for 18–36% of N<sub>2</sub> production at several ammonium-contaminated groundwater sites examined across Canada, and Wang *et al.* (2017) found anammox contributed 38–67% of the N-loss measured in phreatic aquifers examined in China and Switzerland. Conventional practice for assessing anammox reactivity is to use <sup>15</sup>N radiolabelled isotopes combined with molecular characterisation techniques, targeting the hydrazine oxyreductase *hzs* and/or hydrazine synthase *hzs* genes that are diagnostic of anammox bacteria (e.g., Schmid *et al.*, 2005; Harhangi *et al.*, 2012).

As with denitrification, acetylene and sulphide are effective inhibitors of anammox (Jin *et al.*, 2012). Anammox activity is also suppressed by alcohols, in contrast to denitrification for which reaction is promoted by alcohol substrates (e.g., Jensen *et al.*, 2007). Jin *et al.* (2012) describe conversion

of alcohols to inhibitory aldehydes by the hydroxylamine oxidoreductase enzyme present in anammox bacteria as the likely inhibition mechanism.

### **Dissimilatory nitrate reduction to ammonium**

Dissimilatory nitrate reduction to ammonium (DNRA) is a further respiratory process that can occur in the absence of oxygen. Tiedje (1988) noted DNRA is highest under conditions where the C/N ratio is much higher than required for denitrification, and more recent research continues to support this observation (e.g., Kraft *et al.*, 2014; Robertson and Groffman, 2015). There are few published accounts of DNRA occurring in groundwater systems, other than where groundwater interfaces with benthic sediments, such as in estuarine environments (Ibanhez and Rocha, 2017) and wetlands (Jahangir *et al.*, 2017). Because N is converted to ammonium in DNRA, it is not a true sink for nitrogen.

### **Approaches for measuring denitrification *in situ***

In this section a variety of approaches and practical methods for gaining evidence of denitrification in groundwater settings and determining reaction rates, *in situ*, are reviewed. It is helpful to note that the biochemical parameters and associated analytical methods applicable to denitrification studies are generic to most methods. For the purposes of structuring this review, the investigative methods are presented in two categories, distinguishable primarily by the physical scale at which they interrogate a groundwater system. The first category includes methods and techniques that examine the groundwater system under its natural state (in terms of flow condition and hydrochemical gradients) and can provide nitrate attenuation rate estimates over a large scale. From a resource management perspective this holds advantages over the small-scale practical

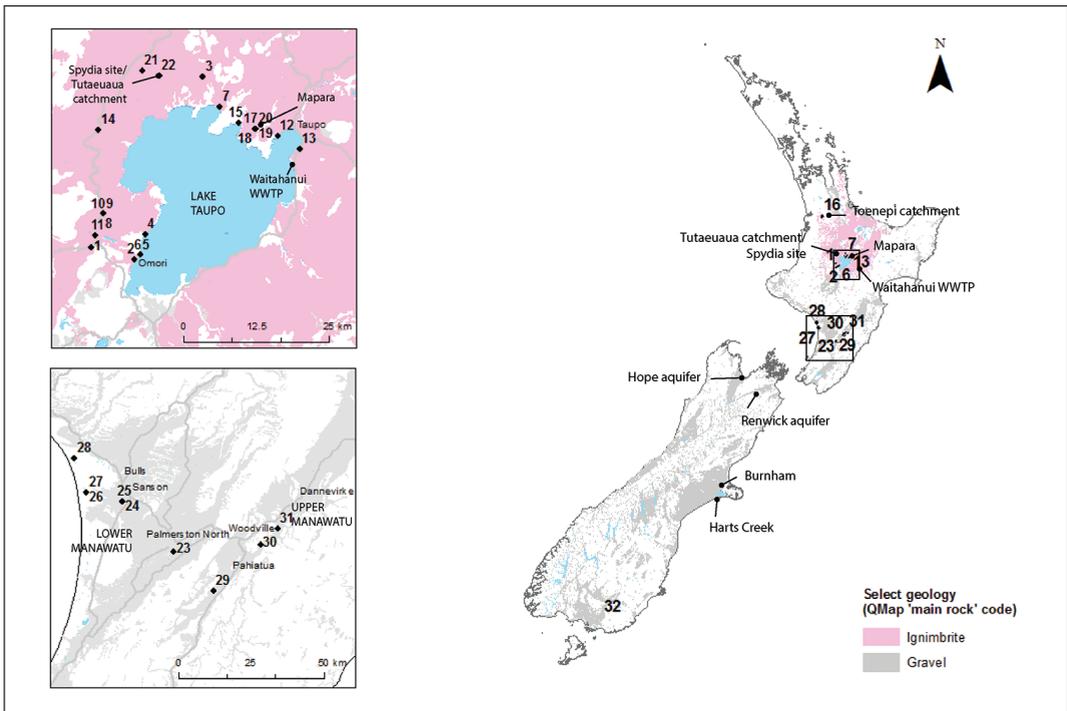
tests that are presented in the second category, which involve a significant artificial (hydraulic and/or chemical) stress applied to the groundwater system and only examine a small, discrete portion of an aquifer in any single test. Consequently, the small-scale tests yield potential nitrate reaction rates, rather than actual rates as occur under natural conditions. The advantages and limitations of differing approaches are discussed, whilst recognising a comprehensive discussion on the problem of scale-effects, as applies to all practical investigative strategies, is beyond the scope of this paper.

Table 1 catalogues all the field sites in New Zealand known to the author where direct *in situ* measurements of groundwater nitrate reactivity have been made. Details of the local site conditions are provided for context and field experiments are described

in the following paragraphs. Figure 2 is a map plotting the geospatial distribution of individual field measurements, and clearly demonstrates how quantitative investigations of nitrate attenuation have focussed mainly on the North Island, namely in the Waikato and Manawatu regions. Geographical places referred to throughout this text are also marked on the map.

**Natural gradient tracer tests, excess  $N_2$  and  $^{15}N$  stable isotope**  
**Natural gradient tracer tests**

Natural gradient tracer tests involve *in situ* measurement of water chemistry from sampling points positioned along groundwater flow paths. Denitrification rates are inferred from environmental or injected tracer concentration gradients observed along such flow paths, after accounting for



**Figure 2** – Map showing geographical distribution of test sites listed in Table 1, identifiable by site number. Other locations mentioned in the paper are marked for reference. Distribution of geologies recorded as ‘gravel’ or ‘ignimbrite’ according to GNS QMap (Heron, 2014) are also shown.

solute travel times. Such an investigative approach requires multiple monitoring wells from which to draw samples and between which observational datasets can be interpolated. Where there are few wells from which to make useful observations along a discrete flow path, additional wells will need to be installed. Obviously, some prior understanding of the local hydrogeological regime is required to inform design of such a monitoring network and having a functional mathematical groundwater flow model of the system to be studied in advance is beneficial for this purpose. Such a model will be required to make quantitative interpretation of findings, regardless. It is the financial expense associated with these aspects that is often the main drawback of natural gradient tracer tests applied for investigative purposes. At the extreme end of the scale, such an investigative strategy might sample along a groundwater streamline, from its point of aquifer recharge to its point of discharge. Such large-scale sampling permits quantitative effects of groundwater denitrification to be characterised at the regional scale and under the natural flow regime, which is commensurate with the decision-making objectives of most applied water resource management. In most cases, some major assumptions are required regarding the historic inputs of nitrate to the groundwater system.

Historically, large-scale denitrification tracer studies were limited to measurement of major ions, nitrogen species and redox parameters and nitrate reaction rates were determined by solving the inverse problem of multi-species reactive transport. The 80-hectare field study of Wang *et al.* (2003) at Waitahanui wastewater treatment plant in Waikato region (inset Fig. 2) was of this nature and employed FEMWATER-N as the mathematical modelling tool. Being the site of a controlled industrial discharge, nitrogen loads to shallow groundwater

in the ignimbrite deposits at Waitahanui were reasonably well constrained. The modelling efforts relied on observations of ammoniacal-N and nitrate-N in the effluent plume, along with chloride, which they treated as a conservative reference tracer. It was assumed denitrification was responsible for the observed reduction in nitrate-N in the anoxic contaminant plume, and the 3-D multi-component transport model supported this viewpoint, although no direct measurements were made to verify this assumption. Measurement of gaseous N-species (as a product of denitrification) or stable nitrogen isotopes conceivably would have been of benefit in this regard.

#### *Excess N<sub>2</sub>*

Being the end-product of both complete denitrification and anammox, N<sub>2</sub> gas measurement can be used to quantify these reaction processes. Ar/N<sub>2</sub> ratios are routinely used as a measure of “excess air” (Heaton and Vogel, 1981), applied to compensate for recharge temperature of dissolved gas tracers, such as CFC and SF<sub>6</sub> used in groundwater dating. In groundwater systems where denitrification or anammox is active, the Ar/N<sub>2</sub> ratio deviates from the excess air ratio as it accumulates N<sub>2</sub> produced from N-respirers – referred to as ‘excess N<sub>2</sub>’. Excess N<sub>2</sub> concentrations are primarily used to evaluate how much of the recharged nitrate-nitrogen has been denitrified in the saturated zone, but if excess N<sub>2</sub> can be correlated with groundwater age it is possible to determine a zero-order *in situ* denitrification reaction rate. This concept has been demonstrated in several groundwater studies overseas (Vogel *et al.*, 1981; Tesoriero *et al.*, 2000; Böhlke *et al.*, 2002; Green *et al.*, 2008; Tesoriero and Puckett, 2011), but has not yet been applied in New Zealand.

Estimation of excess N<sub>2</sub> from Ar/N<sub>2</sub> ratios alone requires making a gross assumption that either an average air temperature applies

**Table 1** – List of New Zealand field sites where *in situ* field measurements of nitrate reactivity have been made. Units of reported reaction rates signify assumed reaction model fit to field data: T<sup>-1</sup> implies first-order reaction; M/L<sup>3</sup>/T implies zero-order reaction rate assumed.

Region	#	Well i.d.	Site name	Depth (m)	NZTM Easting	NZTM Northing	Location	Redox State	Method (see below)	Reaction rate	Geology	Reference (see below)
Waikato	1	72_4094	Kuratau Hall	6	1832124	5692945	Taupo	anaerobic	PPT	0.06 d <sup>-1</sup>	Taupo tephra	1,4
	2	72_4958	Omori	25.5	1839516	5690840	Taupo	anaerobic	PPT	0.07 d <sup>-1</sup>	Taupo tephra	up
	3	72_4952	Otake (Murphy)	14	1851286	5722100	Taupo	anaerobic	PPT	0.02 d <sup>-1</sup>	Oruanui Ignimbrite	up
	4	72_4956	Whareroa	12	1841353	5695070	Taupo	anaerobic	PPT	0.03 d <sup>-1</sup>	Taupo tephra	up
	5	72_4959		24	1840529	5691616			PPT	0.19 d <sup>-1</sup>	Taupo Ignimbrite	4
	6	68_964/ 72_4960	Kuratau	5.0/ 4.0	1840522/ 1840536	5691618/ 5691616	Taupo	anaerobic	RCTWT	0.03 d <sup>-1</sup>	Taupo Ignimbrite	2
	7	72_4087	Kimloch <sup>†</sup>	14.5	1854181	5716943	Taupo	anaerobic	PPT (x2)	0.05; 0.02 - 0.08 d <sup>-1</sup>	tephra	4
	8	72_1087	Rangiatia 'shallow'	6.0-6.6	1834175	5698626	Taupo	aerobic	PPT	nr	Oruanui Ignimbrite	3,4
	9	72_4095		12.0-16.0	1834189	5698631			PPT	0.12 d <sup>-1</sup>	Oruanui Ignimbrite (w/ peat bed)	1,3,4,up
	10	72_4095/ 72_4096	Rangiatia 'deep'	12.0-16.0/ 12.2-16.6	1834189/ 1834189	5698631/ 5698632	Taupo	anaerobic	RCTWT	0.02 d <sup>-1</sup>	Oruanui Ignimbrite (w/ peat bed)	2
	11	72_4093	Waituhi <sup>†</sup>	21.9	1832835	5694924	Taupo	anaerobic	PPT (x2)	0.07 d <sup>-1</sup>	Oruanui Ignimbrite	4
	12	72_4970	Acacia Bay	23	1864080	5712032	Taupo	intermediate	PPT	nr	Oruanui Ignimbrite	up
	13	72_4949	Wharewaka	25	1867879	5709699	Taupo	anaerobic	PPT	0.15 d <sup>-1</sup>	Oruanui Ignimbrite	up
	14	72_4980	Waihaha	6.8	1833318	5713051	Taupo	anaerobic	PPT	nr	Taupo tephra	3
	15	72_4951	Whakaipo Bay	22	1857377	5714219	Taupo	aerobic	PPT	nr	Taupo tephra	1,3
	16	72_2859	Reeker	4.3	1798686	5810863	Rukuhia	anaerobic	PPT	0.67 d <sup>-1</sup>	peat	3,4
	17	72_6553		6.1	1860148	5713212			ISM	nr	Taupo Ignimbrite	up
	18	72_1007		7.4	1860347	5713181			PPT	nr	Taupo Ignimbrite	1,3,4
	19	72_4085	Mapara <sup>†</sup>	7.0-9.8	1860350	5713179	Taupo	anaerobic	PPT (x2)	0.37; 0.48 d <sup>-1</sup>	Taupo Ignimbrite (w/ peat bed)	4
	20	72_4083/ 72_4085		7.0-9.8/ 6.9-9.8	1860345/ 1860350	5713183/ 5713179			RCTWT (x2)	0.09; 0.26* d <sup>-1</sup>	Taupo Ignimbrite (w/ peat bed)	2
	21	72_4089	Waihora	19.5	1840847	5723170	Taupo	aerobic	PPT	nr	Oruanui Ignimbrite	up
	22	72_6552	Spydia	5.93	1843781	5722258	Taupo	anaerobic	ISM	0.006 d <sup>-1</sup>	Oruanui Ignimbrite	5

23	336277	Dairy Farm <sup>†</sup>	6.5-7.0	1820881	5526439	Palmerston Nth (lower Manawatu River)	anaerobic	PPT (x3)	1.2; 0.55; 0.44 mg/l/hr	alluvium	6,7
24	323141	Sanson-A	2.5-3.0	1803362	5543571	Sanson (lower Rangitikei River catchment)	anaerobic	PPT	nr	alluvial gravel	
25	323143	Sanson-B	5.5-6.0	1803361	5543569	Bulls (lower Rangitikei River catchment)	anaerobic	PPT	0.13 mg/L/hr	alluvial gravel	
26	322075	Bulls-A	2.5-3.0	1790830	5546704	Bulls (lower Rangitikei River catchment)	anaerobic	PPT	1.57 mg/L/hr	sand (aeolian)	8,9
27	322073	Bulls-B	5.5-6.0	1790829	5546705	Santoft	anaerobic	PPT	0.12 mg/L/hr	alluvial gravel	
28	312048	Santoft-A	2.5-3.0	1786756	5558337	(lower Rangitikei River catchment)	anaerobic	PPT	0.04 mg/L/hr	sand (aeolian)	
29	357121	Pahiatua <sup>†</sup>	4.9-5.4	1834381	5512928	Pahiatua (upper Manawatu River)	aerobic	PPT (x2)	nr; nr	alluvial gravel	
30	339058	Woodville <sup>†</sup>	5.5-6.0	1850540	5528733	Woodville (upper Manawatu River)	anaerobic	PPT (x2)	0.04; 0.12 mg/L/hr	alluvium (flood deposits)	10,up
31	430021	Dannevirke <sup>†</sup>	7.0-7.5	1856456	5534385	Dannevirke (upper Manawatu River)	anaerobic	PPT (x2)	0.04 mg/L/hr; nr	alluvium (flood deposits)	
32	F46/0847 F46/0868	Matai Road	14.0-17.0/ 12.0-15.0	1275719/ 1275713	4858763/ 4858763	Etdendale	aerobic	RCTWT	nr	alluvial gravel	2

<sup>†</sup> site where a test was repeated; number of tests denoted in brackets in 'method' column.

\* physical scale of repeat test different from initial test.

nr denotes no measurable reaction.

PPT = push pull test; RCTWT = recirculating tracer well test; ISM = in situ mesocosm

<sup>1</sup> Hadfield and Gibbs (2007) <sup>2</sup> Burbury *et al.* (2013) <sup>3</sup> WRC (2007)

<sup>4</sup> Hadfield (2010) <sup>5</sup> Korom *et al.* (2016) <sup>6</sup> Rivas *et al.* (2014) <sup>7</sup> Rivas *et al.* (2015)

<sup>8</sup> Collins *et al.* (2016) <sup>9</sup> Collins *et al.* (2017) <sup>10</sup> Rivas *et al.* (2016)

up = unpublished/pers. comm. John Hadfield, Waikato Regional Council & Aldrin Rivas, Lincoln Agritech.

to the recharge condition of the groundwater being characterised, or all samples were recharged at the same temperature, but with varying amounts of excess air. Applying such assumptions to New Zealand's hydrogeological landscape is not without risk, and is in part the reason why excess  $N_2$  measurements have not been applied extensively to examine denitrification here. Notwithstanding this limitation, Stenger *et al.* (2018) integrated excess  $N_2$  measurement in their multi-faceted investigation of nitrate attenuation in shallow groundwater in the Tutaeuaua catchment on the northern shores of Lake Taupo where it provided a qualitative positive indicator of denitrification occurring in anoxic groundwater.

More accurate excess air evaluations can be achieved if multiple atmospheric noble gas components are analysed (i.e., any combination of Ne, Ar, Kr, Xe) (e.g., Kipfer *et al.*, 2002, Aeschbach-Hertig *et al.*, 2008, Jung *et al.*, 2013). It is therefore worth noting that GNS Science has recently developed the analytical method for measuring neon gas in water samples (Martindale *et al.*, 2017). Expectations are that the ability to determine Ar/Ne/ $N_2$  ratios will provide for a more robust means for determining excess  $N_2$  in New Zealand groundwater, from which *in situ* denitrification reaction rate measurements might be possible in the future.

### **$^{15}N$ stable isotope**

Stable isotopes provide a powerful analytical aid for interpreting the fate and transport of nitrogen species in the environment (Kendall, 1998; Kendall and Aravena, 2000). Not only can  $\delta^{15}N_{NO_3}$  information provide information about the likely origin of nitrate contamination, but when evaluated against  $\delta^{18}O_{NO_3}$  it is also possible to infer whether denitrification has occurred in sampled groundwater. With regard to dissolved nitrate, nitrate derived from fertilizer typically has  $\delta^{15}N_{NO_3}$  values of -8 to +7‰. Nitrate

formed from animal and human waste is slightly more enriched in  $^{15}N$ , typically +5‰ <  $\delta^{15}N_{NO_3}$  < +35‰. Bridging these two categories is nitrogen mineralized from soil organic matter, which has a typical  $\delta^{15}N_{NO_3}$  signature of +2 to +8‰ (Bedard-Haughn *et al.*, 2003; Heffernan *et al.*, 2012; Xu *et al.*, 2016; Nikolenko *et al.*, 2018). Whilst there is much overlap in isotope signature classes, the differences theoretically provide a means for distinguishing the source of nitrate in groundwater samples, provided mixing processes and source history are also considered. Improved interpretations can be made when the  $\delta^{18}O_{NO_3}$  composition of nitrate is also evaluated, because the  $\delta^{18}O/\delta^{15}N$  ratio differs substantially in synthetically-produced nitrate versus natural nitrate (Clark and Fritz, 1997; Nikolenko *et al.*, 2018).

There are examples in New Zealand where  $\delta^{15}N_{NO_3}$  values measured in groundwater have successfully identified probable point sources of groundwater nitrate contamination, such as confirming chicken manure as the main source of elevated nitrate concentrations in groundwater of the Hope region of the Waimea Plains, Nelson (Stewart and Morgenstern, 2001), and identifying animal wastes as the source of nitrate impacts observed at one specific well within the Renwick aquifer, Marlborough (Davidson and Wilson, 2011). However, quite often mixed  $\delta^{15}N_{NO_3}$  signatures are recorded, from which it is difficult to isolate discrete contaminant sources (e.g., Scott *et al.*, 2011; Clague *et al.*, 2015a; Stenger *et al.*, 2018). While application of  $^{15}N$  stable isotope methods for nitrate source identification is advantaged in New Zealand by a lack of sources of atmospherically-derived nitrogen, the method is compromised in pastoral farming settings, which comprise much of the New Zealand agricultural landscape. Pastoral farming in New Zealand involves use of N-fertilizer as well as production of

animal waste, and soil and animal N-pools have overlapping signatures, which gives rise to mixed  $\delta^{15}\text{N}_{\text{NO}_3}$  signatures (Sheppard and Lyon, 1996). It is useful to note also that indistinction between  $\delta^{15}\text{N}$  signatures for human and animal wastes further restricts the usefulness of  $^{15}\text{N}$  stable isotope methods for detecting nitrogen impacts from septic tanks in most New Zealand rural settings.

Being a microbially-mediated process, denitrification causes fractionation of nitrogen isotopes, resulting in enrichment of both  $^{15}\text{N}_{\text{NO}_3}$  and  $^{18}\text{O}_{\text{NO}_3}$  in the residual nitrate pool (Amberger and Schmidt, 1987). Systematic variation of  $\delta^{18}\text{O}$  with  $\delta^{15}\text{N}$  produces a linear relationship on a plot of  $\delta^{18}\text{O}_{\text{NO}_3}$  with  $\delta^{15}\text{N}_{\text{NO}_3}$  that has been used as supporting evidence that denitrification is occurring (Kendall, 1998). Net fractionations are in the range of  $-40\text{‰} < \epsilon^{15}\text{N} < -5\text{‰}$  and  $-18\text{‰} < \epsilon^{18}\text{O} < -8\text{‰}$  (Xue *et al.*, 2009). The fractionation effect is reciprocated in the gaseous N-products of denitrification ( $\text{N}_2\text{O}$ ,  $\text{N}_2$ ) that are depleted in  $^{15}\text{N}$ . Hall *et al.* (2016) highlight limitations in use of nitrogen isotope methods and advise caution when applying them over the catchment scale, due to sensitivity of enrichment factors to scale effects. Nonetheless, there is a general consensus in the scientific literature that in groundwater systems where nitrate gradients are observed along a flow path, a linear relationship of between 0.48 and 1.04 in  $\delta^{18}\text{O}_{\text{NO}_3}$  and  $\delta^{15}\text{N}_{\text{NO}_3}$  values measured in the residual nitrate pool is diagnostic of denitrification (Chen and McQuarrie, 2005; Hall *et al.*, 2016; Xu *et al.*, 2016).

An example application of the  $\delta^{15}\text{N}_{\text{NO}_3}/\delta^{18}\text{O}_{\text{NO}_3}$  dual isotope method in New Zealand is provided in a study of denitrification in shallow groundwater of the Toenepi catchment, Waikato (Clague *et al.*, 2015a). Some limitations were noted concerning the interpretation of the isotopic signatures determined at three multi-level well sites. Firstly, a high proportion of the

strongly reduced groundwater samples had nitrate-nitrogen concentrations below the threshold (approx. 0.03 mg/L  $\text{NO}_3\text{-N}$ ) for reliable isotopic analysis without sample pre-treatment. Secondly, insufficient understanding of the groundwater flow path at the study sites, combined with temporal variation, prevented the isotopic signatures of oxic and anoxic groundwater samples to be conclusively related to each other. While the multi-level well sampling did not yield clear insights, repeated sampling of a saturated Gley soil profile with suction tubes installed at three depths (0.4 m, 0.7 m, and 1.0 m) proved successful. At this site, seasonally-occurring denitrification was reflected in a linear relationship between  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  isotopic enrichments with a slope of 0.73. Denitrification appeared to be more active in the spring months of August/September, compared to in the winter months of June/July. The denitrification capacity of sediments in the catchment was independently confirmed by laboratory-based  $^{15}\text{N}$ -enrichment experiments that revealed relict organic carbon (tree roots, wood, soil layers) buried by alluvium or volcanic ash deposits was sufficient to induce nitrate reductions (Clague *et al.*, 2015b).

Analysing  $\delta^{15}\text{N}_{\text{NO}_3}/\delta^{18}\text{O}_{\text{NO}_3}$  data from 11 multi-level wells at the Waihora well-field (aka Spydia) site on the north-western edge of Lake Taupo (see inset Fig. 2), Stenger *et al.* (2018) also calculated an enrichment slope of 0.75 in their overall dataset, indicating denitrification. However, despite the small size of the Waihora well field (approx. 6000 m<sup>2</sup>) and the uniform land use and management, it did not prove feasible to identify a distinct source signature for nitrate in oxic groundwater. Individually analysing multi-level well sites where samples were taken across a redoxcline did not yield any stronger evidence for denitrification. Spatiotemporal variation of inputs, time lags, and uncertainty about flow paths were

noted as likely reasons (Stenger *et al.*, 2018). Korom *et al.* (2016) similarly determined a  $\delta^{18}\text{O}_{\text{NO}_3}/\delta^{15}\text{N}_{\text{NO}_3}$  fractionation ratio of 0.76 in residual nitrate they monitored as part of an *in situ* denitrification experiment in Oruanui ignimbrite deposits at the same site, details of which are provided later.

Recently, Wells *et al.* (2016) made monthly measurements of  $\delta^{18}\text{O}_{\text{NO}_3}$  and  $\delta^{15}\text{N}_{\text{NO}_3}$  in the spring-fed Harts Creek in Canterbury, over a 2-year duration. Despite the attenuation measurements they derived being imprecise because of gross assumptions required of likely denitrification enrichment factors, the general isotope pattern indicated active denitrification within this sub-catchment, on the shores of Lake Ellesmere/Te Waihora. Net nitrate attenuation was found to be progressively less efficient with increasing distance downstream, corresponding to a transition in soil type from clay-rich to sand-rich. Furthermore, the high frequency sampling results revealed rainfall-driven shifts in isotopic signatures and the discovery that both nitrate discharge and attenuation correlated positively with the quantity of rainfall over the previous 24 hours, independent of antecedent soil moisture conditions or season. Wells *et al.* (2016) speculate this response could reflect hydrologic flushing of partially denitrified nitrate from the hyporheic zone into the surface water.

### **Direct reactive tracer tests made at the point scale**

#### ***Single-well push-pull test***

Where either large-scale natural gradient tracer tests and  $^{15}\text{N}$  natural abundance methods are not feasible, or at specific sites where a process-level understanding of nitrate reactivity is warranted, small-scale tracer tests provide an alternative practical means for examining nitrate reactivity. The single-well push-pull test (Trudell *et al.*, 1986; Istok *et al.*, 1997; Kim *et al.*, 2005)

has developed into a useful method for this purpose and enables examination at a point scale. The concept of a push-pull test is to inject ('push') a tracer solution comprising a conservative tracer (e.g., bromide,  $^3\text{H}$ ,  $\text{SF}_6$ ) and a reactive tracer (typically nitrate in the case of denitrification measurements) into the aquifer being investigated, via a well. Aliquots of the tracer solution are periodically extracted ('pulled') from the same well over time, and the disappearance of nitrate relative to the conservative reference tracer is measured, sometimes in conjunction with accrual of products of denitrification and other biogeochemical indicators. Comparison of reactive tracers to the conservative tracer permits some estimation of reaction rates (e.g., Snodgrass and Kitanidis, 1998; Schroth and Istok, 2006). To confirm denitrification reactions are responsible for any observed nitrate losses, tests often employ  $^{15}\text{N}$  enrichment methods, microbiological analyses and/or the acetylene block method that involves measurement of  $\text{N}_2\text{O}$  gas amassing in the tracer solution as it is recovered from the test-well (Jahangir *et al.*, 2013; Baldwin *et al.*, 2008; Sánchez-Pérez *et al.*, 2003).

Unlike natural gradient tracer tests, nitrate dual isotope analysis, and excess  $\text{N}_2$  calculation, which all provide an assessment under ambient hydraulic and biogeochemical conditions, push-pull tests require augmentation of the system with nitrate. As a consequence, the biogeochemical conditions established during a test are not strictly representative of the natural state, and one must be mindful that reaction rates potentially won't be either. The effects of acclimatisation of denitrifiers in the aquifer and artificial mixing induced by the pumping regime inherently contribute to the overall observed reaction (Burbery, 2005; Eschenbach and Well, 2013), yet in practice are rarely accounted for in interpretations. Push-pull tests have been applied to examine

nitrate reactivity in several groundwater settings in New Zealand. In all those examples, nitrate reaction rates were derived with simplified analytical models, assuming first- or zero-order kinetics.

Waikato Regional Council (WRC) was first to use push-pull tests in New Zealand, in their investigation of shallow groundwater in the Lake Taupo catchment (Hadfield and Gibbs, 2007; WRC, 2007). The tests made by WRC were limited in scope – in so much that interpretations relied solely on measurement of nitrate and bromide along with standard groundwater chemistry parameters (dissolved oxygen, temperature, pH, conductivity) – yet enabled a direct observation of nitrate reactivity. WRC have now performed twenty push-pull tests at seventeen different site locations within the Waikato region, covering a range of redox states. Positive nitrate reduction has been recorded at eleven of the field sites and results tend to indicate a positive correlation between apparent nitrate reactivity and the presence of relict organic material in the aquifer profile (Hadfield, 2010; Hadfield and Korom, 2012) (Table 1). Subsequent studies at the Waihora well-field have affirmed that relict organic matter in the form of palaeosol layers, and to a lesser extent woody debris remaining from the vegetation destroyed by volcanic activity 1.8 ka before present, play a key role in fuelling denitrification in shallow groundwater on the North Island's Volcanic Plateau (Clague *et al.*, 2013; Stenger *et al.*, 2018). Various forms of organic material (peat layers, tree roots and trunks, former topsoil layers covered by alluvium) were noted as saturated zone electron donors in the Toenepi lowland catchment near Hamilton (Clague *et al.*, 2015a, 2015b).

Rivas *et al.* (2014, 2015, 2016) and Collins *et al.* (2016, 2017) have also used push-pull tests to examine the spatial distribution of potential nitrate attenuation within the Manawatu River catchment and Rangitikei

River catchment of the Manawatu-Wanganui region, respectively. In those examples the tests incorporated the acetylene block method, and whilst none of the resulting measured nitrate losses could be reconciled with observed rates of  $N_2O$  gas production,  $N_2O$  amassed in tests conducted in anoxic groundwater settings confirmed denitrification was active. Reasons for the stoichiometric imbalance between  $NO_3$  reactant and  $N_2O$  product were not investigated, but it tends to imply that a process other than denitrification may have been responsible for some of the observed nitrate reactivity, and/or the acetylene block method was not completely effective in the tests. Another limitation with the push-pull tests so far performed in the Manawatu-Wanganui region was their short duration—the nitrate tracer solution resided in the aquifer for less than seven hours. Retrieval of the injected tracer was made in a single continuous “pull” event, rather than a staged extraction conducted over several days, as was the method practised by WRC. Burbery (2005) has shown that reaction rates derived from push-pull tests based on analysis of data obtained from a single abstraction event often yield higher rates than those that might alternatively be estimated if the test were extended over a longer time period involving multiple abstraction events. He suggested that push-pull tests made with long incubation periods provide a more representative assessment of potential reactivity under natural hydraulic conditions than rapid tests that are affected by a lot of forced pumping. Considering the style of the push-pull tests made by Rivas *et al.* (2014, 2015, 2016) and Collins *et al.* (2017), the nitrate reaction rates reported from the Manawatu-Wanganui region (sites 23-31 in Table 1) likely over-estimate actual on-going nitrate reactivity under the natural regime.

### ***Re-circulating tracer well test***

Single-well push-pull tests are not practicable in fast-flowing aquifers due to the high potential for the slug of tracer injected into a test well to drift beyond the capture zone of the well, resulting in insufficient tracer mass recovery from which to measure any reactions (Burbery *et al.*, 2004). This is partly why there are no reports of push-pull tests having been conducted in the South Island, New Zealand, where the hydrogeology is characterised by fast-flowing alluvial outwash gravel aquifer systems, which are also generally aerobic (i.e., inhibitory to nitrate reduction). As a slightly up-scaled alternative to the single-well push-pull test, Burbery and Wang (2010) conceived a recirculating tracer well test (RCTWT) method for measuring nitrate reaction rates *in situ*, which functions on the principle of creating a hydraulic dipole between two wells. The shallow alluvial gravel aquifer at Edendale, Southland, where background nitrate concentrations were in the order of 10 mg/L NO<sub>3</sub>-N, constituted a testing ground for the method (Burbery *et al.*, 2013). Despite a closed dipole system between two wells being sustained in the Edendale aquifer for a period of two weeks, no measurable reduction of nitrate was detected. The finding tends to support the notion that aerobic alluvial gravel aquifer systems have effectively no natural capacity to attenuate nitrate. The reason behind this is because they are carbon-limited and lack suitable electron donors to drive redox reactions (e.g., Rivas *et al.*, 2017). It is useful to note that attempts to conduct a re-circulating tracer well test in a portion of the Canterbury Plains aquifer at the Burnham experimental well-field (e.g., Dann *et al.*, 2008) were not successful, due to failure to establish effective dipole flow between two wells separated less than 5 m apart. The test failure reflects the highly heterogeneous nature of alluvial gravel aquifers that are susceptible to preferential flow phenomena caused by open framework

gravel facies (Dann *et al.*, 2008; Burbery *et al.*, 2017).

Burbery *et al.* (2013) also trialled the RCTWT method in ignimbrite deposits at Mapara, in the Lake Taupo catchment, where WRC had previously performed a push-pull test that had revealed potential for nitrate attenuation (Hadfield and Gibbs, 2007) (Fig. 1; Table 1). At that site, *in situ* nitrate reaction rate estimates determined with the re-circulating tracer test were found to be sensitive to the pumping rate of the test (which affects the effective volume of aquifer examined) and were marginally lower than the rates determined from push-pull tests.

### ***Buried mesocosms***

Bates and Spalding (1998) describe using mesocosm apparatus vibrated into the ground to isolate a portion of aquifer and facilitate small-scale *in situ* batch experiments of denitrification rates in the USA – the approach being akin to a push-pull test in which the tracer solution is shielded from any drift effects. A long-term tracer test of this sort is currently being carried out at the site in Mapara, where nitrate reactivity in groundwater has previously been examined using push-pull tests and RCTWTs. A portion of the saturated Ōruanui ignimbrite has been isolated using a stainless steel chamber into which nitrate has been added (Korom *et al.*, 2016). Based on the evolution of water chemistry (including  $\delta^{15}\text{N}_{\text{NO}_3}$  and  $\delta^{18}\text{O}_{\text{NO}_3}$  signatures) so far monitored in the mesocosm, evidence indicates denitrification reactions are proceeding. More importantly, the comprehensive hydrochemical analyses made by Korom *et al.* (2016) have revealed that while organic carbon appears to play some role in fuelling denitrification in the Ōruanui ignimbrite deposits, indications are that ferrous iron is acting as the major electron donor fuelling nitrate reaction. Korom *et al.* (2016) propose that pyroxene minerals, and amorphous minerals, maybe

associated with volcanic glass, are likely to be acting as the sources of reactive  $\text{Fe}^{2+}$ .

## Summary

There is no question that across New Zealand large amounts of nitrate are leached below the soil zone, providing substantial loads of reactive nitrogen to underlying groundwater systems. However, how much of this groundwater nitrate is naturally attenuated in the subsurface by processes such as denitrification remains unknown, since only a handful of studies have attempted to directly examine the fate of nitrogen in the vadose zone-groundwater continuum. While one can conjecture that denitrification is likely to be the main process by which any nitrate attenuation occurs, there is growing evidence from studies abroad that in the natural world anammox plays a greater role in attenuating nitrate than had previously been considered. Anammox reactions have not been studied in any detail in New Zealand.

A fundamental requirement for any natural attenuation of nitrate to occur is a low redox condition, i.e., low to no dissolved oxygen—at least less than 2 mg/L  $\text{O}_2$ . Recognising this criterion, there is an ongoing drive in New Zealand to create groundwater redox maps as a geographical aid for predicting vulnerability of groundwater systems to nitrate impacts. Whilst maps are useful, translation of such geospatial data into nitrate attenuation information requires some knowledge of effective nitrate reaction rates, which can only be obtained through direct field measurement, using the practical methods described in this paper. Furthermore, whilst the topic was not explicitly addressed in this review, effective resource management requires knowledge of nitrate fluxes. So, nitrate reactivity data ultimately needs to be integrated with groundwater flow data to quantify nitrogen fluxes. For example, Hadfield and Gibbs (2007) may have

demonstrated nitrate disappears rapidly in organic peaty deposits within the Waikato region, but peat is not a particularly permeable substrate, so the potential groundwater flux through such reactive material is conceivably slow, relative to fluxes through alternative sand and gravel alluvium. Such knowledge of reactive transport pathways is needed to give confidence to nitrate attenuation factors applied at the catchment scale, which to date have only been calculated for a few locations and only on the simple basis of nitrogen budget models that have failed to incorporate any hydrogeological component.

Out of all the investigative methods described in this paper, the excess  $\text{N}_2$  method is perceivably the most promising for estimating nitrate attenuation factors at the catchment scale, particularly considering it is now possible to analyse dissolved Ar/Ne/ $\text{N}_2$  in one groundwater sample, and thus obtain more accurate measures of excess air and recharge temperatures. The method depends on being able to sample groundwater along a flow path and to determine its age. Assuming this ternary dissolved gas assessment sees more application in New Zealand then, perceivably, the largest errors associated with its use will pertain to assumptions about groundwater flow paths and historic nitrogen loadings. These are not trivial problems.

Only in the Waikato and Manawatu-Wanganui regions are there any reports of significant field investigation to characterise the fate of nitrate in New Zealand groundwater. This is biased by the fact these regions have flow conditions and aquifer materials that have proven more conducive to nitrate attenuation than the alluvial aquifers of the South Island. Single-well push-pull tests have proven a useful tool for studies at the point-scale in largely sandy sediments of these regions where groundwater velocities are relatively slow. Test results have demonstrated denitrification is active in most, but not all, places where anoxic groundwater conditions

prevail. The few tests conducted to date have highlighted that redox state alone is not necessarily a guarantee of nitrate reaction, at least over the time-scale of the practical tests so far completed, which were in the order of seven hours to several days in duration. A strong dependency, however, has been observed between relict organic matter and nitrate reactivity. The latest evidence obtained from the mesocosm experiment in the Taupo catchment where the groundwater hydrochemistry is being comprehensively examined in response to a controlled nitrate loading to the shallow anoxic groundwater system has revealed that, in addition to heterotrophic denitrification, autotrophic denitrification plays a potentially significant role in nitrate reduction, in the Ōruanui ignimbrite deposits at least. Considering the availability of iron minerals in New Zealand geologies one could speculate from Korom *et al.*'s (2016) findings that there is potential for autotrophic denitrification in many groundwater systems of New Zealand where reduced conditions prevail.

From the suite of push-pull tests so far conducted in the Manawatu catchment, no obvious nitrate reaction has been detected in alluvial gravel aquifer substrate where oxic conditions prevail. Similar unresponsive results were obtained in the RCTWT trialled in outwash gravels of the Edendale aquifer, Southland. Whilst few in number, both results support the notion that there is very low potential for any effective nitrate attenuation in aerobic alluvial gravel aquifer systems, such as constitute the most significant groundwater systems in New Zealand. The recent stable isotope data gathered by Wells *et al.* (2017) has provided evidence of some active denitrification in the groundwater-fed Harts Creek, at the margin of Lake Ellesmere/Te Waihora, and which marks part of the discharge zone of the Canterbury Plains gravel aquifer system. It is suspected, however, that denitrification

activity is focussed in land drains and the heavy soils that cap the gravel aquifer system, which are limited to the margins of the coastal lagoon. Consequently, the capacity for nitrate attenuation within the gravel aquifer system proper appears limited.

Clague *et al.* (2015a) noted some seasonal pattern to isotope enrichment factors in very shallow groundwater at a Gley soil site in the Toenepi catchment, indicating more denitrification in the spring months of August/September versus the winter months of June/July. On the other hand, Wells *et al.* (2017) did not detect any seasonal trend in denitrification activity from the isotope signatures they studied in Harts Creek, Canterbury. Rather, in the Harts Creek system, denitrification activity was discovered to be rainfall event-based. Both studies highlight the dynamism of denitrification and interplay between hydrological and biological activity that will be unique for different groundwater settings and will require characterisation at a local scale. One can conjecture that shallow groundwater systems will express more dynamism than large or deep systems that have more capacity to dampen-out such effects. In hydrological catchments containing shallow groundwater systems the potential for temporal variation in nitrate attenuation should be considered when deriving nitrate attenuation factors.

One key conclusion from this paper is that there have been few direct measures of nitrate attenuation rates across New Zealand. Nitrate fate and transport in the vadose-groundwater zone continuum is not adequately considered in the attenuation factors that have been reported to date, calculated from comparing landscape yield to catchment yield. Hydrogeological conditions differ considerably across the country and so it is inappropriate to transpose attenuation factor values from one catchment to another. There is scope for the practical investigative methods described in this paper to be used

more extensively, to examine groundwater nitrate reactivity *in situ* and across a variety of scales. To make meaningful predictions of natural attenuation rates required for resource management decision making, however, reaction rates need to be integrated with groundwater flow data. Functional groundwater flow and transport models are required to achieve this, and the entire process requires careful consideration of scaling issues. Determining groundwater ages, flow paths and historic N-loadings present ongoing challenges in this field.

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