Denitrification in the shallow groundwater system of two agricultural catchments in the Waikato, New Zealand

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Juliet Catherine Clague

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Intensification and expansion of pastoral farming in New Zealand has resulted in increased nitrate (NO_3^-) leaching. Nitrate leached from the root zone into the underlying groundwater may travel to surface waters and result in eutrophication. Denitrification in the groundwater system may help reduce the impact of intensification on the receiving waters by converting reactive NO_3^- to inert dinitrogen (N_2) gas. Little information exists about the occurrence of denitrification in groundwaters within New Zealand. This project investigates the extent of and limitations on denitrification in two contrasting small catchments within the Waikato region of New Zealand. The lowland Toenepi catchment is under high intensity dairying and features fine textured, alluvial and old volcanic ash deposits. The Waihora well field is an upland catchment under low intensity pastoral agriculture and is formed from coarse, young volcanic deposits.

Isotopic analysis of NO_3^- in reduced groundwater samples from the Toenepi catchment showed temporal and spatial variation in the degree of $\delta^{15}N$ and $\delta^{18}O$ enrichments. Insufficient knowledge on groundwater flow paths at the study site as well as low NO_3^- concentrations made interpretation of the isotopic results difficult. Thus push-pull tests were performed but these, except one, were unable to demonstrate denitrification under *in situ* conditions, even when a carbon (C) substrate was added. However, laboratory incubations of aquifer material taken from the vicinity of the well screens demonstrated denitrification capacity was present. Addition of a C substrate (glucose) generally resulted in an increase in total N gas production. This response to C addition indicates that C availability limits denitrification *in situ*. However, even the low rates of denitrification measured could have a significant impact on the NO_3^- leaching into the shallow groundwater, provided flow paths, and therefore reaction time, are long enough.

The isotopic analysis of NO_3^- in ground water samples proved ineffective at determining whether denitrification occurred at the Waihora field site, as most reduced groundwater samples had NO_3^- concentrations too low for analysis. However, laboratory incubations demonstrated that denitrification could occur below the root zone and was related to the presence of relict organic matter, in the form of buried soils and vegetation. Further experiments determined the denitrification potential of samples from below the A horizon, and indicated that much of the profile was C-limited as addition of glucose or hot-water extractable C resulted in an increase in total N gas fluxes. Despite the lower denitrification capacity at the Waihora well field (compared to Toenepi), the reduced portion of the profile has the potential to make a significant reduction in the NO_3^- concentrations in the shallow groundwater as N inputs from the land are much lower.

This research has demonstrated that denitrification does occur in shallow groundwater systems in New Zealand, and that despite being limited by C-availability, significant reduction of NO_3^- leached from the root zone could occur as a result of relict organic matter located well below the soil zone in the groundwater system.

Keywords: denitrification, shallow groundwater, aquifer, nitrate, ^{15}N isotope, relict organic matter, palaeosol, redox, capacity, potential, incubation, push-pull tests

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Chapter 1

Introduction

1.1 Background

The New Zealand (NZ) economy is dependent on pastoral agriculture. This poses significant challenges for the future: increasing productivity and maintaining (or improving) freshwater quality. It is well known that land use and management practices can have a detrimental effect on the quality of ground and surface waters (Vitousek et al., 1997). However, there is a dearth of knowledge on the pathway of contaminants, such as nitrate (NO_3^-), from source to impact in NZ's groundwater systems (Dymond et al., 2013).

Using an agricultural nutrient budgeting model (Overseer®) Lincoln Agritech Ltd (formerly Lincoln Ventures Ltd) found discrepancies between the estimated NO_3^- leaching losses from the root zone and the subsequent NO_3^- concentrations measured in nearby surface waters within the Toenepi and Waihora catchments (Stenger et al., 2008; Stenger et al., 2009). The Toenepi catchment is located in the eastern part of the Waikato region and contains profiles characterised by alluvial and volcanic ash deposition with lenses of silt, clay and peat (Figure 1.1). There is a lack of both international and national research focussing on denitrification in shallow groundwater systems containing these types of aquifer materials. The Waihora field site is located in the upper part of the Waikato region, close to Lake Taupo and the Taupo volcanic zone and the subsurface profile contains relict organic matter in the form of buried soils (palaeosols) and vegetation. Very little information exists regarding denitrification under volcanic profiles. As a consequence, this current project was initiated to investigate the occurrence and significance of denitrification in the shallow groundwater of these two small catchments in the Waikato region of NZ.

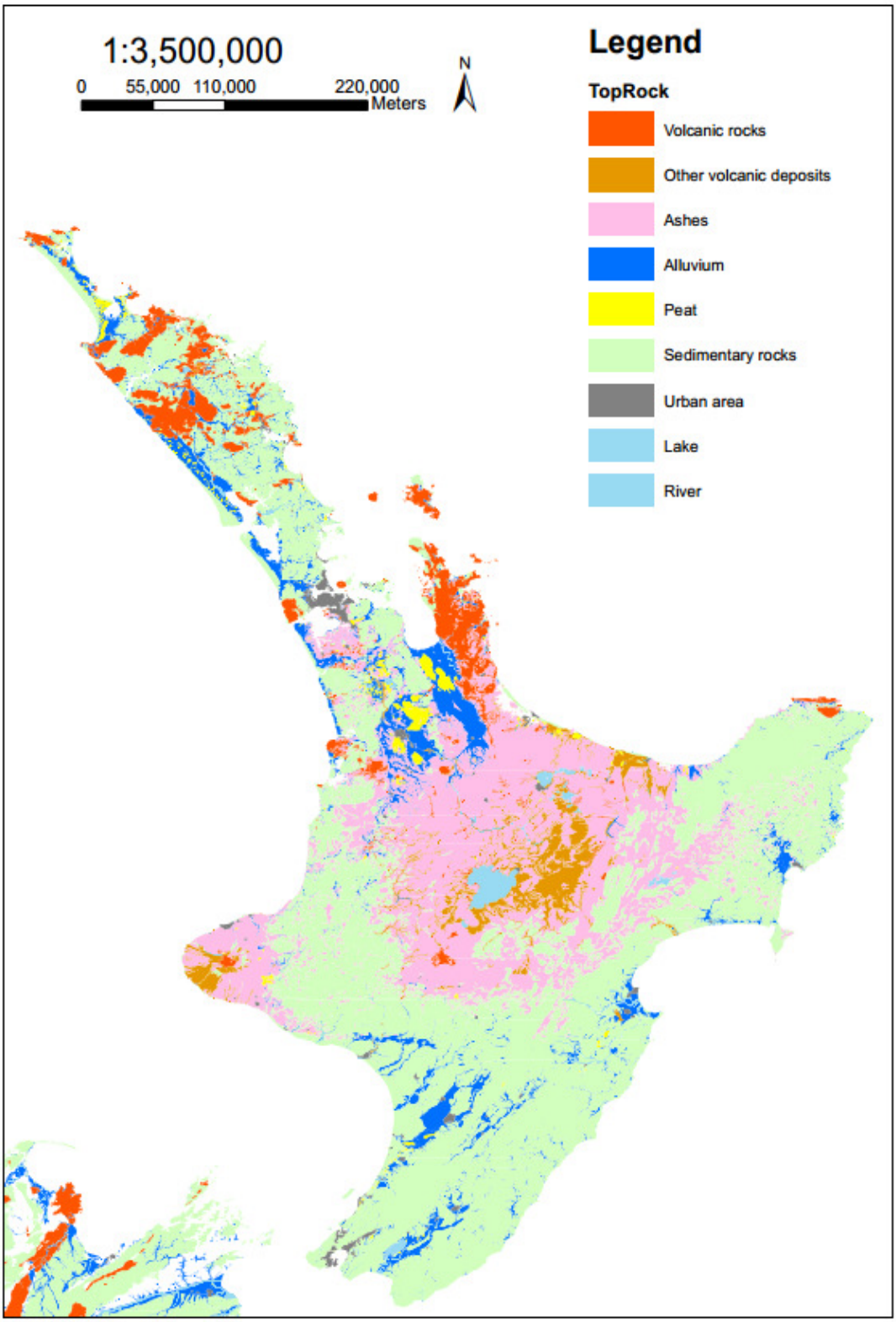

Figure 1.1: Surficial geology of the North Island, New Zealand. Individual toprock classification, according to the New Zealand Land Resource Inventory, has been aggregated for simplification.

When the current project was conceived, research initially focussed on using NO_3^- stable isotopes to identify and quantify denitrification in shallow groundwater samples. This was because the literature showed that NO_3^- stable isotopes could potentially be used as a tool for this purpose. Initial results, based on isotopic analysis did not provide definitive answers and so this necessitated *in situ* and laboratory experiments be performed to further evaluate the role of denitrification in the shallow groundwaters.

1.2 Research Objectives

The objectives of this project were to:

- determine if denitrification was occurring in the shallow groundwater system;
- if denitrification occurred, determine where and when; and
- identify the factors controlling denitrification.

1.3 Thesis Structure

This thesis is divided into 8 chapters and due to the manuscript style used, there will be some duplication of introductory comments and methods between chapters.

Chapter 1 This provides a general introduction, a brief rationale and the outline of the research performed.

Chapter 2 Literature on denitrification in aquifers including various methodologies to identify and quantify denitrification are reviewed and the research gaps are demonstrated.

Chapter 3 Groundwater samples were taken from two small catchments (Toenepi and Waihora) in the Waikato region of NZ and NO_3^- isotopes were analysed to try and assess the role of denitrification in attenuating NO_3^-. Methodologies are described and the results are reported and discussed.

Chapter 4 Following the results of Chapter 3, it was apparent that the isotopic analysis of NO_3^- did not provide conclusive evidence for denitrification, so *in situ* push-pull tests were performed in the Toenepi catchment to determine if denitrification occurred in the aquifer matrix close to the screened zones of the shallow wells. Results are again reported and the implications discussed.

Chapter 5 As *in situ* push-pull tests could not be performed in the Waihora catchment due to the relatively fast groundwater velocities measured, an incubation experiment was performed *in vitro* to determine denitrification capacities of the vadose and aquifer materials. Methodologies and results from this multi-layered, volcanic site are reported and discussed.

Chapter 6 Following the results of Chapter 5, further *in vitro* experiments were carried out to elucidate the factors limiting denitrification in the shallow aquifer materials of the multi-layered volcanic site. Results are again reported and the implications discussed.

Chapter 7 Due to the lack of denitrification capacity and the lack of any significant increase in the denitrification rates following C additions using push-pull tests (Chapter 4), a further experiment was performed *in vitro* to determine both denitrification capacities and potentials of the shallow groundwater materials from the Toenepi catchment. Methodologies are described and the results are reported and discussed.

Chapter 8 Results from previous chapters are summarised and recommendations for future research presented.

Chapter 2

Literature Review: Denitrification in Aquifers

2.1 Introduction

The focus of this PhD project has been to assess and quantify denitrification in the shallow groundwater of 2 small catchments in Waikato, New Zealand. Therefore this literature review concentrates on research involved in measurement and identification of the factors involved in saturated-zone denitrification.

2.2 The Nitrogen Cycle

Dinitrogen gas (N_2) is the major gaseous component in the troposphere, making up 78% of the air we breathe (Galloway et al., 2004). In the terrestrial biosphere, inorganic-N (ammonium (NH_4^+) and nitrate (NO_3^-)) are used by plants and are key substrates in N transformation processes such as nitrification and denitrification (Figure 2.1). As a consequence of the Haber-Bosch process being invented in 1909, inorganic-N fertilisers became widely available and have been used extensively ever since to increase agricultural production (Galloway et al., 2004). Prior to the industrial revolution, and the subsequent use of the Haber-Bosch process, the N cycle was much 'tighter' than it is today because N inputs occurred naturally (e.g. via lightning fixation and biological N fixation (BNF)) with concurrently low fluxes of N through subsequent transformation pathways. As a consequence of synthetically manufactured N forms, the anthropogenic use of fertilisers and manures in agricultural systems has led to higher flows through the N cycle with concomitantly increased rates of N 'leakage', creating higher levels of reactive N in the environment (Galloway et al., 2004; Vitousek et al., 1997).

2.2.1 Transformations of N

Most transformations of N which occur in the soil are microbially-facilitated reactions. Mineralisation of organic N transforms complex organic compounds into plant-available NH_4^+, the bacteria or fungi involved gain energy and assimilate the NH_4^+. Excess NH_4^+ is excreted, and becomes available for plant uptake or is further transformed to NO_3^- via nitrification. Nitrification is a two step oxidation process, carried out by different genera of bacteria: *Nitrosomonas* oxidise NH_4^+ to NO_2^-, while *Nitrobacter* convert nitrite (NO_2^-) to NO_3^- (Figure 2.1). Various factors such as pH, temperature and concentration of other ions, can affect the rate of these transformations, but generally, they proceed to completion, releasing readily-mobile NO_3^- for plant uptake, denitrification or transport though the vadose zone to the water table (McLaren and Cameron, 1990).

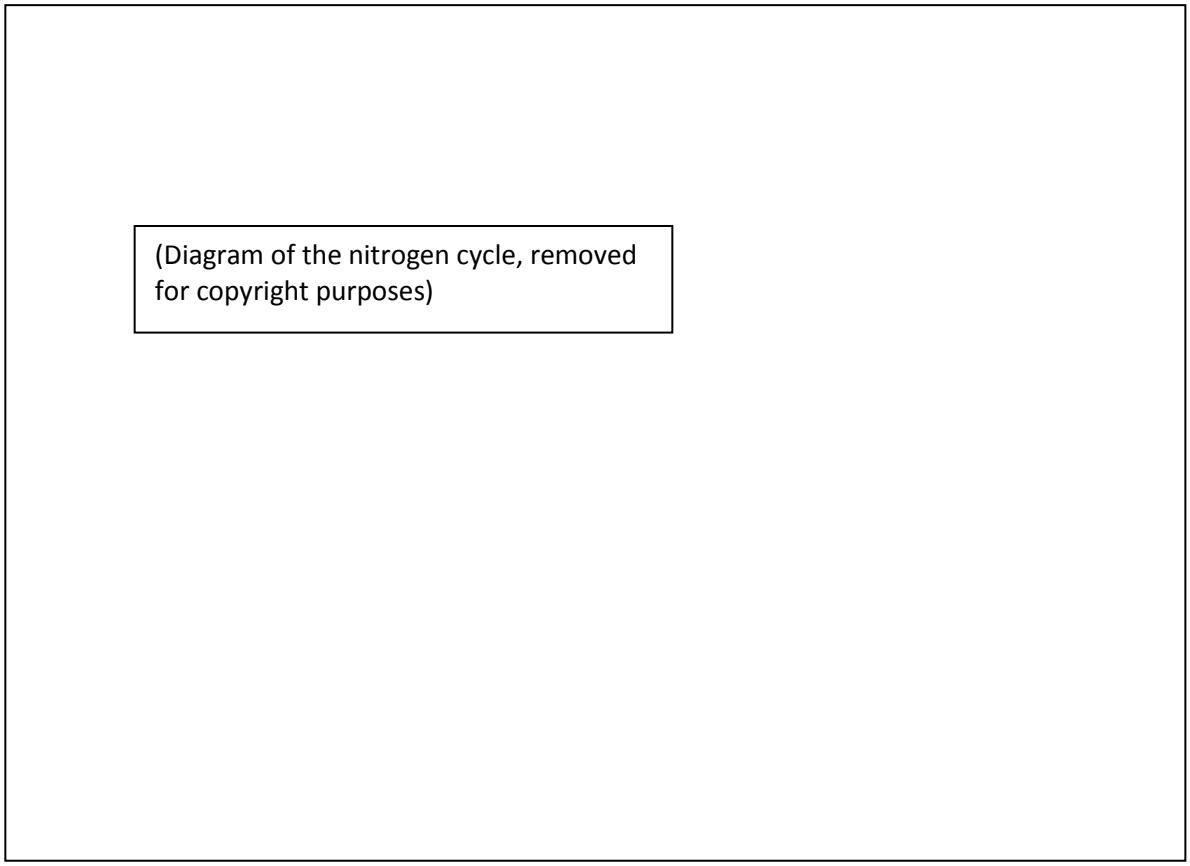

Figure 2.1: The Nitrogen Cycle (source: Rivett et al., 2008)

2.2.2 Nitrate in the environment

Higher levels of reactive N (i.e. NO_3^-) in the environment can lead to eutrophication of rivers, lakes and streams, and contaminate groundwater-sourced drinking water supplies (Vitousek et al., 1997). In the North Island of NZ, lakes are typically considered to be N-limited due to high concentrations of phosphorus (P) of geogenic (i.e. natural) origin. Therefore, these lakes are more susceptible to eutrophication via NO_3^- leaching than similar lakes overseas (Abell et al., 2010; White, 1983). New Zealand guidelines for the maximum acceptable concentration of NO_3^--N in drinking water is 11.3 mg N L^{-1} while ecological trigger values have been set at 0.17 mg N L^{-1} (ANZECC, 2000). The large difference in values highlights how susceptible aquatic ecosystems are to contamination and potential eutrophication. Intensive agricultural practices have increased the amount of reactive N in the environment, with more than 73% of groundwater samples in New Zealand exceeding the ANZECC ecological trigger value and almost 5% exceeding the drinking water standard during the period 1995 – 2006 (MfE, 2007).

2.2.3 The Denitrification Process

Denitrification is the reduction of NO_3^- to N_2 which proceeds through a series of microbial reactions and it is the major pathway for completing the N cycle (Knowles, 1982).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Denitrifying bacteria can be either heterotrophic (use organic C as an energy source) or autotrophic (use reduced inorganic species such as iron (Fe) or sulphur (S) and are facultative anaerobes, meaning they can function in low or no-oxygen environments (Knowles, 1982). Microbes utilise the NO_3^- (or other intermediary reduction products in the denitrification cascade, e.g. nitrous oxide: N_2O) as a terminal electron acceptor and couple the reaction with a suitable electron donor (e.g. C or S, depending on the organism), gaining energy in the electron transfer (Böttcher et al., 1990). For example:

Pseudomonas denitrificans uses a C source:

$$5CH_2 + 4NO_3^- + 4H^+ \rightarrow 5H_2CO_2 + 2N_2 + 2H_2O$$

While *Thiobacillus denitrificans* uses a S source:

$$5FeS_2 + 14NO_3^- + 4H^+ \rightarrow 10SO_4^{2-} + 7N_2 + 5Fe^{2+} + 2H_2O$$

Microorganisms favour reactions which generate the maximum energy for the least amount of energy invested, and they therefore couple the most efficient electron donors and acceptors available. Each step in the process can be arrested, as many microbes are incapable of carrying out the whole pathway and specialise in just one reduction reaction. As oxygen (O_2) produces the most energy per mole of organic C oxidised, it is preferentially utilised by bacteria until scarce (McMahon and Chapelle, 2008). Facultative anaerobes take over from aerobic bacteria when O_2 concentrations drop below a threshold level (which can depend on the populations involved, but a general rule of thumb in water is <2 mg L^{-1} dissolved O_2 (Böhlke et al., 2002)), whereupon NO_3^- becomes energetically favourable for reduction. In aquifers, as O_2 (and NO_3^-) concentrations decrease, obligate anaerobes (those which can only survive in the absence of O_2) utilise manganese (Mn) then Fe and S compounds. While technically this thermodynamic cascade of electron acceptors takes place sequentially, in reality, multiple reactions may be occurring simultaneously in microsites associated with particular minerals and conditions (Korom, 1992; Korom et al., 2012). The presence of elevated concentrations of reduced Fe and Mn, hydrogen sulphide (H_2S), or methane (CH_4) indicates that the redox sequence has passed the stage where NO_3^- is an attractive electron acceptor and denitrification has occurred if NO_3^- was originally present.

Factors which regulate denitrification are:

- Anaerobic status
- Substrate availability (electron donors and NO_3^-)
- A microbial population capable of utilising the NO_3^-
- Trace elements
- pH
- Temperature
- Inhibitory compounds

In aquifers, denitrifiers and NO_3^- are considered ubiquitous, especially in agricultural environments (Seitzinger et al., 2006). The low O_2 environment required occurs in aquifers when gas exchange between the unsaturated zone and atmosphere continuum is limited, and when O_2 initially present in recharge waters is consumed in microbial reactions. This often occurs when organic C (or another electron donor) is abundant. Nutrients (C, S, P) and micronutrients (e.g. boron, copper and zinc) are required by microbes for metabolism and while most environments provide enough, some oligotrophic systems may be limited (Champ et al., 1979). The optimal pH range is generally considered to be 5.5 – 8.0, but depends on the individual species as denitrification has been observed outside this range (Rust et al., 2000). Like pH, the temperature range of microbial operation depends on the species involved, but generally, low temperatures yield low denitrification rates (Bijay-Singh et al., 1989). Naturally occurring substances such as salt (unless a marine environment) (Kana et al., 1998) and H_2S (Myers, 1972) inhibit various steps in the denitrification pathway, while the inhibitory effect on the reduction of N_2O of acetylene is exploited in laboratory incubation experiments (Yoshinari et al., 1977). It is also documented that high concentrations of NO_3^- can alter the denitrification process, either by inhibiting the reduction of N_2O, which results in the accumulation of this greenhouse gas (Blackmer and Bremner, 1978) or inhibition of the whole process (Lalisse-Grundmann et al., 1988). The concentration at which NO_3^- becomes inhibitive differs with different species and conditions, but can be as low as 113 µg N g^{-1} dry soil (Lalisse-Grundmann et al., 1988).

2.3 Measurement of Denitrification in Aquifers

A large number of methods exist for the measurement and quantification of denitrification. Many are more suitable for analysis of soils or lake sediments than groundwater. Initial studies discounted the occurrence of denitrification in aquifers due to low concentrations of dissolved organic C (DOC) in the groundwater (which leaches through the vadose zone) being unable to provide the necessary electrons for heterotrophic denitrification (Smith and Duff, 1988; Starr and Gillham, 1993). Later authors; however, found resident, relict organic matter (or other electron donors) as part of the

aquifer matrix being able to fuel the process (Hill et al., 2004; Jørgensen et al., 2009; Kellogg et al., 2005; Korom et al., 2012; Tesoriero and Puckett, 2011). Studies focussing on denitrification in aquifers often observe the process by monitoring 'natural' concentrations along a flowpath or in a catchment and analyse particular geochemical species which indicate that the process is occurring (e.g. Tesoriero and Puckett, 2011). Others involve the experimental manipulation of the process, either by amending the groundwater directly with tracers or additional substrate, or by the removal of sediments and groundwater for laboratory-based experiments (e.g. Eschenbach and Well, 2013).

2.3.1 Acetylene Inhibition Method

This method is often used in laboratory-based experiments investigating the denitrification capacity or potential of aquifer sediments (Yoshinari et al., 1977). Sediment samples are placed in gas-tight environments with either acetylene-infused water or acetylene in the headspace gas mixture. Acetylene inhibits the microbial conversion of N_2O to N_2 which results in an accumulation of easily-measured N_2O. Many problems exist with this method, as the acetylene can also inhibit the nitrification process, which reduces the amount of NO_3^- available for denitrification. The acetylene can be slow to diffuse into fine-textured sediments and soil respiration can be enhanced. Parallel experiments with and without acetylene are also required to deduce the natural flux of N_2O. Despite these factors, the method does provide a basis for comparison between different aquifers, nutrient regimes and amendment rates. It also demonstrates that denitrification can occur (under optimum conditions), which can sometimes be hard to infer under *in situ* conditions. A series of experiments by Smith and Duff (1988) were used to explain the changes in concentration of N observed in a sewage-contaminated aquifer. To test whether the system was C or N-limited, different amounts of NO_3^- and glucose (a readily-available source of C) were added to samples. Results suggested that denitrification rates depended on the extent of priming the bacteria had previously undergone. Samples which had low NO_3^- concentrations *in situ* responded to NO_3^- additions while those conditioned to high NO_3^- concentrations showed a positive response only when glucose was added. This study by Smith and Duff (1988) demonstrated that different parts of the aquifer would respond differently.

2.3.2 Denitrification Capacity and Potential

This method is similar to the acetylene inhibition method in that samples are incubated with and without various amendments. It is based on the work of Yeomans et al. (1992) and involves replacing the headspace air with an inert gas (helium (He) or argon (Ar) are commonly used), thus making conditions anaerobic. Acetylene is not required as the evolved N_2 is easily measured against the background He or Ar gas. Frequently, an enriched source of N is used, in order to more successfully determine the evolved gases. A *denitrification capacity* experiment seeks to quantify the rate of

denitrification possible when only a source of NO_3^- is added, allowing microbes to utilise any native substrate (C, Fe, S etc) while a *denitrification potential* experiment adds a source of readily available electrons, usually as glucose, sometimes other C substrates (starch, cellulose, DOC etc) (Jahangir et al., 2012). This latter type of experiment shows the maximum potential of the microbial population when substrates are non-limiting. Several authors have used this method successfully to either demonstrate that the system is substrate limited (Yeomans et al., 1992), or microbe limited (Barkle et al., 2007) or a combination of both (Clague et al., 2013). The advantages over the acetylene inhibition method are that the incubation period can be extended, as acetylene-based experiments should be short-term since the acetylene can over time be used as a source of C, and both N_2 and N_2O can be measured off the same gas sample. The main disadvantage of the conventional capacity or potential experiment is the duration of incubation, with most incubation periods not exceeding 5 days. This has led to criticism and potential under-estimation of the reported denitrification rates (Eschenbach and Well, 2013) since the reaction may occur slowly, especially when reduced inorganic species are the main electron donors. These authors suggest a long incubation period of up to 365 days (at a low temperature of 10°C) in order to more accurately estimate a process which may take place over decades *in situ*.

2.3.3 Push-Pull Tests

This technique was first used by Trudell et al. (1986) to document denitrification in aquifers. During such a test, water amended with a conservative tracer, e.g. bromide (Br^-) and a measureable concentration of NO_3^- is injected into the groundwater, left for a period of time, and then the water is withdrawn from the same point, with aliquots of extracted water sampled over time for analytes and water chemistry. A decrease in the levels of NO_3^- (after correction using the conservative tracer) is attributed to denitrification (Istok et al., 1997; Trudell et al., 1986). This procedure uses *in situ* conditions to measure denitrification and it requires relatively slow-moving groundwater flow and relatively high microbial response rates in order to measure a reduction in NO_3^- before the plume has left the well vicinity. Such a test may not be representative of the entire aquifer as variable hydraulic conductivities, location of resident electron donors and flowpaths can affect these tests. A variety of push-pull test modifications have emerged as researchers try to measure the denitrification process more accurately.

A series of push-pull tests were conducted by Istok et al. (1997) with the objective of determining the presence and rate of several microbial-facilitated reactions that occur under anaerobic conditions (denitrification, sulphate reduction and methanogenesis) in a petroleum-contaminated aquifer in Oregon. By injecting an electron acceptor (NO_3^-, NO_2^-, sulphate (SO_4^{2-}) or carbonate(CO_3^{2-}), along with Br^- as a conservative tracer, the authors were able to plot breakthrough curves and calculate reaction

rates. Increased microbial activity in the anaerobic portion of the aquifer was attributed to the presence of petroleum serving as the electron donor (C source). Acetylene has also been used during push-pull experiments. Hill et al. (2000) used this technique in an aquifer near Waterloo in southern Ontario. Acetylene was injected in piezometers and the accumulated N_2O concentrations were then determined. This experiment also amended the aquifer with glucose or NO_3^- to determine which substrate was the limiting factor. A strong inverse relationship was detected between NO_3^-, N_2O and dissolved organic C (DOC) concentrations with the authors concluding that DOC concentrations > 8 mg L^{-1} were required for denitrification.

Occasionally, researchers have found that the denitrification capacity of an aquifer appears to increase over the course of a series of experiments and this is the effect of priming. Sometimes when microbial populations are first exposed to (often) high concentrations of NO_3^-, a lag time occurs as a sufficient population capable of utilising the NO_3^- develops. If the measurement period extends long enough, an increase in denitrification rate with time will be observed, otherwise, the next experiment conducted using that well will have a quicker response. Kellogg et al. (2005) overcame this issue by performing pre-tests 2 weeks before their push-pull tests in a Rhode Island aquifer. They also used enriched $^{15}N\text{-}NO_3^-$ and measured the isotopic composition of N_2 and N_2O in the 'pulled' water to determine denitrification rates (rather than simply looking for the disappearance of NO_3^-).

2.3.4 Tracer Experiments

In situations where the groundwater flow is too fast or microbial reactions are too slow for push-pull tests to be meaningful, tracer tests offer an alternative means of measuring denitrification either under 'natural' or amended conditions. An injection well is installed so that the tracer can be added to the groundwater, and subsequently sampled via a series of monitoring wells at various points along a flowpath. This type of experiment is ideal for monitoring the rate of progression of denitrification and often the injection well is up-gradient of a known redoxcline (transition zone from aerobic to anaerobic conditions).

A combination of field and lab measurements performed by Smith et al. (1996) compared small-scale natural gradient tracer tests in an aquifer in Massachusetts, with incubated sediment core analyses. Treated sewage had contaminated the aquifer with NO_3^- concentrations of up to 50 mg L^{-1} and previous work had observed denitrification in the plume (Smith et al., 1991a). The tracer test involved removing groundwater, amending it with Br^-, acetylene and N_2 (to make the water anoxic) and then reinjecting it. This plume was monitored and sampled using observation wells located 10 m down gradient from the injection well. Calculations of the rate of denitrification were made based on the reduction in NO_3^- levels and the increase in N_2O concentrations. In contrast to previous studies, the reduction rates calculated for the lab-based incubation part of the study were much higher than

the tracer test estimate. This was attributed to spatial heterogeneity of the aquifer material being pronounced in the small cores used in the laboratory study whereas the *in situ* measurements provided an averaged activity level.

2.3.5 Observational Studies

Observational studies include those which involve mass balance calculations and the monitoring of various geochemical parameters. Denitrification rates are determined by measuring the various N components and assuming the difference in concentrations between inputs and outputs is due to denitrification. This approach is difficult to use successfully in large-scale aquifers and catchment studies because of the long time scales often involved with respect to water movement from recharge to discharge areas (Groffman et al., 2006). A further complicating factor in observational studies is temporal variability. The measured NO_3^- (or other N component) in the discharge may be quite different to that in recharge waters because it is not related temporally, i.e. that particular water was recharged many years prior, under a different landscape use or management regime and may or may not have undergone attenuation along the way. Age dating of the water samples and a thorough knowledge of the historic land uses helps to mitigate these issues. In aquifers where the denitrification process has gone to completion, or near to, low NO_3^- concentrations left in groundwater make interpretation difficult. Researchers can instead, measure the product of denitrification: N_2. Excess N_2 is the term used for N_2 formed during denitrification and must be calculated from the total N_2 concentration measured in the groundwater. Some assumptions must be made about the recharge temperature to calculate the proportion of N_2 that is due to the equilibrium with the atmosphere at recharge, and also any excess air which results from air bubbles becoming dissolved during infiltration (Heaton et al., 1983; Vogel et al., 1981).

Possibly the earliest study which sought to document denitrification in aquifers is that of Gillham and Cherry (1978). The authors observed a redoxcline, a thin transition zone between aerobic, high NO_3^- bearing water and anaerobic, low NO_3^- bearing water, using limited geochemical analyses of dissolved oxygen, redox potential, NO_3^- and methane (CH_4). Non-point source contamination of the study area was attributed to intensive agricultural activities (tobacco, corn, small grains) and livestock, resulting in NO_3^- concentrations of up to 50 mg N L^{-1} in upper levels of the groundwater, and declining to 0.3 mg N L^{-1} with depth. The electron donors responsible for facilitating denitrification were not investigated but Gillham and Cherry stated that DOC (transported from the soil zone to groundwater) or material in the aquifer matrix may have performed this role.

Pedersen et al. (1991) sampled groundwater, porewater and sediment cores as they sought to identify the potential electron donors that were part of the aquifer matrix as opposed to being solely in solution with the NO_3^-. The study site was a Danish aquifer contaminated with agricultural sources

of NO_3^- and the authors used a method called total reduction capacity (TRC) to evaluate the potential of the sediments to contribute to denitrification. This analysis did not provide detail about which specific element was the main donor, rather all potential donors were assayed together. However, geochemical analysis of the water samples allowed deduction of the main donor. The authors were justified in looking for donors in the sediment as DOC concentrations in the groundwater were too low to contribute significantly and TRC concentrations showed a significant correlation with the redoxcline. As SO_4^{2-} concentrations declined with depth, S was discounted as an electron donor and solid organic C was assumed to provide the electrons necessary for denitrification. In contrast, a study by Tesoriero and Puckett (2011) showed that increasing concentrations of SO_4^{2-}, combined with increasing age, and decreased O_2 and NO_3^- concentrations supported the hypothesis that sulphide minerals were supplying the necessary electrons for denitrification at the Sumas Aquifer in Washington.

In order to correctly interpret the fate of NO_3^-, many researchers have identified an understanding of the flowpath as being of prime importance in aquifer studies. Tesoriero et al. (2005) used water age dating methods (tritium and CFCs) to document the different layers of water in an aquifer in North Carolina. Old water (>30 y) contained little NO_3^- and no excess N_2, while recent water (<10 y) had high NO_3^- concentrations and no excess N_2. In contrast, a layer of water 10 – 30 years in age contained low NO_3^- and significant excess N_2. Age-dating and excess N_2 concentrations were also used by Puckett and Cowdery (2002) to show that although measured denitrification rates were relatively slow (0.07 - 0.7 mg L^{-1} y^{-1}), a residence time of up to 50 years meant that significant denitrification could take place, protecting the water quality of the nearby Otter Tail River.

Others have found that the land use practices have a significant effect on the attenuation potential. High concentrations of DOC (up to 82 mg C L^{-1}) were found in groundwater under intensive agricultural production, including areas of irrigated sugarcane, in tropical Queensland, Australia (Thayalakumaran et al., 2008). Here, the authors were interested in the denitrification capacity of the aquifer as moderate NO_3^- contamination had occurred and the surface waters in the catchment drained towards the Great Barrier Reef World Heritage Area. Management practices which were thought to contribute to the high DOC in the aquifer included burning of sugarcane and the leaching of sugar-juice during harvest. Areas with both low NO_3^- and dissolved O_2 (DO) concentrations had high Fe^{2+} and Mn^{2+} (up to 15 and 12 mg L^{-1} respectively), indicating that redox conditions were anaerobic, and that there was a significant potential for Fe to also act as an electron donor. However, the distribution of such electron donors demonstrated high spatial variability. The authors produced a map showing the denitrification potential of selected sites in the aquifer and concluded that a change in the management of the sugarcane, which would result in less DOC transported to

groundwater, may have a negative impact on the protection of the groundwater, unless N-loading was also reduced.

2.3.6 Natural Abundance Isotopic Methods

Isotopic analysis of the NO_3^- molecule offers the possibility to not just observe denitrification, but to quantify how much has occurred *in situ* under natural conditions.

2.3.6..1 Background

Isotopes are two or more forms of an element with the same number of protons but a differing number of neutrons. For example, isotopes of N have 7 protons, but can have either 7 or 8 neutrons, giving an atomic mass of 14 or 15. These are denoted as ^{14}N or ^{15}N. Likewise, O isotopes ^{16}O, ^{17}O and ^{18}O all have 8 protons, but 8, 9 or 10 neutrons (Kendall and Caldwell, 1998). Stable isotopes are generally of low atomic mass, with roughly equal numbers of protons and neutrons and do not decay to other isotopes (Kendall and Caldwell, 1998). In the atmosphere, ^{14}N accounts for 99.6337% of N atoms, while ^{15}N makes up the remaining 0.3663%. The main O isotope (^{16}O) has an abundance of 99.759% with ^{17}O and ^{18}O comprising 0.037% and 0.204% respectively.

The stable isotopic compositions are usually expressed in delta (δ) values, which are reported in units of parts per thousand (‰) relative to a standard of known composition:

$$\delta_{\text{sample}}\ (‰) = \left[\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}\right] \text{x } 1000$$

Where R is the ratio of heavy to light isotope (e.g. $^{15}N/^{14}N$)

International standards for N and O are atmospheric air (AIR) for $\delta^{15}N$ and Vienna Standard Mean Ocean Water (VSMOW) for $\delta^{18}O$ (Xue et al., 2009), respectively.

Stable isotope fractionation occurs because of differences in the rates of reactions as a consequence of atomic mass. There are two types of reaction that occur in chemical, physical and biological processes: kinetic and equilibrium reactions. When the reaction or phase change is reversible and products and reactants remain in contact because of a closed, well-mixed system, equilibrium reactions occur. In this reaction, the forward and backward reaction rates are identical and fractionation occurs because the heavier isotope accumulates in the compound with the higher oxidation state. Thus, the products of the reaction can be either enriched or depleted in the heavier isotope, relative to the original substrate.

In contrast, kinetic reactions are unidirectional and are mainly dependent on the bond energies of the isotopes involved. This results in the remaining substrate or reactant becoming enriched in the heavy isotope relative to the products of the reaction. This enrichment factor can be defined as:

$$\varepsilon = 10^3(\propto - 1)$$

With α being the fractionation factor, which is the ratio of reaction constants for the light (K_L) and heavy (K_H) isotopes ($\propto = K_L/K_H$).

A biologically mediated process like denitrification is a kinetic reaction since microorganisms prefer to use the lighter isotope (^{14}N) as it is energetically more favourable. This results in a fractionation effect as the residual NO_3^- becomes enriched in ^{15}N, relative to the product e.g. N_2, until the reaction goes to completion, when the evolved N_2 will have the same isotopic signature as the original NO_3^- (Figure 2.2).

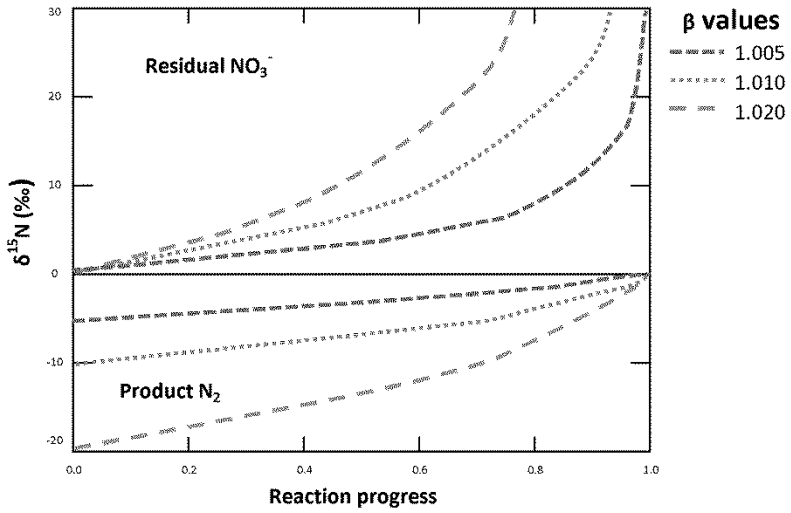

Figure 2.2: Rayleigh fractionation diagram of reaction progress vs. the $\delta^{15}N$ values of residual NO_3^- and product N_2. Fractionation factors (β) of 1.005, 1.010 and 1.020 illustrate that the higher the $\delta^{15}N$ of the NO_3^-, the lower the $\delta^{15}N$ of the N_2. (Source: Kendall, 1998)

Isotopic analysis for identification and quantification of denitrification utilises the isotopic fractionation effect by comparing the $^{14}N/^{15}N$ (or $^{16}O/^{18}O$) ratio of samples to a known standard. Denitrification results in the residual NO_3^- composition becoming enriched in a documented ratio of two N atoms to one O atom, or a slope of 0.5 when $\delta^{15}N$ v.s. $\delta^{18}O$ is plotted – note the denitrification vector shown in Figure 2.3 (Kendall, 1998).

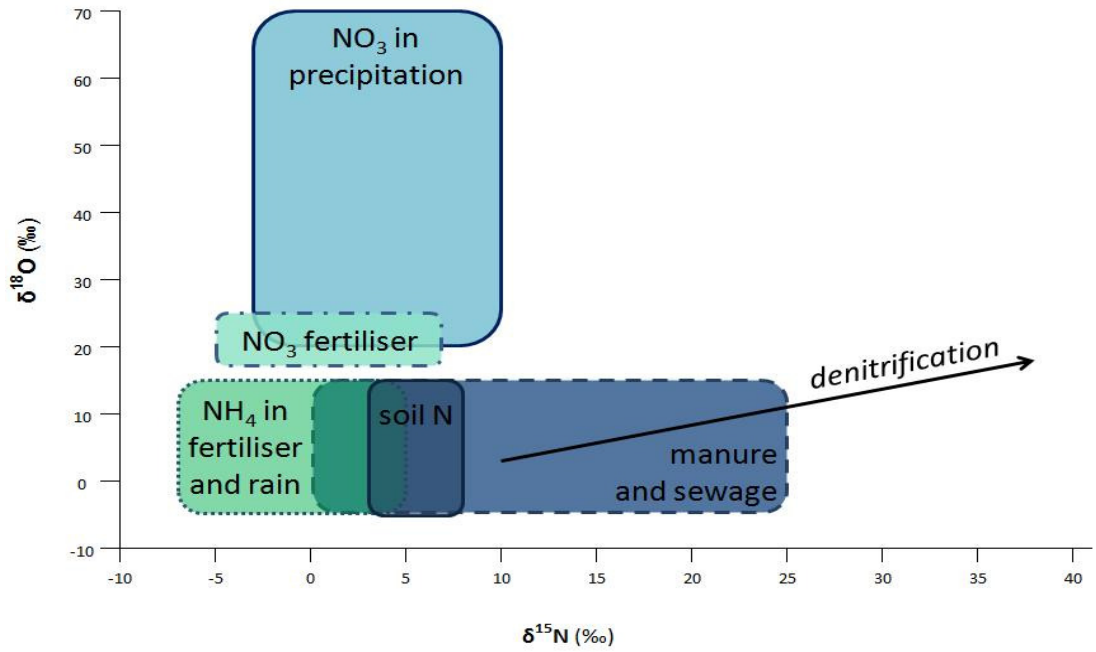

Figure 2.3: Typical isotopic composition of different sources of nitrate. The denitrification vector, with a slope of 0.5 is also shown. (Source: Kendall, 1998)

Due to various fractionation effects, different sources of NO_3^- tend to have identifiable isotopic signatures (Figure 2.3). This means it is theoretically possible to identify the source of the NO_3^- in groundwater samples based on the $\delta^{15}N$ and $\delta^{18}O$ values of the NO_3^-. Although, N transformation processes such as ammonia volatilisation and denitrification confound the original isotopic signature, making positive identification difficult, this systematic change in signature is exploited in denitrification studies.

2.3.6..2 Analytical Methods

There are two groups of methods used to measure the isotopic signature of NO_3^-: chemical and biological conversion.

Chemical Conversion

Early methods of analysis were time consuming, expensive and required a reasonably large NO_3^- concentration. More recently (Silva et al., 2000), anion exchange columns have become popular as a large volume of water can be processed through the column in the field, after which, the columns can successfully be stored for up to 2 months before analysis. This enables much smaller concentrations to be analysed, and is a less labour-intensive procedure than methods such as steam-distillation (Bremner and Keeney, 1966). However, the consumables involved can be expensive, and the required mass of N 'captured' on the resin often requires large quantities of water, which is often not possible in low-conductivity aquifers. Furthermore, to analyse both $\delta^{15}N$ and $\delta^{18}O$, separate samples are required. A recent alternative has been proposed by McIlvin and Altabet (2005). In this

procedure, various chemicals are used to convert NO_3^- to N_2O, with both $\delta^{15}N$ and $\delta^{18}O$ being analysed off the same sample. This method is much quicker and cheaper to run, and requires much less sample volume, but there are human health concerns over the use of some chemicals involved (Xue et al., 2009).

Biological Conversion

A popular alternative to the various chemical procedures is the bacterial denitrification pathway. This method uses bacteria which lack N_2O reductase so when NO_3^- in the sample is denitrified, its isotopic composition is reflected in the readily-measureable N_2O. Sigman et al. (2001) and Casciotti et al. (2002) developed this method to enable much smaller concentrations of NO_3^- to be analysed (62 µg L^{-1}), and both $\delta^{15}N$ and $\delta^{18}O$ can be measured on the same sample. The disadvantages of this method are that the bacteria need to be cultured and maintained, but the main advantage is that a much smaller volume of sample is required with typical volumes of 1 – 5 mL, compared to the 1 – 5 L required for the anion exchange or steam distillation methods.

2.3.6..3 Source Identification Studies

The focus of many early studies utilising isotopic methods has been the identification and quantification of the contributions made by different NO_3^- sources (e.g. inorganic fertiliser, manure, sewage etc.) and the implementation of strategies for mitigating the pollution (Flipse and Bonner, 1985). An example of this is the study by Kreitler and Jones (1975) who used the $\delta^{15}N$ values of NO_3^- to identify whether animal manure or soil N was the main contributor to extremely high groundwater concentrations (up to 250 mg N L^-) in Texas. Natural soil N was found to be the main source of contamination due two factors. Firstly, inadequate rainfall and over 50 years of cultivation resulted in the accumulation of NO_3^- in the soil. Secondly, a landscape modification (terracing) resulted in a rise in the water table by as much as 6 m. This allowed the stored NO_3^- to be leached into the aquifer, causing widespread contamination.

Studies which try to differentiate between soil-derived NO_3^- and atmospherically-deposited NO_3^- are generally successful if they analyse both $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ (Hales et al., 2007; Pardo et al., 2004; Spoelstra et al., 2001). Figure 2.3 shows that while the $\delta^{15}N$ signature for these forms can be similar and overlapping (-3‰ to +7‰), the $\delta^{18}O$ values are very different. Precipitation-derived $\delta^{18}O$ values are representative of air (+23.5‰) and are likely enriched due to fractionation processes associated with thunderstorms, fossil-fuel combustion and photo-chemical reactions in the atmosphere (Kendall, 1998). The much less enriched $\delta^{18}O$ values in soil-N derived NO_3^- reflect the mixture of O_2 sources incorporated into the NO_3^- ion (1/3 from soil atmosphere, 2/3 from soil water) (Andersson and Hooper, 1983; Kumar et al., 1983).

2.3.6..4 Denitrification Studies

Studies utilising isotopic methods can be either observational (i.e. measurement of groundwater parameters *in situ*, under natural conditions) or experimental (i.e. amendment of the system with a combination of tracers). Bates and Spalding (1998) used *in situ* microcosms installed 0.5 m below the water table in a similar way to a push-pull test in a well. Groundwater amended with Br^-, NO_3^- and ethanol (as an electron donor) was injected, and analysis of the changing $\delta^{15}N\text{-}NO_3^-$ composition was undertaken. Isotopic fractionation during denitrification was observed as the $\delta^{15}N$ values of the residual NO_3^- became enriched over time (from +6‰ to >+20‰, within 40 hours) and N removal rates of up to 32 mg N kg^{-1} day^{-1} were calculated. A similar experiment was conducted by Korom et al. (2012), but with deeper mesocosms (5 – 6 m below ground surface) in a glaciofluvial aquifer in the USA. A previous experiment (Korom et al., 2005) had shown evidence of very slow denitrification rates and so the sampling period was over 2 years. Nitrate concentrations declined over time and became enriched (e.g. from +0.92‰ to +15.97‰ for ISM-S2), indicating that denitrification was occurring.

More common than experiment-based denitrification studies are those which have simply documented the process by observing changes in chemical composition of groundwater along a flowpath or vertical gradient. Early work by Böttcher et al. (1990) used the concentration of NO_3^- and the $\delta^{15}N$ and $\delta^{18}O\text{-}NO_3^-$ values to document denitrification in an aquifer in Germany. This type of study relies on active denitrification along a well-defined flowpath, and for a consistent source composition. Knowledge of the time lags and approximate age of the water are also crucial for correct interpretation. The authors found that samples with high NO_3^- concentrations had isotopic compositions similar to their source values, while further along the flowpath samples with low NO_3^- levels were enriched in both $\delta^{15}N$ and $\delta^{18}O$ and in a ratio of 2:1 N:O.

An investigation by Smith et al. (1991b) observed denitrification in an aquifer in Massachusetts along a well defined effluent plume using multiple isotopes ($\delta^{15}N\text{-}NO_3^-$, $\delta^{15}N\text{-}N_2$, $\delta^{13}C$-dissolved inorganic C (DIC)) as well as routine geochemical analyses. Dissolved O_2 and NO_3^- concentrations decreased sharply with depth while excess N_2 concentrations increased with depth. The $\delta^{15}N\text{-}NO_3^-$ values became highly enriched with depth (up to +42‰) while $\delta^{15}N\text{-}N_2$ values were also heavier (maximum of +5.5‰). This is because the NO_3^- being reduced was itself enriched (manure source), therefore any N_2 produced would be heavier than the N_2 in air, which by definition is 0‰. Another line of evidence for denitrification was the DIC content and isotopic composition in the groundwater. Presuming C from the effluent was the electron donor, then CO_2 should be seen as a by-product of denitrification. The authors found this was in fact the case with concentrations of DIC increasing down the profile as well as the $\delta^{13}C$-DIC values becoming less depleted with depth.

2.4 Electron Donors Involved in Denitrification

In many studies, an important question after "is denitrification occurring?" is "what is the electron donor fuelling this reaction?" While early research (Smith and Duff, 1988; Starr and Gillham, 1993) speculated that denitrification could not occur in aquifers due to low concentrations of DOC (<5 mg L^{-1}), many studies (Green et al., 2008; Mehnert et al., 2007; Pauwels et al., 2000) have shown otherwise, due to electron donors either moving with the NO_3^- (e.g. effluent plumes) or comprising a component of the aquifer matrix. Relict organic matter (Hill et al., 2004; Kellogg et al., 2005), lignite (Jørgensen et al., 2009; Strebel et al., 1990), or reduced S compounds (Korom et al., 2005; Schwientek et al., 2008) can often provide the electrons necessary for denitrification.

2.4.1 Carbon

A common source of electrons is the buried organic matter associated with streams and rivers as a result of deposition and meandering. These riparian zones are ideal hotspots for denitrification as groundwater flow lines generally converge towards the surface and slower hydraulic conductivities enable sufficient contact time between reactants. Work by Gold and others (Addy et al., 2002; Addy et al., 1999; Gold et al., 2001; Kellogg et al., 2005) has shown that buried layers of organic-rich material up to 3 m below the water table can contribute significantly to denitrification. While no significant correlation could be found between C content (% C) and denitrification rates, a significant relationship was observed between distance from stream and denitrification rate ($p<0.01$) (Kellogg et al., 2005). This could be due to under-representation of C content in the samples taken for analysis and highlights the difficulty in understanding an aquifer with a patchy distribution of electron donors. Kellogg et al. (2005) also observed a temporal difference in denitrification rates, with some sites showing a significant difference ($p<0.05$) between spring and autumn samplings. These temporal variations are often described as 'hot moments' (McClain et al., 2003) and they reinforce the idea that careful consideration of sampling periods, frequency and interpretation of results must be made.

2.4.2 Reduced Iron and sulphur

Research concluding that non-carbon sources of electrons are the primary donors involved in denitrification reactions has become more common in the last decade. Several studies from Europe have found that pyrite (FeS_2) contributes electrons during autotrophic denitrification. In France, Pauwels et al. (2000) investigated whether denitrification was an important NO_3^- reducing mechanism in a pyrite-bearing schist aquifer under intensive agriculture. The alternative NO_3^- reducing process was that dilution with deeper, uncontaminated groundwater in the fractured rock was causing NO_3^- concentrations to decline with depth. The denitrification capability of the aquifer had been investigated previously (Pauwels et al., 1998) and isotopic analysis of NO_3^- showed $\delta^{15}N$

enrichment with depth. Sulphate concentrations increased with depth, but concentrations analysed were not sufficient to account for the amount of NO_3^- that had been denitrified (as calculated by $\delta^{15}N\text{-}NO_3^-$ concentrations and excess N_2). However, further analysis of the sediment also revealed SO_4^{2-} precipitated in minerals such as amorphous iron sulphate. While DOC concentrations in the upper part of the aquifer enabled heterotrophic denitrification to take place, the authors concluded that FeS_2 oxidation was the primary mechanism. The study was expanded to cover a number of sites (Pauwels et al., 2010) with the intention of using the isotopic composition of SO_4^{2-} ($\delta^{34}S\text{-}SO_4^{2-}$ and $\delta^{18}O\text{-}SO_4^{2-}$) to identify the source and fate of SO_4^{2-} and NO_3^- in the groundwater. Denitrification was observed in wetland areas (organic C as electron donor) and in the fissured part of the aquifers (FeS_2 as electron donor). The isotopic composition of SO_4^{2-} was beneficial to use when denitrification had proceeded to almost completion meaning insufficient NO_3^- remained for analysis. This is because the NO_3^- and SO_4^{2-} were coupled so that the amount of depletion seen in $\delta^{34}S\text{-}SO_4^{2-}$ could be related to the extent of denitrification.

Schwientek et al. (2008) also used the isotopic composition of SO_4^{2-} to provide evidence of denitrification in a German aquifer. Low concentrations of DOC (< 1 mg L^{-1}) and resident organic C meant that heterotrophic reduction of NO_3^- was unlikely, but declining concentrations of both O_2 and NO_3^- combined with enriched $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values indicated that denitrification could be occurring. Isotopic measurement of sediment-derived FeS_2 revealed isotopically light $\delta^{34}S$ values of -30.4 to -11.8‰, while other S sources such as atmospheric deposition or fertiliser had $\delta^{34}S$ values ranging from -1.6 to +7‰. Analysis of the groundwater $\delta^{34}S\text{-}SO_4^{2-}$ found at the sites where denitrification was suspected, gave depleted results of around -15‰.

2.4.3 Multiple donors

The composition of aquifer sediments is often a mixture of components. For example, pyrite (FeS_2) is often associated with lignite. Postma et al. (1991) observed a sharp redox gradient and denitrification in an aquifer in Denmark which contained both lignite and FeS_2. Based on concentrations of SO_4^{2-} and DIC in the reduced zone of the aquifer, the authors concluded that sulphur was the dominant electron donor, but that C could be contributing to as much as 15% of the NO_3^- reduction. High concentrations of Fe^{2+} found in the water samples indicated that the iron from the FeS_2 was not being used for denitrification. In contrast, Korom et al. (2012) found C to be the dominant electron donor in a glacial-fluvial aquifer in North Dakota, contributing from 43 to 92% of the denitrification observed, with pyrite contributing 4 - 18%. Also, non-pyritic Fe^{2+} contributed to 2 - 43% of denitrification, as the sediments studied had reasonable quantities of amorphous Fe^{2+} (up to 0.23%).

2.5 Denitrification Research in New Zealand

Very few studies have been carried out to investigate the occurrence of groundwater denitrification in New Zealand. Much of the New Zealand specific research has focussed on soil, vadose or riparian zones. Research performed in the soil zone (up to 40 cm depth) by Luo et al. (1996; 1998; 1999; 2000) examined denitrification potential under grazed pastures in the Manawatu using a series of laboratory experiments. Denitrification rates declined with depth, but increased with N and C additions.

The denitrification capacity of vadose zone material around Lake Taupo (up to 10 m deep) was investigated by Barkle et al. (2007). An incubation experiment was used to quantify the capacity of the microbial population to denitrify following the addition of isotopically-labelled NO_3^- (no C was added). Results showed denitrification was possible at depth under ideal laboratory conditions and the authors recommended field-based observations and experimentation to fully understand the process. The denitrification potential (i.e. with C additions) of vadose zone material in Canterbury (to 4.8 m deep) was analysed by Peterson et al. (2013). In this experiment, C extracted from the soil was added, along with NO_3^- and incubated under anaerobic conditions. Denitrification rates declined with depth, but were significantly enhanced by the addition of DOC and the authors recommended further research into the bioavailability of DOC *in situ* and the occurrence of hot moments.

Another vadose zone study in Canterbury was conducted by Dann et al. (2013) who investigated the dynamics of NO_3^- and N_2O in alluvial gravel sediments after urine application. This field study was designed to track the movement and production of N_2O through gas samplers and suction cups installed to 5 m depth found that the greatest N_2O production occurred in the soil zone, but measureable fluxes were observed close to the water table (4 – 5 m depth).

A study by Schipper et al. (1993) documented denitrification in a riparian zone (<1 m deep) in a pine forest (*Pinus radiata*) subject to irrigation with effluent. The authors took soil cores and measured denitrification using incubation with NO_3^- and acetylene and found high rates of NO_3^- removal in sediments where the organic matter content was high (C as electron donor). A similar study by Zaman et al. (2008b) showed that the addition of lime increased the gaseous N fluxes in both wetland and pasture soils (0 - 0.1 m deep) from the Waikato and that wetland soils produced more N_2O, due to conditions more suitable for denitrification (saturated, higher total C).

Work by Bruesewitz et al. (2011) showed that Rotorua lake sediments contain substantial denitrification potential, especially in catchments where increased agricultural activities have led to increased N and C loads.

Two studies have investigated denitrification in the shallow groundwater around Lake Taupo where the presence of relict organic matter at depth is presumed to supply the necessary electrons for denitrification. Both studies use *in situ* methods like push-pull tests (Hadfield and Gibbs, 2007) or re-circulating tracer well tests (Burbery et al., 2013) to induce a denitrification response in the system by adding a source of NO_3^- and a conservative tracer (Br^-). Denitrification was observed in some wells, where the bore logs had indicated relict organic matter, but aquifer heterogeneity was a major factor in the loss of the tracers as the hydrology of each site was not well understood. Another study using the push-pull technique was conducted by Zaman et al. (2008a) who investigated the denitrification capacity of wetland sediments. To avoid the problem of advection and dispersion of the push solution, each piezometer was installed within a lysimeter casing, effectively isolating the subsurface zone of interest (0 – 0.7 m depth). While NO_3^- concentrations declined rapidly, only 7% of the lost N was recovered as gaseous N, leading the authors to presume other processes like plant uptake were responsible for the majority of the NO_3^- removal.

Some work investigating NO_3^- contamination of aquifers in NZ has focussed on the potential sources of contamination and briefly identified some locations where, based on geochemical parameters, denitrification could be occurring (Kensington et al., 2004; McLarin et al., 1999). Since the main objective of these studies was to connect land use and groundwater quality, denitrification was only mentioned as a possible pathway for lower-than-expected groundwater NO_3^- concentrations.

Similarly, a field observation study by Stenger et al. (2008) found that denitrification was the main mechanism responsible for low concentrations of NO_3^- in groundwater despite being in an intensive dairying catchment. Sampling of shallow groundwater (<5 m deep) showed that reduced conditions were frequently encountered (low DO, measureable Fe^{2+} and Mn^{2+}) with concomitantly low NO_3^- concentrations. The source of the electrons was not discussed, although the authors stated that the bacteria responsible could be heterotrophic, autotrophic, or both and that regardless of the pathway, the models commonly used to estimate farm nutrient losses did not take this process into account.

2.6 Conclusions

Our understanding of the denitrification process and our ability to identify and quantify its occurrence has progressed in the past 40 years with the help of an increased awareness of environmental consequences of various land management practices. Improved technology has become available to a wider variety of researchers and allowed for more accurate analysis of NO_3^-, the products of denitrification and potential electron donors, as well as classification of microbial populations. However, research on denitrification in groundwater systems in New Zealand is sadly lacking.

With a high proportion of agriculture and a high value placed on New Zealand's '100% pure' image, there is already conflict and regulation regarding nutrient loading to land, and the potential effects on surface waters. Identifying and quantifying locations where denitrification does or does not occur in aquifers will enable strategic land management to minimise the effects of agriculture on receiving waters.

This project aims to address some part of this research gap by identifying flowpaths and reactive zones where N transformations could occur in two Waikato catchments. Field and laboratory-scale experiments will help identify the electron donors involved, and quantify actual and potential denitrification rates.

Chapter 3

Using dual isotopes of nitrate to detect denitrification in two shallow groundwater systems

3.1 Abstract

Denitrification in the shallow groundwater of agricultural catchments can provide a substantial 'ecosystem service' by reducing the ecological impact of farming activities. Therefore, assessing whether denitrification is occurring would enable strategic farming practices and protect the water quality of local streams and rivers. Evidence for denitrification is usually a combination of geochemical indicators such as declining dissolved oxygen and nitrate (NO_3^-) concentrations. The isotopic analysis of NO_3^- is another tool that can provide evidence, and also estimate the extent of the process, provided flowpaths are well understood. In this study, shallow groundwater samples (maximum 8 m below ground surface) were taken from two small agricultural catchments in the Waikato region of New Zealand. The dual isotopic composition of NO_3^- was analysed to determine where and how much denitrification was occurring. Results indicate that denitrification was occurring at some locations, but interpretation was hampered by unknown flowpaths and low concentrations of NO_3^-. Seasonal denitrification was observed at one site where the soil profile was periodically saturated with $\delta^{15}N$ values up to +28.5‰ and $\delta^{18}O$ values of +19.6‰. A wide scatter of values were measured in oxidised samples, indicating that the source of the NO_3^- was variable, making calculation of the extent of the process problematic.

3.2 Introduction

Nitrate (NO_3^-) contamination of groundwater is a global issue due to contamination of potable water supplies and eutrophication of groundwater, streams and rivers (Vitousek et al., 1997). Water quality guidelines set the maximum acceptable value (MAV) for NO_3^- in drinking water at 11.3 mg NO_3^- -N L^{-1} (WHO, 2004). However, the trigger level at which ecological change occurs in streams and rivers is much lower (0.17 mg N L^{-1}) (ANZECC, 2000). Recent statistics (1995 – 2008) for groundwater quality in New Zealand (NZ) show that almost 5% of the sites monitored exceeded the WHO drinking water standard, while 73% exceeded the ecosystem trigger level (Daughney and Randall, 2009). In NZ, year-round grazing of intensively managed pasture has resulted in elevated NO_3^- concentrations in ground and surface waters of many agricultural catchments (MfE, 2007). Haynes and Williams (1993) and Moir et al. (2011) have estimated that 23 - 25% of a paddock grazed by dairy cows is covered in urine spots annually, and it is these high volume, high N rate (ca. 600 - 1000 kg N ha^{-1}) spots which are the

main source of NO_3^- in groundwater in NZ. In contrast, the direct leaching of fertiliser-N is often negligible as application timing and rates are matched to meet plant demand.

In many catchment studies NO_3^- concentrations measured in streams are lower than would be expected based on estimated NO_3^- leaching losses from the root zone (Dymond et al., 2013). Nutrient management tools like Overseer®, which estimate root zone losses, are often used to predict the effect of landuse change on local groundwater and surface water bodies. However, this approach does not take into account specific flow paths (including their lag times) and potential N transformations which may occur below the bottom of the root zone in the vadose and saturated zones. An understanding of NO_3^- transit times through groundwater systems and accompanying N transformation processes such as denitrification are essential if stream monitoring data is to be related to NO_3^- root zone loss estimates.

Denitrification is the microbial reduction of NO_3^- through the obligatory intermediates nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) to dinitrogen (N_2). Denitrification requires oxygen (O_2) depleted conditions, which occur *in situ* when the dissolved oxygen (DO) concentration in groundwater drops below approximately 2 mg O_2 L^{-1} (Rivett et al., 2008). Electron donors fuelling denitrification may be carbon (C) compounds either residing in the aquifer matrix or dissolved C being recharged from the soil zone, in which case the process is known as heterotrophic denitrification. Alternatively, the donors may be reduced iron (Fe) and sulphur (S) compounds, enabling autotrophic denitrification. Early research focussed on dissolved organic C as the electron donor (Smith and Duff, 1988; Starr and Gillham, 1993) while subsequent studies recognised the role of resident organic material buried within aquifers (Hill et al., 2004; Kellogg et al., 2005; Mehnert et al., 2007). Others (Robertson et al., 1996; Schwientek et al., 2008; Strebel et al., 1990) have investigated the role of reduced S compounds as the main electron donor. Research at the Fuhrberger Feld in Germany has shown that both C and S occur in the aquifer matrix and that this results in a zone of heterotrophic denitrification overlying autotrophic denitrification (Weymann et al., 2010). In contrast, research by Korom et al. (2012) has shown that multiple electron donors in an aquifer may result in both auto- and heterotrophic denitrification occurring simultaneously in the same zone.

Many previous studies have inferred that denitrification is occurring, based on declining NO_3^- concentrations under reduced conditions (Gillham and Cherry, 1978; Hendry et al., 1983; Pedersen et al., 1991; Postma et al., 1991; Starr and Gillham, 1993). Groffman et al. (2006) highlights the difficulty of accurately measuring a process like denitrification since declining NO_3^- concentrations indicate denitrification is occurring and the end product, N_2, cannot be directly determined in a groundwater sample, as groundwater already contains a significant amount of N_2 of atmospheric origin.

Calculating the 'excess N_2' that can only be explained by denitrification *in situ* requires supplementary analyses (e.g. noble gases like argon and neon) and assumptions about the groundwater recharge temperature. An alternative method used to identify and quantify denitrification is to measure changes in the stable isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) and concentrations of the initial and residual NO_3^- pool over changing distance or time (Groffman et al., 2006).

Kinetic reactions like denitrification are unidirectional and fractionation occurs because the strength of chemical bonds involving ^{15}N are slightly stronger than those where ^{14}N occur and microbes will selectively discriminate and use the most energetically favourable isotope (^{14}N). Therefore, the product (N_2) is isotopically light, relative to the residual NO_3^-, which becomes progressively enriched in ^{15}N (Kendall, 1998). As seen in the Rayleigh diagram (Figure 3.1), as the reaction progresses, a gradual change in $\delta^{15}N$ occurs for both the residual NO_3^- and produced N_2. Different fractionation factors (β) shown in Figure 3.1 illustrate how the changing $\delta^{15}N$ values of N_2 affect the remaining NO_3^- composition and that as denitrification proceeds to completion and the NO_3^- is used up, the produced N_2 will have the same isotopic signature as the original NO_3^-. The degree of fractionation, and thus β, varies and depends on the microbial community and environmental conditions present. Difficulties arise, when using the method if mixing of multiple NO_3^- sources occurs (Böhlke et al., 2002).

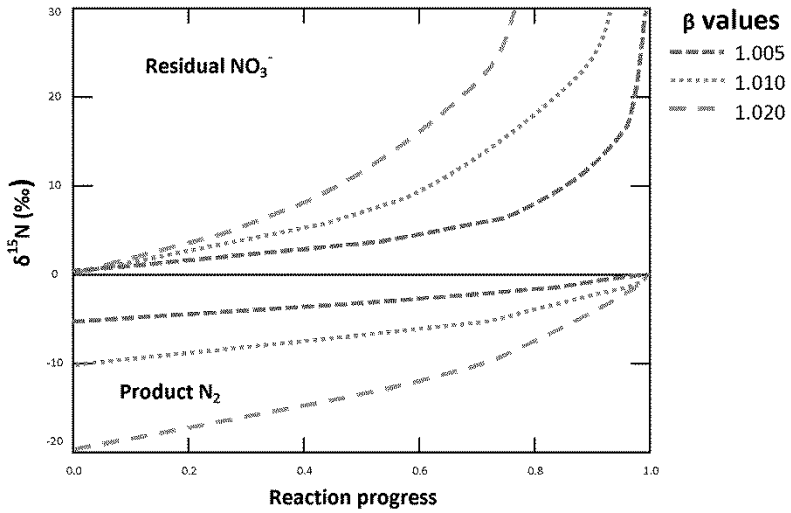

Figure 3.1: Rayleigh fractionation diagram of reaction progress vs. the $\delta^{15}N$ values of residual NO_3^- and product N_2. Fractionation factors (β) of 1.005, 1.010 and 1.020 illustrate that the higher the $\delta^{15}N$ of the NO_3^-, the lower the $\delta^{15}N$ of the N_2. (Source: Kendall, 1998)

Figure 3.2 illustrates that some sources of NO_3^- have isotopically distinct signatures and it is theoretically possible to identify the source of NO_3^- in a groundwater sample based on the NO_3^-

molecule's $\delta^{15}N$ and $\delta^{18}O$ signature. However, there is also considerable overlap in source signatures (Kendall, 1998). Many studies have used this approach to identify NO_3^- sources in aquifers or rivers (Aravena et al., 1993; Burns and Kendall, 2002; Einsiedl et al., 2005; Fukada et al., 2004; Kaown et al., 2009; Widory et al., 2004). However, transformation processes such as mineralisation, ammonia volatilisation and denitrification confound the original signatures, making definitive identification difficult (Ostrom et al., 1998). In NZ's pastoral agricultural systems, most of the NO_3^- in groundwater has undergone various N transformations in the soil-plant-animal system before being leached, potentially obscuring the original source signature. While these changes are problematic for source identification studies, the systematic change in $\delta^{15}N$ and $\delta^{18}O$ values arising from denitrification can be exploited, since the enrichment of both isotopes typically falls along the denitrification vector, where a plot of $\delta^{18}O$ vs. $\delta^{15}N$ has a slope of between 0.48 and 0.76 (Böttcher et al., 1990; Fukada et al., 2003; Mengis et al., 1999). Figure 3.2 shows an idealised slope of 0.5.

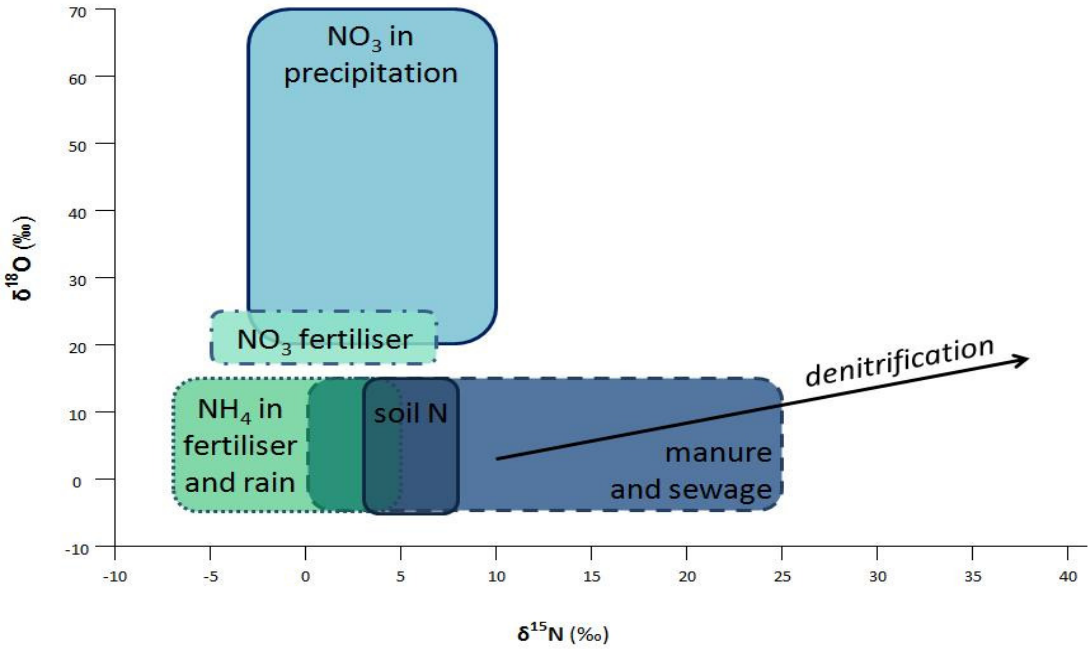

Figure 3.2: Typical isotopic composition of different sources of nitrate. The denitrification vector, with a slope of 0.5 is also shown. (Source: Kendall, 1998)

Assuming no N transformations have occurred, the NO_3^- in groundwater samples from the oxidised zone of an aquifer should have a $\delta^{15}N/\delta^{18}O$ signature similar to its source (Figure 3.2). If denitrification subsequently occurs down gradient then samples taken from the reducing zone of the aquifer should have a heavier isotopic signature ($\delta^{15}N$ and $\delta^{18}O$) and plot along the denitrification vector that starts at the isotopic signature of the NO_3^- in oxidised groundwater (Aravena and Roberston, 1998; Baily et al., 2011; Böttcher et al., 1990; Cey et al., 1999; Devito et al., 2000; Mengis et al., 1999). The resulting degree of enrichment will depend on the original NO_3^- source and what fraction of the NO_3^- pool has been denitrified (see Figure 3.1). If the NO_3^- in the aquifer is from

multiple sources or sampling flowpaths are not well defined, the $\delta^{15}N/\delta^{18}O$ plot will be difficult or impossible to interpret (Baily et al., 2011; Chen et al., 2009).

Previous work in the Toenepi catchment (Stenger et al., 2008) showed a discrepancy between estimated NO_3^- concentrations leaching from the root zone and NO_3^- concentrations measured in the stream draining this catchment. Analysis of shallow (1 – 5 m below ground surface) groundwater revealed declining concentrations of DO and NO_3^- with depth, indicating denitrification may have been occurring. Similar groundwater conditions had been documented at the Waihora well field near Lake Taupo (Stenger, 2011). The purpose of this study was to use NO_3^- isotopic analysis to identify the extent and location of the denitrification in these shallow groundwater systems.

3.3 Materials and Methods

3.3.1 Study Area – Toenepi

The Toenepi catchment is located in the eastern part of the Waikato region in the North Island of NZ (Figure 3.3) and features gently rolling topography with an elevation of 30 – 130 m above mean sea level. The annual rainfall during 2003 – 2012 was 1306 mm y^{-1} and the mean air temperature was 14.1°C. The predominant land use in the 15 km^2 catchment is intensive dairying, and has been for over 50 years. The average stocking rate in 2003 was 3.1 cow ha^{-1} and N fertilisers are widely applied (average rate 100 kg N ha^{-1} y^{-1}).

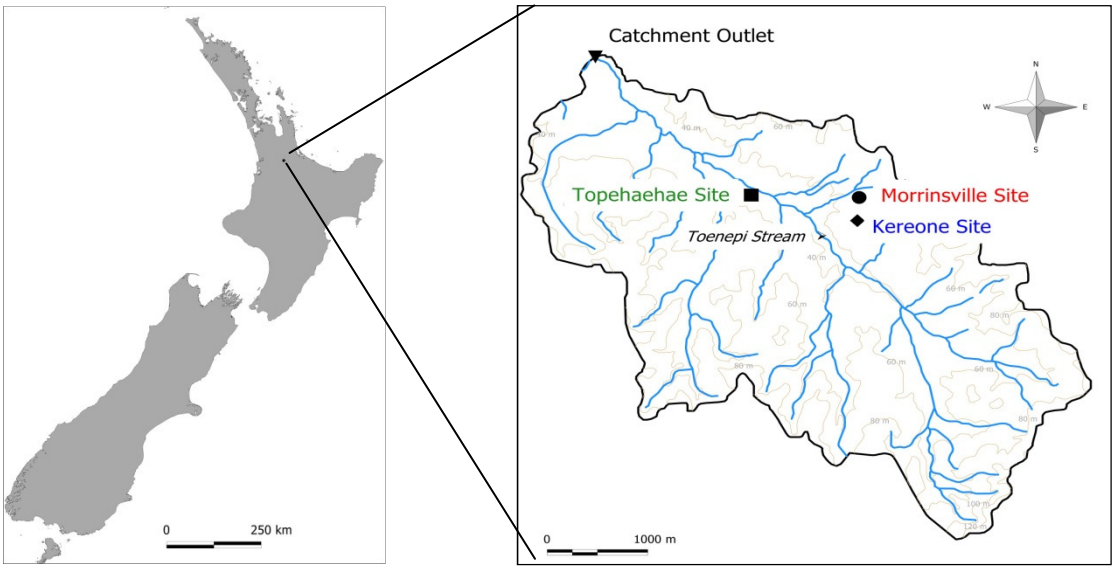

Figure 3.3: Location of the Toenepi catchment, and map showing the location of the study sites.

Three sites in the catchment were investigated and are named according to their soil type: Topehaehae, Morrinsville and Kereone.

Prior to this investigation, slug testing was carried out on wells in the catchment following the guidelines of Butler (1998). Briefly, this involved measuring the water level (to gain an initial reading) then inserting a water level sensor (Diver®, Schlumberger, USA) set to 0.5 sec recording interval. When the water table had recovered, a solid slug, designed to increase the head by 0.5 m was rapidly introduced in order to achieve as instantaneous an increase as possible. This is termed a 'slug in' test. The slug was then removed once the water level had recovered to the initial reading, resulting in a 'slug out' test. Once the water level had again returned to the initial value, the sensor was removed. Both the 'slug in' and 'slug out' data were analysed using the Bouwer and Rice (1976) method for unconfined aquifers.

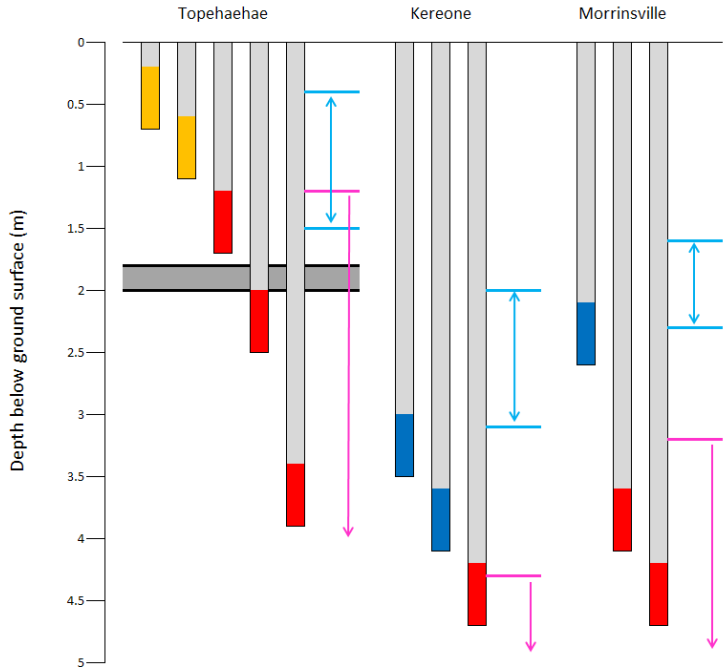

Figure 3.4: Diagram of well layout at the three sites: Topehaehae, Kereone and Morrinsville. Length of well indicates depth below ground surface, coloured zone indicates screened zone. Red wells always draw reduced water (dissolved oxygen < 2 mg L^{-1}), blue are always oxidised. Orange wells are sometimes oxidised, sometimes reduced. The blue lines indicate average water table fluctuation; the pink line indicates a positive response to the Childs Test (presence of reduced iron (Fe^{2+})). The dark grey zone at 2 m depth at the Topehaehae site indicates the aquitard layer.

3.3.1..1 Topehaehae Site

The Topehaehae soil is a Typic Recent Gley Soil (NZ soil classification) (Hewitt, 1998), located on a river floodplain and derived from recent alluvial deposits. Gley soils account for only 9% of the catchment and are restricted to locations close to the Toenepi stream. The soil is characterised by a thin A horizon (0.1 – 0.15 m) over poorly drained sandy loam and silt loam materials (Wilson, 1980).

Being located only 75 m from the stream and 36 m above mean sea level, the water table at the site reaches the surface in winter under natural conditions, but artificial drainage (mole and tile drains approximately 0.4 m and 0.7 - 1.0 m below ground surface, respectively) results in an average winter maximum water table depth of 0.4 m below ground surface. During late summer, the water table typically drops to approximately 1.5 m depth. Despite the artificial drainage, reducing conditions prevail over much of the profile, which is characterised by pale grey loams and a positive response to the Childs test (indicating the presence of reduced iron (Fe^{2+})) (Childs, 1981). An aquitard of dense silt material at 1.8 - 2 m depth forms the lower boundary of the shallow groundwater system (Figure 3.4). At this site, wells were installed to the following depths: 0.7 m, 1.1 m, 1.7 m, 2.5 m and 3.9 m (Figure 3.4). All wells in the catchment were constructed of 50 mm diameter PVC pipe, with 0.5 m long wells screens. Hydraulic conductivities (K_{sat}) derived from slug tests using the Bouwer and Rice method (Bouwer and Rice, 1976) ranged from high values of 1422 mm d^{-1} (at 1.1 m) to low values of 1 mm d^{-1} (at 2.5 m).

Due to the high water table conditions experienced during winter, suction tubes were installed at this site to capture the movement of nutrients close to the source. Aiming to reduce the variability typically observed when using conventional suction cup samplers (Weihermüller et al., 2007), substantially larger suction tube samplers with ceramic sections of 0.25 m length and 40 mm diameter were used. These were installed horizontally in 3 sides of a pit excavated to 1.2 m depth. A 0.3 m long PVC tube glued to the ceramic section ensured that the sampling zone was in the undisturbed soil profile at least 0.25 m away from the pit wall. Samplers were installed in 6 replicates at three depths: 0.4, 0.7 and 1.0 m below the ground surface.

3.3.1..2 Kereone Site

The Kereone site is located approximately 400 m from the Toenepi stream (Figure 3.3) and at an elevation of 48 m above mean sea level. The soil is classified as a Typic Orthic Allophanic Soil (NZ soil classification) (Hewitt, 1998) and is a deep, well drained silt loam soil overlying a fine-textured sandy clay loam. Allophanic soils cover 47% of the catchment. The soil has developed from Holocene to late Pleistocene deposits of rhyolitic and andesitic volcanic ash (Wilson, 1980). A positive response to the Childs test only occurs in the lower part of the profile (approximately 4.3 m depth) (Figure 3.4) after an abrupt transition to a dark brown organic matter layer. Average water table depth in early autumn is 3.1 m below the ground surface while the late winter/spring maximum occurs at 2 m depth. At this site, three wells were installed at 3.5, 4.1 and 4.7 m below the ground surface (Figure 3.4). Slug tests in these wells revealed hydraulic conductivities ranging from very low (0.2 mm d^{-1} at 3.5 m) to medium values (178 mm d^{-1} at 4.7 m).

3.3.1..3 Morrinsville Site

The Typic Orthic Granular Soil (NZ soil classification (Hewitt, 1998)) found at the Morrinsville site is characterised by a well drained clay loam soil overlying material that is less well drained than the Kereone site. The Morrinsville site is located approximately 250 m from the Kereone site and 600 m from the stream (Figure 3.3), at an elevation of 45 m above mean sea level. Granular soils cover 40% of the catchment. These materials are strongly argillised Pleistocene rhyolitic and andesitic volcanic ash layers which have formed pale coloured clay, often mottled with bright orange iron concretions reflecting changes in redox conditions around the water table (Wilson, 1980). A sharp redox boundary occurs between 3 and 3.5 m below the ground surface, depending on the water level. Figure 3.4 shows the positive response to the Childs test in core samples taken in September 2006, beginning at a depth of 3.2 m. The average minimum water table occurs in early autumn (2.3 m depth) while maximum spring water table can reach 1.6 m below ground surface. Wells at the Morrinsville site extended 2.6, 4.1 and 4.7 m below the ground surface (Figure 3.4). Slug tests in these wells revealed hydraulic conductivities ranging from low (5 mm d^{-1} in 4.7 m) to high values (1140 mm d^{-1} in 2.6 m).

3.3.2 Study Area – Waihora

The Waihora field site is a 6,000 m^2 site located in the headwater of the Tutaeuaua catchment at the north-western edge of Lake Taupo (Figure 3.5). The gently rolling land slopes down to a wetland area which feeds into the Tutaeuaua stream. The site has an elevation of 525 – 536 m above mean sea level, an annual air temperature of 11.2 ± 0.3°C and average rainfall of 1470 ± 232 mm (between 2006 – 2012). Prior to 2009, sheep grazing occurred at the site, and since then low-intensity calf and heifer grazing has occurred. Fertiliser inputs remain low with no N fertiliser applied.

The proximity to Lake Taupo (a super volcano which most recently erupted in 186 AD) has resulted in a multi-layered geological profile. The modern soil (Podzolic Orthic Pumice Soil) generally has a thin A horizon (0.1 – 0.15 m) and free-draining, sand-textured material to approximately 0.7 m depth, overlying coarse-sand textured unwelded Taupo Ignimbrite (TI) (Figure 3.6). This material was deposited during the Taupo eruption 1.8 ka BP and can vary in thickness between 1 – 3 m at the site. The soil horizon present at the time of the eruption now forms a Palaeosol (P) (buried soil) which has a heterogeneous distribution and thickness over the site (0 – 2 m thickness). Woody debris remaining from the vegetation at the time of the eruption has occasionally been found in the base of the TI, in locations where the groundwater table fluctuates above these layers, presumably reflecting the lower mineralisation rates possible under saturated conditions. Coarse-sand textured Oruanui Ignimbrite (OI) occurs under the P layer and was deposited during the Oruanui eruption approximately 26.5 ka BP (Figure 3.6).

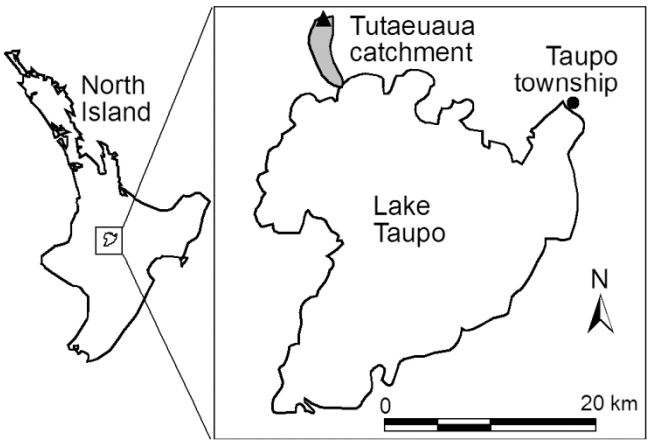

Figure 3.5: Location of Lake Taupo in the North Island. The Waihora well field is indicated by the triangle in the top of the Tutaeuaua catchment.

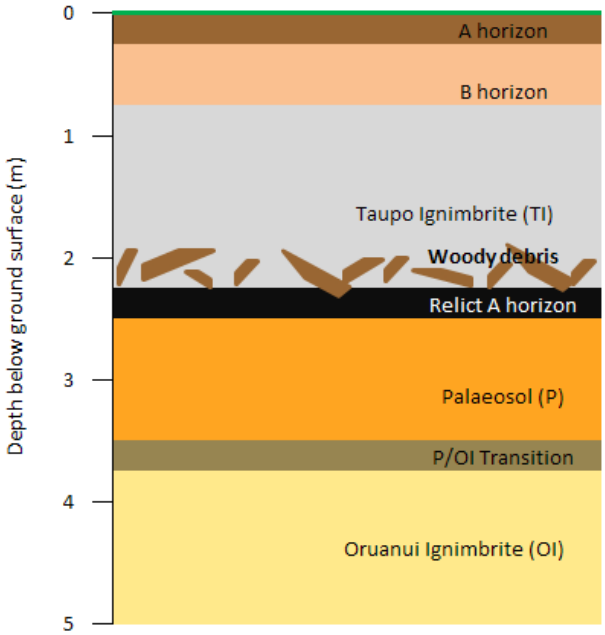

Figure 3.6: Schematic of the stratigraphic profile at the Waihora well field site.

At the Waihora site, 26 wells were installed between May 2007 and April 2008, and were constructed from the same 50 mm diameter PVC pipe as the Toenepi wells, with screens 0.5 m long. Multiple wells to different depths were often installed at the same location in the well field, for example, the WR20 well cluster has 5 wells, ranging between 1.4 and 5 m below ground surface. A transect between WR21 and WR20 (Figure 3.7) was established to characterise a cross section down the slope to the wetland. Slug tests (Bouwer and Rice, 1976) were performed on all wells and revealed a wide range of hydraulic conductivities. The K_{sat} values for the TI layer ranged from 0.15 – 8.83 m d^{-1}, the P layer had values between 0.103 and 4.5 m d^{-1} while the OI layer had the lowest conductivity values ranging from 0.029 to 1.55 m d^{-1}.

The depth to the water table and its seasonal fluctuation vary between the well clusters, reflecting their location on the slope and distance to the wetland at the bottom of the slope. At the upper edge of the site (e.g. WR21 (Figure 3.7)), the early autumn minimum can fall to 6.5 m below the ground surface, while the depth in spring can rise to 3 m below ground surface. In contrast, the water table near the wetland (e.g. WR20 (Figure 3.7)) only fluctuates between 0.2 and 1.2 m below the ground surface. Oxidised conditions prevail over much of the site with 15 wells always drawing oxidised water; however, patchy distribution of relict organic matter (in the palaeosol and woody debris layers) can cause hot spots of reducing conditions. Hot moments also occur with water table fluctuations as one well in the WR19 well cluster sometimes has reduced groundwater and at other times, draws oxidised groundwater.

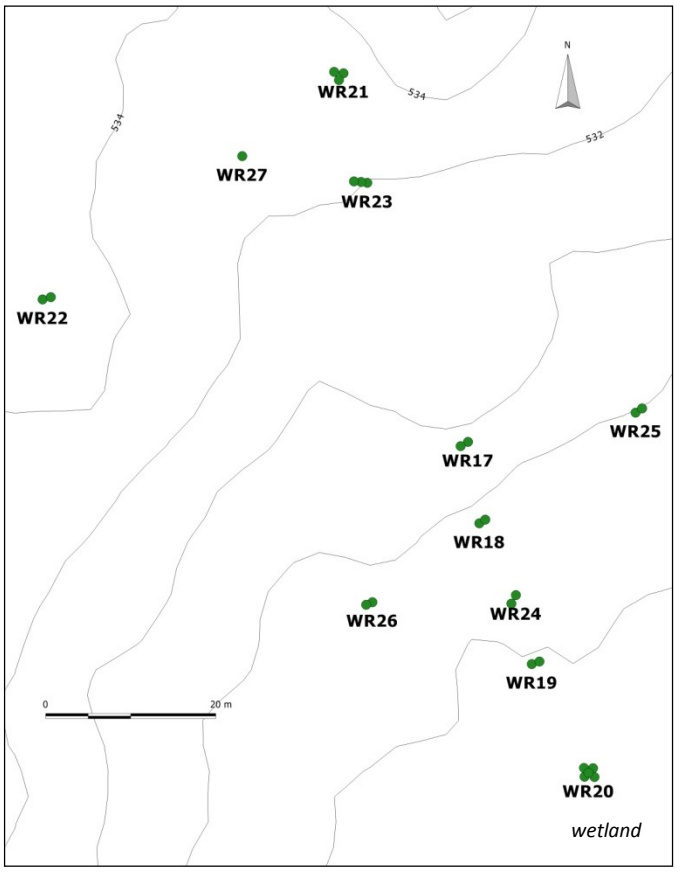

Figure 3.7: Well cluster layout at the Waihora well field near Lake Taupo.

3.3.3 Sampling and Analytical Methods

3.3.3..1 Suction Tubes

Suction tubes were prepared for sampling by first removing any residual water, then a 50 kPa tension was applied by removing a set volume of air (350 mL) with a 60 mL plastic syringe (Terumo®, Japan). Samples were collected the following day using a 60 mL plastic syringe. A 50 mL sample was taken for determination of NO_3^--N concentrations using the standard methods (APHA 4500-NO_3 I) of

automated cadmium reduction and Azo dye colourimetry and a flow injection analyser (Lachat Instruments, WI, USA) (APHA, 2005).

Another 50 mL sample was taken for NO_3^- isotopic analysis, with $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values determined using the bacterial methods of Sigman et al. (2001) and Casciotti et al. (2002) at UC Davis, California, USA. All samples (n = 123) were stored frozen, shipped cool and filtered to 0.45 µm before analysis. Although the methodology of Sigman et al. (2001) states that samples with NO_3^- concentrations as low as 0.014 mg N L^{-1} could be analysed, initial analysis indicated that a minimum of 0.03 mg N L^{-1} yielded more reliable results. As a consequence, subsequent samples with NO_3^- concentrations <0.03 mg N L^{-1} were not submitted for analysis.

Suction tubes were sampled 2 – 5 times per year from June 2008 to Sep 2010 during high water table periods so that all samples (n = 130) were taken during saturated conditions.

3.3.3..2 Well Sampling

Standard procedure was to remove a water volume equivalent to 3 times the standing water column (Daughney et al., 2006) and this was done on the same day as sampling at the Waihora site. However, some wells in the Toenepi catchment recharged so slowly that wells were purged the day before sampling, and removal of more than one standing water column was unfeasible. In this instance, particular attention was given to the stability of the online readings before samples were taken on the following day. Low flow sampling methods (Daughney et al., 2006) were used to minimise water level changes, using a variable speed pump and flow controller, limiting flow rates to 0.1 – 0.15 L min^{-1}. A flow cell was used with probes to measure DO, pH and electrical conductivity (EC), connected to a data logger (TPS 90-FLMV Field Lab) and logged every 30 sec. Samples were taken after readings had stabilised to the following criteria: DO ± 0.3 mg L^{-1}, pH ± 0.1 pH units, EC ± 3% of reading.

The Toenepi wells were sampled to up to 3 times per year (during winter and spring) between 2008 and 2011, resulting in a total number of samples of 64. The Waihora wells were sampled twice in 2008 (September and November), in March 2009 and November 2011 (n = 72).

3.3.3..3 Well Sample Analysis

Well water samples (250 mL) were taken and analysed for NO_3^--N, NO_2^--N, ammoniacal N (NH_4^+-N) and dissolved reactive phosphorus (DRP) according to standard methods (APHA, 2005). Then 50 mL samples were taken for isotopic analysis of NO_3^-. Most samples (n = 100) were again analysed using the bacterial methods of Sigman et al. (2001) and Casciotti et al. (2002) at the Stable Isotope Facility at UC Davis, California, USA. However, some samples (n = 26) were analysed by the Stable Isotope

Centre at GNS Lower Hutt, NZ using the chemical method of McIlvin and Altabet (2005). Samples for isotopic analysis were stored frozen, shipped cool and filtered to 0.45 µm before analysis.

At selected sampling dates, the Toenepi wells were also sampled for silica (SiO_2) analysis. A 50 mL sample was field-filtered using 0.45 µm syringe tip filters and analysed using ICP-OES according to standard methods (APHA 3120-B) (APHA, 2005).

3.3.3..4 Age dating

The concentration of SiO_2 was used as a proxy for tritium age dating, since a good relationship was observed between these two variables in samples taken in 2011 (Figure 3.8). The longer the groundwater is in contact with the aquifer, the higher the SiO_2 concentration and the lower the TU value. Due to the different geology and presence of the aquitard at 2 m depth, the Topehaehae (To) SiO_2 data cannot be grouped with the Kereone (Ke) and Morrinsville (Mv) results and forms its own dataset. The three To data points located close to 0 TU are from the To-390 well, below the aquitard, and are older (estimated at > 200 years). Other To data points, from above the aquitard, are younger (<3 years), despite relatively similar SiO_2 concentrations (80 – 110 mg L^{-1}). All the Ke data is classified as young (being recharged within the last 5 years) while only the uppermost layer of groundwater at the Mv site (2.6 m below ground surface) is classified as young. The groundwater from the 4.1 m depth is relatively old, with an estimated age of 50 years, while water at 4.7 m depth is older, at around 150 years.

Taking into account the relationships observed in Figure 3.8, samples were classified as being young or old. The Ke and Mv samples with SiO_2 concentrations > 70 mg L^{-1} were classed as 'old', while To data was handled differently; all samples from the To-390 well were classified as 'old' and all those taken from above the 2 m aquitard were denoted as 'young'.

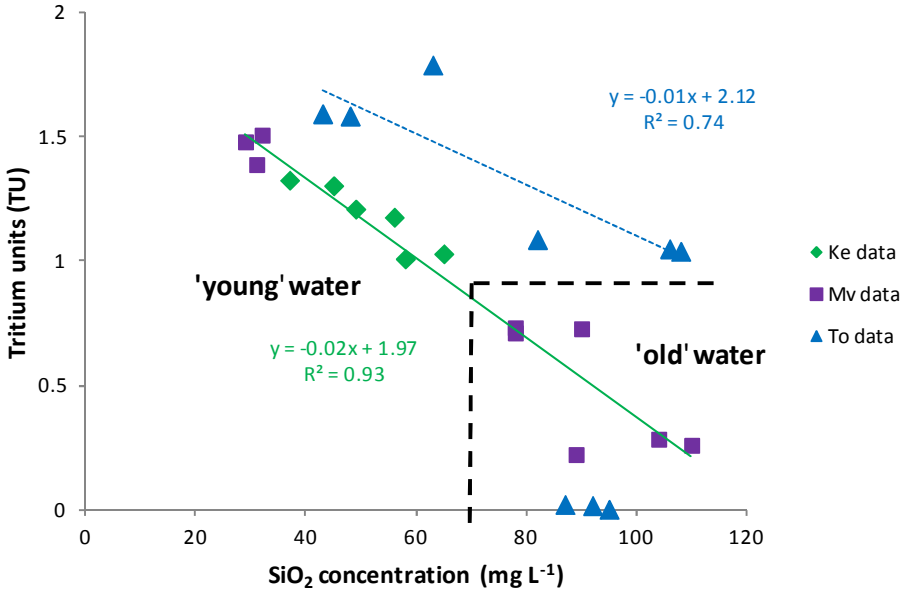

Figure 3.8: Linear relationships between SiO_2 concentration (mg L^{-1}) and tritium units (TU) for Toenepi groundwater samples. The solid green trendline is for the Kereone (Ke) and Morrinsville (Mv) data only, the dotted blue line is for the Topehaehae (To) results from wells shallower than 2 m depth. The three To data points close to zero TU are not included in either dataset. The groundwater was classified as either 'young' or 'old' based on this data.

3.3.3..5 Statistical Analysis

All statistical analyses were conducted using Microsoft Excel 2007. Means, standard deviation and standard error were calculated for different parameters, and where appropriate, significant differences between means were determined using Student's t-test.

3.4 Results and Discussion

3.4.1 Temporal Variation

3.4.1..1 Kereone Site

The very low hydraulic conductivities in the material surrounding the Ke-350 well, at 3.5 m depth resulted in only one sample being taken for this well. The DO and NO_3^- concentrations for the other two wells were similar over time; the average NO_3^- concentration ± standard deviation for the Ke-410 well was 8.35 ± 0.44 mg N L^{-1} while the Ke-470 well had 0.53 ± 1.06 mg N L^{-1}. The isotopic composition of the young, oxidised groundwater at this site (Ke-350 and Ke-410 wells) was also fairly similar over the sampling period (Figure 3.9), with an average $\delta^{15}N\text{-}NO_3^-$ value of +6.9‰ and $\delta^{18}O\text{-}NO_3^-$ value of +6.8‰. This is well within the range of values expected for NO_3^- derived from soil N (Figure 3.2).

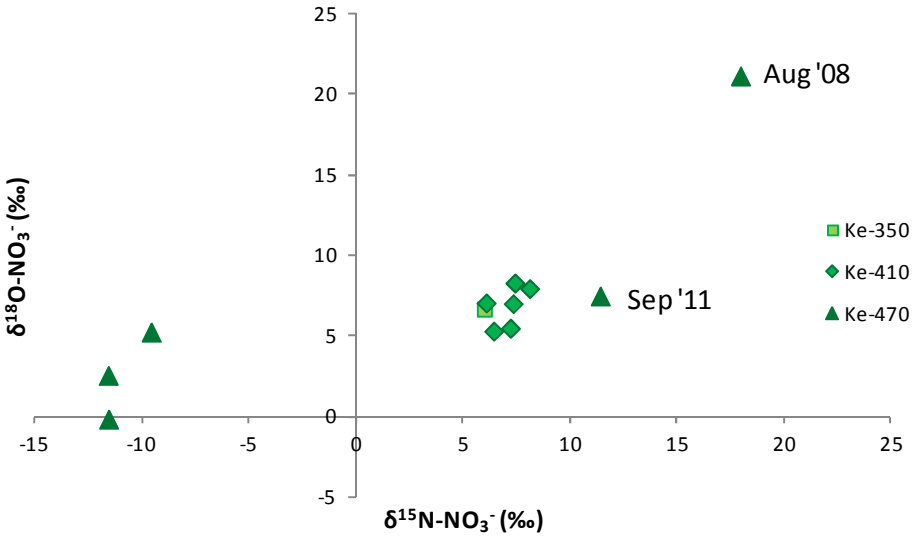

Figure 3.9: Isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^- collected from the Kereone wells. Values are in per mil (‰) relative to atmospheric air ($\delta^{15}N$) or Vienna standard mean ocean water ($\delta^{18}O$).

The wide range of isotopic compositions measured in samples from the Ke-470 well was surprising, given the relatively stable NO_3^- and DO concentrations. One sample, from August 2008, shows enrichment indicating denitrification has occurred, with a $\delta^{15}N$ enrichment factor (ε) of -3.1‰. This small $\delta^{15}N$ ε factor is close to the range found by Mariotti et al. (1988) (-4.7 to -5.0‰) and Sebilo et al. (2003) (-1.5 to -3.6‰) but quite different to those found by others (-15.9 to -30‰) (Böttcher et al., 1990; Mengis et al., 1999; Vogel et al., 1981). Mariotti et al. (1988) proposed two explanations for such small enrichment factors, that denitrification rates were rapid or that the small pores of an aquifer provided a sink for NO_3^-. As denitrification proceeds to completion in small pores, a concentration gradient occurs, and results in more NO_3^- diffusing from the fast flowing system into the small pores, resulting in a smaller ε factor.

The sample from September 2011 had the highest NO_3^- concentration of the Ke-470 samples (2.7 mg N L^{-1}), perhaps indicating that denitrification had not occurred, or not to the same extent as at previous sampling dates. The cause of the three isotopically light Ke-470 samples is unknown. While $\delta^{15}N$ values of -10 to -15‰ in precipitation have been found in atmospheric studies e.g. Zhang et al. (2008), and theoretically, nitrification should form isotopically light NO_3^- (Kendall, 1998), no likely mechanism could be found that could explain the sources of the NO_3^- measured in groundwater 4.7 m below the ground surface. The concentration of all three Ke-470 samples were <0.09 mg N L^{-1}, so perhaps measurement uncertainty was a factor. It is also possible that these samples were much older than what was expected for this site, as SiO_2 concentrations of 79 and 96 mg L^{-1} were also measured on two of these samples, classifying them as 'old' (Figure 3.8). However, this type of negative $\delta^{15}N$ value was not seen in other 'old' samples.

3.4.1..2 Morrinsville Site

The Morrinsville site showed declining NO_3^- and DO concentrations with depth, with only the upper well at 2.6 m below the ground surface being oxidised (Table 3.1). This young water had the highest NO_3^- concentrations (up to 11 mg N L^{-1}) in the catchment and a range of isotopic values between 4.87 and 9.13‰ for $\delta^{15}N$ and 4.6 to 8.33‰ for $\delta^{18}O$, within the range for soil and manure sources (Figure 3.2). As the next deepest well, at 4.1 m depth always yielded reduced water, more enriched samples from this well were expected, similar to the Ke-470 well sample (with $\delta^{15}N$ of 17.95‰ and $\delta^{18}O$ of 21.17‰). However, the average $\delta^{15}N$ and $\delta^{18}O$ values were 10.05 and 8.82‰, respectively (Table 3.1).

Table 3.1:Characteristics of groundwater samples taken from the Morrinsville site at Toenepi from six sampling events between 2008 and 2011.

Average ± std dev	Mv-260	Mv-410	Mv-470
NO_3^- (mg N L^{-1})	9.87 ± 0.90	1.25 ± 0.49	0.06 ± 0.04
DO (mg L^{-1})	6.93 ± 0.98	1.09 ± 0.29	1.53 ± 0.35
$\delta^{15}N$-NO_3^- (‰)	7.26 ± 1.78	10.05 ± 2.50	5.74 ± 4.39
$\delta^{18}O$-NO_3^- (‰)	6.51 ± 1.56	8.82 ± 2.03	11.56 ± 7.01

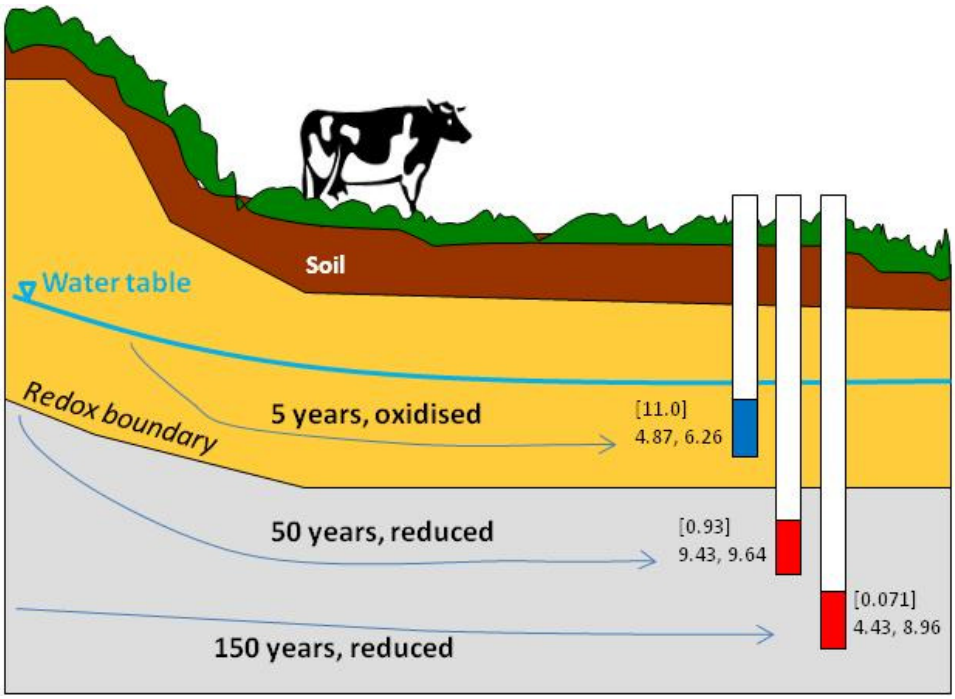

Figure 3.10: Flowpath schematic for the Morrinsville site in the Toenepi catchment. The three wells, at 2.6, 4.1 and 4.7 m below ground surface are indicated by their redox status; blue for oxidised, red for reduced. The approximate age of groundwater sampled at the site is also given, as are the NO_3^- concentrations (in square brackets) and isotopic signature ($\delta^{15}N$, $\delta^{18}O$) for the August 2008 sampling.

This slight enrichment between the 2.6 and 4.1 m wells results in a $\delta^{15}N$ ε value of -1.3‰. This pattern of young, oxidised groundwater overlying older, reduced water has been documented elsewhere (Böhlke et al., 2007; Puckett and Cowdery, 2002). However, both of these studies concluded that a significant vertical component of groundwater movement was occurring, meaning that the oxidised water was the source of the NO_3^-, and that denitrification was occurring within the vertical profile between the sampling depths of the oxidised and reduced groundwater. It is unlikely that this is the case for the Morrinsville site, given the great age difference between the oxidised and reduced waters and that the lateral flowlines involved are unknown. Figure 3.10 shows a schematic of possible flowlines for the different ages of water at this site.

The deepest well, at 4.7 m depth showed the widest spread of isotopic values (Figure 3.11), despite relatively similar DO and NO_3^- concentrations being measured over time (Table 3.1).

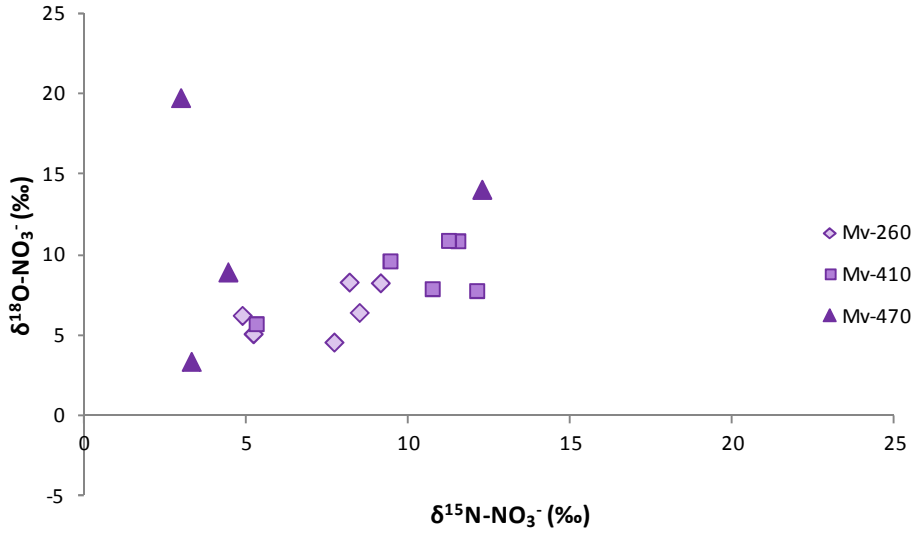

Figure 3.11: Isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^- collected from the Morrinsville wells. Values are in per mil (‰) relative to atmospheric air ($\delta^{15}N$) or Vienna standard mean ocean water ($\delta^{18}O$).

Some of this isotopic variability may be from measurement uncertainty as many of these samples were close to analytical limitations (0.03 mg N L^{-1}). Despite refinement of the analytical methods used to determine the isotopic composition of NO_3^- over the past 40 years, significant limitations still exist. Compared to many northern hemisphere studies (Vitòria et al., 2008; Wassenaar, 1995), NO_3^- concentrations in groundwater in NZ are low, but still present a major ecological problem for surface waters at discharge points (Daughney and Randall, 2009). For the purpose of identifying and quantifying denitrification under relatively low N landuse applications, the analysis of samples with low NO_3^- concentrations is of paramount importance. Studies from overseas have revealed evidence for denitrification at NO_3^- concentrations as high as 5 mg N L^{-1} (Aravena and Roberston, 1998) while others typically find enrichment in samples with concentrations of 0.5 mg N L^{-1} (Baily et al., 2011; Böttcher et al., 1990). However, with much of the data set (Figure 3.12), NO_3^- concentrations (both initial and final) were much lower than these previously reported studies and thus restricting isotope analysis to concentrations >0.03 mg N L^{-1} severely limits the applicability of the isotopic method. The use of anion exchange resins such as those used by Chang et al. (1999) and Silva et al. (2000) could potentially overcome this limitation if enough sample could be collected. However, in many cases, 40 – 50 L of sample would be required to give sufficient mass of N on the column, which would be impractical for many of the wells. The anion exchange method is also a more difficult procedure than either the bacterial or chemical conversion methods, and requires large quantities of expensive silver nitrate, resulting in a high analysis cost.

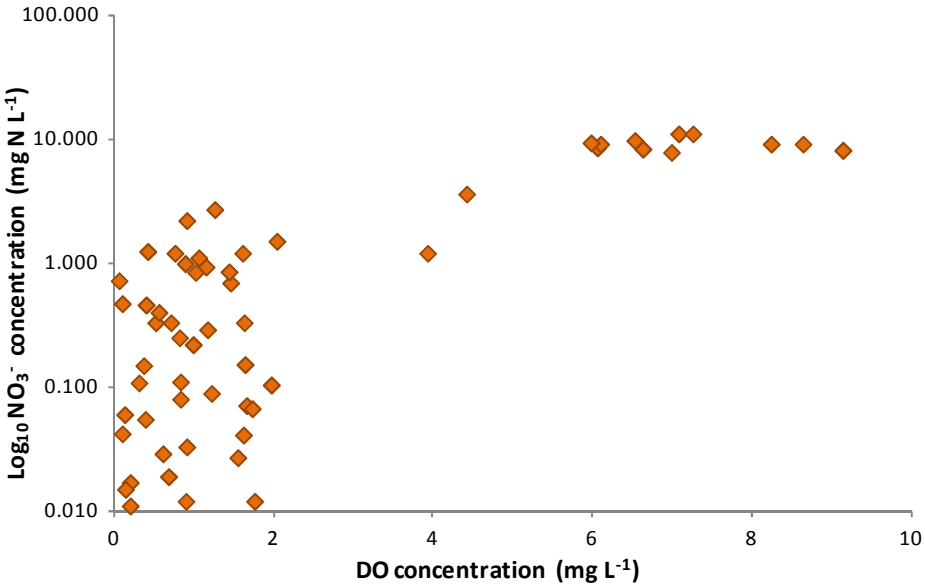

Figure 3.12: Range of NO_3^- concentrations (Log_{10} scale) (mg N L^{-1}) and DO concentrations (mg L^{-1}) found in the Toenepi well samples, n = 62.

3.4.1..3 Topehaehae Site

Soil profile NO_3^- concentrations measured using the suction tube samplers varied widely. During the 10 sampling events, NO_3^- -N concentrations ranged from below the detection limit (<0.005 mg L^{-1}) to 131 mg L^{-1}. The data presented in Figure 3.13 is a collation of data over 3 years (2008 – 2010). The Jun/Jul data represent 4 sampling dates in early winter while the Aug/Sep data combines 6 sampling events in late winter - early spring. This temporal bulking was done to simplify data presentation as sampling took place at different times each year. Two very high concentrations were measured in the same sampler at 0.4 m depth on consecutive sampling dates (12 days apart in 2008) and are denoted by the ** symbols in Figure 3.13. It is assumed these concentrations resulted from a remanent urine spot near the sampler as no fertiliser was applied at this time and grazing had occurred 2 months prior. Nitrification, the conversion of ammonium N to NO_3^- can take up to 60 days for full conversion during winter, at temperatures of 7.5 – 10°C (Holland and During, 1977). In general, higher soil solution NO_3^- concentrations were measured at all depths, in early winter (June and July) presumably due to NO_3^- building up in the soil over summer (during unsaturated conditions) and being flushed out as soil drainage occurred, while lower concentrations were measured later in the season (August and September samplings), presumably when the stock of NO_3^- in the soil profile becomes depleted.

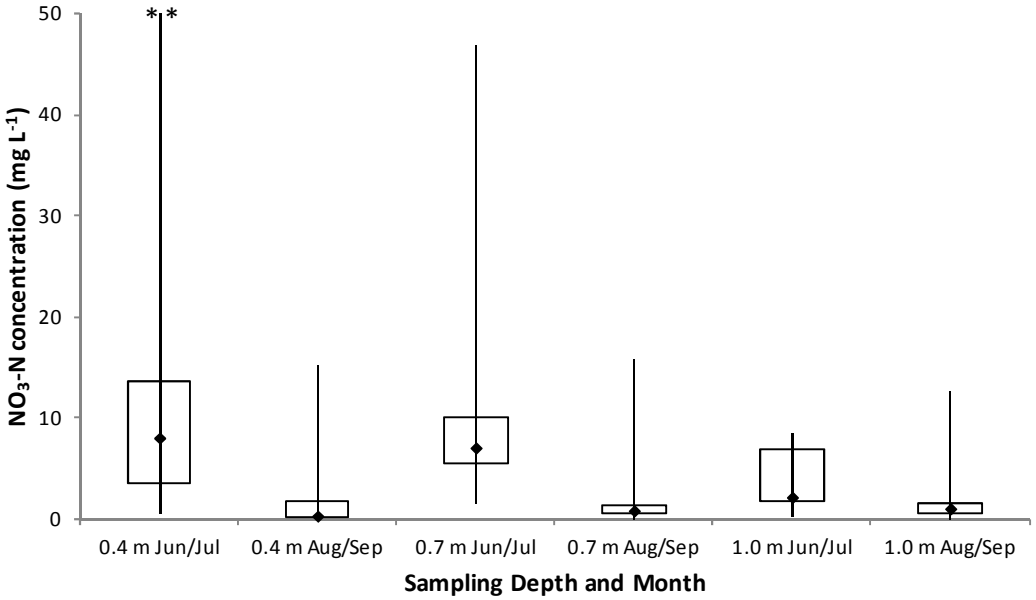

Figure 3.13: The range of NO_3^- concentrations (mg N L^{-1}) found in samples taken from the Topehaehae suction tube samplers. The diamond symbol indicates the median value, n = 130. Box = 25 and 75 percentiles. The ** indicates 2 outlier values of 120 and 130 mg N L^{-1} in the 0.4 m depth samplers.

This temporal variability in NO_3^- concentrations was reflected in the isotopic composition of NO_3^-. Figure 3.14 shows the linear relationship between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ (R^2 = 0.77). This gives an enrichment of $\delta^{15}N$ relative to $\delta^{18}O$ by a factor of 1.4:1 which is within the range reported in the literature (1.3:1 to 2.1:1, or slope of 0.77 to 0.48) as being indicative of denitrification (Aravena and Roberston, 1998; Baily et al., 2011; Böttcher et al., 1990; Chen et al., 2009; Fukada et al., 2003; Mengis et al., 1999).

A clear temporal change can be seen in Figure 3.14 with the August and September samples generally being more enriched than those from June and July. The average increase in $\delta^{15}N$ values ranges from 3‰ for the 0.4 m samplers to more than 7‰ for the 0.7 and 1.0 m samplers. The $\delta^{18}O$ values from the August and September samplings are slightly less enriched, with average increases of 2.7‰, 4.6‰ and 5.6‰ for the 0.4, 0.7 and 1.0 m samplers respectively.

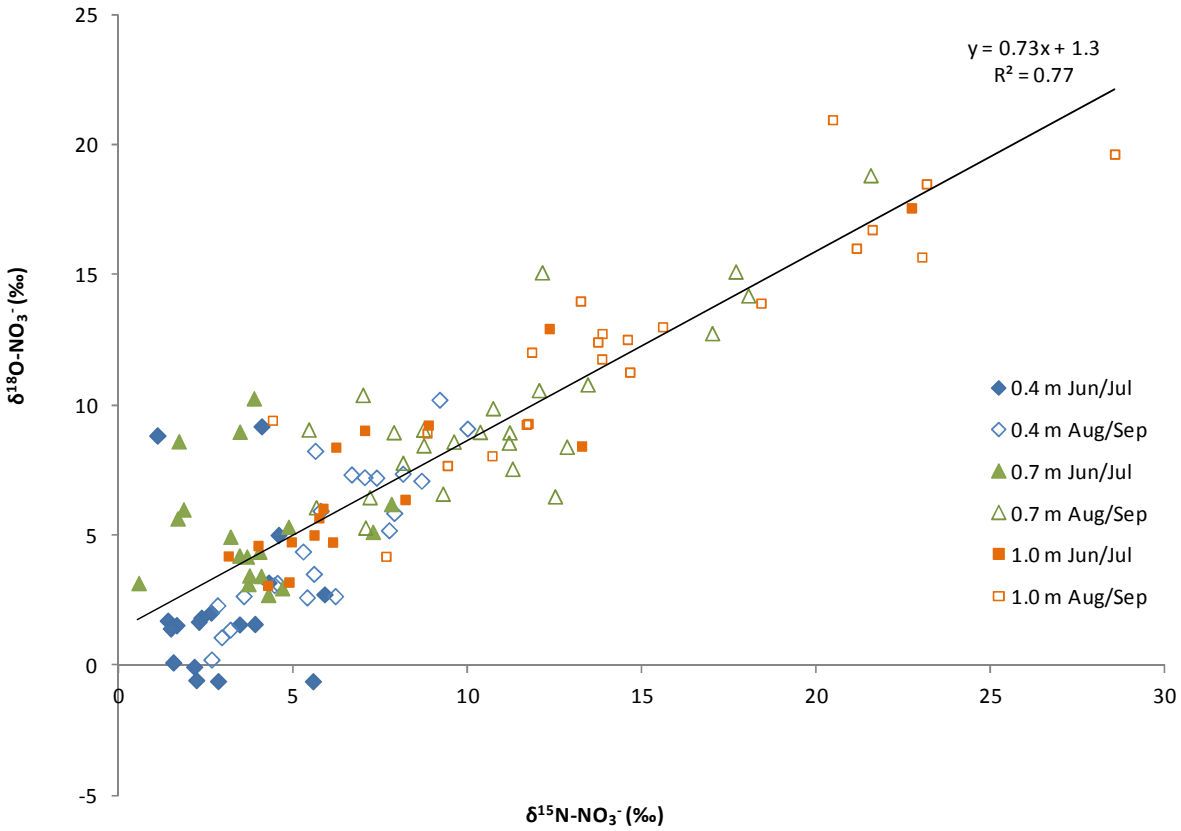

Figure 3.14: The linear relationship between the $^{15}N\text{-}NO_3^-$ and $^{18}O\text{-}NO_3^-$ values sampled from the Topehaehae suction tube samplers. Closed symbols are from samplings in June or July; open symbols are from August or September samplings, n = 121.

However, a consistent isotopic source signal was not obvious, as early winter data from the 0.4 m samplers ranged from +1‰ to +6‰ $\delta^{15}N\text{-}NO_3^-$ and from -0.5‰ to +9‰ for the $\delta^{18}O\text{-}NO_3^-$ values. Based on knowledge of the catchment, the NO_3^- sources are a combination of urea fertiliser, soil N (including N fixed by clover) and ruminant excreta. Even though the heavier signatures tended to occur at greater depth (0.7 m and 1.0 m), it is difficult to connect these $\delta^{15}N/\delta^{18}O$ signatures with the 'source' signatures at 0.4 m depth since the exact flowpaths involved are unknown. As the profile was saturated at all sampling dates, water flow will have had a strong lateral flow component as K_{sat} hydraulic conductivities were relatively fast at 1.1 m depth (1422 mm day^{-1}), and mole (at 0.4 m depth) and tile drains (around 1.0 m depth) facilitate transfer of water (and NO_3^-) to the nearby stream. Accordingly, NO_3^- measured in a given sampler at 0.4 m at one sampling date is unlikely to be picked up at a later sampling date at the corresponding sampler in 0.7 m depth. It is rather more likely that this nitrate gets transported past a neighbouring sampler in 0.7 m depth. Despite the unknown flowpaths, an attempt was made to calculate the enrichment factor (ε) as done by Mengis et al. (1999) using a simplified Rayleigh equation based on time (seasonal enrichment) and distance (vertical enrichment). Seasonal enrichment compares the average $\delta^{15}N$ values for the June/July

period with the Aug/Sep values at the same depth. This presumes either no movement through the profile (vertical or lateral), or that the source signal is constant. Vertical enrichment compares the average $\delta^{15}N$ results for the 0.4 and 0.7 m depths, 0.4 and 1.0 m depths and 0.7 and 1.0 m depths, with the initial concentration and $\delta^{15}N$ value from the Jun/Jul sampling period and the residual values taken from the Aug/Sep samplings. This method does not take lateral flow into account.

Table 3.2: Enrichment factors (ε) calculated for the Topehaehae suction tube samples in the Toenepi catchment.

Seasonal Enrichment (Jun/Jul – Aug/Sep)	**$\delta^{15}N$ enrichment factor (ε)**
0.4 m depth	-1.12‰
0.7 m depth	-4.15‰
1.0 m depth	-9.57‰
Vertical Enrichment	
0.4 – 0.7 m depth	-3.36‰
0.7 – 1.0 m depth	-6.26‰
0.4 – 1.0 m depth	-4.91‰

The data in Table 3.2 shows that enrichment factors become more pronounced with depth but are much smaller than those presented by Mengis et al. (1999) ($\delta^{15}N$ ε = -27.6‰) and are of a similar magnitude to those calculated by Mariotti et al. (1988) who found isotopic enrichment factors between -4.7 and -5.0‰. The smaller ε values are often reported for partially denitrified groundwater and may be due to the denitrification reaction occurring primarily in the small pores, rather than in the freely moving bulk of the groundwater system, thus limiting exchange between these two NO_3^- pools (Groffman et al., 2006; Mariotti et al., 1988).

It is likely that this enrichment occurs later in the season as conditions become optimal for denitrification with continuous periods of saturation leading to declining DO and growth of microorganisms capable of denitrification.

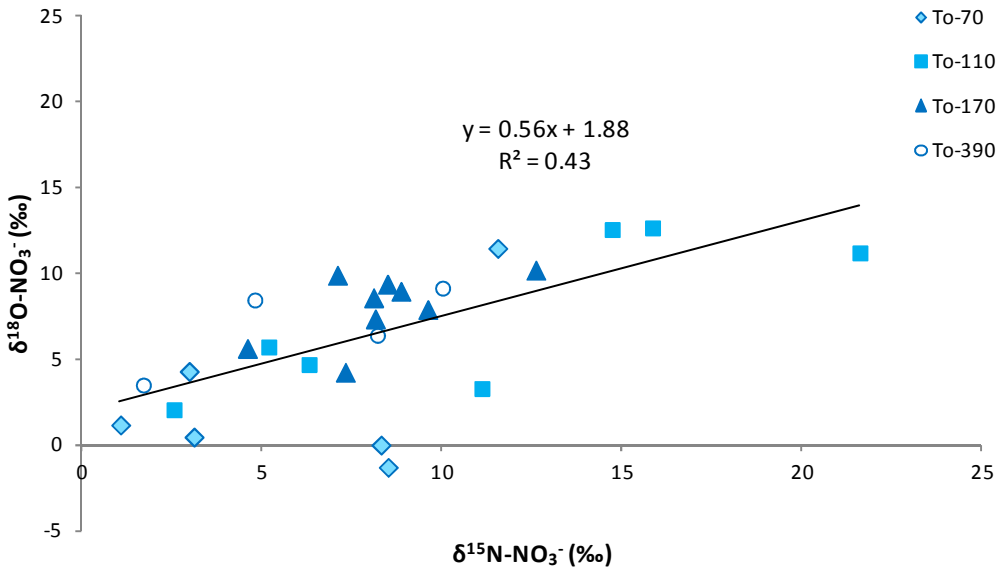

Figure 3.15: Isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^- collected from the Topehaehae wells. Values are in per mil (‰) relative to atmospheric air ($\delta^{15}N$) or Vienna standard mean ocean water ($\delta^{18}O$).

A wide spread of isotopic values were also found in the well samples at the Topehaehae site (Figure 3.15) with a temporal pattern of enrichment also evident for the two shallowest wells. For example, the To-70 well, at 0.7 m below ground surface, had a $\delta^{15}N$ range of 1.07 – 11.54‰ and $\delta^{18}O$ values ranged from -1.3 to 11.46‰. The more enriched isotopic values were from sampling in September, and were in the range of the 0.7 m suction tube samples at the same date. The deeper suction tube samplers, at 1.0 m depth generally yielded more enriched values than the To-110 well samples, at 1.1 m depth. Two factors contributed to this. Firstly, as the well screen was 0.5 m long, the well sampled water from the 0.6 – 1.1 m depth, therefore a mixture of different groundwater flowpaths were sampled. Secondly, the suction tube samplers take more of the small-pore water compared to the well which primarily takes water freely moving in large pores, and conditions for denitrification are likely to be more suitable in such small pores. Despite this, seasonal enrichment in the To-110 well provided the strongest indication of denitrification (Figure 3.16) with ε factors of -12.99‰ for 2009 data (July and September) and -6.36‰ for 2010 values (June and September).

Contrary to expectation, the To-170 well did not show enrichment trends consistent with season or depth, with average $\delta^{15}N$ and $\delta^{18}O$ values of 8.31 ± 2.14‰ and 8.02 ± 1.99‰. From this data, it is presumed that the NO_3^- at 1.7 m depth had not moved vertically from the 1.1 m depth, and it appears to have a consistent source signature that is different to the shallower wells. It is possible that some degree of denitrification has occurred, since average NO_3^- and DO concentrations were low (0.4 mg N L^{-1} and 0.9 mg O_2 L^{-1}), but it appears to occur without seasonal influence.

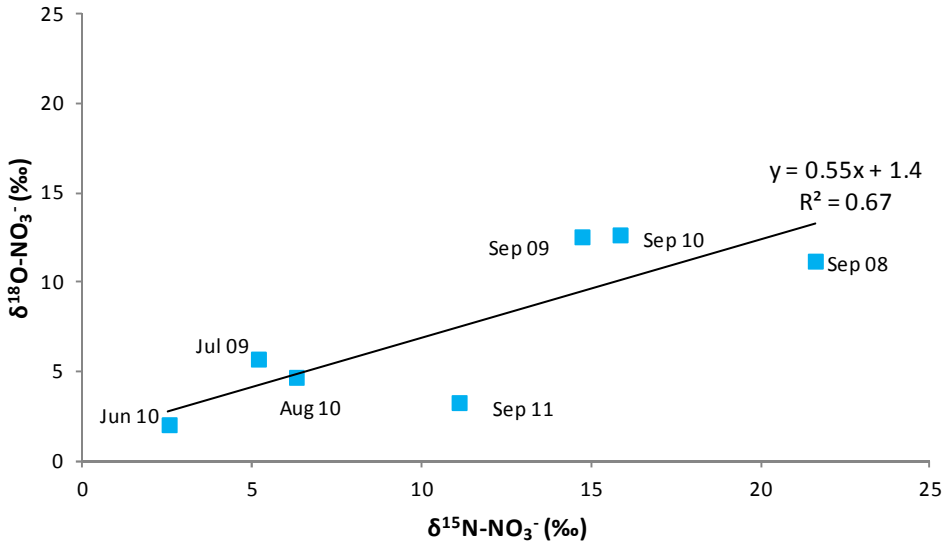

Figure 3.16: Isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^- collected from the To-110 well at the Topehaehae site in the Toenepi catchment.

The four samples from the To-390 well in Figure 3.15 show a wide range of isotopic values, similar to the Mv-470 well. It is likely that measurement uncertainty was partly responsible for the spread of values as the average NO_3^- concentration measured for this well was 0.021 mg N L^{-1}, with more than 50% of samples having concentrations too low for isotopic analysis.

3.4.1..4 Waihora Site

The Waihora wells showed a wide range of NO_3^- concentrations, ranging from below detection limits (<0.002 mg N L^{-1}) to 10.2 mg N L^{-1}. The NO_3^- and DO concentrations were temporally consistent, with individual wells yielding similar concentrations over time.

The redox status of these well samples was categorised using the DO concentrations. Wells with DO <2 mg L^{-1} were denoted as 'reduced' and those >2 mg L^{-1} were labelled 'oxidised'. Figure 3.17 shows that when the isotopic results were grouped by redox status, a small degree of enrichment was seen in the reduced samples. On average, the reduced wells had $\delta^{15}N$ and $\delta^{18}O$ values 3.3‰ higher than the oxidised samples; however, this data was affected by analytical bias at low NO_3^- concentrations as 60% of reduced samples collected could not be analysed.

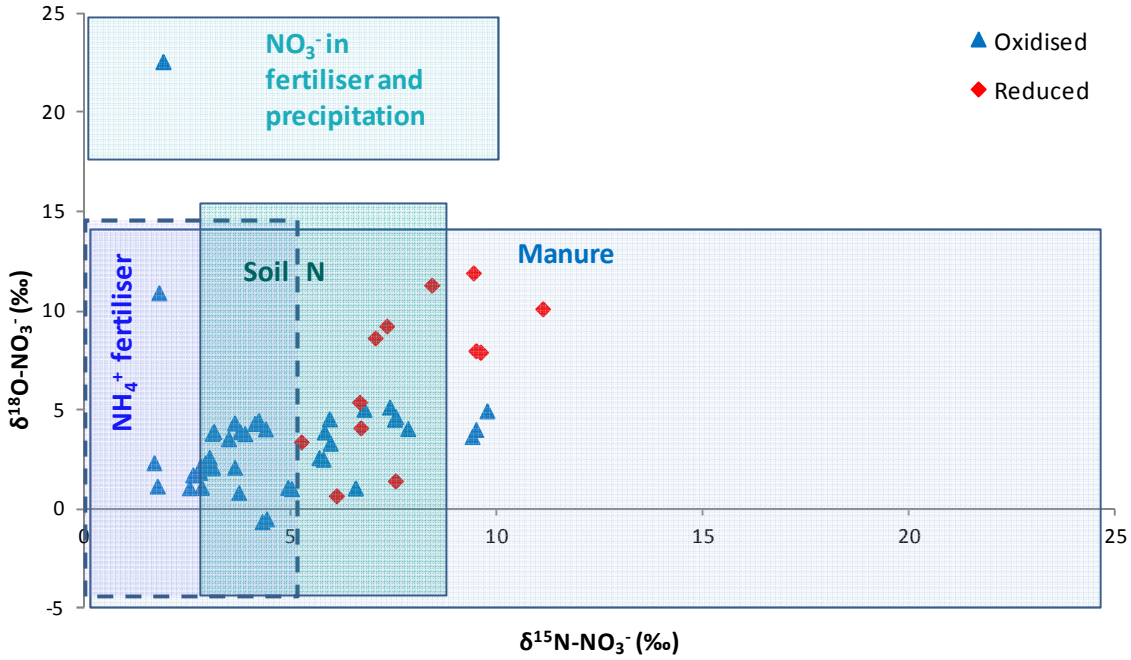

Figure 3.17: Isotopic composition of groundwater at the Waihora well field, categorised by redox status. Wells with DO concentrations measured in the well at the time of sampling <2 mg L^{-1} are denoted as reduced. The theoretical source signatures are also indicated, adapted from Kendall (1998).

Due to this complication of low NO_3^- concentrations, only a few well clusters sampling both oxidised and reduced groundwater could be analysed. The WR24 cluster, located near the bottom of the transect (Figure 3.7), shows a small degree of enrichment between the shallow (3.4 m below ground surface) and deep (5.6 m depth) wells at the three dates sampled (Figure 3.18) with $\delta^{15}N$ ε values of -0.7‰, -1.1‰ and -3‰ for the September 2008, November 2008 and November 2011 samplings, respectively. The WR20 well cluster, on the edge of the wetland (Figure 3.7) had similar $\delta^{15}N$ ε values for the September 2008 and November 2011 samplings of -2.8‰ and -3.0‰ respectively. It was anticipated that the three deeper wells at this site would show more enriched signatures, but NO_3^- concentrations were too low (0.002 – 0.021 mg N L^{-1}), for isotopic analysis to be performed.

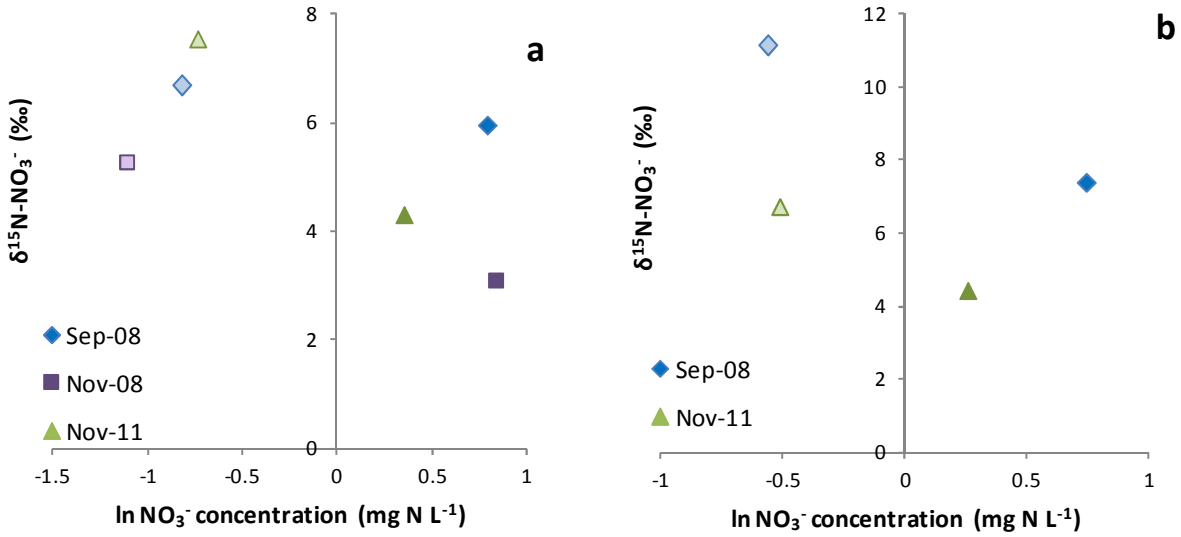

Figure 3.18: The natural log of NO_3^- concentrations (mg N L^{-1}) vs. the $\delta^{15}N$ values (‰) for the WR24 (chart a) and WR20 (chart b) well clusters at the Waihora site between September 2008 and November 2011. The WR24 chart has solid symbols from the oxidised WR24-1 well (3.4 m depth) and open symbols are from the reduced WR24-2 well (5.6 m depth). The WR20 chart shows the oxidised WR20-1 well at 1.4 m depth as solid symbols and samples from WR20-2 (2.4 m depth and reduced) with open symbols.

3.4.2 Spatial Variation

3.4.2..1 Toenepi Catchment

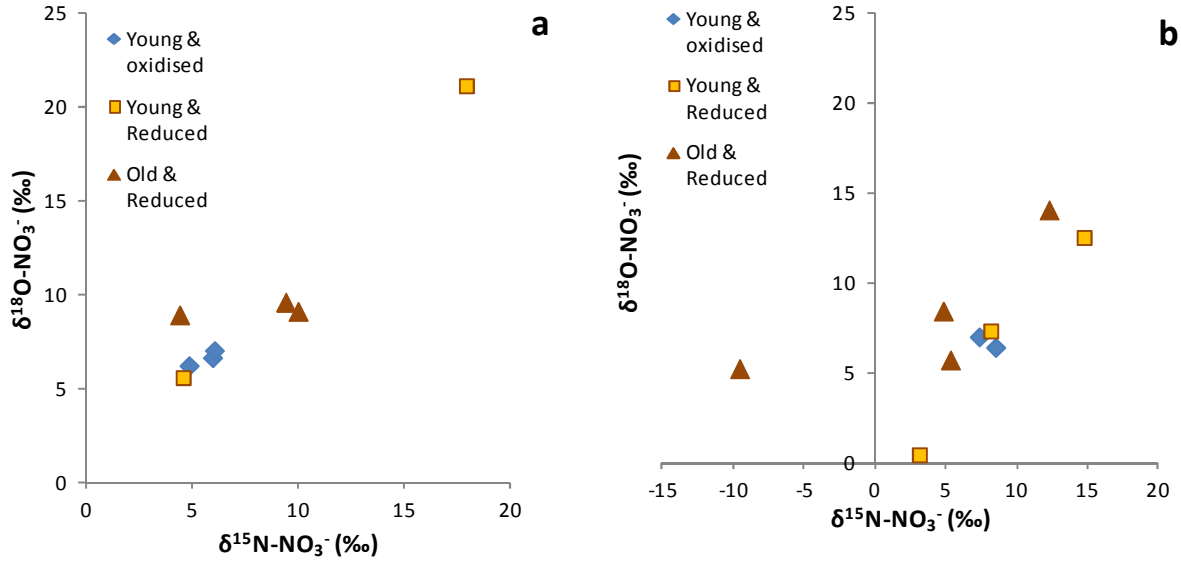

Figure 3.19: Isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^- collected from the wells in the Toenepi catchment in a) August 2008 and b) September 2009.

The spatial variability of isotope composition between well samples in the Toenepi catchment was large, even for samples with similar age and redox conditions (Figure 3.19). The well samples were again categorised by age using SiO_2 data and DO concentration (<2 mg L^{-1} as 'reduced') for the August 2008 and September 2009 data. There was strong agreement between the young and oxidised samples, but the reduced samples with a similar age had a wide range of isotopic values.

Figure 3.20 provides an example of the spatial variability in NO_3^- concentrations measured in each suction tube sampler at the 2 sampling dates in 2009 (tube # 41 – 46 are replicates at 0.4 m, 71 – 76 are at 0.7 m and 101 – 106 at 1.0 m depth). This data illustrates how variable NO_3^- concentrations were over space and time as a high NO_3^-concentration at one sampling date (e.g. 19 mg N L^{-1} measured in # 41) was followed by a relatively low concentration (1.5 mg N L^{-1}) on a subsequent sampling (2 months later). The results demonstrate that a high degree of spatial (and temporal) variability existed in the saturated soil profile, even over very small distances (suction tube replicates were approximately 0.5 m apart). This variability in NO_3^- concentrations affected the isotopic composition as shown by the range of $\delta^{15}N$ and $\delta^{18}O$ values analysed in Figure 3.21.

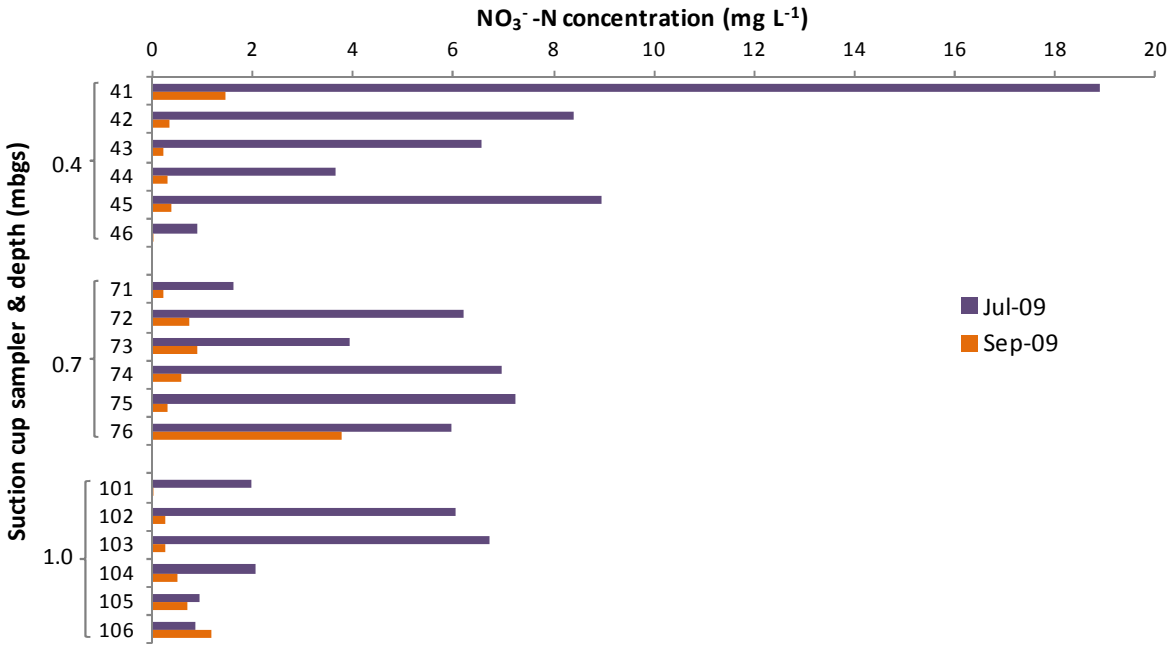

Figure 3.20: Nitrate concentrations measured in each suction tube sampler at the July and September 2009 samplings. Replicates at 0.4 m depth are denoted suction tube # 41 – 46; at 0.7 m depth tube # 71 – 76 and at 1.0 m depth with tube # 101 – 106.

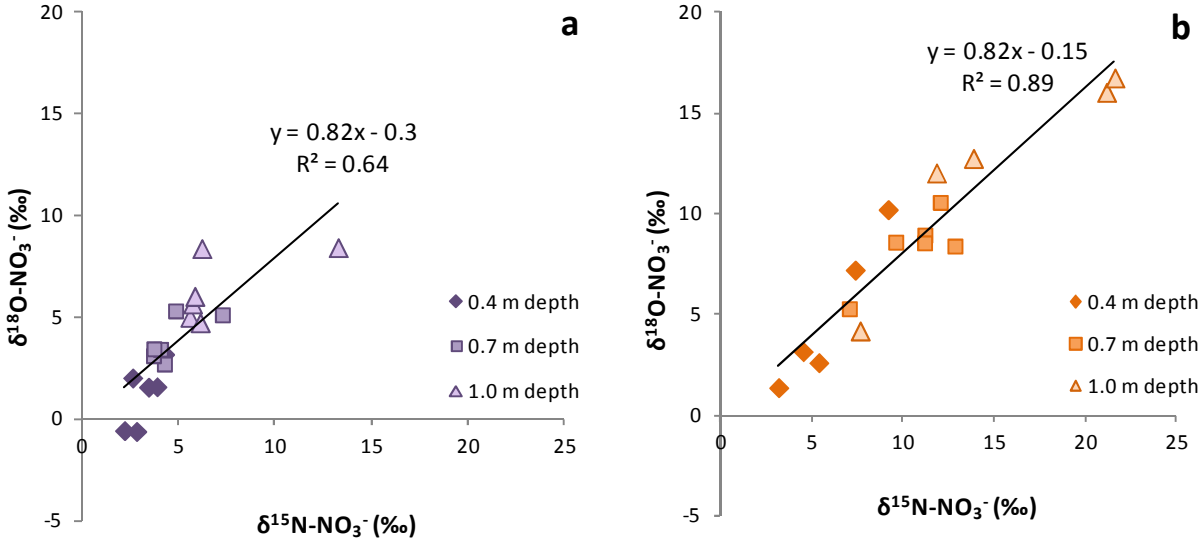

Figure 3.21: Isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^- collected from the suction tube samplers at the Topehaehae site in the Toenepi catchment in a) July 2009 and b) September 2009.

The spatial variability seen in the NO_3^- concentrations and $\delta^{15}N/\delta^{18}O$ signatures was much greater than initially expected, as based on the small scale of the experiment (a few square metres) and similar N inputs, a more narrowly defined source signal (at least in the 0.4 m depth samplers) had been anticipated.

3.4.2..2 Waihora Well Field

The oxidised groundwater at the Waihora site showed more variability in the $\delta^{15}N$ values than $\delta^{18}O$ values (Figure 3.22). In this example, data from the well sampling in September 2008 were categorised based on the measured DO concentration. This amount of spatial variability was surprising given how close together these sample sites were (maximum of 90 m between wells), and possibly indicates several sources of N and different flow paths within the catchment.

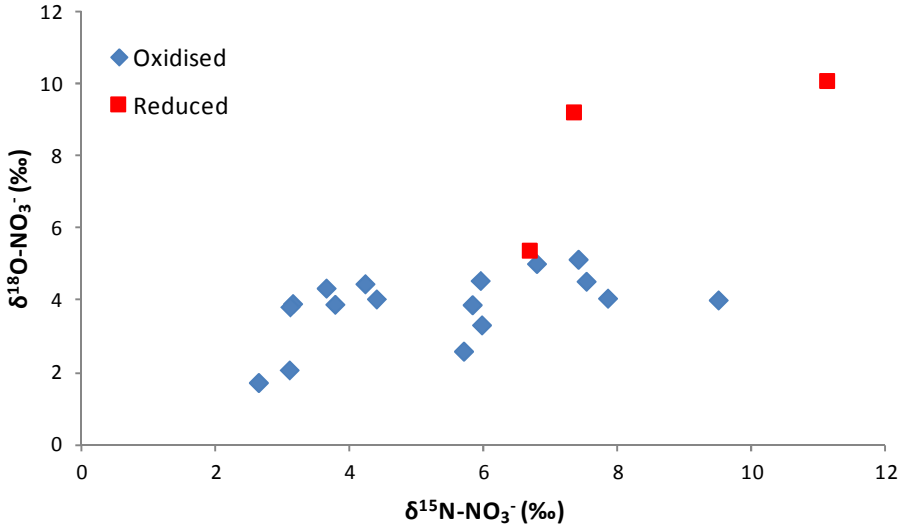

Figure 3.22: Isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) of NO_3^- collected from the wells at the Waihora well field in September 2009.

Isotopic data is most useful for determining denitrification sites and rates when the flowpaths involved are well understood (Böhlke, 2002). When the data from 2008 is presented in a cross section showing the longitudinal transect of wells at the Waihora site, it becomes easier to see the relationship between the occurrence of reduced conditions at the site (Figure 3.23). This schematic shows the stratigraphy of the site, the depth to each well screen, the water table dynamics (blue dotted lines) and redox status of each well. Reduced conditions are represented in red, oxidised in blue and one well (WR19-2), which sometimes samples oxidised groundwater and sometimes reduced water, is shown in orange. The presence of the woody debris at the base of the TI, and the palaeosol can produce reduced conditions when the water table is above these layers, providing ideal conditions for denitrification. This diagram also illustrates how limiting the isotopic method is for determining the occurrence of denitrification when NO_3^- concentrations are low. For example, the three deepest wells at the WR20 cluster, where it was expected to that the greatest degree of enrichment would occur, could not be analysed.

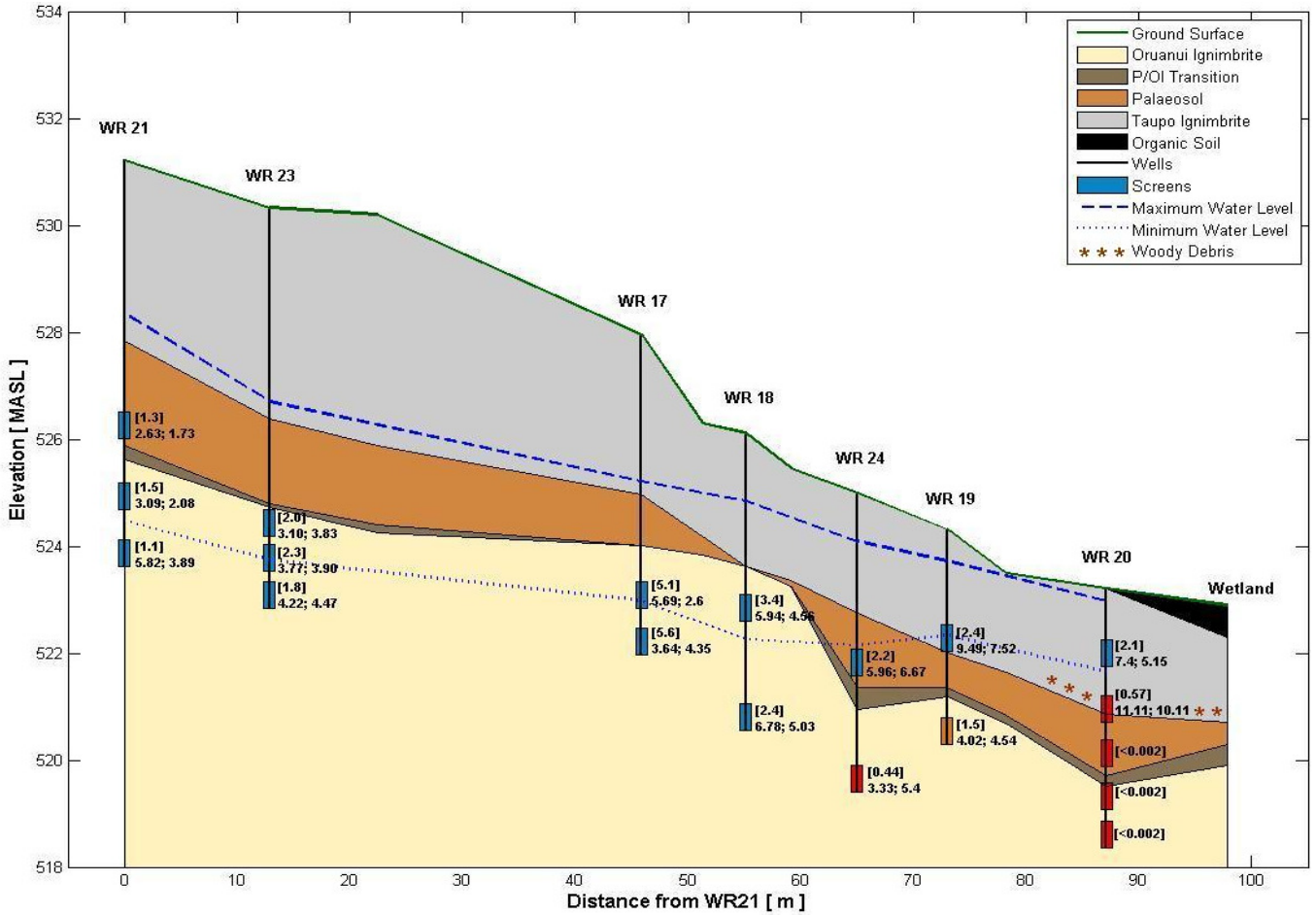

Figure 3.23: Longitudinal cross section of the Waihora well field showing the stratigraphy, the depth of wells and redox status (red = reduced, blue = oxidised, orange = sometimes reduced). The NO_3^- concentration (mg N L^{-1}) is in square brackets with the isotopic signature below ($\delta^{15}N$; $\delta^{18}O$). Results are from the September 2008 sampling, samples with NO_3^- concentrations <0.03 mg N L^{-1} were not submitted for isotopic analysis. The Y axis shows the elevation of the site in metres above sea level (masl).

3.5 Conclusions

The isotopic analysis of NO_3^- in these two shallow groundwater systems provides an indication of where and when denitrification might be occurring. In situations where the flowpaths between oxidised and reduced zones are well defined, $\delta^{15}N$ and $\delta^{18}O$ values can provide insight into the rate of denitrification occurring *in situ* in a way that is useful when examining the effects of land use and management practices on receiving waters. However, where flowpaths are unknown, or low NO_3^- concentrations occur, isotopic information may be less valuable or unavailable. In these situations, other methods, such as excess N_2 may be more appropriate. Despite these challenges, the data points to seasonal denitrification at the Topehaehae site in the Toenepi catchment, and it is likely that the Kereone, Morrinsville and Waihora sites also undergo denitrification, even though the degree of enrichment observed is comparatively low. For these sites, it may be possible to determine whether denitrification is occurring directly around the well by using push-pull, tracer tests or laboratory incubation experiments.

Chapter 4

In situ experiments to identify denitrification in a shallow groundwater system

4.1 Abstract

Denitrification in the shallow groundwater system of a small Waikato lowland catchment was assessed using push-pull techniques. Groundwater was extracted and then amended with nitrate (NO_3^-) and bromide (Br^-), which served as a conservative tracer. This amended groundwater was pushed (i.e. injected) back into the shallow groundwater system and subsequently small samples were pulled (i.e. extracted) after incubation periods of increasing lengths (up to 5 days). Both the denitrification capacity and the denitrification potential were assessed *in situ* at three well locations in the catchment where reduced groundwater had previously been found. No denitrification was observed in the push-pull experiments unless glucose was added. This could indicate that denitrification does not occur in the vicinity of the well screens and the groundwater redox status is due to reduction processes occurring somewhere upgradient along the groundwater flow path to the well screens. Alternatively, the injected substrate pool may have been too small to allow the microbial population to respond to a measureable extent in the short time period before the substrate plume became depleted due to dilution of the pulled sample.

4.2 Introduction

An understanding of how, where, and when denitrification occurs and what the denitrification capacity is in groundwater systems is needed in order to promote NO_3^- removal and to protect ground and surface water quality. Results from groundwater monitoring networks that show declining dissolved oxygen (DO) and nitrate (NO_3^-) concentrations with depth indicate that denitrification may be occurring. However, determining the location and extent of denitrification from such data sets can be problematic. In more recent years, the isotopic signature of NO_3^- in the groundwater system has been used to calculate the degree of denitrification (Baily et al., 2011; Böttcher et al., 1990; Mengis et al., 1999), but flowpaths and source signatures need to be well defined for this method to work. However, as discussed in Chapter 3, at sites where NO_3^- inputs are low, or denitrification has progressed to near-completion, the NO_3^- concentrations are often too low for isotopic analysis to be used. Therefore additional information acquired using compatible techniques is required.

In situ experiments offer a means of studying the denitrification process under field conditions. One technique for groundwater systems where hydraulic conductivities are relatively slow is the push-pull test which can be used to ascertain denitrification rates (Trudell et al., 1986) and rates of other microbial processes (Istok et al., 1997) *in situ*. In this method, a solution containing a conservative tracer, often bromide (Br^-), and reactive NO_3^- is injected into a well to create a plume in the aquifer matrix surrounding the well screen (Figure 4.1). After a pre-determined period of time, the groundwater is extracted and the concentration of the remaining solutes measured (Figure 4.1).

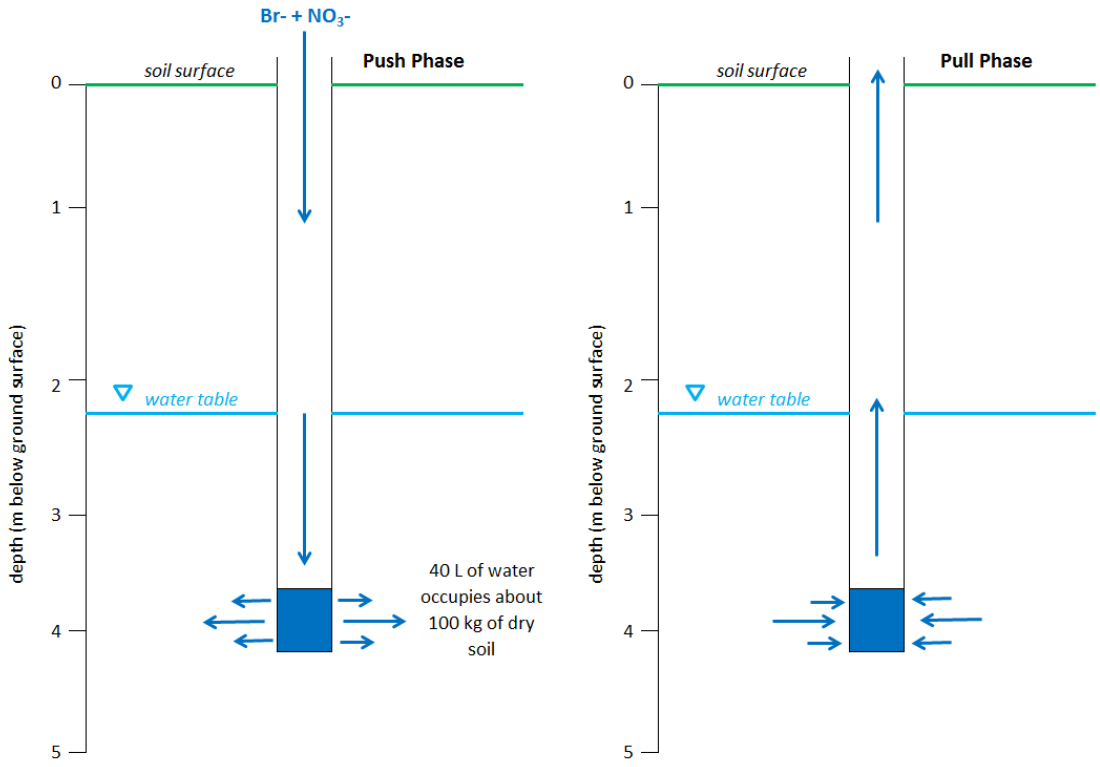

Figure 4.1: Diagram of the push-pull test showing the push phase where tracer is injected, and the pull phase where the tracer is removed.

The effect of dilution of the pulled sample is quantified by measuring the decrease in concentration of the conservative tracer. Any more rapid decline in the NO_3^- concentration is attributed to denitrification. The tracer solution may be enriched in ^{15}N (Addy et al., 2002; Smith et al., 2004) which enables inputs of NO_3^- and its removal to be determined, or amended with a source of carbon (C) (Mehnert et al., 2005) to ascertain if the system is C limited and to determine absolute denitrification potentials *in situ*. In denitrification potential experiments, both C and N are non-limiting, and thus maximum rates of heterotrophic denitrification are measured (Yeomans et al., 1992). Experiments using only NO_3^- and a conservative tracer determine only the existing

denitrification capacity, utilising resident electron donors to complete reactions, and not the denitrification potential of the system.

The push-pull method is particularly useful for determining whether denitrification is actively occurring around a well screen, or if the NO_3^- leached from the root zone may already have been denitrified further up the groundwater flowpath. However, the potential effects of priming (whereby repeated injections improve the microbial response) must also be considered (Kellogg et al., 2005).

In the current study, push-pull tests are used in an attempt to better document and quantify denitrification under *in situ* conditions for the Toenepi study site, after $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ analysis of groundwater samples (Chapter 3) yielded only limited information about this process. Both denitrification capacity and denitrification potential were measured in selected wells within the catchment in order to understand the factors limiting denitrification *in situ*.

4.3 Materials and Methods

4.3.1 Study Site – Toenepi Catchment

The Toenepi catchment has an area of 15 km^2 and is located in the eastern part of the Waikato region (Figure 4.2). The predominant land use is dairying with an average stocking rate (in 2003) of 3.1 cows ha^{-1} and annual fertiliser-N inputs of 100 kg N ha^{-1} y^{-1}. The underlying geology of much of the catchment has formed from Holocene to late Pleistocene andesitic and rhyolitic ash deposits. In locations close to the Toenepi stream, recent alluvial deposits predominate. Relict organic material has been found in core samples taken at the three sites shown in Figure 4.2, reflecting the depositional nature of the materials.

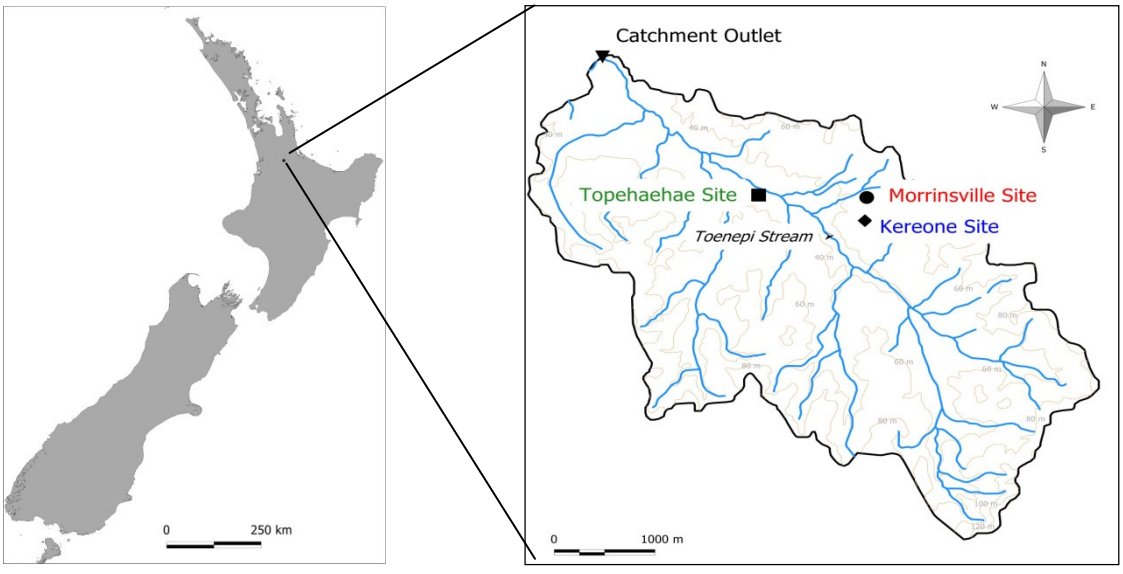

Figure 4.2: Location of the Toenepi catchment and the three study sites.

Shallow wells (maximum of 4.7 m depth) were installed at three sites in the catchment: Kereone, Morrinsville and Topehaehae (Figure 4.2). Three wells were installed at the Kereone and Morrinsville sites, and four wells installed at the Topehaehae site. The wells were installed using an 80 mm diameter corer and were constructed from 50 mm diameter PVC pipe, with 0.5 m long well screens. The annular gap was filled with pea-sized gravel and a bentonite plug near the soil surface prevented preferential flow during rainfall. The depth of each well was chosen to cover a range of possible conditions including proximity to the water table and redox status (Table 4.1). The Childs test was used to determine the redox status of the core material. When the Childs test solution is sprayed on vadose or aquifer material, a red colour forms on material containing reduced iron (Fe^{2+}); an indication of anaerobic conditions (Childs, 1981). To estimate hydraulic conductivities, slug tests were also performed following the recommendations of Butler (1998).

Briefly, this involved measuring the water level (to gain an initial reading) then inserting a water level sensor (Diver®, Schlumberger, USA) set to 0.5 sec recording interval. When the water table had recovered, a solid slug, designed to increase the head by 0.5 m was rapidly introduced in order to achieve as instantaneous an increase as possible. This is termed a 'slug in' test. The slug was then removed once the water level had recovered to the initial reading, resulting in a 'slug out' test. Once the water level had again returned to the initial value, the sensor was removed. Both the 'slug in' and 'slug out' data were analysed using the Bouwer and Rice (1976) method for unconfined aquifers.

Some wells (e.g. Ke-410 and Mv-470) had very slow conductivities (K_{sat} < 0.01 m d^{-1}), while others were relatively fast (e.g. To-110) with K_{sat} values >1 m d^{-1} (Table 4.1). Five wells were selected for push-pull experiments (in blue in Table 4.1; Figure 4.3), and focussed on those where groundwater with low DO and NO_3^- concentrations had been sampled. However, one well (Ke-410) which always draws oxygen-rich, NO_3^--bearing groundwater was tested to investigate whether denitrification could be induced, if low DO water was introduced (in the push solution). The groundwater drawn at the To-110 well was reduced at the time of testing (September) but the redox status can vary throughout the year, and the well can be completely dry during water table minimums (summer and autumn). Two of the Topehaehae wells (To-70 and To-170) could not be tested using push-pull methods as the water table was below the top of the screen at the time of testing. The To-390 well was not selected for a push-pull test as this well is separated hydrologically from the upper wells by an aquitard.

Table 4.1: Summary of well characteristics for all Toenepi wells. The five wells used in the push-pull tests are indicated in blue.

Well	Ke-350	Ke-410	Ke-470	Mv-260	Mv-410	Mv-470	To-70	To-110	To-170	To-390
Length (m below ground surface)	3.5	4.1	4.7	2.6	4.1	4.7	0.7	1.1	1.7	3.9
Well screen length (m)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Estimated hydraulic conductivity (m d^{-1})[b]	0.0002	0.009	0.18	1.14	0.025	0.005	1.5	1.42	1.05	0.009
Average NO_3^- concentration[a] (mg N L^{-1})	5.9	8.1	0.9	9.7	1.2	0.1	1.1	0.7	0.4	0.02
Average DO concentration[a] (mg L^{-1})	8.8	7.6	0.8	7.1	1.1	1.5	1.8	1.2	0.9	0.5

[a] monitoring period 2008 – 2011

[b] Bouwer and Rice (1976)

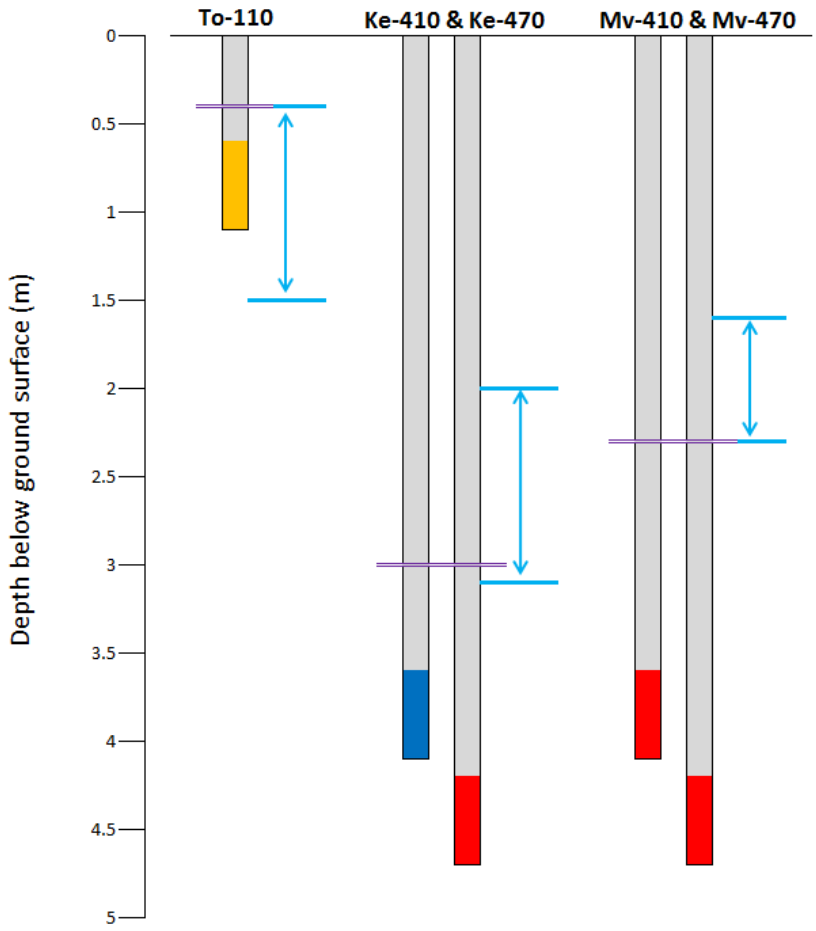

Figure 4.3: Wells used in the Toenepi push-pull tests. The well screen depth and redox status is indicated by colour (red = reduced, blue = oxidised, orange = occasionally oxidised). The seasonal water table dynamics are indicated by the blue lines and arrow while the water table at the time of the push-pull determination is indicated by the purple double line.

4.3.2 Push-Pull Denitrification Capacity Experiments

The push-pull methods of Trudell et al. (1986) and Istok et al. (1997) were used to determine denitrification rates within the vicinity of the well screens. For this procedure, groundwater from each well was collected several days before each experiment, analysed for dissolved oxygen (DO) using an optical DO sensor (YSI, Yellow Springs, OH, USA) and then stored in polypropylene plastic containers in cooler boxes (<10°C) if the DO was low (< 2 mg L^{-1}). Because individual wells could not supply the required push volume of 40 L in a timely manner, all low-DO wells were used to yield sufficient water and mixed together. This volume of water was much smaller than volumes used by others (90 – 270 L) (Istok et al., 1997; Vandenbohede et al., 2008) as the wells were screened for only 0.5 m compared to 1 – 5 m. A 1 L subsample of groundwater was taken and the Br^- and NO_3^- were added. The push solution was then made up in a 50 L polypropylene plastic container by adding the stored groundwater to the concentrate. The push solution was carefully stirred in a smooth motion without creating bubbles to ensure the solution was well mixed, without increasing the DO of the

groundwater. The final concentration of the 40 L volume was 100 mg Br^- L^{-1} (as KBr), 40 mg N L^{-1} (as KNO_3) and 100 mg L^{-1} brilliant blue. The brilliant blue was used as a visual tool to monitor the degree of dilution in the field. Samples of the push solution were taken before injection to account for the contribution of NO_3^- in the stored groundwater so that the actual concentration of NO_3^- injected varied from well to well (41 – 47 mg N L^{-1}).

Prior to the push phase, the water level, DO and temperature of the well were measured. The standing water column was then removed (1.4 – 4.8 L depending on the well) to avoid dilution of the push solution. Duplicate samples of this groundwater were taken for analysis of background concentrations of Br^- and NO_3^-.

The push solution was injected using a 12 V diaphragm pump. The injection rate ranged from 2 – 5 L min^{-1}, depending on the hydraulic conductivity of the surrounding aquifer, and was adjusted using a release valve so that the pressure did not exceed 50 PSI (344 kPa).

Sampling began by pulling the injected water back after 2 to 3 hours after injection. This time depended on the well's characteristics with pulling commencing when the water level had recovered to pre-injection levels. During the pull phase, the pumping rate was between 0.25 and 0.5 L min^{-1}, with samples taken every 0.5 L of water pulled, resulting in 5 – 20 samples per 'batch'. Due to the low hydraulic conductivities of the materials surrounding the well screens, the pull phase consisted of pumping until the well was dry followed by a recovery time, when the water level was allowed to return to within 0.5 m of the original water level before pumping began again. Generally, 2 – 3 batches of samples were taken in the first two days after injection. Thereafter, sampling occurred once a day for up to 3 more days, dependent on the colour of brilliant blue in the sample, i.e. how much dilution had occurred. This was assessed by eye and comparison with previous samples, in the field. Samples (50 mL) were taken for determination of Br^- and NO_3^- concentrations. Samples were stored in cooler boxes in the field, and upon being returned to the lab, were chilled at 4°C until analysis. These experiments took place in February and March 2012.

The Br^- and NO_3^- concentrations are reported as relative concentrations (C/C_o) where the concentrations measured in each sample are divided by the initial concentration in the push solution. Corrections were made for the background concentrations of Br^- and NO_3^-.

4.3.3 Push-Pull Denitrification Potential Experiments

A second phase of push-pull tests were carried out in order to assess the denitrification potential of the shallow groundwater system in contact with the screened zone of each well. These tests were carried out at least 1 year after the phase I experiments (September 2013), so any priming occurring as a result of the initial push-pull tests was considered minimal. In these experiments, three wells

were chosen (Ke-470, Mv-410 and To-110 (Figure 4.3)). Push solutions contained Br^-, NO_3^-, brilliant blue and glucose as a source of readily available C for denitrification. The concentration of glucose used was 400 mg C L^{-1} while the other solute concentrations were the same as the previous push-pull experiments. Therefore, the C:N ratio of applied tracer was 10:1. This ratio was chosen to supply C in excess and is similar to the ratio commonly found in top-soils (Ghani et al., 2007; Sparling and Schipper, 2004). Preparation, injection and extraction of the pushed solution were performed in the same manner as the first experiments. However, the push solution was only left for 1.5 – 2 hours before the pull phase began, and the sampling interval was increased to every 1 L.

4.3.4 Analytical methods

Groundwater DO and temperature were measured in the standing water column using the YSI ODO meter (YSI, Yellow Springs, OH, USA), which was calibrated daily. Groundwater samples were first filtered to remove any sediment (Whatman 42) before being analysed for Br^- using an ion selective electrode and meter (ISTEK, Korea). A standard curve (1 – 1000 mg Br^- L^{-1}) was constructed to convert these readings to Br^- concentrations. The concentration of NO_3^- -N was determined using standard methods for ion chromatography (APHA 4110B) (APHA, 2005).

4.4 Results and Discussion

4.4.1 Push-Pull Denitrification Capacity Experiments

The concentrations of both NO_3^- and Br^- tended to decline rapidly at all five sites where push-pull denitrification capacity tests were conducted (Figure 4.4). Active denitrification was not observed at any site since declining NO_3^- concentrations mirrored those of the Br^- tracer. This does not necessarily mean that denitrification cannot occur at each site. It may simply be that the rate may be too slow to measure in a short push-pull experiment of up to 5 days. Or it may mean that the process does not happen directly at the screened zone of aquifer material, but further up the groundwater flowpath, where relict organic matter (or other electron donors) resides.

Based on the hydraulic conductivities measured for each well (Table 4.1), it was expected that the wells with the slowest conductivities would exhibit the lowest rates of tracer dilution. This expectation applies only if the hydraulic gradients are similar; a reasonable assumption for the Kereone and Morrinsville sites, given that they occur only 250 m apart. It was assumed that the hydraulic gradient at the Topehaehae site would be lower, as the floodplain is flat.

However, the Mv-410 well (with a K_{sat} value of 0.025 m day-1 (Table 4.1)), had C/C_o values <0.05 after only 0.5 volumes had been pulled (Figure 4.4), which was about 8 hours after injection (Figure 4.5). In contrast, the To-110 well, with the highest K_{sat} value of 1.42 m day^{-1} had a C/C_o value of 0.3 after this

volume, 23 hours after injection (Figure 4.5). Therefore, the hydraulic gradient at the Morrinsville site was much greater than the gradient at the Topehaehae site, as the Morrinsville plume moved more quickly despite a lower hydraulic conductivity of aquifer material.

This dilution effect reflects the inadequacy of slug test data in estimating and describing aquifer characteristics; however, they do provide a rough guide to potential flow rates. Previous studies using the push-pull method (Kellogg et al., 2005) do not specify K_{sat} values of the aquifer studied and arrive at a suitable rest period between the push and pull phases by pre-testing. It was assumed that having the hydraulic conductivity information would avoid this initial testing phase.

It is unclear how the pattern shown in the Mv-470 chart in Figure 4.4 could occur as Br^- and NO_3^- theoretically move together (being about the same size and having the same charge) and any mechanism which would retard NO_3^- movement and dilution should also retain Br^-. It is unlikely that the pool of NO_3^- is being replenished by the background groundwater, as routine sampling of this well has revealed consistently low NO_3^- concentrations (0.01 – 0.11 mg N L^{-1} between 2008 and 2011). Also, based on the age dating work in Chapter 3, groundwater from this well is approximately 140 years old and should have naturally low NO_3^- concentrations. It is conceivable that the repeated pumping until dry action might have pulled water into the well from above this layer; however, the Mv-410 well also has consistently low NO_3^- concentrations (Table 4.1). Furthermore, a water level pressure sensor in the Mv-410 well recorded minimal drawdown effects (approximately 0.025 m) during the pull phase of the Mv-470 test.

The mass recoveries of Br^- and NO_3^- were <40% for all wells since most tests resulted in a smaller total volume being pulled than was pushed (Figure 4.4) and the injected plume was transported out of reach with the groundwater flow. These mass recoveries are much smaller than those found previously. Kellogg et al. (2005) measured conservative tracer recoveries of at least 70%, while Istok et al. (1997) reported 88 – 99 % mass recoveries of Br^-. As already reflected in the C/C_o charts, the Br^- and NO_3^- masses recovered at each well were similar, indicating that dilution rather than denitrification was the cause of the declining concentrations. The rapid dilution may also have been in part, induced by the amount of sampling and the short rest period between the push and pull phases causing more resident groundwater to interact with and dilute the tracer plume. However, the rest period chosen (2 – 3 hours) was within the timeframe given by others (0 – 24 hours) (Istok et al., 1997; Kellogg et al., 2005; Mehnert et al., 2005).

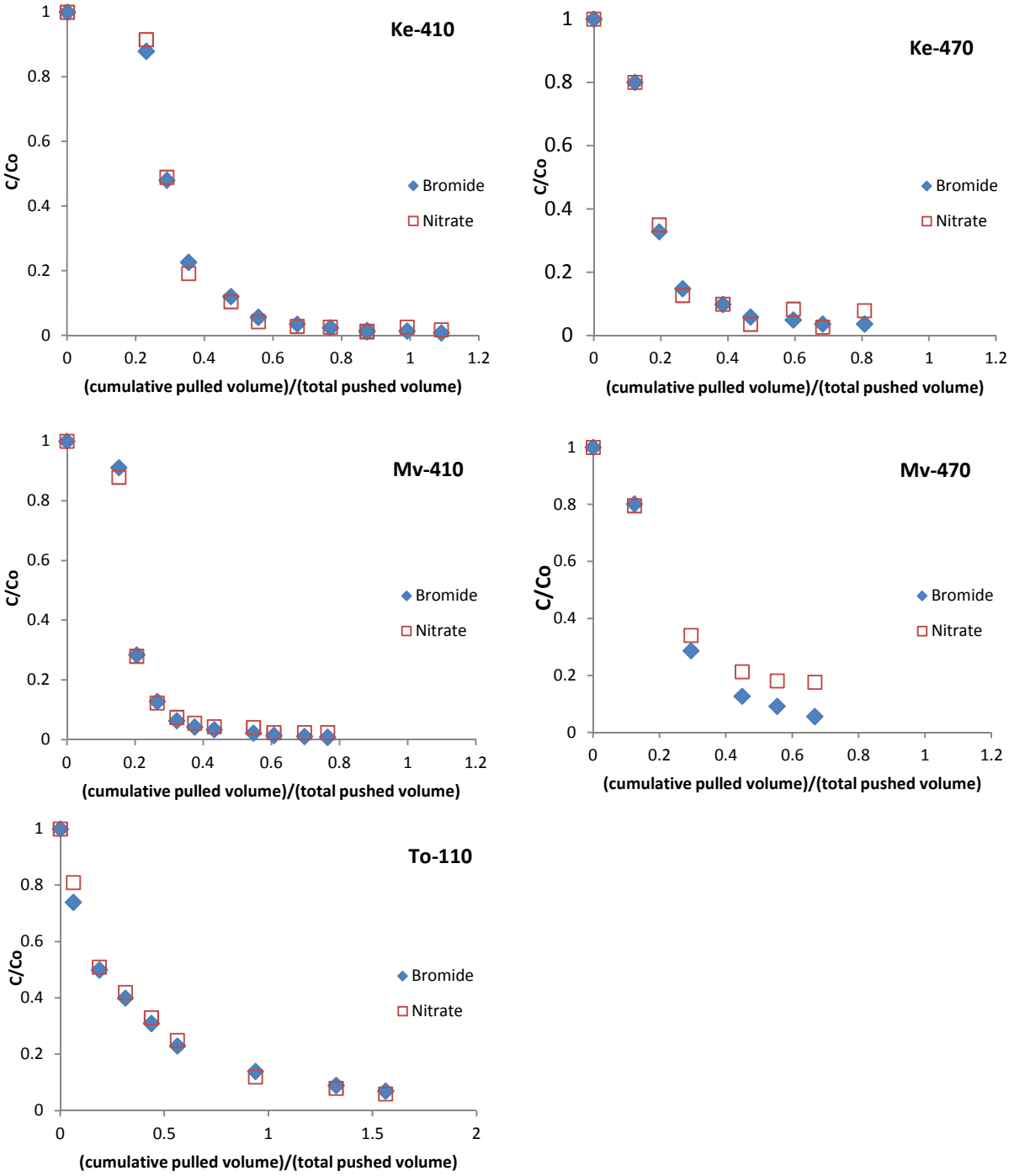

Figure 4.4: Relative concentration profiles of bromide and nitrate against volume from the push-pull denitrification capacity tests at the 5 wells in the Toenepi catchment. Data points are single samples.

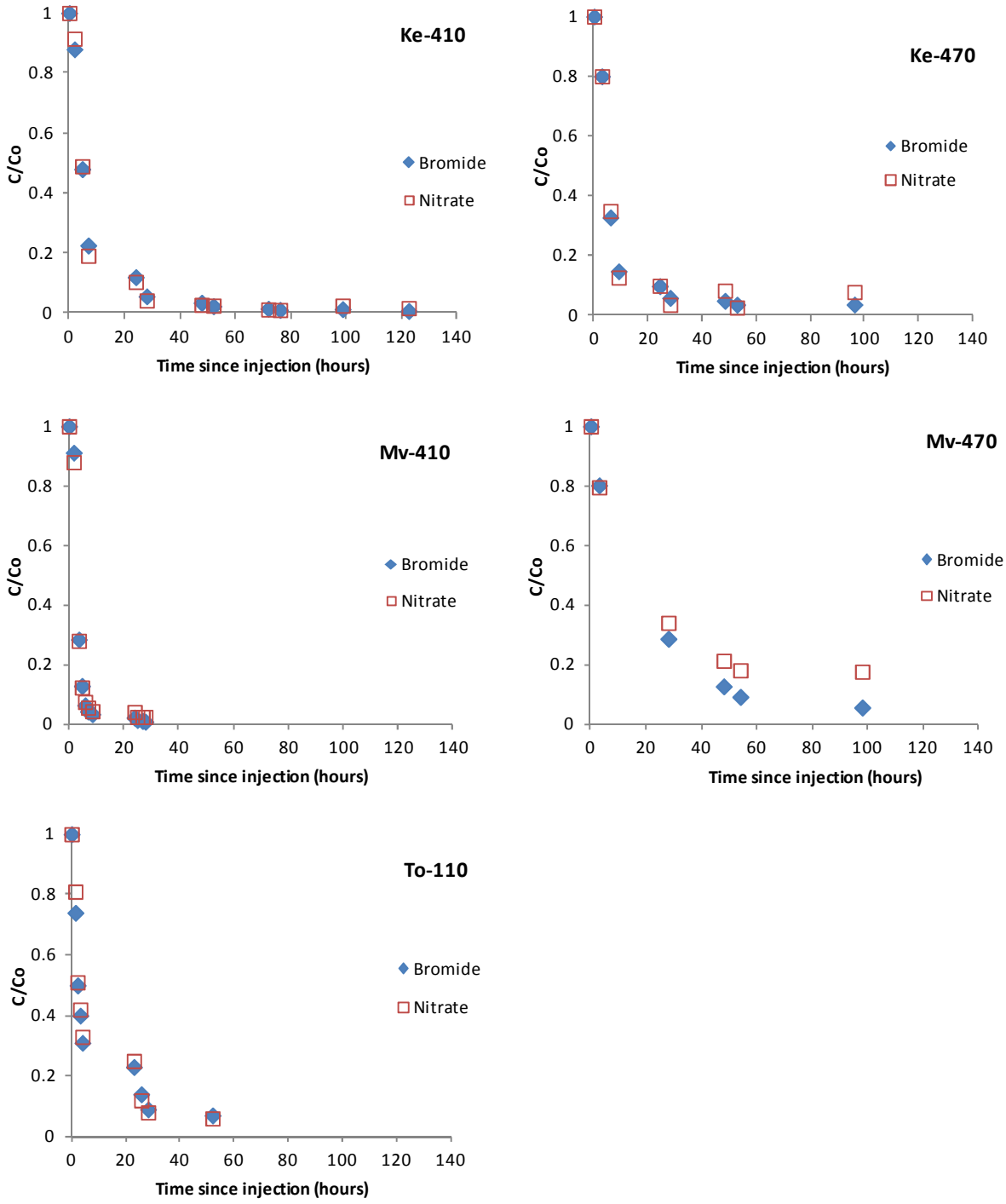

Figure 4.5: Relative concentration profiles of bromide and nitrate vs. hours since injection for the push-pull denitrification capacity tests at 5 wells in the Toenepi catchment. Data points are single values.

4.4.2 Push-Pull Denitrification Potential Experiments

Push-pull denitrification potential experiments were undertaken on the Ke-470, Mv-410 and To-110 wells. The rationale behind these denitrification potential experiments was to ascertain the maximum denitrification rates that could occur under *in situ* conditions. For this reason, the Ke-410

well was not tested with glucose as groundwater was always oxidised and had high NO_3^- concentrations (Table 4.1). The unusual and unexplained C/C_o trends shown in the Mv-470 data (Figure 4.4) also made this well an unsuitable candidate for determining denitrification potentials.

Previous studies (Bates and Spalding, 1998; Hill et al., 2000; Mehnert et al., 2005) have shown denitrification being induced following C addition to the tracer solution in aquifers, when denitrification had been previously undetected in the absence of a C substrate addition. Similarly, denitrification was detected at the To-110 well site following glucose addition (Figure 4.6). However, no denitrification was detected at the Ke-470 or Mv-410 well sites (Figure 4.7).

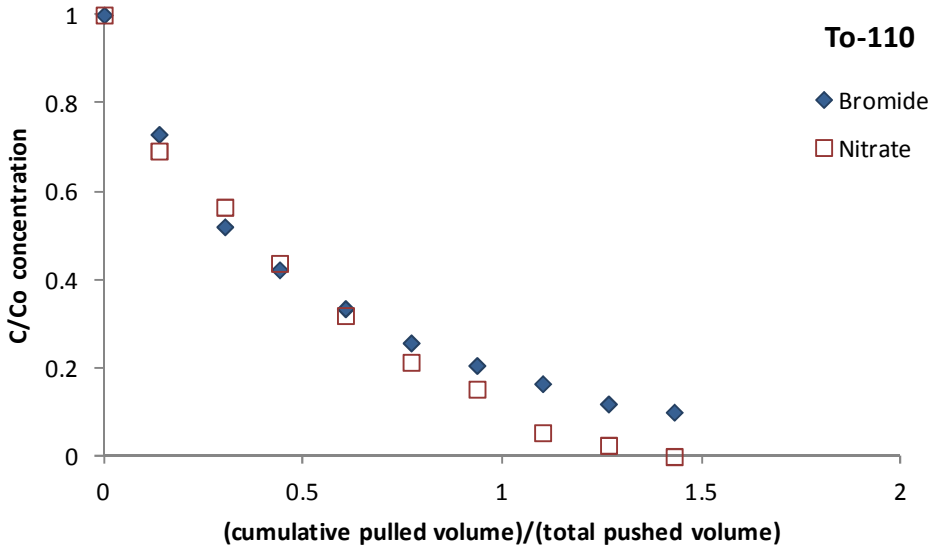

Figure 4.6: Relative concentration profile of bromide and nitrate for the denitrification potential push-pull experiment at the Topehaehae site (To-110 well).

As expected, based on the C/C_o charts (Figure 4.7) the total mass recoveries of Br^- and NO_3^- were low for the Ke-470 and Mv-410 wells (<30%). The To-110 well had total mass recoveries of 44% and 38% for Br^- and NO_3^-, respectively.

The lack of response to glucose in the Ke-470 well (Figure 4.7) was unexpected for several reasons; core samples from the installation of this well showed layers of organic material at this depth (4.2 – 4.7 m below ground surface); groundwater samples from this well had been categorised as young (mean residence time approximately 8 years) but generally had low NO_3^- concentrations (0.012 – 2.7 mg N L^{-1}, 2008 – 2011) and low DO concentrations occur at well screen depths (Table 4.1).

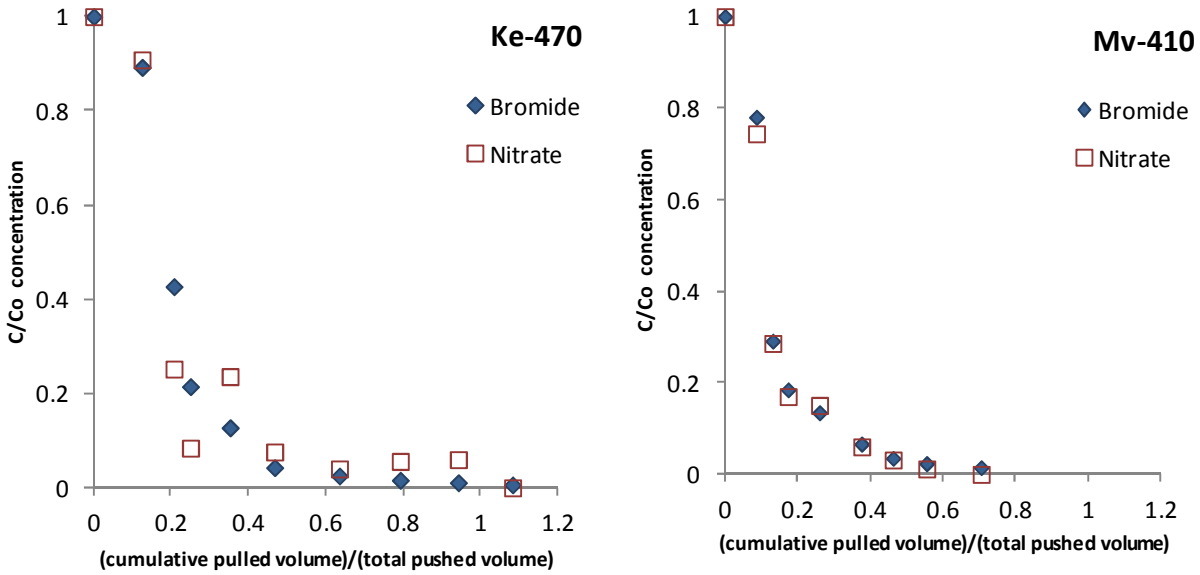

Figure 4.7: Relative concentration profiles of bromide and nitrate from the denitrification potential push-pull tests at the Ke-470 and Mv-410 wells in the Toenepi catchment. Data points are single measurements.

The most likely reason for gaining no evidence of denitrification from this experiment is that the injected plume moved too fast away from the well screen, thus preventing sampling of a measureable difference. However, based on the relative concentrations measured over time (Figure 4.8), the hydraulic velocity must be much faster than estimated as less than 10% of the original push solution remained only 24 hours after injection. While this is the major disadvantage of the push-pull technique it did not impede denitrification detection at the To-110 site, which, despite having a much faster K_{sat} value (1.4 m day^{-1}, Table 4.1), had a low hydraulic gradient, resulting in slower velocities as more than 30% of the push solution remained in the aquifer after 24 hours (Figure 4.8).

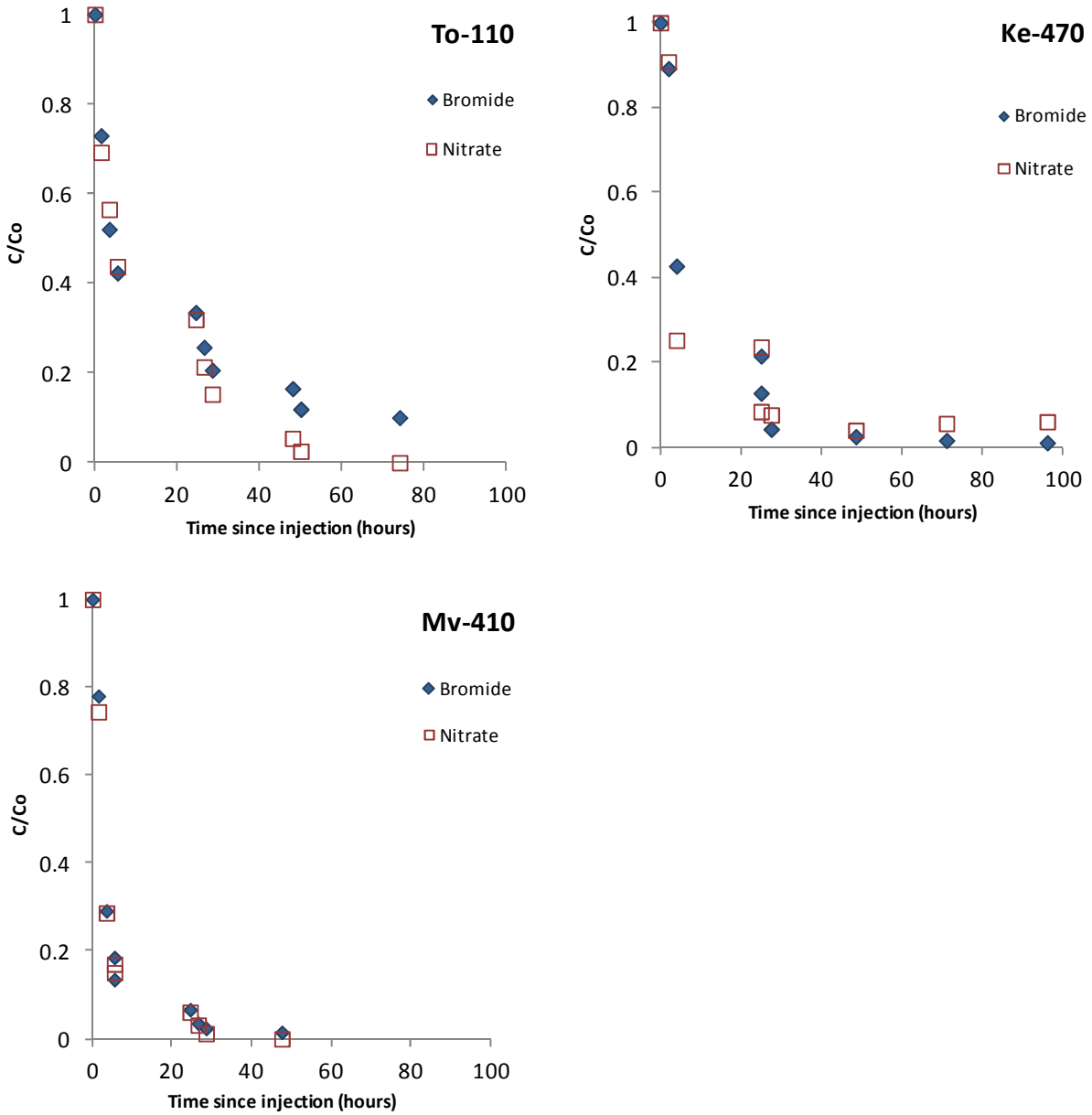

Figure 4.8: Relative concentration profiles of bromide and nitrate vs. hours since injection for the denitrification potential push-pull experiments in three Toenepi wells. Data points are single measurements.

Another reason for the lack of response is the short time scale of the experiment. The 1 – 3 days generally taken could be too short for the resident bacteria to either make a measureable response (as they need time to activate) or grow a population sufficient enough to make a measureable difference to the NO_3^- concentration. Research by Addy et al. (2002) and Kellogg et al. (2005) demonstrated the benefit of priming; however, repeated use of the same well might result in an over-estimation of the 'natural' capacity of the aquifer system.

4.5 Conclusions

Although no denitrification capacity response was observed at any of the well sites, this may be because denitrification rates are too low under natural conditions to be detected with the push-pull method used. When glucose was added as an electron donor for heterotrophic denitrifiers, only the To-110 well showed a response. Rapid movement of the push solution and dilution with up-gradient groundwater may be responsible for the lack of any observed denitrification potential in both the Mv-410 and Ke-470 wells, where relative concentrations of Br^- were <10% of what was added after 24 hours, whereas the To-110 well had a Br^- C/C_o value of 34%. This was contrary to expectations based on the slug test estimated K_{sat} values.

It is also possible that denitrification does not occur in the material directly in contact with the well screen, but further up the groundwater flowpath. Further studies need to assess the aquifer matrix materials in closer detail, to determine whether microbes are absent or too low in numbers for significant denitrification rates to occur.

Chapter 5

The impact of relict organic materials on the denitrification capacity in the unsaturated – saturated zone continuum of three volcanic profiles

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5.1 Abstract

The denitrification capacity of wetlands, riparian zones and aquifers in glacial outwash areas is well documented, but little or no information exists for volcanic profiles, particularly those containing relict organic matter contained in or on top of palaeosols (old soils buried by volcanic deposits) below the groundwater table. Relict carbon (C) contained in these layers could provide the necessary electrons to fuel heterotrophic denitrification. To the best of our knowledge, this is the first study investigating the denitrification capacity in both the unsaturated and saturated zone of volcanic profiles. Samples from three profiles types with differing organic matter distribution were amended with ^{15}N-enriched nitrate (NO_3^-) and incubated in the laboratory under anaerobic conditions. Dinitrogen (N_2) dominated the ^{15}N gas fluxes; averaged across all samples it accounted for 96% of the total ^{15}N (nitrous oxide ($^{15}N_2O$) and $^{15}N_2$) gas fluxes. Dinitrogen fluxes were generally highest in the A horizon samples (4.1 – 6.2 nmol N g^{-1} h^{-1}), but substantial fluxes were also observed in some palaeosol layers (up to 0.72 nmol N g^{-1} h^{-1}). A significant correlation ($p < 0.001$) was found between the concentration of extractable dissolved organic C and the total ^{15}N gas flux produced in samples from below the A horizon, suggesting that heterotrophic denitrification was the dominant NO_3^- attenuation process in this study. Extrapolation of lab-derived denitrification capacities to field conditions suggests that the denitrification capacity of profiles containing relict soil organic matter in the saturated zone exceeds the estimated N leaching from the root zone.

5.2 Introduction

Pastoral farming, the dominant agricultural land use under New Zealand's (NZ) temperate climate conditions, has experienced tremendous intensification during the last few decades. In the past thirty years, the number of dairy cattle has doubled to 6.2 million (Statistics New Zealand, 2011), with a concomitant increase in the use of nitrogen (N) fertilisers. It is well documented that large

quantities of nitrate-N (NO_3^--N) can leach out of the root zone of intensively grazed pastures (Ball et al., 1979; Di and Cameron, 2002a; Di and Cameron, 2002b), particularly from the large number of urine patches that arise from the prevailing year-round grazing regimes. These leaching losses have the potential to contaminate the underlying groundwater and subsequently lead to eutrophication of groundwater-fed surface waters (Böhlke et al., 2007). While eutrophication of streams, rivers and lakes is a world-wide problem, it has become a particularly critical issue in NZ, as land use intensification has begun in recent years in catchments that currently still have near-pristine water quality (e.g. Lake Taupo) (MfE, 2007).

Many studies have observed declining NO_3^- concentrations with depth in the unsaturated (Cannavo et al., 2004) or saturated zones (Gillham and Cherry, 1978; McMahon and Böhlke, 1996; Robertson et al., 1996; Spalding and Parrott, 1994; Trudell et al., 1986) while others (Pfeiffer et al., 2006; Stenger et al., 2008) have found that there are often discrepancies between the estimated concentrations of NO_3^- leaching out of the soil zone and the concentrations measured in the underlying groundwater and groundwater-fed streams and rivers, even when lag times and mixing of water from various sources are taken into account. This apparent NO_3^- attenuation has usually been ascribed to microbially mediated denitrification. The removal of NO_3^- from groundwater through denitrification is an environmentally benign attenuation process, provided complete reduction to inert dinitrogen gas (N_2) occurs. However, incomplete denitrification can result in the release of nitrous oxide (N_2O). Nitrous oxide is both a global warming gas and a precursor to catalysts involved in ozone depletion (Ravishankara et al., 2009). The contribution of groundwater-derived N_2O to global N_2O fluxes is still poorly understood. Most denitrifiers are facultative anaerobes, using NO_3^- as the terminal electron acceptor in oxygen-depleted environments. For heterotrophs, the electron donor is carbon (C), while autotrophs utilise reduced iron (Fe) or sulphur (S) compounds (Rivett et al., 2008).

While there is an ample body of research on denitrification occurring in the soil zone (usually not more than 1 m deep), research on denitrification occurring below the soil zone is still relatively scarce. Only a few studies have investigated denitrification in the deeper part of the vadose zone, i.e. between the soil zone and the saturated zone (Barkle et al., 2007; e.g. Siemens et al., 2003). Studies on saturated zone (or aquifer) denitrification are somewhat more common and review papers have been presented by Korom (1992) and more recently by Rivett et al. (2008). Apart from measurement challenges (e.g. Groffman et al., 2006) the relative scarcity of such studies is presumably partly due to the perceived lack of available electron donors below the soil zone (Siemens et al., 2003). Many studies have revealed that dissolved organic C (DOC) leaches out of the soil zone at relatively low concentrations to groundwaters (<5 mg L^{-1}) and that it is often of low bioavailability (Smith and Duff, 1988; Starr and Gillham, 1993). Recent research has focussed on assessing the availability and reactivity of immobile electron donors residing in the aquifer matrix, often in alluvial or glacial

outwash aquifers with relict organic matter (Hill et al., 2004; Kellogg et al., 2005; Mehnert et al., 2007), lignite (Jørgensen et al., 2009; Strebel et al., 1990) or reduced S compounds (Jørgensen et al., 2009; Korom et al., 2005; Robertson et al., 1996; Schwientek et al., 2008; Strebel et al., 1990). It has increasingly become evident that more than one electron donor can contribute to denitrification occurring in an aquifer. Recently, Korom et al. (2012) used a combination of monitoring, experimentation and modelling to estimate the proportions of major electron donors contributing to denitrification in a glacial-fluvial aquifer in North Dakota. Organic C was the dominant electron donor (43 – 92%); however, pyrite (4 – 18%) and non-pyritic ferrous iron (2 – 43 %) contributed significantly to denitrification. In volcanic landscapes, there may be potential electron donors residing below the depth of the modern soil, namely the remains of vegetation buried by volcanic deposits, and relict soil organic matter (SOM) contained in palaeosols (buried soils). The potential environmental benefits arising from denitrification occurring in the subsurface environment of volcanic landscapes are substantial, as volcanic soils are often intensively managed and can be densely populated, with more than 10% of the world's population living on them (Ping, 2000).

New Zealand is a tectonically active country, and the present-day Lake Taupo was formed in the caldera of a super-volcano. Native vegetation and mountains cover 56% of the catchment area and plantation forestry 22%. Pastoral farming accounts for the remaining 22% of the area. In spite of the relatively low land use intensity of the prevailing sheep and beef operations, pastoral farming has been estimated to be responsible for 36% of the total N load to the lake (equating to 93% of the load that can be managed by human action), (Waikato Regional Council, 2005). Responding to early signs of deteriorating water quality and the identification of a considerable potential for dairy expansion, Waikato Regional Council initiated in 2001 the development of policies to restrict N losses from the land (Waikato Regional Council, 2005).

Concurrent with the policy development, the Waihora research site was established on the north-western side of Lake Taupo to investigate nutrient movement under pastoral farming through the vadose zone (Barkle et al., 2011) and the groundwater system. Our earlier study in the Lake Taupo catchment (Barkle et al., 2007) and groundwater quality monitoring data from the Waihora field site (Stenger, 2011) suggested that NO_3^- leached from the soil zone could potentially be attenuated along the unsaturated – saturated zone continuum before groundwater discharges into Tutaeuaua Stream or directly into the lake.

To test the hypothesis that relict organic materials could provide electron donors for heterotrophic denitrification, a denitrification capacity laboratory experiment was performed. Apart from our earlier work (Barkle et al., 2007) we are not aware of any other study investigating the denitrification capacity of volcanic profiles. While our previous work focussed on unsaturated zone profiles of up to

12 m depth, the current study had a maximum unsaturated zone thickness of 2 m. The greater portion of each profile (approx. 4 m deep) was consequently located below the groundwater table, which in highly porous volcanic profiles often has a strong effect on redox conditions and the long-term preservation of organic matter buried by volcanic deposits. This makes the current study, to the best of our knowledge the first one to address the denitrification capacity in the unsaturated - saturated zone continuum of volcanic profiles.

5.3 Materials and Methods

5.3.1 Lake Taupo Catchment

The present-day Lake Taupo was formed in the caldera of a super-volcano and is located in the middle of the North Island of New Zealand (NZ) (Figure 5.1). The two most significant eruptions from the Taupo volcano occurred approximately 26.5 ka before present (BP), known as the Oruanui eruption, and 1.8 ka BP (Taupo eruption). Both eruptions were massive events, ejecting 1170 km^3 (Oruanui) and 60 km^3 (Taupo) of material across much of the North Island (Molloy, 1988), covering forests and tussock land, reforming the landscape, and creating NZ's largest lake (622 km^2). The lake is highly valued, from both a tourism and recreational point of view. With average concentrations (1999 – 2003) of 70.3 mg m^{-3} total Nitrogen (N), 5.57 mg m^{-3} total Phosphorus (P), 1.18 mg m^{-3} Chlorophyll a, and an average Secchi depth of 14.6 m, its water quality is still near-pristine (Waikato Regional Council, 2005).

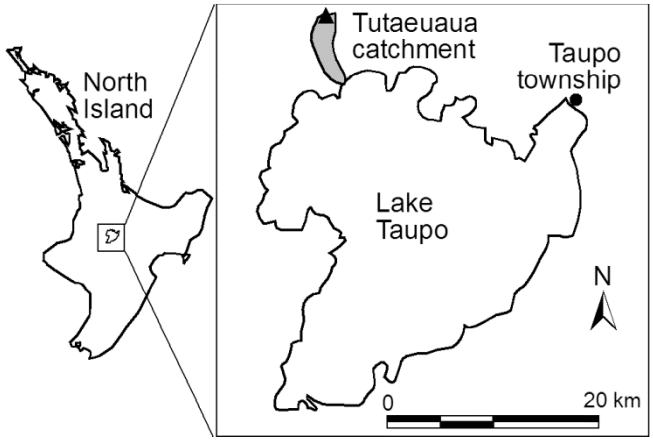

Figure 5.1: Location of Lake Taupo in the North Island of New Zealand. The Waihora Well Field is indicated by the triangle in the top of the Tutaeuaua catchment.

5.3.2 Waihora Field Site

The Waihora field site is located in the headwater area of the Tutaeuaua catchment (175°74.997' E, 38°36.863' S) at the north-western edge of Lake Taupo, NZ (Figure 5.1). The 6000-m^2 site is

characterised by gently rolling land (3 - 8%) sloping down to a wetland area feeding into the Tutaeuaua Stream, with a mean annual air temperature of 11.2°C, average rainfall of approx. 1470 mm and an elevation of 525 – 536 m above mean sea level (Stenger, 2011). The land use changed in 2009 from sheep grazing (8 stock units ha^{-1}) to low intensity calf and heifer grazing. Fertiliser inputs have remained low (250 kg ha^{-1} Sulfur Super (8.7% phosphorus, 14.7% sulfur)) with no N fertiliser applied.

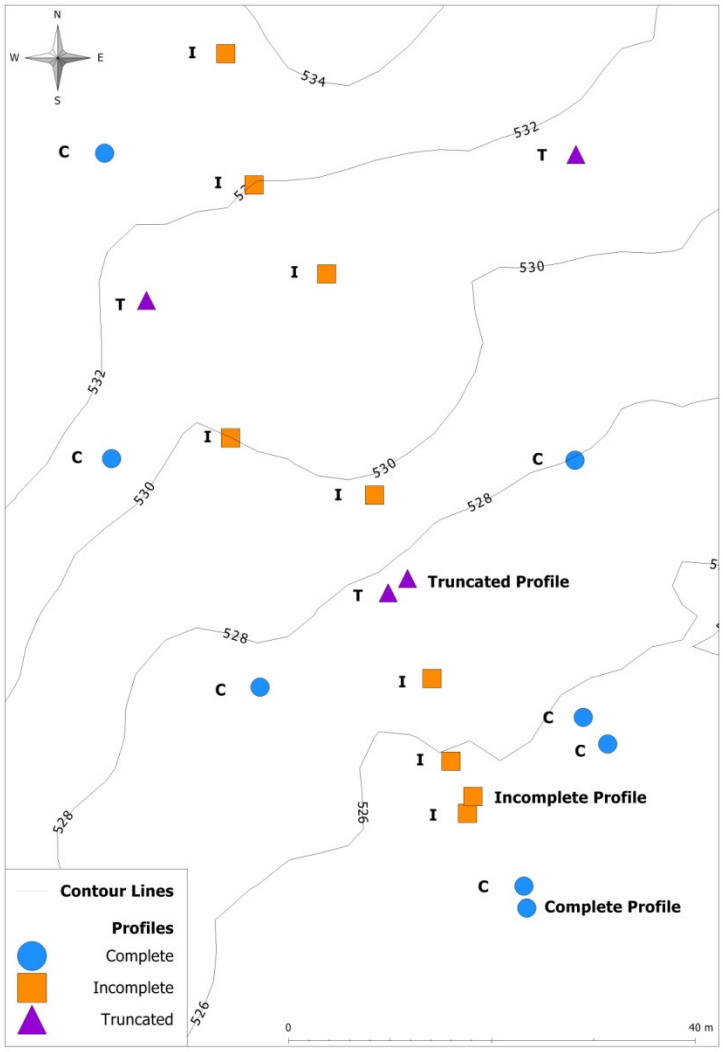

Figure 5.2: Spatial distribution of the three typical profiles in the Waihora Well Field, Tutaeuaua catchment, NZ. Complete profiles = C, Incomplete profiles = I, Truncated profiles = T, boreholes used for experiment indicated by full name of profile.

Three profile types had been differentiated prior to this study, when a series of groundwater monitoring wells (max. depth 8.6 m) were installed at the Waihora field site. These profile types have been categorised as *Complete*, *Incomplete*, or *Truncated.* As shown Figure 5.2, seven of the described profiles belonged to the Complete type (C), eight were Incomplete (I), and three were Truncated (T).

Samples for this laboratory study were taken from three new boreholes of up to 4.5 m depth that were cored at the lower slope of the research site in June 2010, adjacent to previously described profiles (and are labelled Complete Profile, Incomplete Profile, and Truncated Profile in Figure 5.2). The upper part of the three profiles, consisting of the modern soil and its parent material, are quite similar in the three sampled profiles. The modern soil belongs to the Oruanui loamy sand series within the Podzolic Orthic Pumice Soil subgroup (NZ soil classification) and is a mesic Andic Haplorthod according to U.S. soil taxonomy (Rijkse, 2005). The soil depth at the three sampled profiles was between 0.8 and 1.0 m (Figure 5.3). The parent material of the modern soil is the underlying, coarse-sand textured, unwelded Taupo Ignimbrite (TI) deposited during the Taupo eruption approx. 1.8 ka before present (BP). The thickness of the TI layer varied between 1.2 and 1.5 m (Figure 5.3) among the different profiles. Fine textured Palaeosols (buried soils) of late Pleistocene to early Holocene origin (Wilson, 2004) were found at the Complete and Incomplete profiles, but were absent in the Truncated profile.

The Complete and Incomplete profiles differ with respect to the thickness of their Palaeosol layers (P) and the amount and vertical distribution of relict organic materials. The Complete profile has a thick Palaeosol layer (1.0 m) including a relict A horizon, and woody debris from the vegetation destroyed by the Taupo eruption is present at the base of the TI. The Complete profile type has only been found at locations (Figure 5.2) where the base of the TI and the P layers are located below the groundwater table, presumably reflecting lower rates of organic matter mineralisation under saturated conditions. The Incomplete profile contains no woody debris, no relict A horizon and a thinner Palaeosol layer (0.6 m). This profile type was predominantly found where the P layers are located above the groundwater table (Figure 5.2), although in the profile sampled, the P layer was saturated. The Truncated profile has neither a Palaeosol layer nor particulate organic material residing below the depth of the modern soil. It is not known how and when the palaeosol layers were removed at the locations having a Truncated profile. The underlying coarse sand textured Oruanui Ignimbrite (OI) was deposited during the Oruanui eruption approx. 26.5 ka BP (Wilson, 2004).

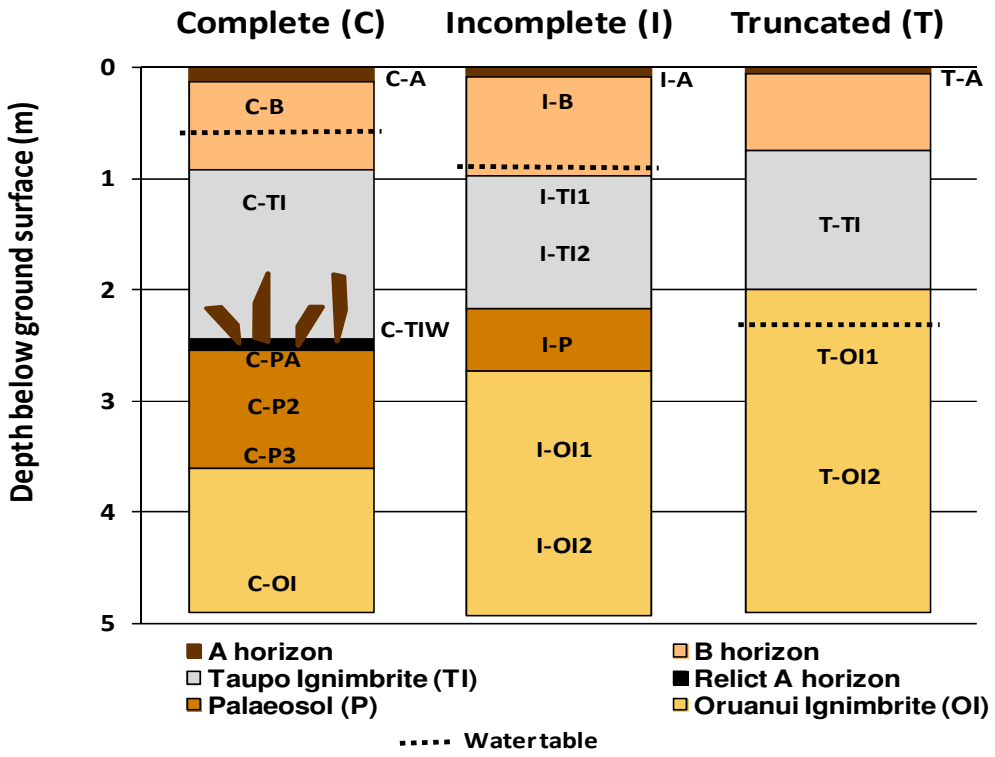

Figure 5.3: Schematic of the three profile types showing the approximate depth of the samples taken. The Complete profile (C) has woody debris in the bottom of the Taupo ignimbrite (TI) and a relict A horizon at the top of the Palaeosol. The Incomplete profile (I) is without the woody debris, and the Truncated profile (T) has no debris or palaeosol. Water table at the time of sampling is indicated by the dotted line.

Volcanic profiles like the ones sampled are characterised by low dry bulk densities and corresponding high total porosities. Detailed analysis of another Incomplete profile at the Waihora field site showed total porosity ranging from 61 to 68% within the modern soil (A and B horizons) and their TI parent material, with higher porosities in the P (70-74%), and lower values (57-59%) in the OI (Barkle et al., 2011).

5.3.3 Sample Collection

The samples for the laboratory study were obtained in June 2010 at the three borehole locations shown in Figure 5.2, using a corer with 80-mm diameter. The depths from which samples were taken were chosen to cover the different material layers (soil A and B horizons, TI, P, OI) and taking differences in the saturation status (unsaturated vs. saturated) and the presumed redox status (indicated by Childs test) into account. This approach resulted in a total of 19 samples, eight from the

Complete profile, seven from the Incomplete profile, and four from the Truncated profile (Table 5.1, Figure 5.3). The fresh weight of the samples ranged from 0.5 to 0.8 kg.

Note that the two uppermost samples from each profile originated from the unsaturated zone, while all deeper samples were from below the groundwater table (Table 5.1). The Childs test (Childs, 1981), for reduced iron (Fe^{2+}), was used as a field indicator of the redox conditions existing throughout the profiles at the time of sampling. Facilitated by their high water content, the samples were bagged tightly after retrieving the cores from the boreholes, thus minimising their exposure to atmospheric air. The samples were immediately put into cooler boxes at the field site before being returned to the laboratory and stored at 2°C for 10 days before analysis.

Note that all samples taken below the A horizon are described as subsoils, although only the B horizon and the palaeosol samples are technically subsoil samples in the narrow sense.

Table 5.1: Sampling depth range and description of the samples taken from the three typical profiles. Unsaturated or saturated conditions and Childs test response (positive = reduced iron (Fe^{2+}) present, negative = Fe^{2+} absent) also noted.

Material	Complete	Incomplete	Truncated
A horizon	**C-A**	**I-A**	**T-A**
	0 – 0.13 m	0 – 0.09 m	0 – 0.06 m
	Unsaturated	Unsaturated	Unsaturated
B horizon	**C-B**	**I-B**	
	0.20 – 0.40 m	0.40 – 0.60 m	
	Unsaturated	Unsaturated	
Taupo Ignimbrite	**C-TI**	**I-TI1**	**T-TI**
	1.00 – 1.20 m	1.00 – 1.20 m	1.55 – 1.75 m
	Saturated, negative	Saturated, negative	Unsaturated
	C-TIW	**I-TI2**	
	2.35 – 2.45 m	1.50 – 1.70 m	
	Contains woody debris, saturated, negative	Saturated, negative	
Palaeosol	**C-PA**	**I-P**	
	2.46 – 2.66 m	2.26 – 2.43 m	
	Includes relict A horizon, saturated, positive	Saturated, negative	
	C-P2		
	3.00 – 3.20 m		
	Middle, saturated, positive		
	C-P3		
	3.30 – 3.50 m		
	Base, saturated, positive		
Oruanui	**C-OI**	**I-OI1**	**T-OI1**
Ignimbrite	4.20 – 4.40 m	3.30 – 3.50 m	2.50 – 2.70 m
	Saturated, positive	Saturated, negative	Saturated, negative
		I-OI2	**T-OI2**
		3.90 – 4.10 m	3.40 – 3.60 m
		Saturated, positive	Saturated, negative

5.3.4 Profile Characterisation

To characterise the C, N and S contents of the different profiles, a range of analyses were undertaken on all samples. Gravimetric moisture content was determined so that all subsequent analyses could be determined using equivalent dry weights (Blakemore et al., 1987). The extractable C and N components of the materials were determined in triplicate by weighing 30 g (equivalent dry weight) of material into a 250 mL extraction bottle and adding 120 mL 0.01 mol L^{-1} $CaCl_2$ solution. Samples were shaken on an end-over-end shaker for 1 hour before being filtered through Whatman 42 filter paper. Extracts were analysed for dissolved organic C (DOC) using an Elementar Hi-TOC II instrument (Elementar Analysensysteme GmbH, Hanau, Germany). Elemental analysis of the total C composition in dried and ground (500 µm) samples was performed using a LECO combustion method (Leco CNS2000 Analyzer, LECO Corporation, St Joseph, USA), while S concentrations were determined by XRF. For this analysis, samples were dried and ground (75 µm) and analysed using a Philips X'Unique XRF Spectrometer fitted with a rhodium tube (Philips Scientific and Industrial, Eindhoven, The Netherlands).

5.3.5 Denitrification Capacity Experiment

An incubation experiment using ^{15}N enriched NO_3^- and based on the method of Yeomans et al. (1992) was conducted to measure the denitrification capacities of the profiles under anaerobic conditions. Denitrification capacities rather than potentials (where NO_3^- and a C-source are added) were measured to enable investigation of the effect the different types and quantities of native C had on the denitrification process. As noted above, it was hypothesised that the woody debris at the base of the TI layer of the Complete profile and the relict soil organic matter in the palaeosol layers of the Complete and the Incomplete profiles would provide the necessary electron donors for denitrification to occur.

The laboratory incubations were carried out in triplicate. 100 g (equivalent dry weight) samples of chilled, field moist soil were weighed into 0.5 L mason jars, and lids fitted with rubber septa, were used to seal the jars. Air-tightness was checked by applying a small amount of pressure and immersing the sealed jar in a waterbath. If no air bubbles were observed, the jar was considered sealed. The atmosphere in each jar was made anaerobic by purging three times for 2 minutes with zero-grade argon, gently shaking the jar in-between to minimise the occurrence of any air pockets.

The jars were allowed to equilibrate for 24 hours at 25°C before 50 mL of 49 atom% ^{15}N-KNO_3 solution (10 µg N g^{-1} dry weight) was added via the septa using a syringe. The relatively low concentrations of native extractable DOC (all horizons < 40 µg C g^{-1} dry weight) required a low N amendment rate to prevent incomplete reduction (Firestone, 1982) or inhibition of the

denitrification process (Lalisse-Grundmann et al., 1988). Lalisse-Grundmann et al. (1988) demonstrated inhibition of the entire denitrification process (not just N_2O reductase activity) when the NO_3^- concentration in an incubation study was increased from 36 to 113 µg N g^{-1} dry weight. Given the small quantities of N occurring in situ, and low concentrations of native C, it was decided that a low N amendment rate as used by Barkle et al. (2007) (who used 5 – 13 µg N g^{-1} dry soil) was more appropriate than rates of 300 – 500 µg N g^{-1} dry soil as used by Bijay-Singh et al. (1989) and Yeomans et al. (1992). Using purged and evacuated Exetainers® (Labco Ltd, High Wycombe, UK), 10 mL samples of the headspace gas were taken at 24 and 48 hours for determination of N_2O concentrations, using GC analysis (SRI 8610, SRI Instruments, Torrance, USA) and at 48 hours (15 mL) for analysis of $^{15}N_2$ and $^{15}N_2O$ by isotope ratio mass spectrometry (PDZ Europa Ltd, Crewe, UK) using the methods of Stevens et al., (1993).

5.4 Results and Discussion

Total C concentrations in each profile decreased very rapidly from their maximum in the A horizon to very low concentrations below the B horizon (Figure 5.4a). Exceptions to this pattern were the palaeosol layers in the Complete and Incomplete profiles, which had elevated total C concentrations (Figure 5.4a). The total C concentration (67,000 mg kg^{-1}) at the top of the Palaeosol layer of the Complete profile (C-PA), which contains a relict A horizon, was within the range found in the three modern A horizon samples (46,000-94,000 mg kg^{-1}). The total C content of the other three Palaeosol samples (two from deeper in the Complete profile (C-P2, C-P3), one from the Incomplete profile (I-P)) ranged from 24,000-33,000 mg kg^{-1}, i.e. they had concentrations substantially higher than found in the modern B horizons of these profiles (max 8,500 mg kg^{-1}). With 13,000 mg kg^{-1}, the sample from the woody debris containing TI base layer of the Complete profile (C-TIW) had a total C concentration lower than the palaeosol samples, but still higher than all other samples from below the modern A horizon.

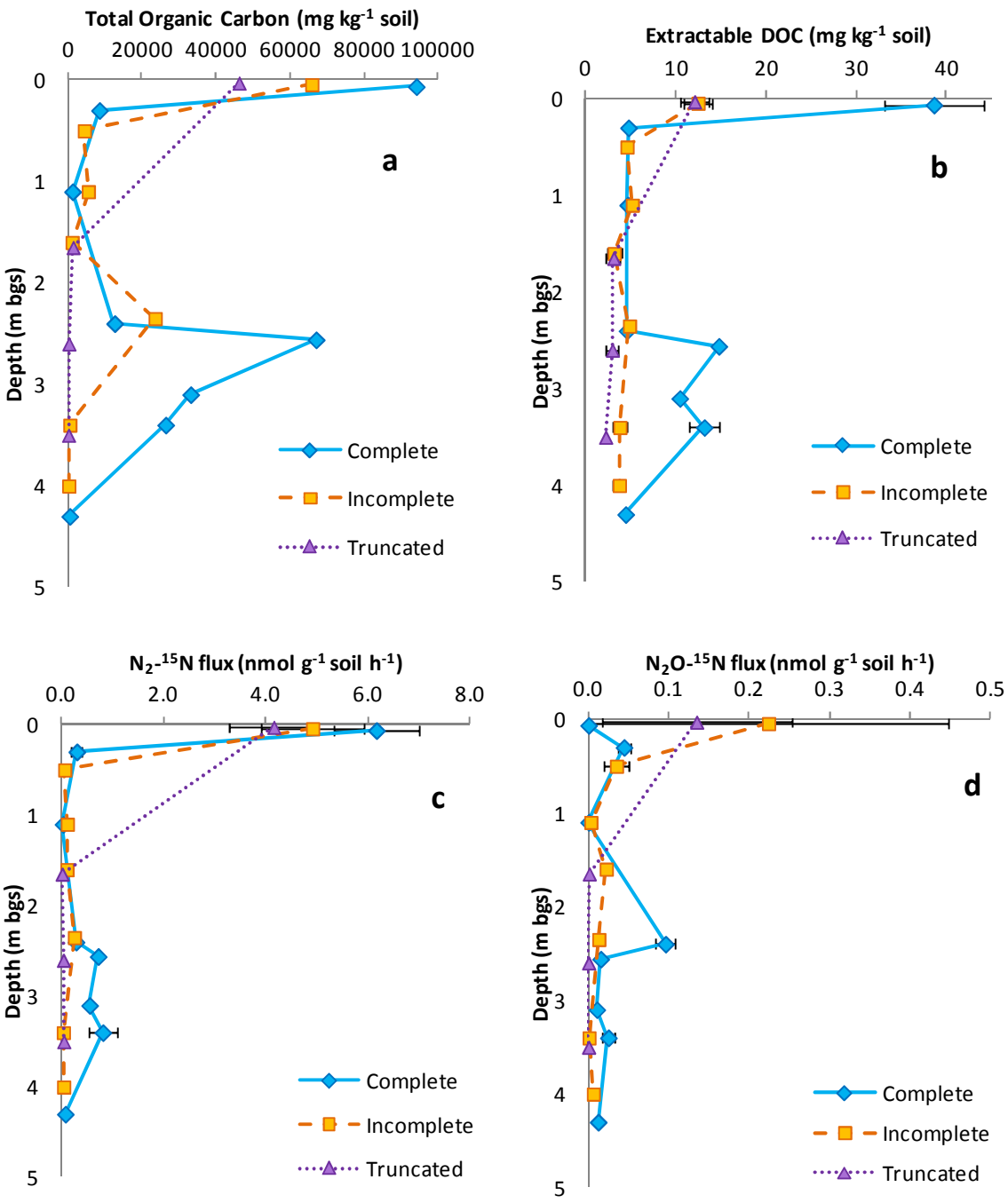

Figure 5.4: Depth profiles of the total organic carbon (C) (mg kg^{-1}) content (a), extractable dissolved organic carbon (DOC) concentration (mg kg^{-1}) (b), hourly $^{15}N_2$ fluxes (nmol N g^{-1} dry soil h^{-1}) (c) and hourly $^{15}N_2O$ fluxes (nmol N g^{-1} dry soil h^{-1}) (d) measured in the three profiles sampled (*n*=3, error bars ± s.e.m, except for total C, where *n*=1).

The depth profiles of extractable dissolved organic carbon (DOC) (Figure 5.4b) had a pattern very similar to the total C profiles. However, in contrast to the distribution of total C, neither the C-TIW, nor the I-P had enhanced concentrations of extractable DOC. Moreover, the peak in extractable DOC

concentration in the C-PA sample was much less pronounced than the corresponding total C peak. This is likely due to the age and recalcitrant nature of the C remaining after 1800 years.

These results confirm that substantial amounts of organic matter can occur in volcanic profiles in the saturated zone well below the depth of the modern soil. The spatially heterogeneous distribution of organic matter, as evident in the differences between the sampled profiles, suggests that denitrification hotspots may occur at the field site.

Dinitrogen was the dominant ^{15}N gas produced in the denitrification capacity experiment, demonstrating that complete denitrification was prevailing. Averaged over all profiles and sampling depths, $^{15}N_2$ accounted for 96% of the Total ^{15}N ($^{15}N_2$+$^{15}N_2O$) gas flux, while $^{15}N_2O$ accounted for only 4% (Figure 5.4c, d). The highest $^{15}N_2O$ flux rate of 0.40 nmol N g^{-1} h^{-1} was observed in the A horizon sample from the Incomplete profile (I-A). However, this high average was caused by one particularly high replicate; the other two had substantially lower fluxes (0.0016 and 0.0017 nmol N g^{-1} h^{-1}) similar to those observed in the samples from the A horizon of the Truncated profile (T-A).

Regression analysis showed that C contents of the samples (total C or extractable DOC) explained a significant proportion of the variability in either $^{15}N_2$ or total ^{15}N gas production when the whole data set was split into the A horizon samples and all other samples (labelled subsoils, Figure 5.5). In contrast, the much lower $^{15}N_2O$ fluxes did not correlate with soil C contents. Accordingly, regression coefficients were generally higher for $^{15}N_2$ fluxes than for total ^{15}N gas fluxes (Table 5.2).

Table 5.2: R^2 of linear regression equations for relationships between ^{15}N fluxes and C contents.

		A horizon	Subsoils
y	**x**	**R^2**	**R^2**
Total gaseous ^{15}N	Extractable DOC	0.81	0.85*
Total gaseous ^{15}N	Total C	1.00	0.72*
$^{15}N_2$	Extractable DOC	0.87	0.89*
$^{15}N_2$	Total C	1.00	0.74*
$^{15}N_2O$	Extractable DOC	0.27	0.00
$^{15}N_2O$	Total C	0.02	0.00

*significant at the 0.001 level

In spite of their initially presumed higher C contents, the I-P and C-TIW samples had denitrification rates only moderately higher than those subsoil samples without a previously identified C source (relict SOM, woody debris; Figure 5.4c, d). However, the low ^{15}N gas flux measured on the I-P sample is in agreement with the lower total C, and particularly the lower extractable DOC concentration that

was measured in that particular Palaeosol sample as compared to the three Palaeosol samples from the Complete profile (Figure 5.4a, b). Similarly, the only moderately enhanced ^{15}N gas flux in the C-TIW sample is in agreement with the C data from that sample. Total C (Figure 5.4a) was only slightly higher than in TI samples without woody debris, suggesting that the debris, due to its size, may have been under-represented in the samples used for the lab experiment. Concentrations of extractable DOC were even more similar between TI samples with and without debris, which presumably reflects the recalcitrant nature of the total C contained in the woody debris. The observation that oxygen (O_2) and NO_3^- -depleted groundwater has consistently been observed in this zone at the Waihora field site supports the idea that the small sample volume used in the lab study may have contained less organic matter than available in situ. The importance of woody debris as an electron donor might thus have been underestimated in the lab incubation.

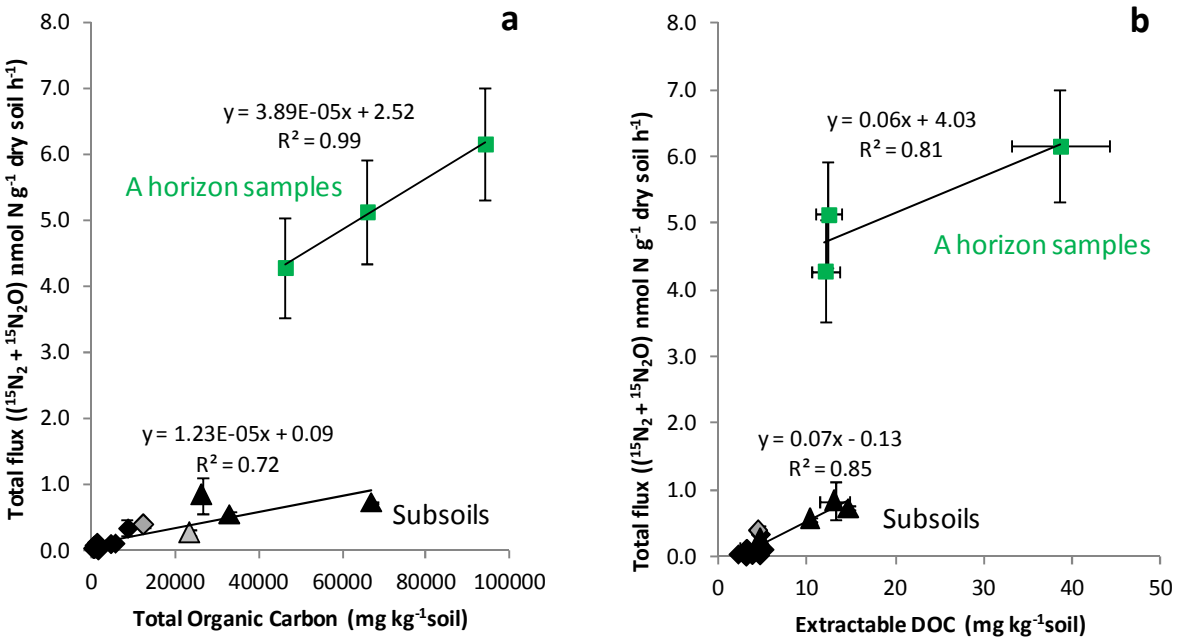

Figure 5.5: Linear relationships between the total organic carbon (mg kg^{-1} soil) content (a) or the extractable dissolved organic carbon (DOC) concentration (mg kg^{-1}) (b) of the soils sampled, and the Total ^{15}N flux ($^{15}N_2$ + $^{15}N_2O$) produced (nmol N g^{-1} dry soil h^{-1}). Square symbols are the A horizon samples from each profile, triangle symbols in the subsoil category denote palaeosol samples from the Complete profile (black) and Incomplete profile (grey). Diamond symbols denote all other subsoil samples, with the Taupo ignimbrite with woody debris sample from the Complete profile highlighted in grey (*n*=3, error bars ± s.e.m, except for total C, where *n*=1) The relationship between total carbon and the total ^{15}N flux is not statistically significant for the A horizon, but is highly significant (p<0.001) for the subsoil samples.

The A horizon samples produced by far the highest total denitrification fluxes ($^{15}N_2$+$^{15}N_2O$) despite some Palaeosol samples from the Complete profile having comparable total C or DOC contents (Figure 5.5a, b). This lower total ^{15}N gas flux per unit of C observed in the subsoil samples compared to the A horizon samples could be due to three factors, either separately or in combination. Firstly, denitrification could be restricted by the low microbial biomass existing in the subsoils. Hot water-extractable C, which is closely related to microbial biomass (Ghani et al., 2003; Sparling et al., 1998), had previously been determined on samples from a different Incomplete profile at the Waihora field site. It dropped by 95% from the A horizon to TI in 1.0 m depth and was only slightly enhanced in the palaeosol layers (Stenger et al., 2006). Secondly, the organic matter residing in the subsoils may have a chemical composition that makes it less available for microbial decomposition than the organic matter in the A horizon. The ratio of extractable DOC to Total C ranged from 2 – 4% in the A horizon samples, but was < 1% in the subsoil samples, presumably reflecting the more recalcitrant nature of subsoil C. Thirdly, the capacity of the microbial communities to decompose organic matter may differ between the A horizon and the subsoils, either due to different activity status or different community composition. Further research, including a denitrification potential experiment, would be required to ascertain the relative importance of these three factors.

In contrast to the A horizon, the denitrification gas fluxes from the sixteen subsoil samples were more closely related to extractable DOC concentrations (Figure 5.5a, b). This result is in agreement with our expectation that extractable DOC should be a better estimate of the C available to microbes than total C. Some 99.7% of the variation in total ^{15}N gas fluxes in the A horizon samples and 85% in the subsoil samples could be explained by the variation in C contents (total C and extractable DOC, respectively) (Table 5.2). While the correlation between the total C content and total ^{15}N gas flux is extremely high in the A horizon samples (r=0.98), the relationship is not statistically significant due to the small number of samples limiting the degrees of freedom to 1. In contrast, the relationship between total C and total ^{15}N gas produced in the subsoil samples is highly significant ($p<0.001$). This observation supports our hypothesis that the ^{15}N gas production is most likely caused by heterotrophic denitrification, while autotrophic denitrification presumably makes at most a minor contribution in many horizons of the profiles sampled. The low total S concentrations measured in most samples (Figure 5.6), which indicate that reduced S compounds cannot be a major electron donor, also support this conclusion. However, enhanced total S concentrations were measured in the C-TIW sample and to a lesser degree in those from the underlying palaeosol layers. It is conceivable that autotrophic denitrification has contributed to the ^{15}N gas production in these samples, particularly in the C-TIW sample, which had the highest S concentration (> 800 mg S kg^{-1}) and the highest $^{15}N_2O$ gas production of all subsoil samples (Figure 5.4d). Böhlke et al. (2002) and Zhang et al. (2009) found reduced S fuelling denitrification in sediments with much higher S contents (up to 8470

mg S kg^{-1}), but Postma et al. (1991) and Jorgensen et al. (2009) both reported denitrification occurring in materials with similar or lower S contents (max. 480 mg S kg^{-1}) than measured in our samples.

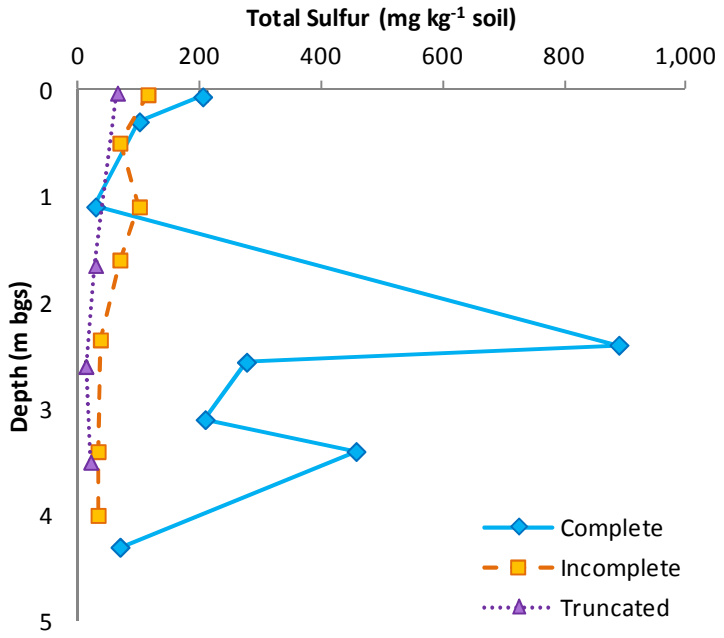

Figure 5.6: Depth profiles of the sulfur (S) content (mg kg^{-1}) measured in the three profiles.

The decrease in available C and the concomitant decline in denitrification rates with depth as seen in the profiles sampled, has been documented in previous studies (Barkle et al., 2007; Jarvis and Hatch, 1994); however, these studies only focussed on the vadose zone. Concerning C availability in the saturated zone, Smith and others (Smith and Duff, 1988; Smith et al., 1991b; Smith et al., 1996) focussed on a sand and gravel aquifer receiving effluent discharged from a sewage treatment facility. It was assumed that denitrification was due to the presence of mobile DOC in the effluent, rather than the result of any interaction with solid-phase C in the aquifer matrix. A denitrification potential experiment (Smith and Duff, 1988) reported a significant increase in N_2O production in response to glucose addition, leading the authors to conclude that the system was C-limited. A similar conclusion was reported by DeSimone and Howes (1996) who found a marked increase in N_2O production when glucose was added to aquifer sediments but also that in situ denitrification was unlikely due to consumption of the effluent-derived DOC in the unsaturated zone and very low concentrations of solid-phase C. Our data show that denitrification in the saturated zone is possible due to the presence of relict organic material commonly found in volcanic landscapes (woody debris, relict SOM) but, as the comparison of the Complete and Incomplete profiles shows, the heterogeneous distribution of that relict OM would result in a spatially-variable amount of denitrification.

The $^{15}N_2$ and $^{15}N_2O$ fluxes measured (over all depths) are within the range reported by Jarvis and Hatch (1994) and Barkle et al. (2007), but the A horizon sample fluxes are substantially lower than those reported by Bijay-Singh et al. (1989) and Hill and Cardaci (2004). Yeomans et al. (1992) also report much larger fluxes, even at 3 m depth. However, direct comparisons are hampered by varying experimental conditions between these experiments (e.g. NO_3^- additions in these experiments ranged from 300 – 500 µg N g^{-1} dry soil, much greater than the 10 µg N g^{-1} dry soil added in this experiment). The study by Barkle et al. (2007) used similar N amendment rates (9 – 13 µg N g^{-1} dry soil), and experimental conditions, yet the authors found no $^{15}N_2$ in samples deeper than 0.9 m, in contrast to our experiment. These findings may reflect the different populations of microbes existing under unsaturated vs. saturated conditions.

While the ^{15}N gas fluxes measured on the subsoil samples are low compared to the A horizon samples, anaerobic conditions will rarely occur in situ in the highly porous A horizon (and in the unsaturated zone in general). However, anaerobic conditions are much more common below the groundwater table, as gas diffusion is reduced by a factor of 10^4 under saturated conditions, effectively preventing replenishment from the atmosphere of any oxygen consumed below the groundwater table. Correspondingly, O_2-depleted groundwater (< 2 mg DO L^{-1}) has been shown to occur at several locations at the Waihora field site (Stenger, 2011). The Childs test undertaken in the field when the samples for the denitrification capacity experiment were collected, demonstrated the presence of Fe^{2+} in the P and OI samples from the Complete profile and the deeper OI sample from the Incomplete profile (I-OI2) (Table 5.1). Given that denitrification precedes ferric iron (Fe^{3+}) reduction in the thermodynamic redox sequence (e.g. Stumm and Morgan, 1995), redox conditions at the field site were apparently suitable for denitrification to occur (at least) in these saturated subsoil samples.

To evaluate what these lab-derived denitrification capacity estimates may mean for NO_3^- attenuation at the Waihora field site, three main factors need to be considered: temperature effect, anaerobic conditions, and long-term C depletion. To account for the difference in temperatures, the total ^{15}N gas fluxes determined at 25°C were adjusted to the mean annual air temperature at the field site (11°C) using a Q_{10} value of 2.0, which resulted in a denitrification capacity rate reduction by 62%. Oxygen-depleted groundwater is commonly found at the field site; almost always in TIW layers and in the majority of Palaeosol and underlying Oruanui Ignimbrite layers, but not at the sites with the Truncated profile (Stenger, 2011). These field observations suggest that in many instances, the denitrification capacity estimated in the lab under anaerobic conditions would not be limited in situ by O_2 availability.

However, in contrast to the short-term lab incubation, gradual depletion of available C would occur in the long term in the subsoils and consequently reduce the fraction of the lab-derived denitrification capacity estimate that can be realised in situ. Using the stoichiometric equation given by Pedersen et al. (1991), the reduction of 1 mg of NO_3^- requires 1.1 mg of organic C. If we assume that extractable DOC is an approximation of the readily available C fraction, the potential amount of DOC used during the 48 h lab experiment can be back-calculated based on the mass of gaseous ^{15}N produced. These results indicate that the C consumption in the subsoil samples equated to a maximum of 6% of the extractable DOC, which would correspond to 2.4% at the lower temperatures prevailing at the field site. Thus, assuming that extractable DOC represents the C fraction available to denitrifiers and that this fraction does not get replenished in the subsoils, in situ denitrification might become limited after a minimum of 10 months of denitrification occurring at capacity level. However, this is unlikely to happen as much less NO_3^- is actually available in situ for denitrification and the microbial community is unlikely to be confined to only using extractable DOC.

To facilitate comparisons with leaching loss estimates, annual denitrification capacities were calculated for each sample using bulk densities measured previously at the Waihora field site (Barkle et al., 2011) and a nominal thickness of 0.1 m. In situ temperature-adjusted total ^{15}N fluxes were then extrapolated to the hectare (ha) scale (kg N ha^{-1} y^{-1}). Denitrification capacity estimates for subsoil materials were 14 kg N ha^{-1} y^{-1} at the base of the TI, and 18 – 27 kg N ha^{-1} y^{-1} in the Palaeosol layers of the Complete profile (per 0.1 m layer). The highest subsoil denitrification capacity estimated at the Incomplete profile amounted to 9 kg N ha^{-1} y^{-1} in the Palaeosol, with TI values being only somewhat lower (5 – 7 kg N ha^{-1} y^{-1}). In the Truncated profile, denitrification capacity estimates below the A horizon did not exceed 2 kg N ha^{-1} y^{-1}. Annual N leaching losses were estimated using the nutrient budgeting model OVERSEER (Ver. 5.4.8, http://www.overseer.org.nz). Reflecting the low land use intensity at the field site, only 9 kg N ha^{-1} y^{-1} were estimated to leach below the base of the root zone (Betteridge and Power, personal communication, 2010). Accordingly, locations with Complete or Incomplete profiles should have sufficient denitrification capacity to quantitatively denitrify the leached NO_3^-, while very little, if any, denitrification is to be expected at locations with the Truncated profile type.

5.5 Conclusion

This experiment demonstrated that a strong relationship exists between the C content of a sample and the ^{15}N gas flux produced, supporting our hypothesis that heterotrophic denitrification is the dominant process in this experiment. A large difference in ^{15}N gas production per unit of C was observed between the A horizon samples and all other subsoil samples, even those with C concentrations similar to the A horizon samples. This indicates that either the quality of the available

C or the metabolic capacity of the microbial community (due to different activity status or composition) was substantially greater in the A horizon samples than in the remainder of the profile. The palaeosol samples from the Complete profile gave the highest subsoil total ^{15}N gas fluxes and calculations extrapolated to annual capacities in the field (18 – 27 kg N ha^{-1} y^{-1} per 10 cm layer) are greater than the estimated N leaching from below the root zone (9 kg N ha^{-1} y^{-1}). Therefore, this research demonstrates that denitrification in the saturated zone of volcanic profiles containing relict organic matter could result in a significant reduction in NO_3^- concentrations. However, little or no denitrification capacity seems to exist where there is no palaeosol present in the subsoil zone. It is worth noting that this lab incubation study may have under-represented the denitrification capacity of the woody debris layer as field observations frequently show reduced, low-nitrate water above the palaeosol layer and this warrants further investigation.

Acknowledgements

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Chapter 6

Denitrification potential in the shallow groundwater of a layered volcanic profile: laboratory incubation experiments

6.1 Abstract

Volcanic profiles with buried organic matter in the form of palaeosols and remnant vegetation offer potential sites for denitrification in shallow groundwater subject to nitrate (NO_3^-) contamination. Previous studies (Chapter 5) showed that denitrification without added carbon (C) was possible at depths of up to 3.5 m below ground surface. It was hypothesised that the relict organic material was not readily available to microbes and that denitrification was C-limited. This was tested in a denitrification potential incubation experiment by comparing the denitrification response to different sources of C: cold water extractable C (CWEC), hot water extractable C (HWEC) and glucose-C (GLU) against the denitrification capacity (NoC). In general, the denitrification capacity was limited by the availability of relict C as greater total N ($^{15}N_2 + N_2O$) gas fluxes were observed for samples with the HWEC or GLU treatments. Inhibition of denitrification was observed in the CWEC treatment as high concentrations of NO_3^- (up to 40 µg N g^{-1}) in the CWEC treatment combined with low pH (all samples <5.5) resulted in denitrification fluxes lower than the corresponding NoC samples. A horizon fluxes generally yielded the highest total N gas fluxes (5.3 – 11.7 nmol N g^{-1} h^{-1}); however, substantial gas fluxes were observed in some palaeosol samples (3.5 – 5.4 nmol N g^{-1} h^{-1}). The microbial population of the deepest samples (4 m below ground surface) limited denitrification as no treatment effects and very low fluxes (0.01 – 0.23 nmol N g^{-1} h^{-1}) were observed. The results suggest that if required, natural rates of denitrification could be enhanced if a readily available source of C were added to the shallow groundwater.

6.2 Introduction

Nitrate (NO_3^-) concentrations in many New Zealand aquifers and surface waters have increased during the last decades, largely reflecting increased leaching losses from increasingly intensive pastoral land use (Daughney and Randall, 2009). Denitrification occurs in groundwater systems and potentially provides a natural attenuation capacity that helps protect the groundwater quality and the quality of groundwater-fed surface waters. It is well documented that incomplete denitrification occurs in the soil/vadose zone (Brown et al., 2001; Höll et al., 2005; Nevison, 2000; Thomas et al., 2012) and to a lesser extent in the saturated zone (Weymann et al., 2008). This can result in substantial nitrous oxide (N_2O) emissions (Nevison, 2000). A lack of information exists regarding the relative importance of factors controlling the dinitrogen (N_2):N_2O partitioning in shallow groundwater

systems. The concentration of dissolved oxygen (DO) and NO_3^-, the pH of groundwater and the bioavailability of electron donors interact and affect the ratio (Blackmer and Bremner, 1978; Firestone et al., 1979; Firestone et al., 1980), but inhibition and expression depend on the individual species involved and local environmental conditions.

The difficulties involved in identifying and quantifying denitrification in groundwater systems are evident in many studies (Böhlke and Denver, 1995; Green et al., 2008; Hendry et al., 1983) and have been elaborated on in several review papers (Groffman et al., 2006; Korom, 1992; Rivett et al., 2008). Knowledge of groundwater flow lines and water ages is important for interpretation of many field observations. However, gaining this information can be both time-consuming and costly. Flow paths may also be subject to temporal variation. Laboratory experiments under controlled conditions offer a means of manipulation not possible in the field, allowing measurement and quantification of denitrification. The use of anaerobic incubation experiments to gain an understanding of the factors limiting denitrification has been well documented (Bijay-Singh et al., 1989; Blackmer and Bremner, 1978; Luo et al., 1996; Yeomans et al., 1992).

Identification of denitrification processes in the subsoil and the saturated zone has also been the subject of recent work in New Zealand (Barkle et al., 2007; Clague et al., 2013; Peterson et al., 2013; Stenger et al., 2008; Stenger, 2011). Much of this previous work has focussed on shallow groundwater systems in agricultural catchments with relict organic matter in or on top of palaeosols (old soils buried by volcanic deposits).

A strong correlation ($p < 0.001$) between extractable dissolved organic carbon (DOC) in the volcanic materials and the total N gas ($N_2O + N_2$) flux generated after 48 hours incubation was found in a previous laboratory-based denitrification capacity experiment (Chapter 5 (Clague et al., 2013)). However, denitrification below the A horizon was shown to be limited either by the availability of the resident (relict) C or the microbial population, as subsoil samples, with similar levels of DOC to some A horizon samples, had much lower N fluxes (Clague et al., 2013).

In Clague et al. (2013) (Chapter 5), and the current experiment, the definitions pertaining to denitrification capacity and potential are as defined by Yeomans et al. (1992). Briefly, the 'denitrification capacity' is defined as the denitrification possible when the microbial community only has the native C available, while the 'denitrification potential' describes the denitrification possible when any substrate-limitation is overcome by adding a C source.

In the current experiment, it was hypothesised that the limited availability of the resident relict C would restrict the denitrification capacity of the profile below the A horizon. This hypothesis was tested in two denitrification potential experiments. Phase I focussed on denitrifier responses to cold-

water extractable C (CWEC) and hot-water extractable C (HWEC) derived from the A horizon of the soil profile sampled. The CWEC was considered a proxy for the C that could leach under field conditions from the A horizon into the subsoil layers and the underlying saturated zone, potentially acting as a recharging electron donor for denitrification occurring in the groundwater zone. The HWEC, which largely consists of lysed microbial biomass (Ghani et al., 2003; Sparling et al., 1998), was chosen to provide a readily available C source. Phase II of the experiment evaluated the bioavailability of the HWEC against glucose, which previous studies have shown to be a readily available source of C (Dendooven et al., 1996; Murray et al., 2004). It was further hypothesised that the HWEC would provide the largest response in terms of N gas fluxes in the first phase, but that glucose addition would produce even larger N gas fluxes in the second phase of the experiment.

6.3 Materials and Methods

6.3.1 Study Site and Sample Collection

Lake Taupo is situated in the middle of the North Island of NZ (Figure 6.1). The lake (622 km^2) formed in the caldera of a super-volcano, and the surrounding catchment comprises native vegetation (56%), plantation forestry (22%) and pastoral farming (22%). Even though the dominant farming type is extensive sheep and beef operations, this low intensity land use has been estimated to contribute 36% of the total N load to the lake (Waikato Regional Council, 2005). The water quality of the lake is still near-pristine, with average concentrations (2007-2011) of 80 mg m^{-3} total N, 5.1 mg m^{-3} total phosphorus, a 16 m Secchi depth and 0.9 mg m^{-3} chlorophyll a. However, rising N concentrations in the highly valued lake prompted the regional council to develop policies that would restrict N inputs into the lake (Waikato Regional Council, 2005).

Samples were collected from the Waihora Well Field, which is located in the upper part of the Tutaeuaua catchment, situated near the north-western edge of Lake Taupo, New Zealand (Figure 6.1). The well field (approximate area of 6000 m^2) is characterised by gently rolling land (3 – 8%) sloping down to a fenced and recently planted wetland area. The site has an elevation of 525 – 536 m above mean sea level, an annual rainfall of 1470 mm and annual air temperature of 11.2°C (Stenger, 2011). The land use has recently changed from sheep grazing in 2009 (8 stock units ha^{-1}) to low intensity calf and heifer grazing; however, fertiliser inputs remain low with no N fertiliser applied.

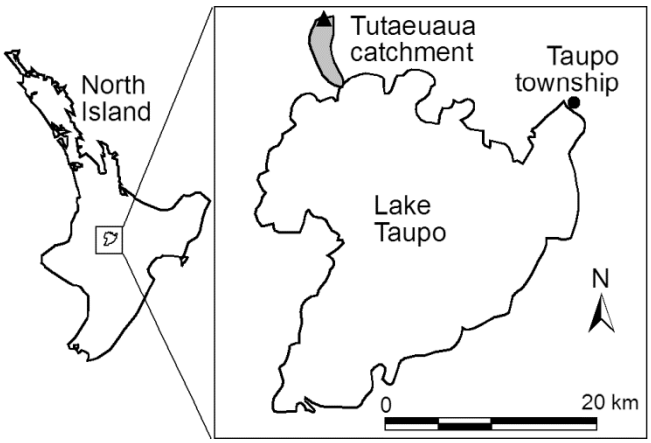

Figure 6.1: Location of Lake Taupo in the North Island of New Zealand. The Waihora Well field is indicated by the triangle in the top of the Tutaeuaua catchment.

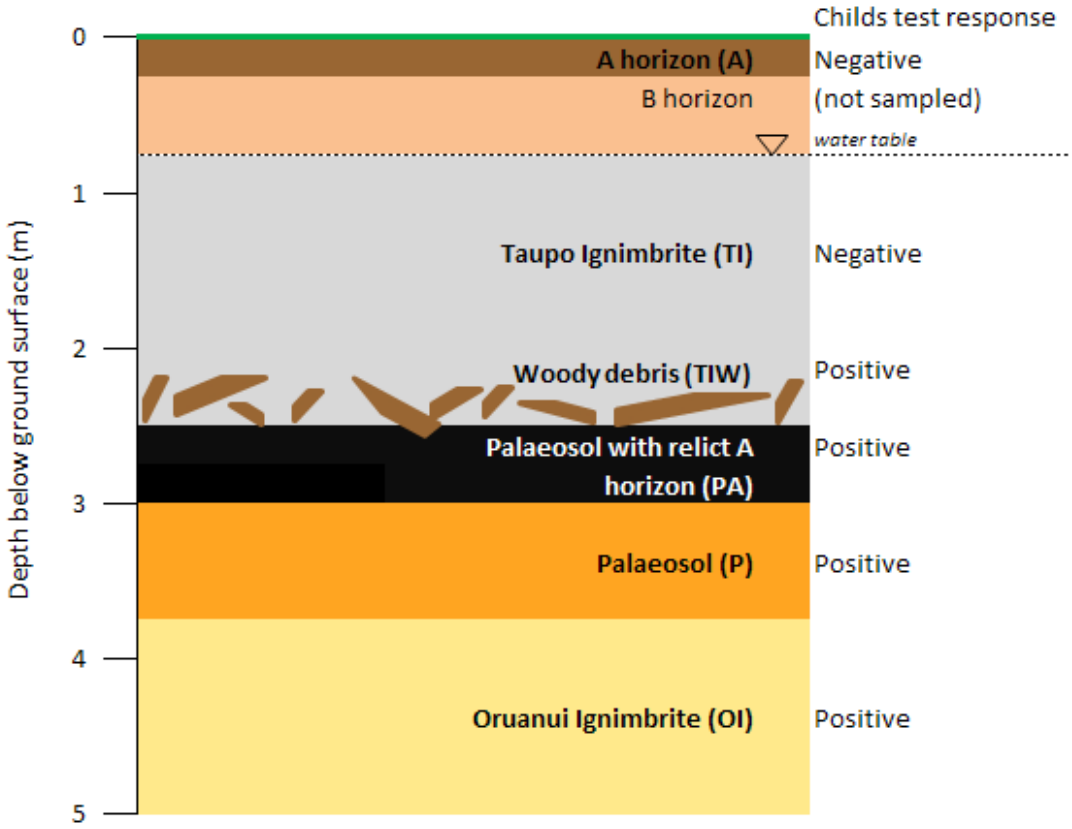

Figure 6.2: Schematic of the lithology of the boreholes sampled showing the layers sampled. Negative response to the Childs test = reduced iron absent, positive = reduced iron present. Note water table at 0.9 m in March 2011.

As described previously (Clague et al., 2013; Chapter 5), three different profile types have been documented at this well field. This study focuses on the 'complete' profile, which at the surface

comprises a modern soil (Oruanui loamy sand) classified as a Podzolic Orthic Pumice Soil (NZ soil classification) or mesic Andic Haplorthod (US soil taxonomy) (Rijkse, 2005). The underlying parent material is a coarse, sandy textured unwelded Taupo Ignimbrite (TI) deposited during the Taupo eruption approx 1.8 ka before present (BP). In this profile, woody debris from the vegetation destroyed during this eruption is present at the base of the TI and overlies the palaeosol (buried soil) (Figure 6.2). The Palaeosol layer is comprised of fine-textured material of late Pleistocene to early Holocene origin with a relict A horizon containing some particulate organic material in the upper part (PA), while the lower Palaeosol layer (P) also contains relict soil organic matter in the matrix, but no wood or roots. The Oruanui Ignimbrite (OI) underlies the P layer (Figure 6.2); this coarse, sand-textured material was deposited during the Oruanui eruption approx 26.5 ka BP (Wilson, 2004).

For the first phase of the experiment, two cores (80 mm diameter) were taken to a depth of 5 m upslope of the wetland, where previous borehole descriptions showed the complete profile occurred. Sampling occurred in March 2011, when the water table was 0.90 m below ground surface. Material from each core, corresponding to the labels A, TI, TIW, PA, P and OI (Figure 6.2) was taken and bagged after testing the redox status by spraying sub-samples of the materials with the Childs reagent (Childs, 1981). This easy-to-conduct field test indicates the presence of ferrous iron (Fe^{2+}) by giving a bright red colour response. Material from the TIW, PA, P and OI layers tested positive at the time of sampling demonstrating the presence of reduced conditions. Note that all but the A horizon samples were taken from below the water table, i.e. from the shallow groundwater system (Figure 6.2).

Samples from the corresponding layers of each borehole were bulked together to give a large, homogeneous volume of material for subsequent analyses. In the TI sample, large (>1 cm) pieces of pumice were removed to allow for more consistent replication. In horizons with relict organic material (TIW and PA), the particulate organic fragments (wood, roots etc) were removed, broken down, and then redistributed into the material, thus ensuring a relatively homogeneous distribution. This process may have changed the availability of the relict C but it was preferable to conducting the experiment with variable replication.

For the second phase of the experiment, a single core (80 mm diameter) was taken in January 2013, close to the previous sampling sites (approximately 10 m apart), when the water table was 0.87 m below ground surface. As the OI layer was not sampled in this experiment, the maximum depth sampled was 2.7 m below ground surface and all samples taken (apart from the A horizon) gave a positive response to the Childs test. These samples were treated the same as the previous samples, with respect to removal of pumice and redistribution of organic matter.

Samples for both phases of the experiment were stored at 2°C until analysis. For the phase I experiment, the storage time was 5 months while the phase II experiment, samples were only stored for 20 days before analysis.

6.3.2 Analytical Methods

6.3.2..1 Profile Characterisation

Gravimetric moisture content was measured on all samples so that subsequent analyses could be calculated on a dry weight basis (Blakemore et al., 1987).

The total N and C contents of the materials were analysed on air-dried and ground (500 µm) samples using the LECO combustion method (Leco CNS2000 Analyzer, LECO Corporation, St Joseph, USA).

The pH of all samples were measured in triplicate using a soil:water ratio of 1:2.5 following the procedure of Blakemore et al. (1987).

A preliminary C extraction investigation was done for both parts of the experiment to ascertain the resident C concentrations. The method of Ghani et al. (2003) was used to determine CWEC (also known as water soluble C) and HWEC for all depths. In brief, four replicate samples of 10 g (equivalent dry weight) were weighed into 250 mL Nalgene® extraction bottles. Then 100 mL of deionised (DI) water was added and samples were shaken on an end-over-end shaker for 1 hour at room temperature (20°C). Samples were then centrifuged for 20 min at 3500 rpm with the supernatant decanted off and filtered through Whatman 42 filter paper, then 0.45 µm syringe-tip filters to yield the cold water extract (CWE). Another 100 mL of water was then added to the sediment in the extraction bottle, washing in any material collected on the filter paper. The bottle was then capped and shaken for 10 sec to re-suspend the material. Bottles were then incubated in an oven at 80°C for 16 hours, removed, shaken to re-suspend the sediment and left to cool for 1 hour before being centrifuged for 20 min at 7000 rpm. A higher spin speed was required to gain a clear supernatant. Samples were then pre-filtered though a Whatman 541 filter, then Whatman 42, then 0.45 µm syringe tip filters to yield the hot water extract (HWE). The extracts were analysed for dissolved organic C (DOC), dissolved inorganic C (DIC) and total dissolved C (TDC) using an Elementar Hi-TOC II instrument (Elementar Analysensysteme GmbH, Hanau, Germany). As all samples had DIC concentrations below the detection limit (1 mg L^{-1}), the total C fraction is entirely comprised of organic C. Therefore, CWEC refers to the DOC concentration of the CWE, and likewise for the HWE. Concentrations of NO_3^- and NH_4^+ were also analysed on each extract using standard methods APHA4500-NO_3^- I and APHA 4500 NH_3 F, respectively (APHA, 2005).

For the bulk extraction of the A horizon, it was calculated that a decrease in the soil:water ratio would be required to extract a sufficient amount of DOC into the CWE, therefore 24 bottles with 50 g (dry weight equivalent) and 100 mL of water were used. After the difficulties in obtaining a clear supernatant in the preliminary investigation, the centrifuge spin speed was increased to 10,000 rpm and 0.45 µm mixed cellulose ester filters (Millipore) under vacuum were used. The greater spin speed resulted in a compaction of the sediment which was difficult to re-suspend by hand-shaking, so samples were placed on the end-over-end shaker for 30 min after the second addition of 100 mL. The bottles were then placed in the oven at 80°C for 16 hours, removed, then shaken and left to cool for 1 hour before centrifugation at 10,000 rpm for 20 min. The supernatant was then filtered using 0.45 µm mixed cellulose ester filters under vacuum to yield the HWE, which was then diluted 6x in order to have similar rates of C amendment as the CWE.

6.3.2..2 Denitrification Potential Experiment

The denitrification potential experiment involved some changes to the procedure outlined by Clague et al. (2013) (Chapter 5), namely the redistribution of fragmented organic material as described previously and an increase in the water:soil ratio, resulting in a slurry to facilitate contact between microbes and substrates.

In the first phase of the experiment, five replicates of 50 g (equivalent dry weight) of chilled, field moist soil were weighed into 0.5 L mason jars, and lids fitted with rubber septa, were used to seal the jars. The seal on each jar was checked for air-tightness by immersion in a waterbath and the application of a small amount of pressure in the jars. Jars were considered sealed if no bubbles were observed. Jars were then made anaerobic by purging the headspace with zero-grade argon for 2 minutes, three times, gently shaking the jar in between to minimise air pockets in the soil and create an anaerobic environment. After a conditioning period of 24 hours (Chirinda et al., 2011) at 25°C, jars were opened and 10 mL of the ^{15}N-enriched (49 atom%) KNO_3 solution was added (10 µg N g^{-1} dry soil). Then, according to the treatment, either 113 mL of DI water (NoC treatment), 90 mL of the CWE and 23 mL of DI water (CWE treatment), or 10 mL of the HWE and 103 mL DI water (HWE treatment) were added and mixed into the material using a spatula. The different extract:water ratios were used in order to add the same mass of C. This gave a slurry with a material:water ratio of 1:3, and a theoretical C:N ratio of 5:1. This is lower than what other authors have previously used and was a compromise between the goal of applying the same mass of C in the two treatments and the unexpectedly low concentrations of CWE-DOC in the A horizon (Table 6.1). Post-experimental analysis of the extracts used showed C additions were lower than intended and CWEC and HWEC concentrations were 33 and 36 µg C g^{-1} soil, respectively. This variation is presumably due to the inherent variability of C and N contents of the soil, and the different soil:water ratios affecting the extraction efficiency. For the HWE treatment, the C:N ratio became 3:1, while an unexpectedly high

concentration of resident NO_3^- in the A horizon (41 mg N kg^{-1}) was extracted into the CWE, and contributed to an overall C:N ratio of 1:1.5. Thus, in the CWE treatments, NO_3^- was in excess of the added C. The remaining HWE was frozen for use in phase II of the experiment.

After the additions, jars were re-sealed, checked and flushed with argon to create an anaerobic headspace and incubated at 25°C. Although the microbial population would be adapted to lower temperatures (average air temperature at the site of 11°C), Bijay-Singh et al. (1989) have shown that a greater denitrification response was observed at higher temperatures than would be found *in situ* (8°C vs. 20°C). Therefore, 25°C was chosen to ensure a rapid and measureable response and is within the range (20 - 30°C) given by others (Barkle et al., 2007; Bijay-Singh et al., 1989; Blackmer and Bremner, 1978; Clague et al., 2013; Luo et al., 1996).

Duplicate headspace samples were taken at 48 hours for $^{15}N_2$, $^{15}N_2O$ and $^{13}CO_2$ analysis by isotope ratio mass spectrometry (15 mL; PDZ Europa Ltd, Crewe, UK) using the methods of Stevens et al. (1993).

The analytical procedure for the second phase of the experiment was very similar; however, some modifications were made to ensure an air-free environment was achieved. Jars were flushed with zero grade argon for 3 min, then evacuated for 5 min, and then filled and flushed with argon for another 3 min to ensure no air remained in the soil or headspace. The experiment was carried out in triplicate, with 25 g (equivalent dry weight) samples. In order to compare the two experiments, the HWE used in phase I was defrosted and re-used for phase II. The glucose (GLU) treatment had the same C:N ratio as the HWE treatment (3:1), as used in the previous experiment, with N provided as ^{15}N-enriched NO_3^-.

6.3.3 Statistical Analysis

Statistical analyses were performed using Microsoft Excel 2007. Means, standard deviations and standard errors were calculated for different parameters and where appropriate, significant differences between means were determined using Student's t-test.

6.4 Results and Discussion

6.4.1 Denitrification Potential Phase I

Soil total C, CWEC, and HWEC concentrations varied within the profile sampled. The soil total C concentration in the PA horizon was approximately 50% of that found in the A horizon (Table 6.1). However, the CWEC concentration in the PA horizon was almost twice that of the A horizon. While the CWE is acknowledged as containing the most labile and water-soluble forms of C, Ghani et al. (2003) found greater variability and a weaker correlation with total soil C, as well as lower

concentrations overall. This is likely due to the high degree of spatial variability associated with A horizon samples as a result of grazing (including urine and faeces deposition) and fertiliser application.

The distribution of HWEC within the profile closely followed total C concentrations, with the exception of the OI layer, which had a much larger proportion of HWEC compared to the soil total C (11% in OI, all other layers were <5%). The HWEC concentration in the A horizon was larger than the values obtained by Sparling et al. (1998) for Pumice Soils (424 – 600 μg C g^{-1}). This could be due to the higher extraction temperature used (80°C vs. 70°C). However, the A horizon HWEC compares well to other reported values. For example, Ghani et al. (2003) found 2139 – 5093 μg C g^{-1} for A horizon soils under sheep and beef farming using 80°C. Our result is at the lower end of this range probably because the sampling depth used was much greater (0 – 180 mm vs. 0 – 75 mm). Ghani et al. (2003) demonstrated that HWEC provided a measure of soil quality while Sparling et al. (1998) showed that HWEC could be used as an approximation of microbial biomass C. Thus the HWE is a source of C considered highly available to microbes. The variable distribution of HWEC through the profile indicates that the resident microbial population varies substantially with depth, with the TI layer having the smallest microbial biomass.

Table 6.1: Soil total C, dissolved organic C (determined from cold water extract (CWE) and hot water extract (HWE)) and soil pH for the profile sampled in phase I of the denitrification potential experiment. Values represent averages of either 3 (Total C and pH) or 4 (CWEC and HWEC) replicates.

Horizon	Total C (μg g^{-1})[a]	CWEC (μg g^{-1})[b]	HWEC (μg g^{-1})[b]	pH[c]
A	96259	54	2929	4.07
TI	1672	12	27	5.24
TIW	12020	8	74	4.11
PA	50899	106	325	4.28
P	27028	17	101	4.38
OI	752	13	89	5.11

[a] Total C on the soil by LECO combustion (Blakemore et al., 1987)
[b] Hot and cold water extracts (Ghani et al., 2003)
[c] pH_{H2O} soil:water 1:2.5 (Blakemore et al., 1987)

The addition of HWEC generated higher ($p < 0.05$) $^{15}N_2$ fluxes than either the CWE or NoC treatments (Figure 6.3) with the exception of the A horizon sample, indicating that samples below the A horizon were C-limited.

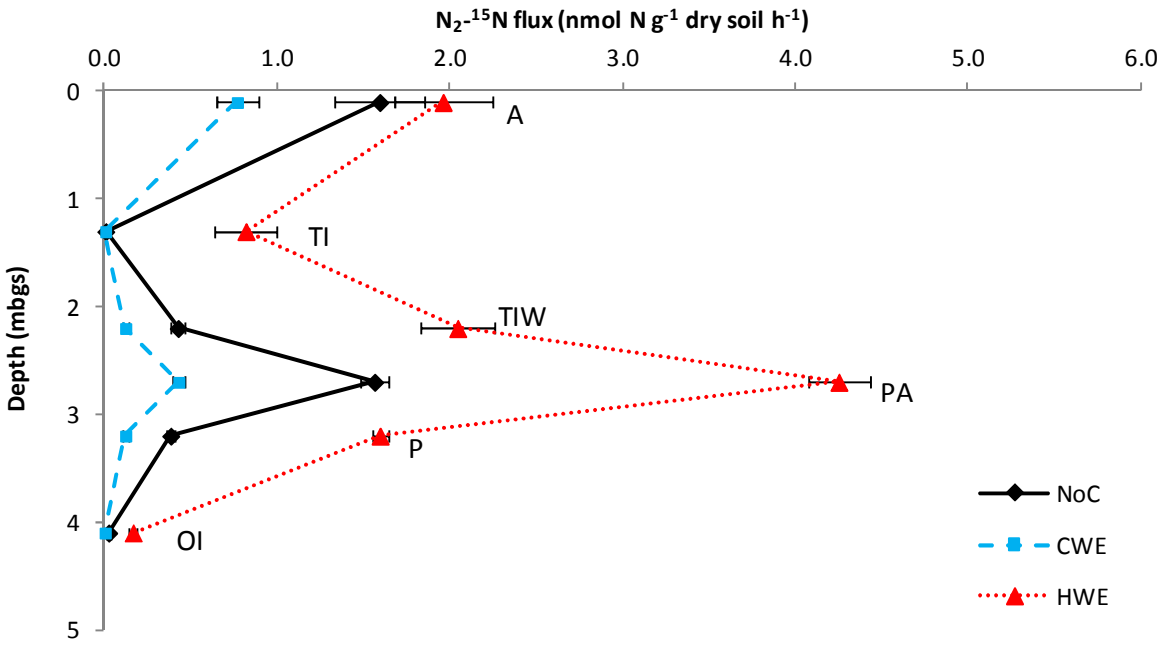

Figure 6.3: Depth profile of the hourly $^{15}N_2$ fluxes (nmol N g^{-1} dry soil h^{-1}) measured in the three treatments after 48 hours incubation in phase I of the experiment (n=5, error bars ± s.e.m.).

Addition of the CWE inhibited $^{15}N_2$ production in the TIW, PA and P samples, which produced lower ($p < 0.05$) $^{15}N_2$ fluxes than the NoC treatment (Figure 6.3). This was presumably due to the high concentration of NO_3^- inadvertently extracted from the A horizon soil and added to these samples, changing the C:N ratio from the calculated 3:1 to 0.66:1. An inhibitory effect of high NO_3^- concentrations on the reduction of N_2O to N_2 has been documented previously (Blackmer and Bremner, 1978; Firestone et al., 1979). However, in these studies, the total N gas production remained unchanged and only the N_2:N_2O ratio changed. In contrast, this study (Figure 6.4) and that of Lalisse-Grundmann et al. (1988) showed a decrease in total N gas production when elevated concentrations of NO_3^- were present. No N_2:N_2O data is available for the experiment by Lalisse-Grundmann et al. (1988) as acetylene was used and only N_2O fluxes were measured. Blackmer and Bremner (1978) also noted an inhibitory effect due to the interaction between NO_3^- concentration and low pH (5.7 – 6.8). The pH was measured (Table 6.1) on all samples at the end of the incubation, as well as non-incubated samples. No differences in pH were seen between the incubated and non-incubated samples, and the pH of the CWE samples were no different to the other treatments. However, the samples were all acidic, with pH values between 4.05 and 5.55 (Table 6.1). Therefore, it is the combination of low pH and high NO_3^- that has inhibited denitrification in the CWE treatment.

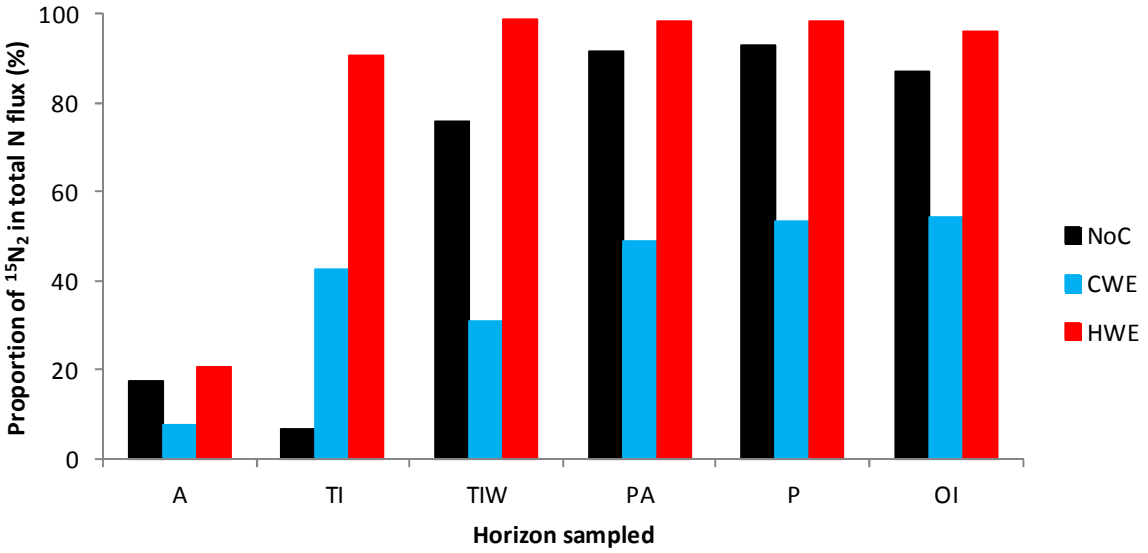

Figure 6.4: The $^{15}N_2$ component (%) of total N gas fluxes ($^{15}N_2$ + N_2O) measured in the three treatments after 48 hours incubation in phase I.

In most samples, the $^{15}N_2$ flux was greater than the N_2O flux, i.e. >50% (Figure 6.4). The exceptions to this were results from the A horizon, the NoC treatment for the TI horizon where the overall fluxes were small, and many samples from the CWE treatment (Figure 6.4).

A variation from the previous experiment (Chapter 5) in the reported total N flux measurements was decided upon due to the high antecedent NO_3^- concentrations (at least 40 mg N kg^{-1}) measured in the A horizon. The total N gas flux represents the $^{15}N_2$ and all N_2O measured, not just the $^{15}N_2O$ component. This was justified by 3 factors: firstly, that microbes will preferentially use the lighter resident NO_3^- rather than the added $^{15}NO_3^-$, resulting in a lower atom % being measured, effectively diluting the added signature. Secondly, N_2O production in these samples contributes a significant flux of N, and it is mostly $^{14}N_2O$. Omission of this data gives a false impression of the actual level of activity of the sample (Figure 6.5). Thirdly, the purpose of adding an enriched NO_3^- source was for effective measurement of N_2 fluxes, which can be difficult to determine as ambient concentrations are high, and a completely air-free environment is difficult to establish. Since N_2O occurs at low levels in the atmosphere, it is presumed that the elevated N_2O concentrations measured are derived solely from denitrification in the sample. It is also presumed that the antecedent NO_3^- affects the composition of N gas production in a similar manner as an equivalent concentration of $^{15}NO_3^-$, i.e. if a large amount of $^{15}NO_3^-$ were added, incomplete denitrification would predominate.

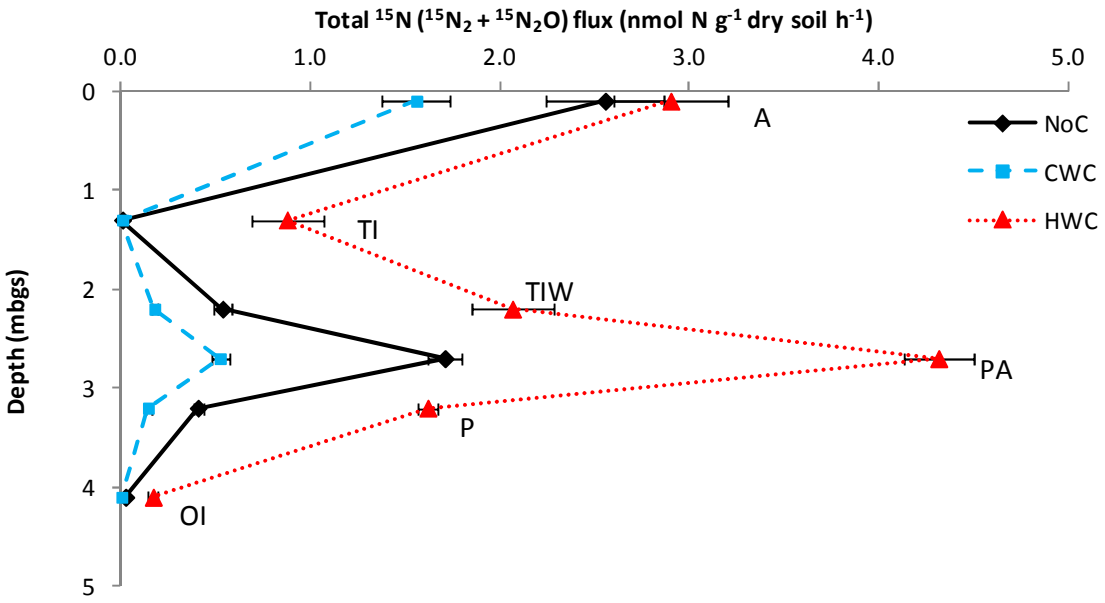

Figure 6.5: The Total ^{15}N fluxes ($^{15}N_2$ + $^{15}N_2O$) (nmol N g^{-1} dry soil h^{-1}) measured in the three treatments after 48 hours incubation in phase I (n=5, error bars ± s.e.m.).

Nitrous oxide production was <0.5 nmol g^{-1} h^{-1} in all depths except the A horizons, where fluxes up to 9 nmol g^{-1} h^{-1} occurred, of which, only 0.8 – 1 nmol g^{-1} h^{-1} was derived from ^{15}N. Contrary to expectation and previous results (Clague et al., 2013) (Chapter 5), the N_2O fluxes measured in the A horizon samples were much greater than the corresponding $^{15}N_2$ fluxes, with only 1 – 2 nmol g^{-1} h^{-1} of $^{15}N_2$ being produced. The cause of this incomplete denitrification is presumably due to the high antecedent NO_3^- concentrations (at least 40 mg N kg^{-1}) in these samples inhibiting the N_2O-reductase activity, as previously observed by Blackmer & Bremner (1978) and Firestone et al. (1979).

This high NO_3^- concentration in the A horizon biased the CWE and therefore is also the cause for the comparatively low $^{15}N_2$:N_2O ratios measured in samples of the CWE treatment (Figure 6.4). As the extraction procedure was sequential, very little NO_3^- remained to be extracted in the hot water procedure (0.22 mg N kg^{-1} soil measured in the HWE). In almost all layers, the CWE treatment had a greater proportion of the total flux generated as N_2O, compared to the NoC and HWE treatments (Figure 6.4).

The low proportion of $^{15}N_2$ measured in the TI-NoC samples was a function of measurement uncertainty as these samples had very low fluxes (<0.1 nmol N g^{-1} h^{-1}) from the $^{15}N_2$ enriched pool.

When both $^{15}N_2$ and N_2O fluxes were considered together (Figure 6.6), the significance of the contribution of N_2O to the A horizon total N flux becomes clear as the largest total N gas fluxes were

generated by the A horizon samples. The A horizon treatments did not differ from each other and were double the next largest flux production (PA-HWE treatment); indicating that the samples were most likely not C-limited. However, it is possible that the HWE did not provide any increase in available C; i.e. it was less bio-available than expected. However, the strong treatment response seen in most of the other depths implies that this was not the case. The increase ($p < 0.05$) in total N fluxes with the addition of HWEC, as seen in the TI, TIW, PA and P samples suggests that these layers were C-limited. In contrast, the very similar and low (<0.2 nmol N g^{-1} h^{-1}) fluxes measured in the OI samples indicates that perhaps the microbial population was limiting denitrification in this layer. Although the HWEC concentration of the OI sample was greater than the TI layer (Table 6.1), denoting a larger microbial biomass (Sparling et al., 1998), this analysis gives no information on the individual microbial species. Therefore, it is likely that the OI sample was specifically denitrifier-limited with respect to heterotrophic processes. This conclusion was supported by the CO_2 flux data (Figure 6.7), which showed very similar CO_2 fluxes generated by the HWE treatments of the TI and OI samples (1.5 and 1.7 nmol C g^{-1} h^{-1} respectively).

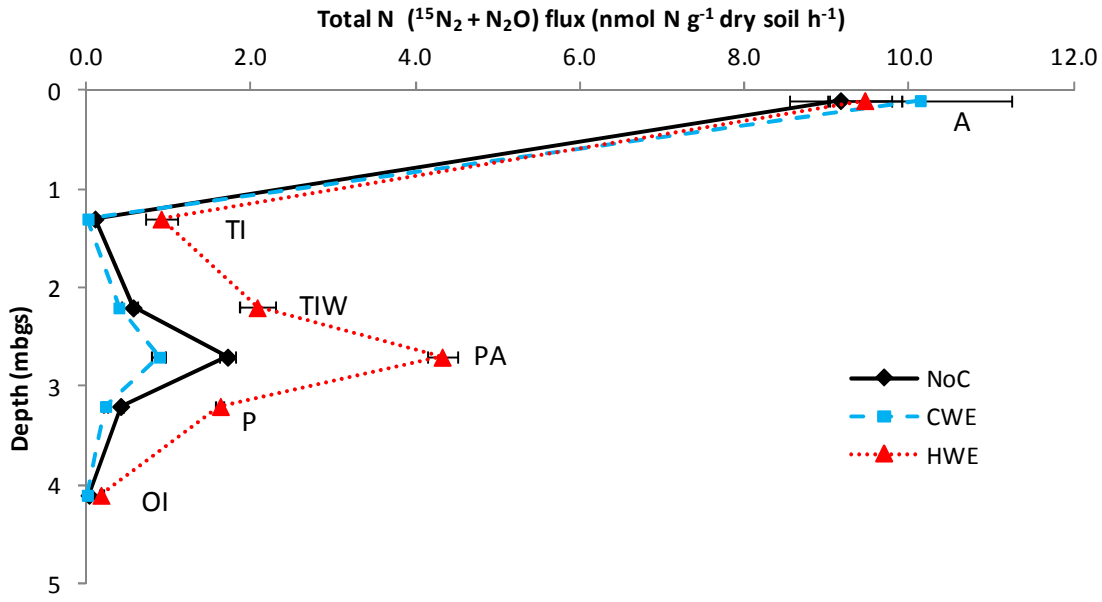

Figure 6.6: Total N fluxes ($^{15}N_2 + N_2O$) (nmol N g^{-1} dry soil h^{-1}) measured in the three treatments after 48 hours incubation in phase I (n=5, error bars ± s.e.m.).

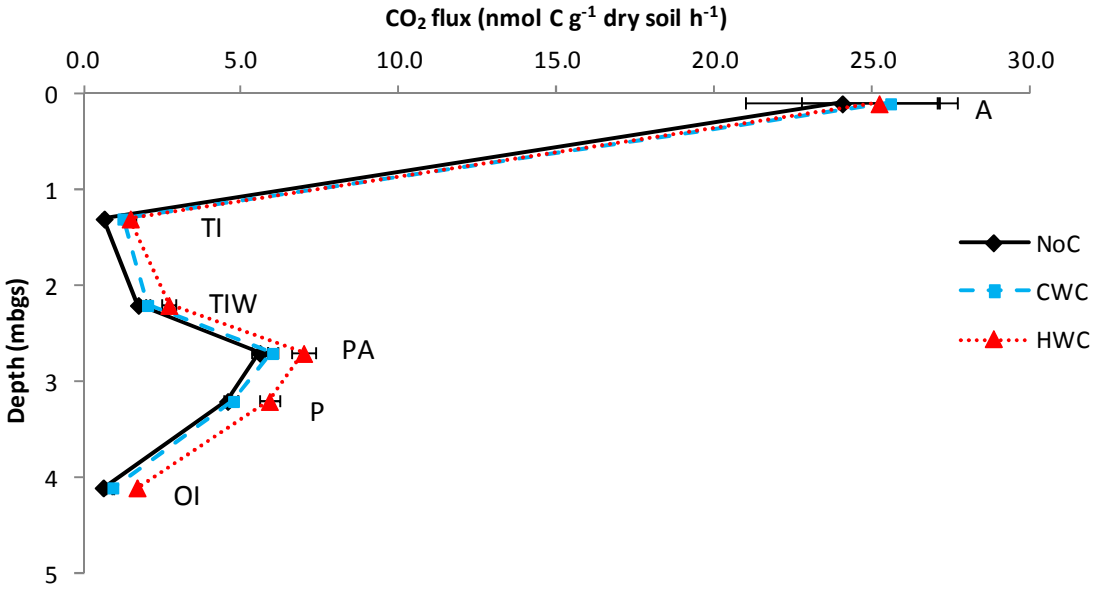

Figure 6.7: CO_2 fluxes (nmol C g^{-1} h^{-1}) measured in the three treatments after 48 hours incubation in phase I (n=5, error bars ± s.e.m.).

6.4.2 Denitrification Potential Phase II

The second phase of the denitrification potential experiment was triggered by the small increase in CO_2 production (Figure 6.7) in response to the HWE treatment in the initial incubation. It was hypothesized that the C in the HWE treatment was not as bioavailable as glucose-C, the most commonly used source of C for denitrification potential experiments (Dendooven et al., 1996; Jahangir et al., 2012; Murray et al., 2004) and that the system was C limited rather than being microbe limited.

Table 6.2: Soil total C and N, dissolved organic C (determined from cold water extract (CWE) and hot water extract (HWE)), NO_3^--N and NH_4^+-N (determined from CWE) for the profile sampled in phase II of the denitrification potential experiment.

Horizon	Total C (µg g^{-1})[a]	Total N (µg g^{-1})[a]	CWEC (µg g^{-1})[b]	HWEC (µg g^{-1})[b]	CWE-NO_3^--N (µg g^{-1})[b]	CWE-NH_4^+-N (µg g^{-1})[b]
A	65200	5190	25	772	1.3	3.1
TI	1200	90	22	23	0.8	4.5
TIW	26300	650	35	76	0.8	4.8
PA	44200	2010	58	109	0.3	4.6
P	39100	1890	51	86	1.9	5.0

[a] Total C and N on the soil by LECO combustion (Blakemore et al., 1987)
[b] Hot and cold water extracts (Ghani et al., 2003)

The total C and N concentrations had similar vertical distributions, but the C:N ratio varied from 13:1 (in the A and TI horizons) to 41:1 in the TIW layer. The high C content of the TIW layer presumably reflects the relict vegetation, which was predominantly found as large (2 – 5 cm) pieces of wood.

The vertical profile distributions of the CWEC and HWEC concentrations from the second phase were similar to the previous incubation, although different C concentrations were measured (Table 6.1; Table 6.2). Low concentrations of NO_3^- were also measured in the CWE samples (Table 6.2), and were similar to those measured in phase I, with the exception of the A horizon where a higher concentration (40 μg N g^{-1}) was measured in the phase I sample. The difference between A horizon samples is most likely due to the occurrence of antecedent urine spots although other possibilities include slight differences in landscape position (e.g humps vs. hollows) and seasonal variations in rainfall, pasture growth and grazing.

Very little N_2O was measured in samples after 48 hours incubation, as shown in Table 6.3, in contrast to the previous experiment (Figure 6.4). This was presumably due to the lower NO_3^- concentrations measured in the CWE for all horizons sampled (Table 6.2), thus enabling complete denitrification to occur. For ease of comparison with the phase I data, the total N flux represents the $^{15}N_2$ flux and all the N_2O flux, rather than the $^{15}N_2O$ component as reported in Chapter 5. There is virtually no difference between the two N_2O values (up to 0.005 nmol N g^{-1} h^{-1}).

Table 6.3: Proportion of $^{15}N_2$ gas flux in total N gas flux (%) measured in the three treatments after 48 hours incubation in phase II.

	Proportion of $^{15}N_2$ in total N gas flux (%)		
Horizon	**NoC**	**HWE**	**GLU**
A	99	99	100
TI	87	98	100
TIW	98	99	100
PA	99	100	100
P	100	100	100

The profile distribution of the total N gas flux (Figure 6.8) was unexpected, as in contrast to previous results, the N gas fluxes produced in the P layer, were for all treatments, similar in magnitude to the A horizon fluxes. The total N gas fluxes generated by the different treatments of the A and P layers did not differ ($p > 0.05$), suggesting that the samples were not C limited, but possibly limited by low microbial populations. However, the CO_2 fluxes produced by these samples differed with horizon (Figure 6.9). The A horizon treatments produced more than twice the CO_2 of the P samples, suggesting that other heterotrophic processes were active in the A horizon, but not in other horizons.

In the PA horizon, a treatment effect ($p < 0.05$) was observed between the HWE and NoC treatments but the GLU treatment fluxes were not significantly different to the NoC samples. Both C

amendments differed ($p < 0.05$) relative to the NoC samples in the TI and TIW layers, indicating these samples were C-limited. The GLU treatment yielded higher ($p < 0.05$) fluxes for both horizons, confirming the hypothesis that the HWEC did not provide as much readily available C as glucose.

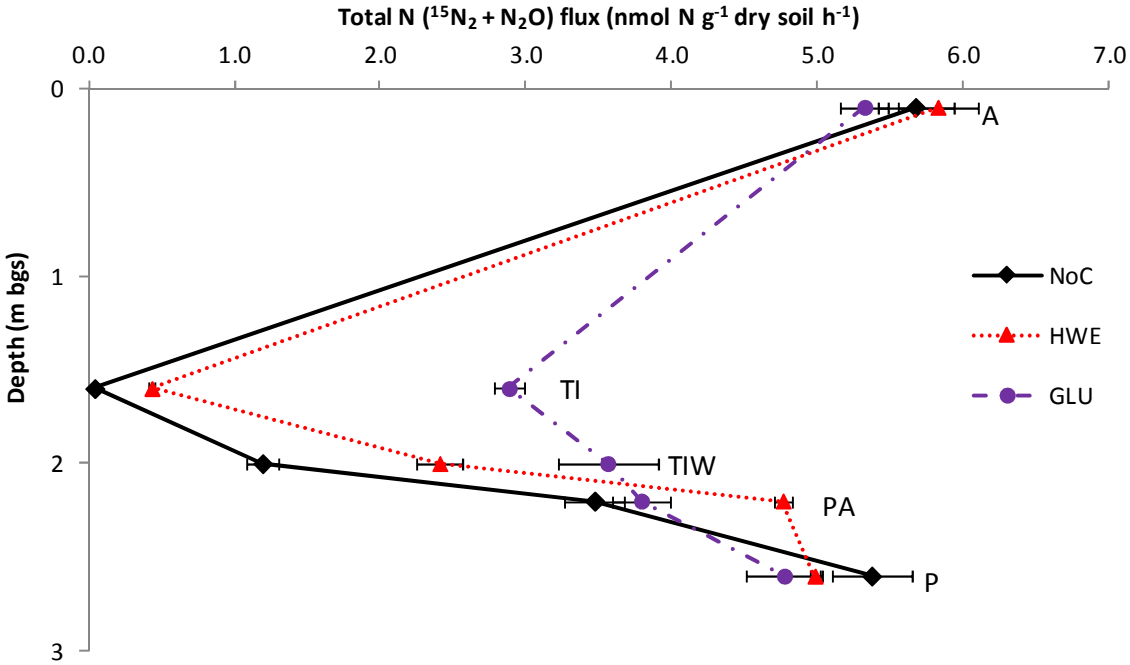

Figure 6.8: Total N fluxes (nmol N g^{-1} h^{-1}) measured in the three treatments of the second denitrification potential experiment after 48 hours incubation in phase II (n=3, error bars ± s.e.m.).

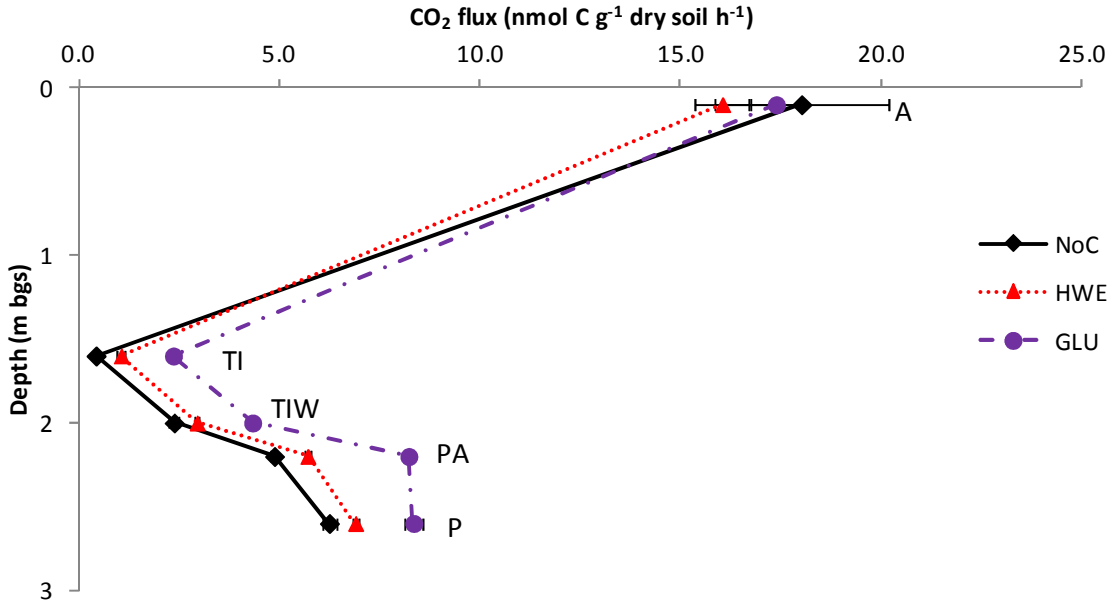

Figure 6.9: CO_2 fluxes (nmol C g^{-1} h^{-1}) measured in samples after 48 hours incubation in phase II (n=3, error bars ± s.e.m.).

The relatively low rate of C addition (36 µg C g^{-1} dry soil, compared to 150 µg C g^{-1} used by Jahangir et al. (2012)) did not limit the denitrification rates as a maximum of 28% of the C applied was generated as CO_2, presuming the 48 hour flux rate was constant and that all the CO_2 came from the C addition. This is in contrast to Dendooven et al. (1996) and Peterson et al. (2013) who found over 80% of the added C (as glucose and HWE respectively) was produced as CO_2 after 72 hours of anaerobic incubation. Even when the 48 hour flux is calculated to cover 72 hours, only 40% of the added C would be produced as CO_2.

6.5 Conclusions

In general, the main hypothesis was supported since denitrification rates measured in the profile sampled were limited by C availability as addition of the HWE or glucose solution increased N gas flux production. The exception to this was the A horizon, where abundant native C resulted in no treatment differences, and the OI layer where very low fluxes were measured for all treatments, indicating a small denitrifier population.

The relative availability of the CWEC could not be assessed reliably since high NO_3^- concentrations in this extract inhibited the denitrification response. Results indicate that in some layers, the HWEC provided a source of C comparable to glucose (PA and P samples) as total N gas fluxes measured were similar while in the TI and TIW layers a significantly larger response to GLU addition was measured.

Variability of C and N concentrations between the two profiles sampled resulted in differing responses to incubation, for example the A horizon samples from phase I generated large quantities of N_2O (80 – 90% of total N fluxes) due to high concentrations of antecedent NO_3^- , while the phase II A horizon samples had about 1% of the total N gas fluxes from N_2O as NO_3^- concentrations were low. The palaeosol horizons of the phase II incubation had similar relict C concentrations and thus gave similar responses, while the corresponding horizons from the initial incubation had smaller C concentrations in the deeper palaeosol layer, and consequently a lower denitrification flux. This small scale variability highlights the difficulty of assessing denitrification capacity across a catchment.

This experiment has reinforced the findings of Chapter 5: denitrification can occur in this volcanic profile under *in vitro* conditions, with greater fluxes generated where relict organic matter exists. The measurement challenges experienced in Chapter 3, where samples with very low NO_3^- concentrations could not be analysed by isotopic methods illustrate how denitrification can proceed to near-completion, even under *in situ* conditions. This laboratory study shows that if required in the future, a C source could be added to enhance the natural denitrification rates, if for example, the

relict organic matter is fully utilised, or NO_3^- concentrations leaching to groundwater increase and lead to inhibition of the denitrification pathway.

Chapter 7

Denitrification potential in the shallow groundwater system of a lowland catchment in the Waikato: a laboratory study

7.1 Abstract

Evidence for the occurrence of denitrification in shallow groundwater systems in New Zealand (NZ) is poorly documented; however, an observational study of the Toenepi dairying catchment in the Waikato region of NZ revealed a prevalence of reduced groundwater with low nitrate (NO_3^-) concentrations. Denitrification in the shallow groundwater system could play an important role in catchments with high NO_3^- leaching losses, by reducing the environmental impact of land use and management practices. Overseas studies have shown that denitrification below the root zone is often limited by the low availability of carbon (C) or other electron donors. This laboratory incubation study sampled three different profiles (to a maximum depth of 4.7 m below ground surface) in the Toenepi catchment (15 km^2). Denitrification capacity was measured following the addition of ^{15}N-enriched NO_3^-, while glucose was used as a readily available C source when ascertaining the denitrification potentials. Results indicate that the profiles were C-limited as depth increased, with more than 80% of samples showing an increase ($p < 0.01$) in total N gas ($^{15}N_2 + N_2O$) production after C addition. The largest total N fluxes were observed in the A horizon samples (4.5 – 10 nmol N g^{-1} h^{-1}); however, fluxes of a similar magnitude (4.0 – 4.5 nmol N g^{-1} h^{-1}) were also measured in several samples from below the A horizon when glucose was added. The composition of the total N gas flux varied with depth as the A horizon samples generally produced more N_2O than samples from all other depths. It is assumed that the high concentration of resident NO_3^- found in these samples inhibited complete denitrification, even when C was in excess. Extrapolation of denitrification capacity rates to field temperatures (14°C) indicates that much of the material found at depth, particularly at the Kereone and Topehaehae sites could contribute towards reducing the estimated NO_3^- leaching losses (29 – 42 kg N ha^{-1} y^{-1} estimated by Overseer®) beyond the root zone.

7.2 Introduction

Intensive agricultural practices can result in a loss of nitrate (NO_3^-) from the root zone. Transport of this NO_3^- through the vadose zone, into the groundwater system can lead to degradation of drinking water supplies and detrimental effects on ecosystem health where the contaminated groundwater discharges into surface waters (Daughney and Randall, 2009). Eutrophication of streams, rivers and lakes is a worldwide problem, but particularly in NZ in areas under intensive dairying. Transformation processes like denitrification limit the extent and impact of NO_3^- contamination as NO_3^- is reduced to

environmentally benign dinitrogen (N_2). In order to predict the effect of landuse practices on the ground and surface water quality, an understanding of where, when and how much denitrification occurs is required (Böhlke et al., 2007).

While changes in the NO_3^- concentration data of groundwater samples along a flowpath can indicate the occurrence of denitrification, it is difficult to determine where or when the NO_3^- was reduced.

Labile carbon (C) in recharge water is often at low concentrations. Studies show that the aquifer matrix often supplies the necessary fuel for microbes, whether C for heterotrophic denitrification (Hill and Cardaci, 2004) or reduced iron (Fe) and sulphur (S) for autotrophic denitrification (Schwientek et al., 2008). The alluvial sediments layered with volcanic ash deposits found in the Waikato region of New Zealand can provide such sources of electrons. Compared to A horizon-derived C, relict organic matter and minerals at depth may be less bioavailable to microbes and thus limit the degree of denitrification that occurs *in situ*. The difficulties encountered when applying *in situ* methods to gain evidence for denitrification have been documented in Chapters 3 and 4. For this reason, a denitrification potential experiment was designed, to obtain potential denitrification rates under idealised laboratory conditions (high temperature, oxygen-free environment, sufficient NO_3^-) in the presence and absence of glucose, a readily-available source of C.

7.3 Materials and Methods

7.3.1 Study Site and Sample Collection

The Toenepi catchment is located in the eastern part of the Waikato region in the North Island of New Zealand (Figure 7.1). Most of the 15 km^2 catchment is under intensive dairying, with an average stocking rate (in 2003) of 3.1 cows ha^{-1}. The geology consists of Holocene to late Pleistocene volcanic ash deposits, with silt loam and fine sandy loam textures overlying older deposits which are characterised by sticky and plastic halloysitic and allophanic clays. Closer to the stream, the subsoil is dominated by layers of sandy volcanic ash, cross bedded with lenses of peat, silt and clay.

Three sites in the catchment were chosen for detailed study in 2006, and named by their soil classification: Kereone, Morrinsville and Topehaehae (Figure 7.1). At the Kereone and Morrinsville sites, three wells were installed while the Topehaehae site had 5 wells installed (Figure 7.2). The wells were installed using a corer with an 80 mm diameter and were constructed of 50 mm diameter PVC pipe, with a screened zone 0.5 m long.

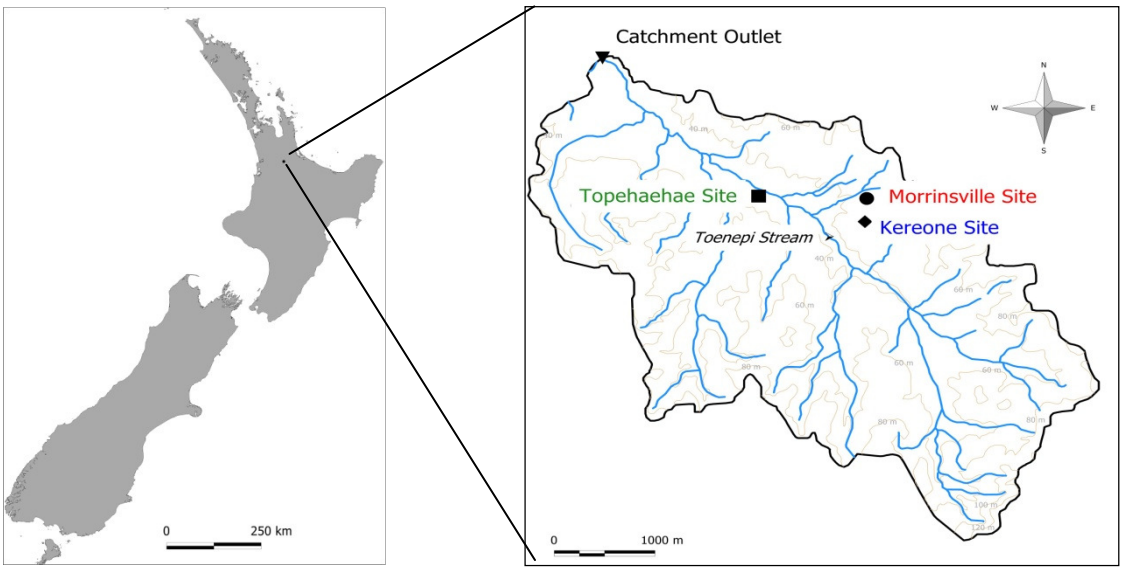

Figure 7.1: Location of the Toenepi catchment and the three study sites.

The Kereone site has an Allophanic Soil (Typic Orthic Allophanic Soil) and is characterised by well drained silt loam and sandy clay loam materials. The profile has layers that contain particulate organic material (including roots and wood) from about 4.2 m below ground surface. A positive result for the Childs Test (presence of reduced iron (Fe^{2+})) indicates reduced conditions from around 4.5 m depth. Three wells were installed at this site in 2006 (Figure 7.2), with the upper two (at 3.5 and 4.1 m depth) consistently drawing oxidised groundwater. A sharp redox boundary occurs below 4.1 m depth as the deeper well, with a screen from 4.2 - 4.7 m depth, always draws reduced water. The water table depth fluctuates seasonally between 2 and 3 m below ground surface (Figure 7.2).

The Morrinsville site (Typic Orthic Granular Soil) is characterised by silt loams, loamy sands and loamy clays which make it less well drained than the Kereone site. The maximum water table depth in spring lies 1.6 m below the ground surface, while the early autumn minimum occurs at 2.3 m depth (Figure 7.2). Reduced conditions at this site are consistently found in the groundwater from the two deeper wells (at maximum depths of 4.1 and 4.7 m) (Figure 7.2) while the well at 2.1 - 2.6 m depth range draws oxidised water.

The small scale spatial variation of the different layers and redox boundaries at this site was revealed when core samples were taken at a maximum distance of 5 m apart on three occasions (June 2006, September 2012 and January 2013). Some of the variation in colours, depth to organic matter, and Childs Test response could be due to seasonal changes, but the water table location at the time of sampling differed by a maximum of 0.25 m (1.95 m in 2006, 2.1 m in 2012 and 2.2 m in 2013). The samples taken for this experiment in January 2013 did not comprise a layer containing particulate organic matter at depth, whereas previous cores had thin layers (3 – 5 cm) at 4.55 m (2006) and 4.80

m (2012). Also, the whole profile had a negative response to the Childs test while a positive response to reduced Fe had previously been documented for 3.65 – 4.75 m (2006) and 2.45 – 2.95 m (2012).

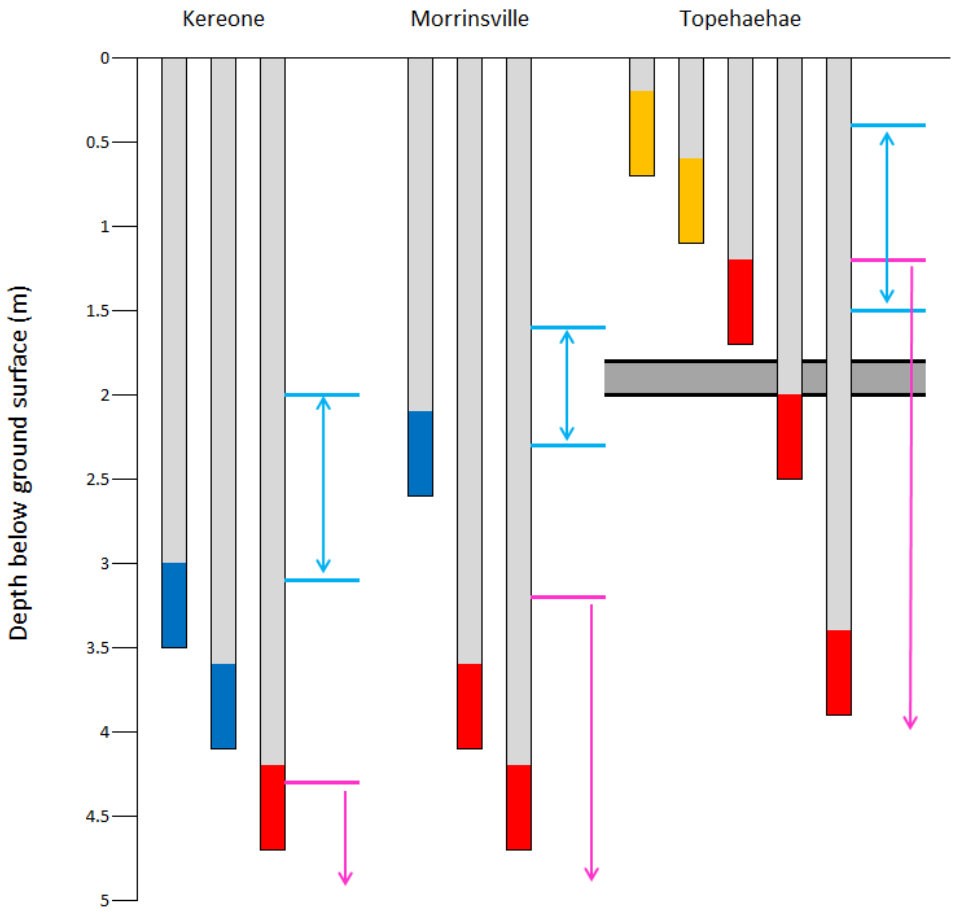

Figure 7.2: Diagram of well layout at the three sites: Kereone, Morrinsville and Topehaehae. Length of well indicates depth below ground surface, coloured zone indicates well screen. Red well screens always draw reduced water, blue always oxidised. Orange well screens draw sometimes oxidised, sometimes reduced groundwater. The blue lines indicate average water table fluctuation; the pink line indicates a positive response to the Childs Test (presence of reduced iron). The horizontal dark grey zone at 1.8 - 2 m depth at the Topehaehae site indicates an aquitard layer.

The Topehaehae site (Typic Recent Gley Soil) is located only 75 m from the Toenepi stream and is derived from recent alluvial deposits. The profile is characterised by poorly drained sandy loam and silt loam layers, caused by a thin aquitard layer at approximately 2 m below ground surface. Under natural conditions, the water table would reach the surface in winter, but artificial drainage enables year-round grazing with mole and tile drains (at 0.4 m and 0.7 – 1.0 m depth respectively) restricting the water table depth to a maximum of 0.4 m below ground surface (Figure 7.2). The minimum water table depth occurs at 1.5 m, in early autumn. Reducing conditions prevail over much of the profile,

with the 5 wells installed drawing either consistently reduced water or temporally reduced water (Figure 7.2).

Samples were collected in January 2013, from the three study sites in the catchment (Figure 7.1), using an 80 mm diameter corer. Approximately 700 g of sample was taken from each depth increment (Table 7.1), up to 4.7 m below ground surface, yielding a total of 21 samples. Many of the samples taken correspond to the screen depths of the wells located at each site (Figure 7.2) to enable comparison of laboratory results with *in situ* observations (Chapters 3 and 4).

Samples were bagged tightly to minimise exposure to air and placed in cooler boxes in the field. Upon returning to the laboratory, samples were stored at 2°C for a maximum of 48 days before analysis.

Table 7.1: Depth and texture of the samples taken from the three sites in the Toenepi catchment. Textural analysis carried out according to Milne et al. (1995) A positive Childs test response, indicating reduced conditions, is given where applicable.

Kereone samples	Morrinsville samples	Topehaehae samples
0 – 0.2 m	**0 – 0.2 m**	**0 – 0.2 m**
A horizon	A horizon	A horizon
Silt loam	Silt loam	Silt loam
3.5 – 3.7 m	**2.4 – 2.6 m**	**0.2 – 0.4 m**
Sandy clay loam	Silt loam	Silt loam
Dark orange mottles	Some pale orange mottles	
3.8 – 4.0 m	**3.8 – 4.0 m**	**0.5 – 0.7 m**
Sandy clay loam	Loamy sand	Silt loam
Orange mottles	Dark orange mottles	Orange mottles
4.0 – 4.2 m	**4.0 – 4.2 m**	**0.8 – 1.0 m**
Silty clay	Loamy sand	Fine sandy loam
Orange and black mottles	Orange and brown mottles	Orange mottles
4.2 – 4.3 m	**4.5 – 4.7 m**	**1.1 – 1.3 m**
Silt	Loamy clay	Silt loam
Dark brown organic matter present	Orange and brown mottles	Orange mottles
4.3 – 4.5 m		**1.4 – 1.6 m**
Silty clay loam		Sandy loam
Sticky and plastic		Positive Childs test
Positive Childs test		
4.5 – 4.6 m		**1.6 – 1.8 m**
Silt loam		Silt loam
Patches of organic matter		Positive Childs test
Positive Childs test		
4.6 – 4.7 m		**2.1 – 2.3 m**
Black organic matter including wood		Silt loam with lenses of
and roots		silt and sandy clay loam
Positive Childs test		Positive Childs test

7.3.2 Profile Characterisation

A range of analyses were undertaken to characterise and compare the three profiles sampled. The gravimetric moisture content was determined for all samples so that subsequent analyses could be determined on an equivalent dry weight basis (Blakemore et al., 1987). The total C and nitrogen (N) contents were determined using LECO combustion methods using air dried and ground (500 μm) samples. Dried and ground (75 μm) samples were also analysed for iron and sulphur using X-Ray Fluorescence (XRF).

Hot and cold-water extractable C was determined on all samples following the method of Ghani et al. (2003) with minor modifications. In this procedure, 10 g (equivalent dry weight) samples were placed in 250 mL Nalgene® extraction bottles, and 100 mL deionised (DI) water was added. Samples were then placed on an end-over-end shaker at room temperature (25°C) for 1 hour before being centrifuged at 12,000 rpm for 10 min. The supernatant was then filtered through Whatman 42 filter paper to yield the cold-water extract (CWE). This C extract represents the water-soluble C in the soil and the potentially readily available pool of C for microbial processes. Following removal of the supernatant, a further 100 mL of DI water was added to the extraction bottle. Samples were shaken by hand to re-suspend the sediment, and then put on the shaker for 1 hour at room temperature. Bottles were then placed in an oven (80°C) for 16 hours prior to centrifugation at 12,000 rpm for 10 min. The supernatant was then filtered through Whatman 42 filter papers to give the hot-water extract (HWE). This extract is an approximation of the microbial biomass (Ghani et al., 2003; Sparling et al., 1998) and also includes labile soil organic matter components such as root exudates and amino acids (Ghani et al., 2003). Prior to analysis, samples were filtered further (0.45 μm) using Millipore mixed cellulose ester filters. Extracts were analysed for total dissolved C (TDC), dissolved organic C (DOC) and dissolved inorganic C (DIC) using standard methods (APHA, 2005). The concentration of NO_3-N and NH_4-N were also determined on these samples (APHA, 2005).

7.3.3 Denitrification Potential Experiment

A denitrification potential experiment based on the method of Yeomans et al. (1992) was conducted with some modification. Incubation of samples with and without glucose enables comparison of N gas production utilising only native C resources with a maximum production possible when C is abundant, under idealised laboratory conditions.

This experiment was performed with 4 replicates of each treatment: NoC samples were only amended with NO_3^- while GLU samples had both NO_3^- and glucose added.

Chilled, field-moist samples (50 g equivalent dry weight) were weighed into 0.5 L mason jars. The jars were then sealed with lids which had been fitted with rubber septa. The air-tightness of the seal was

checked by applying pressure and immersing in a waterbath. If no bubbles were observed, the jar was considered sealed. Jars were then flushed with zero grade argon for 3 minutes to remove ambient air. Previous testing had shown that a 3 minute flush was sufficient to remove all oxygen (O_2); however, traces (2 – 5% N_2) of N_2 remained. For the purposes of pre-conditioning, it was decided that removal of all O_2 was sufficient. After equilibration at 25°C for 24 hours, the jars were re-opened, sufficient water was added to make a slurry with a soil:water ratio of 1:3 and then treatments were added. All jars received N amendment at a rate of 10 µg N g^{-1} dry soil using ^{15}N-enriched KNO_3 (49 atom%). The GLU treatment samples also received glucose added at a rate of 100 µg C g^{-1} dry soil. The comparatively low rate of N amendment was used despite many studies (Bijay-Singh et al., 1989; Jahangir et al., 2012; Luo et al., 1998; Murray et al., 2004; Yeomans et al., 1992) using much higher N amendment rates of 50 – 500 µg N g^{-1}. However, these previous studies have generally used top-soils, with microbial populations capable of utilising high concentrations of NO_3^-, compared to the vadose and saturated zone samples from much greater depth, used in this experiment. Also, a previous incubation experiment (Clague et al., 2013) (Chapter 5) found significant denitrification occurred at low N amendment rates while Lalisse-Grundmann et al. (1988) found that NO_3^- concentrations between 36 and 113 µg N g^{-1} could inhibit N_2O gas production. The rate of C amendment was chosen to ensure that C was non-limiting and provided the deeper samples with a C:N ratio similar to that commonly found in pastoral top-soils (Ghani et al., 2007; Sparling and Schipper, 2004).

Following amendments, the slurry mixture was thoroughly stirred to ensure good contact with substrates; jars were then resealed and checked again for air-tightness. The headspace was again made anaerobic by flushing with argon for 3 minutes, evacuating for 5 minutes and then flushing again for 3 minutes. Samples were then incubated at 25°C. Duplicate headspace gas samples were taken for analysis of $^{15}N_2$, $^{15}N_2O$, and $^{13}CO_2$ and placed in 12 mL Exetainer® vials (Labco Ltd) which had been previously flushed with argon and evacuated (-1 atm). Sampling occurred after 24, 48 and 72 hours of incubation. Analysis was carried out using isotope ratio mass spectrometry (PDZ Europa Ltd) using the methods of Stevens et al. (1993).

7.3.4 Statistical Analysis

Statistical analyses were performed using Microsoft Excel 2007. Means, standard deviations and standard errors were calculated for different parameters and where appropriate, significant differences between means were determined using Student's t-test.

7.4 Results and Discussion

7.4.1 Profile Characterisation

As all CWE and HWE samples had DIC concentrations below the detection limit of the laboratory method (1 mg L^{-1}), the total C content comprised entirely of organic C. Therefore, all subsequent reference to the C concentration of the CWE and HWE refers to the organic fraction. Concentrations of C in the hot and cold water extracts varied with depth in the profiles sampled (Table 7.2). Relatively similar C concentrations were found in the A horizons of the three sites, and all three hot-water samples (HWEC) yielded more C than expected based on the work of Sparling et al. (1998), who found a maximum of 867, 891 or 970 μg C g^{-1} in granular, allophanic or gley soils, respectively, under pasture. However, HWEC values were similar to those recorded by Ghani et al. (2003) who found that the HWEC in soils from dairy farms ranged from 1786 – 4304 μg C g^{-1} soil.

In general, the HWEC distribution reflected the total C contents of the soil profiles with two main exceptions: the Kereone 4.6 – 4.7 m sample and the Topehaehae 2.1 – 2.3 m sample. Both samples had elevated HWEC concentrations of approximately 250 μg C g^{-1}, but much greater total C contents of 285,000 μg C g^{-1} and 44,000 μg C g^{-1} respectively. These values were greater than the corresponding A horizon samples, with the Kereone A having 94,100 μg C g^{-1} and the Topehaehae A yielding 31,900 μg C g^{-1} (Table 7.2). Both of these deep samples had obvious organic matter present, either as wood or leaf matter.

The CWEC profile distributions differed from the HWEC and total C contents with some samples yielding much larger CWEC concentrations than the corresponding HWE, some yielding less, and some with similar CWE and HWE C concentrations (e.g. the Kereone 3.8 – 4.0 m sample with 17 and 19 μg C g^{-1} respectively) (Table 7.2).

Relatively high concentrations of NO_3^- were extracted from the A horizon samples at the Kereone and Morrinsville sites, presumably as a result of previous N inputs such as urine and fertiliser. The Topehaehae site had much lower NO_3^- and NH_4^+ concentrations over all depths, probably reflecting the lower N inputs at this farm (only 67 kg N ha^{-1} y^{-1} applied compared to the catchment average of 100 kg N ha^{-1} y^{-1}) and more rapid movement of nutrients through the periodically saturated and artificially drained site.

Total N contents in the soil profiles followed the pattern of total C for many of the Topehaehae and Morrinsville samples, with the C:N ratio ranging between 7 and 16. The four shallowest Kereone samples (down to 4.2 m depth) also had similar C:N ratios with values of 8 or 9. However, the four deeper Kereone samples had much higher C contents, with C:N ratios between 24 and 48 (Table 7.2).

Similarly, the deepest Topehaehae sample at 2.1 – 2.3 m depth had a much greater proportion of C than the rest of the profile, with a ratio of 22.

Table 7.2: Characteristics of Kereone, Morrinsville and Topehaehae site samples.

Site & Depth sampled (m)	pH[a]	Total C[b] (μg C g^{-1})	CWEC[c] (μg C g^{-1})	HWEC[c] (μg C g^{-1})	Total N[b] (μg N g^{-1})	CWE-NO_3^{-}[c] (μg N g^{-1})	CWE-NH_4^{+}[c] (μg N g^{-1})
Kereone							
0 – 0.2	5.48	94100	129	1430	10000	28.2	5.2
3.5 – 3.7	5.32	1800	116	97	220	6.2	3.3
3.8 – 4.0	5.62	1700	17	19	210	6.8	4.4
4.0 – 4.2	5.61	1800	66	26	200	3.5	4.1
4.2 – 4.3	5.17	38100	51	80	800	0.5	7.9
4.3 – 4.5	5.17	4100	15	30	170	0.4	3.9
4.5 – 4.6	5.18	53600	32	107	1200	0.8	7.8
4.6 – 4.7	5.23	285000	129	252	5990	0.5	8.0
Morrinsville							
0 – 0.2	5.09	87200	127	1313	9170	41.4	8.5
2.4 – 2.6	5.62	1200	95	48	170	6.4	3.4
3.8 – 4.0	6.08	600	101	25	70	0.9	3.9
4.0 – 4.2	5.92	500	92	30	70	1.7	4.1
4.5 – 4.7	6.01	500	16	20	70	0.5	4.0
Topehaehae							
0 – 0.2	5.39	31900	150	1351	3410	4.2	3.0
0.2 – 0.4	5.17	8300	22	179	830	1.8	2.5
0.5 – 0.7	5.53	3400	19	57	350	0.4	2.8
0.8 – 1.0	5.24	1800	22	26	170	0.7	3.1
1.1 – 1.3	5.15	700	26	18	90	1.0	2.9
1.4 – 1.6	4.13	1600	26	29	130	0.1	2.6
1.6 – 1.8	4.39	2600	20	106	160	0.2	2.7
2.1 – 2.3	4.53	44000	89	255	2040	0.2	5.5

[a] pH_{H2O} soil:water 1:2.5 (Blakemore et al., 1987)
[b] Total C and N by LECO combustion (Blakemore et al., 1987)
[c] Hot and cold water extracts (Ghani et al., 2003)

7.4.2 Denitrification Potential Experiment

7.4.2..1 Temporal Variation

As reported earlier (Chapter 6), high concentrations of resident NO_3^- affect the N_2O composition since microbes preferentially use the antecedent NO_3^- rather than the added enriched NO_3^-. For this reason, the total N fluxes reported represent the $^{15}N_2$ flux + N_2O flux rather than the $^{15}N_2$ flux + $^{15}N_2O$ flux. For all samples except the A horizon of the Kereone and Morrinsville profiles, this distinction makes no difference to the total N flux shown.

The timing of the maximum total N gas fluxes ($^{15}N_2$ + N_2O) varied with site, treatment, and depth. However, 55% of samples had peak total N gas production at 48 hours, with 36% still increasing at the 72 hour sampling period. This trend primarily occurred in the GLU treatment of the normally oxidised Kereone and Morrinsville samples and indicates the ubiquitous nature of heterotrophs. It also highlights one of the problems of using an extended period of incubation as stimulation of enzyme production can lead to greater N gas fluxes (Drury et al., 2008). If the purpose of the experiment was to create a 'snapshot' of the capability of the indigenous microbial population, incubation for longer than 5 - 8 hours is not recommended (Drury et al., 2008); however, this experiment aimed to test if denitrification could occur at all, given ideal conditions.

To avoid the time-lag in microbial response seen by others (Chirinda et al., 2011; Peterson et al., 2013; Smith and Tiedje, 1979), a conditioning period of 24 hours was used. This period allows the activation of enzymes in preparation of substrate addition and may explain why many samples yielded peak N gas production at 48 hours while others have found peak production occurs at 65 hours (Murray et al., 2004), 72 hours (Peterson et al., 2013) and 96 hours (Jahangir et al., 2012).

Since many other authors (Barkle et al., 2007; Bijay-Singh et al., 1989; Clague et al., 2013; Lalisse-Grundmann et al., 1988) have sampled at 48 hours, this data will be used for comparison.

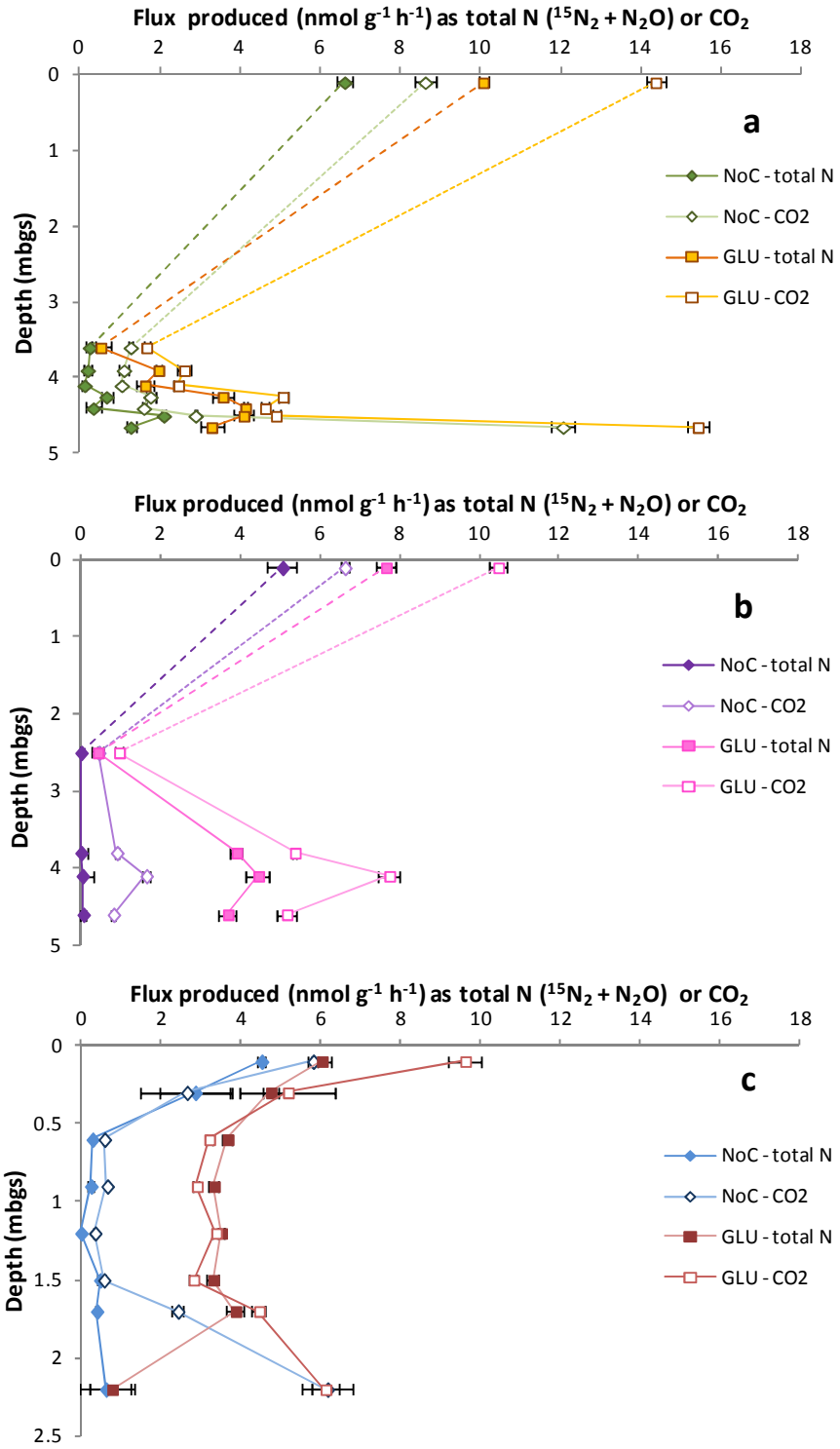

Figure 7.3: Depth profiles of the average total N (nmol N g^{-1} h^{-1}) or CO_2 gas fluxes (nmol C g^{-1} h^{-1}) produced after 48 hours incubation. The dashed line indicates that the denitrification potential of vadose zone material between the A horizon and second sample is unknown, and probably not linear. The Kereone site is shown in chart a, Morrinsville in chart b, and Topehaehae in chart c. Data points are averages based on 4 replicates (error bars = ± s.e.m.).

7.4.2..2 Vertical Variation

As expected, the A horizon samples of both treatments had the greatest total N gas fluxes (Figure 7.3). Unlike the results of other authors who have found a consistent, exponential decline in fluxes with depth (Barkle et al., 2007; Jarvis and Hatch, 1994) the N gas fluxes from samples deeper in the profile varied with depth. Nearly all samples showed a significant ($p < 0.05$) increase in total N gas production when glucose was added, demonstrating that the system was generally C-limited. The range of A horizon fluxes (4.5 – 6.6 nmol n g^{-1} h^{-1}) measured in the NoC treatment were comparable to those measured in similar experiments (Barkle et al., 2007; Clague et al., 2013) but much lower than those measured by Bijay-Singh et al. (1989), perhaps reflecting the lower N amendment as well as different soil and management techniques.

Samples from below the A horizon had a wide range of denitrification capacities (NoC treatment) (0.002 – 2.86 nmol n g^{-1} h^{-1}) and potentials (GLU treatment) (0.53 – 4.75 nmol n g^{-1} h^{-1}) that were in the same range as those found by Jarvis and Hatch (1994) for a soil under grass-clover, cut treatment.

Three samples showed no significant ($p > 0.01$) treatment effect: Kereone 3.5 – 3.7 m, Morrinsville 2.4 – 2.6 m and Topehaehae 2.1 – 2.3 m (Figure 7.3). These samples had very small total N fluxes (<1 nmol N g^{-1} h^{-1}) and differences between the NoC and GLU treatments were <0.5 nmol N g^{-1} h^{-1}. It appears that these samples were limited by the microbial populations present as slightly higher fluxes were observed at 72 hours for these samples, possibly indicating population growth. Two of these samples (Kereone 3.5 – 3.7 m and Morrinsville 2.4 – 2.6 m) also had relatively small CO_2 gas production and minimal treatment effect in the GLU samples. This shows that in general, the microbial population capable of utilising the supplied C was relatively small. Oxidised conditions are consistently found *in situ* at these depths, so it is likely that the majority of microbes present operate best under aerobic rather than anaerobic conditions.

In contrast, the third sample (Topehaehae 2.1 – 2.3 m) had relatively large CO_2 fluxes (Figure 7.3c) and essentially no difference between treatments was detected. This indicates that the sample was not C-limited, or generally microbe-limited, but specifically denitrifier-limited. Total C analysis of this sample revealed a relatively high C content (44,000 µg C g^{-1}) (Table 7.2), which, based on the CO_2 data, was presumably bio-available.

The relationship between relict C and CO_2 fluxes was also found in the deepest Kereone sample, which had a very high total C content (285,000 µg C g^{-1}) and corresponding CO_2 fluxes (12 – 15 nmol g^{-1} h^{-1}). In this sample; however, the total N fluxes were also significant, and showed a positive response to glucose treatment, indicating that despite the high total C content, only a fraction of the relict C was bio-available. As groundwater samples from this depth are always reduced and low in

NO_3^-, it is assumed this relict C provides a source of electrons to fuel the denitrification process *in situ*.

The Topehaehae sample from 0.2 – 0.4 m depth also showed no treatment effects ($p > 0.01$) on total N fluxes due to the large variability between replicates, indicating that denitrification might occur in micro-hotspots within this sample, and that resident organic matter had not been homogenised sufficiently before samples were taken.

The composition of N gases produced varied with depth, with A horizon samples generally producing a greater proportion of N_2O than samples from other depths (Figure 7.4). It is hypothesised that the A horizon samples from the Kereone and Morrinsville sites produced large fluxes of N_2O due to high native NO_3^- concentrations inhibiting the conversion to N_2. Nitrate analysis of the cold water extracts of these two samples yielded 30 – 40 µg N g^{-1} dry soil (Table 7.2); 3 – 4 times greater than the N amendment rate, whereas the Topehaehahe A horizon had NO_3^- concentrations of 5 µg N g^{-1} dry soil. This inhibition of N_2O-reductase activity has been observed elsewhere (Blackmer and Bremner, 1978; Firestone et al., 1979) and Blackmer and Bremner (1978) demonstrated that it was a combination of high NO_3^- and low pH which inhibited complete denitrification to N_2. The pH of both A horizons from the Kereone (5.48) and Morrinsville (5.09) (Table 7.2) sites were more acidic than those used by Blackmer and Bremner (1978) and well below the value at which minimal inhibition occurs (pH 7.0). Although the pH of the Topehaehae A horizon (5.39) was between these two samples, the low native NO_3^- concentration resulted in at least 80% of the total N fluxes being converted to $^{15}N_2$ highlighting the interdependency between NO_3^- and pH.

The relatively low proportion of N_2 determined for the Morrinsville NoC samples from below the A horizon is presumably due to measurement limitations. Very low total N fluxes (<0.5 nmol N g^{-1} h^{-1}) (Figure 7.3b) were measured in these samples, and the $^{15}N_2$ signal was either zero or not significantly higher than the background ^{15}N enrichment (0.3663 atom%), resulting in analytical uncertainty.

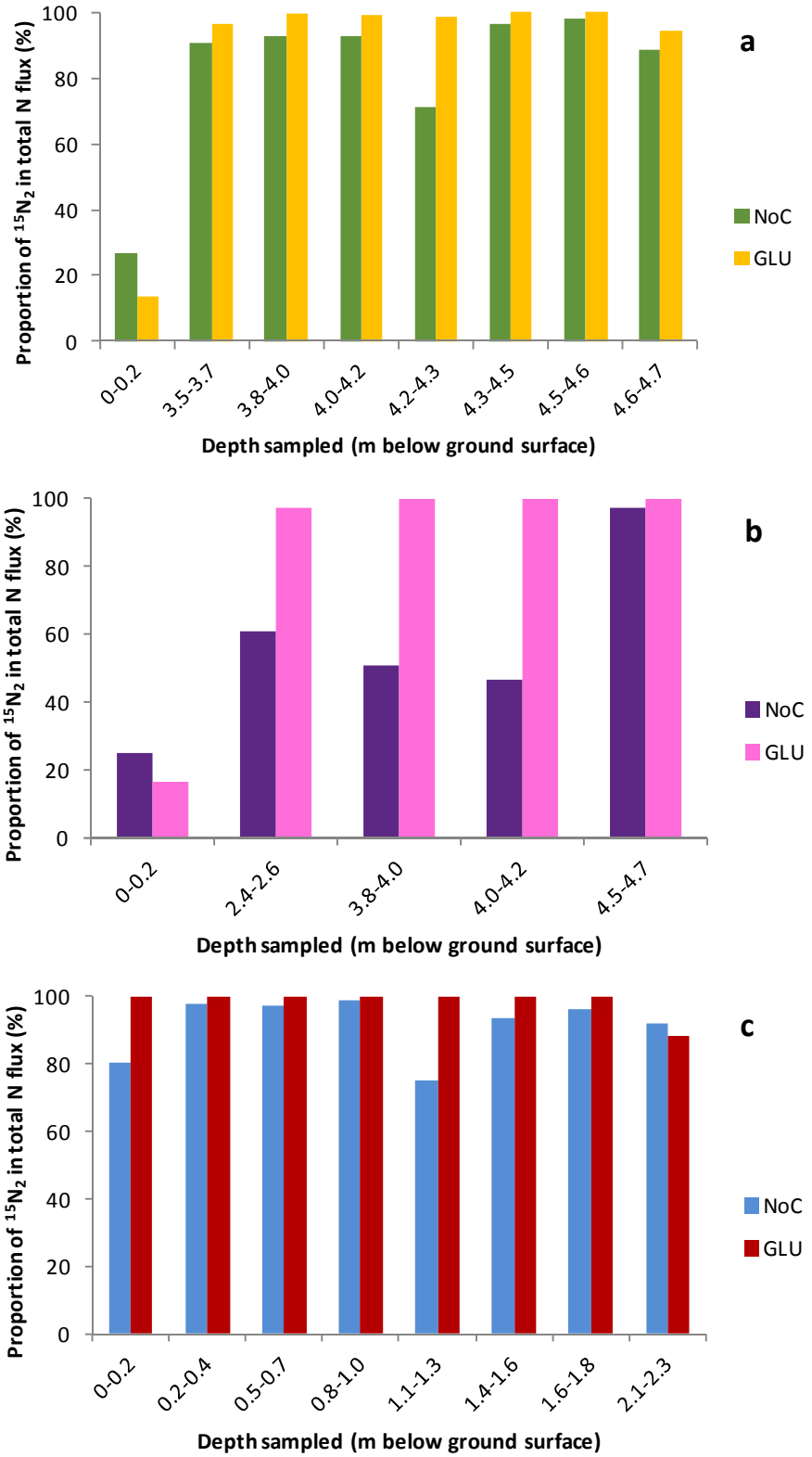

Figure 7.4: The $^{15}N_2$ component (%) of total N gas fluxes ($^{15}N_2 + N_2O$) measured in samples after 48 hours incubation. Kereone samples in chart a, Morrinsville in chart b, and Topehaehae in chart c.

Contrary to the work of Clague et al. (2013) (Chapter 5), no clear relationships were found between the total N gas produced and the cold water extractable C (CWEC) (Figure 7.5a) and a relatively weak relationship occurred between total N gas fluxes and the total C content of each sample (Figure

7.5b). Others (Hill and Cardaci, 2004; Jahangir et al., 2012) have also found weak relationships between CWEC and denitrification potential, reflecting the influence of small scale, highly variable factors such as urine deposition (Ghani et al., 2003) or fragments of relict C on the CWEC content. If the very high C (285,000 μg C g^{-1}) and relatively low N flux (1.3 nmol N g^{-1} h^{-1}) produced by the deepest Kereone sample (at 4.7 m depth) was excluded from the correlation, then 71% of the variation in total N fluxes could be explained by the total C content of the sample (Figure 7.5b). This is lower than the 81 – 85% found by Clague et al. (2013) (Chapter 5) for three volcanic profiles. In contrast, the total N gas fluxes for all samples were more closely related to the hot water extractable C concentrations (HWEC) (Figure 7.5c). Although the HWEC dataset does not comply with the statistical requirements of a normal distribution for a linear regression, the overall slope remains similar when the three A horizon samples are excluded (0.006x); however, the percentage variation explained drops to 37%.

The CWEC is generally considered to be the most labile C and an approximation of the quality of C likely to be transported in solution (via leaching) (Peterson et al., 2013) while the HWEC contains microbial C, amino acids and soluble carbohydrates (Ghani et al., 2003; Sparling et al., 1998). Therefore, the relationship between HWEC and total N flux (Figure 7.5c) is probably not directly related to the C content or its bioavailability, but rather a reflection of the microbial biomass available for conducting denitrification. The three A horizon samples of each profile had the highest HWEC concentrations (and largest microbial populations), and therefore yielded the largest total N fluxes. The two samples with relatively low HWEC but high total N gas fluxes were from the Kereone site at 4.5 – 4.6 m depth and the Topehaehae profile at 0.2 – 0.4 m depth. These samples do not plot close to the trendline, possibly as a reflection of a more labile C composition (compared to the other samples) or because the microbial populations in these samples were more efficient than the others and could produce greater fluxes at lower C contents. The total N to CO_2 ratios of the fluxes produced by these samples are 0.73 and 1.08 for the Kereone and Topehaehae respectively. These ratios are the highest from the NoC treatment samples below the A horizon (within their respective profiles) and close to the A horizon ratios of 0.77 and 0.78, indicating that the relict C in these samples is of a similar availability to the A horizon C. These ratios are much greater than reported from other studies (e.g. ~0.3:1 in Peterson et al. (2013)) and close to the theoretical maximum of 0.99 reported by Burford and Bremner (1975). This ratio assumes all N gas produced as N_2, which is a valid assumption for these samples where 97-98% of the total N gas fluxes were composed of N_2. For samples like the Kereone or Morrinsville A horizon samples, where only 25% of the total N gas flux was as N_2 (Figure 7.4), the ratio becomes 1.12.

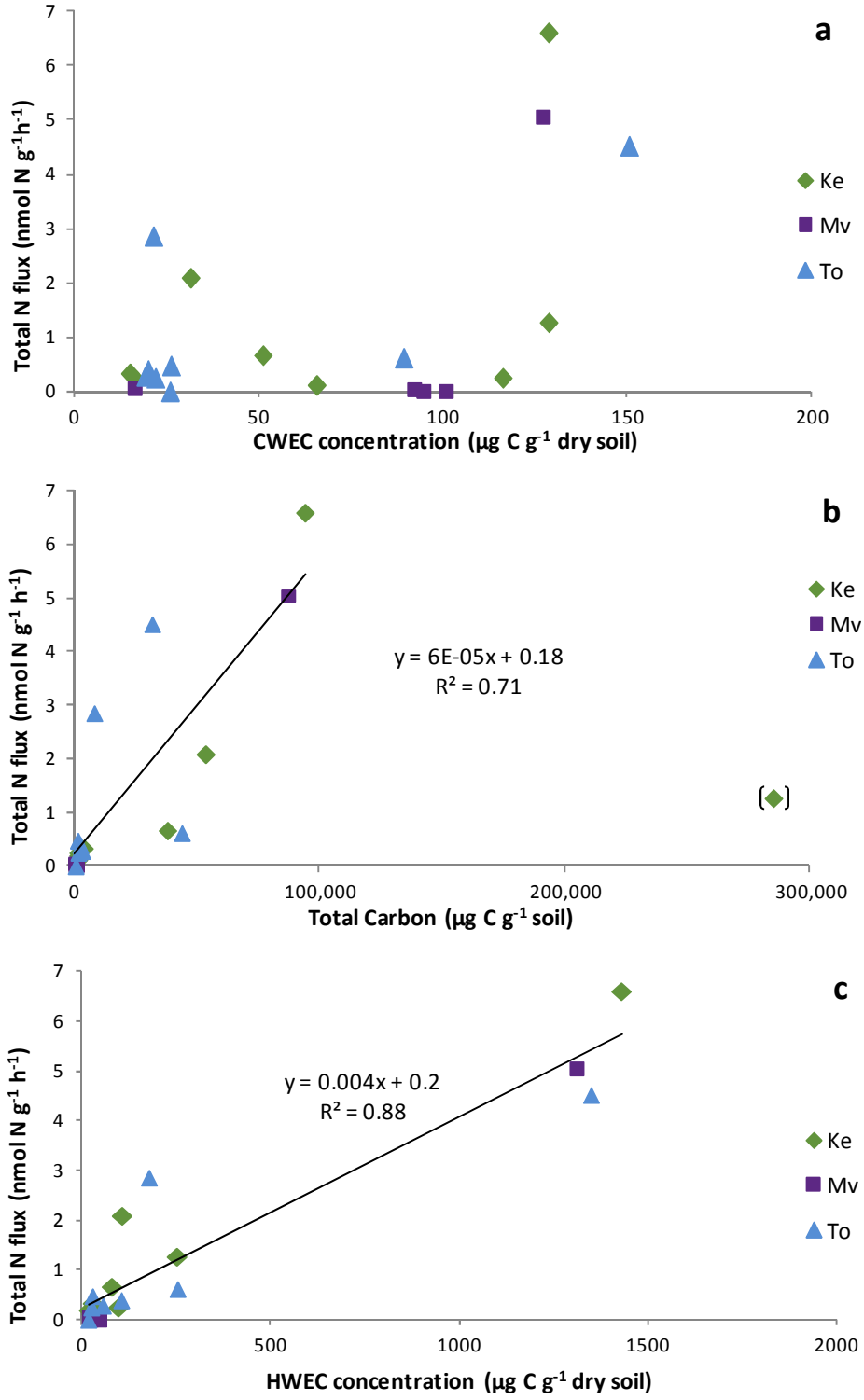

Figure 7.5: Relationships between the total N gas flux ($^{15}N_2 + N_2O$) (nmol N g^{-1} h^{-1}) produced in the NoC samples after 48 hours incubation at the three sites vs. cold water extractable dissolved organic carbon (CWEC) (µg g^{-1}) (chart a); the total C content of each sample (µg g^{-1}) (chart b) or hot water extractable dissolved organic C (HWEC) (µg g^{-1}) (chart c). The linear regression shown in chart b excludes the data point in brackets as an outlier. Data points are averages of three (CWEC or HWEC) or four (total N flux) replicates, or single measurements for total C.

As well as C, reduced Fe and S may be used by microbes to fuel the denitrification process. Autotrophic denitrification is common in aquifers with high pyrite content (Pauwels et al., 2000; Zhang et al., 2009) but has been detected in aquifers with as little as 230 mg S kg^{-1} (Postma et al., 1991). No linear relationships were found between the total N flux produced after 48 hours incubation and the Fe or S content of the sample (Figure 7.6). In general, samples were low in both Fe and S, compared with aquifers where autotrophic denitrification has been observed (Böhlke et al., 2002; Korom et al., 2012; Zhang et al., 2009). As any possibly occurring reduced Fe and S species would have lower concentrations than the total concentration determined by XRF analysis, inorganic electron donors presumably make at most a minor contribution to denitrification, if at all. However, as Korom et al. (2012) have demonstrated, measureable autotrophic denitrification can occur on a much slower timescale than is commonly measured in incubation assays (200 – 400 days compared to 2 days) and if the groundwater flowpaths involve long travel times in contact with aquifer materials bearing active Fe or S minerals for years or decades, a significant contribution could be made to reducing the NO_3^- load. This type of activity could be determined using long-term incubation experiments (with pyrite as an electron donor treatment amendment (Jørgensen et al., 2009)) or *in situ* using specially constructed mesocosms (Korom et al., 2005).

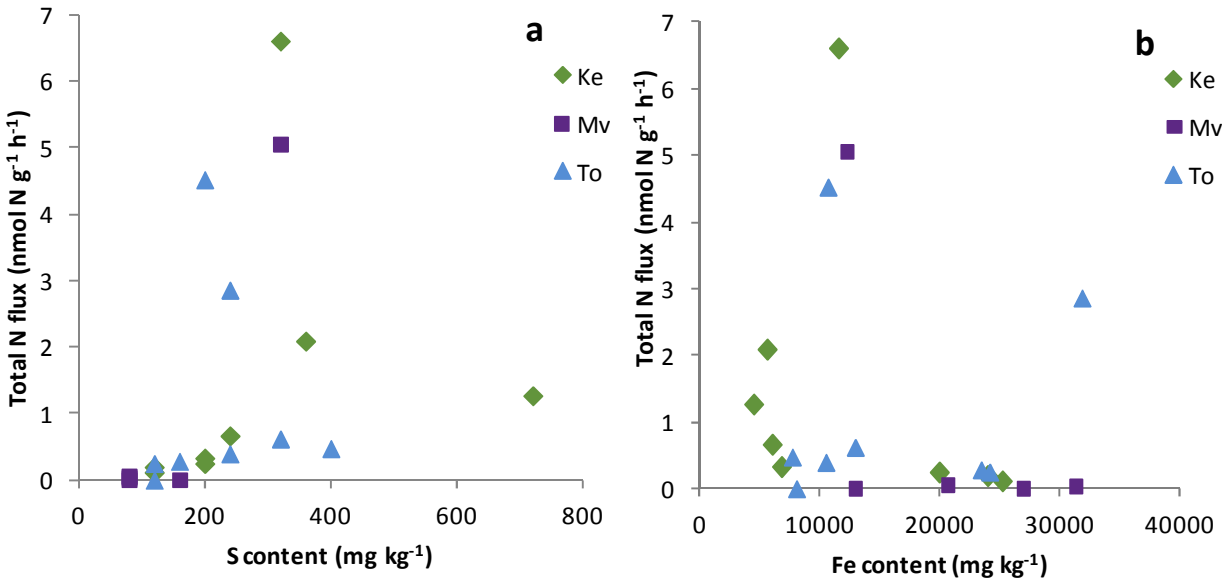

Figure 7.6: Total N flux produced (nmol n g^{-1} h^{-1}) vs. a) sulphur (S) content and b) iron (Fe) content (both as mg kg^{-1}) for the three Toenepi profiles sampled. Data points are single measurement values for the S and Fe data, while the total N flux values are averages of four replicates.

7.4.3 Implications of Research

In order to assess the extent of denitrification possible *in situ*, the NoC treatment total N gas fluxes (at 48 hours) were adjusted for a temperature of 14°C (the average recharge temperature) using a

Q_{10} factor of 2.0. This temperature coefficient is close to the 1.6 measured by Fischer and Whalen (2005) over a temperature range of 7 - 20°C. Fluxes were converted to a kg N ha^{-1} y^{-1} basis using previously determined bulk densities from the soil zone (Table 7.3). Samples which would normally occur under oxidised conditions in the field were not considered, even though microsites of denitrification may occur within these layers.

The calculated denitrification capacities (i.e. without added C) for the total reduced zones of the three sites ranged from 30 kg N ha^{-1} y^{-1} at the Morrinsville site to 426 kg N ha^{-1} y^{-1} for the Kereone profile (Table 7.3). The substantially lower *in situ* capacity estimated for the Morrinsville site is primarily a reflection of the available C found *in situ*, as the Morrinsville profile (excluding the A horizon) was markedly lower in total C and HWEC than the other two profiles (Table 7.2). If a readily available source of C was present at this site, the profile could potentially denitrify over 3000 kg N ha^{-1} y^{-1}, assuming a C amendment rate similar to the one used in the GLU treatment, and that this C source was constantly replenished.

The large difference between the Kereone and Morrinsville denitrification capacities highlights how spatially variable the occurrence of the process is, and how difficult it would be to categorise a catchment into high and low denitrification capacities as these two sites are located within the same paddock, only 250 m apart (Figure 7.1). Overseer® estimates NO_3^- leaching losses for the whole-farm where the Kereone and Morrinsville sites are located as 42 kg N ha^{-1} y^{-1} while for the Topehaehae site, the whole farm leaching estimate is 29 kg N ha^{-1} y^{-1} (personal communication with the farmers). At these leaching rates, even the low denitrification capacity determined for the Morrinsville profile equates to a NO_3^- load reduction by approximately two thirds. This is higher than the 36% reduction in NO_3^- concentrations calculated by the StreamGEM model used by Woodward et al. (2013) for the shallow groundwater component of this catchment. However, this model provides an estimate of the denitrification capacity of the whole catchment, and accounts for areas within the catchment where no denitrification occurs.

Even if the Overseer® estimates were 100% incorrect and the entire amount of N applied as fertiliser (136 kg N ha^{-1} y^{-1} and 67 kg N ha^{-1} y^{-1} for the Kereone and Topehaehae sites, respectively (personal communication with the farmers)) was leached from the root zone, the estimated denitrification capacities of the Kereone and Topehaehae sites would be sufficient to significantly reduce NO_3^- loads to groundwater.

Table 7.3: Denitrification capacity of the three profiles under *in situ* conditions.

Kereone		Morrinsville		Topehaehae	
Depth sampled	N denitrified (kg N ha^{-1} y^{-1})	Depth sampled	N denitrified (kg N ha^{-1} y^{-1})	Depth sampled	N denitrified (kg N ha^{-1} y^{-1})
0 – 0.2 m	oxidised	0 – 0.2 m	oxidised	0 – 0.2 m	oxidised
3.5 – 3.7 m	oxidised	2.4 – 2.6 m	oxidised	0.2 – 0.4 m	oxidised
3.8 – 4.0 m	oxidised	3.8 – 4.0 m	2	0.5 – 0.7 m	64
4.0 – 4.2 m	oxidised	4.0 – 4.2 m	18	0.8 – 1.0 m	55
4.2 – 4.3 m	55	4.5 – 4.7 m	10	1.1 – 1.3 m	0.4
4.3 – 4.5 m	41	**Total**	**30**	1.4 – 1.6 m	73
4.5 – 4.6 m	257			1.6 – 1.8 m	108
4.6 – 4.7 m	73			2.1 – 2.3 m	53
Total	**426**			**Total**	**353**

As age dating and isotopic analysis has shown (Chapter 3), the occurrence of low NO_3^- concentrations in groundwater at the Morrinsville site (Chapters 3 and 4) is most likely not due to denitrification but the lower NO_3^- concentrations leached into the groundwater at the time of recharge 50 – 150 years ago. Given the increase in NO_3^- leaching from the soil under more recent intensive dairying and the relatively low denitrification capacity measured at these depths (Table 7.3), it is foreseeable that NO_3^- concentrations in these wells will increase over time as the 'load to come' arrives, unless significant denitrification occurs further up the groundwater flow path.

7.5 Conclusions

In general, the three Toenepi profiles sampled were C-limited since glucose-C addition resulted in an increase in total N gas ($^{15}N_2$ + N_2O) and CO_2 production. The rate of N gas production was more strongly related to the HWEC than other forms of C; however, this may be due to the good correlation between HWEC and microbial biomass (Sparling et al., 1998). This research demonstrates that even though the denitrification capacities (i.e. without added C) measured were much lower than the potential rates (with C), a significant reduction in NO_3^- concentrations in groundwater may occur as Overseer® estimates that only 30 - 40 kg N ha^{-1} y^{-1} are lost from the root zone. While prediction of catchment denitrification capacities could be problematic, given the unknown prevalence of relict organic matter as found in the Kereone profile, the widespread occurrence of reduced groundwater with low NO_3^- concentrations indicates significant denitrification occurs (Stenger et al., 2008). The difficulties of obtaining evidence for denitrification under *in situ* conditions

(as detailed in Chapters 3 and 4) resulted in uncertainty about the location and extent of denitrification in the Toenepi catchment. The results of this study confirm that at a small scale, denitrification capacities exist and that these can explain the observed low NO_3^- concentrations discussed previously.

Chapter 8

Conclusions

8.1 Overall summary

The literature review and previous work identified a lack of knowledge regarding denitrification in shallow groundwater systems in NZ. Thus the main objectives of this research were to

- determine if denitrification was occurring in the shallow groundwater;
- if denitrification occurred, determine where and when; and
- identify the factors controlling denitrification.

This project has contributed to the gap in knowledge about denitrification in shallow groundwater systems in NZ. The objectives were pursued using a variety of methods in two small catchments in the Waikato region of NZ. This chapter summarises the results obtained in the preceding chapters and identifies future research opportunities.

8.1.1 Denitrification in the Toenepi catchment

The well-defined redox boundaries observed in the shallow groundwater of the Toenepi catchment led to the hypothesis that denitrification was occurring. In order to document and quantify this process under *in situ* conditions, the isotopic signature of NO_3^- in groundwater was analysed (Chapter 3). The most successful application of this method was at the Topehaehae site where both suction tubes and wells sampled the shallow groundwater. A strong temporal variation in isotopic signature was observed for the suction tube data, with late winter and early spring samples being more enriched in ^{15}N and ^{18}O than those taken earlier in the season (Figure 3.14). An enrichment factor of $\delta^{15}N$ relative to $\delta^{18}O$ of 1.4:1 was within the previously reported range (Aravena and Roberston, 1998; Baily et al., 2011; Böttcher et al., 1990; Chen et al., 2009; Fukada et al., 2003; Mengis et al., 1999). Vertical enrichment of $\delta^{15}N$ and $\delta^{18}O$ was also evident at this site: samples deeper in the profile (0.7 and 1.1 m depth) tended to have greater $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ signatures than samples from 0.4 m depth. Fractionation of NO_3^- isotopes with depth was not observed in water samples taken from the To-170 and To-390 wells at 1.7 and 3.9 m depth (Figure 3.15). The main reasons for this were that these wells sampled different groundwater flow lines compared to the shallower wells and suction tube samplers as water (and NO_3^-) movement at this site appears to be predominantly in a lateral direction. Understanding groundwater flow paths (direction, speed and vertical dynamics) is essential to the sound interpretation of isotopic data and to understand redox

dynamics of the groundwater. Age dating of groundwater at the Morrinsville site showed a large difference in ages between the upper oxidised layer (young) and deeper reduced groundwater (50 – 150 years) (Figure 3.8), indicating that they were not connected. This is one of the reasons why the isotopic method was unsuitable for the other two sites in the Toenepi catchment. Despite all well water samples being classified as young and potentially linked hydrologically at the Kereone site, evidence for denitrification at the Kereone site based on isotopic analysis, was hampered by collected samples having NO_3^- concentrations too low for isotopic methods to be applied.

Push-pull tests were then conducted (Chapter 4) at selected wells in the Toenepi catchment in order to quantify denitrification rates under *in situ* conditions. Results indicated that denitrification did not occur in the aquifer matrix directly surrounding the well screen as Br^- and NO_3^- concentrations declined at the same rate (Figure 4.4). However, when C and NO_3^- substrates were added in order to quantify denitrification potentials in the wells, a denitrification response was observed in the To-110 well at the Topehaehae site (Figure 4.6). The lack of a response in the other wells was presumably due to rapid dilution of substrates, as opposed to microbial numbers or suitable electron donors being lacking, as many wells had low concentrations of the tracer solution one day after injection.

This hypothesis was tested using laboratory incubation denitrification potential experiments (Chapter 7). When the denitrification fluxes of the aquifer core samples, corresponding to the well depths, were measured it was found that in general, samples produced greater total N ($^{15}N_2 + N_2O$) fluxes when glucose was added indicating that the system was C-limited (Figure 7.3). As expected, the A horizon samples generated the greatest total N fluxes (up to 6.6 nmol N g^{-1} dry soil h^{-1}); however, two samples from the Kereone site (4.5 – 4.6 and 4.6 – 4.7 m depth) produced significant fluxes even without added C (up to 2 nmol N g^{-1} dry soil h^{-1}) (Figure 7.3). Some samples showed no effect of substrate addition, thus, denitrification was assumed to be limited by the microbial populations present.

In order to extrapolate these *in vitro* results to field conditions, denitrification capacity fluxes were adjusted from the incubation temperature of 25°C to the average recharge temperature of 14°C using a Q_{10} factor of 2.0. The total denitrification capacity for the reduced zones of each profile ranged from 30 kg N ha^{-1} y^{-1} at the Morrinsville site to > 400 kg N ha^{-1} y^{-1} at the Kereone site (Table 7.3), and primarily reflects the available C found *in situ* (Table 7.2). Despite the lower denitrification capacity of the Morrinsville site, it may be sufficient to significantly reduce NO_3^- leaching from the root zone as the agricultural nutrient budgeting model, Overseer®, estimates 42 kg N ha^{-1} y^{-1} may be lost from this site. However, these profile denitrification capacity estimates would be a 'best case scenario' as they assume that the 48 hour total N gas flux is constant, and that the relict C does not get depleted over time.

Overall, this study has shown that the changes in groundwater NO_3^- concentrations observed at redox boundaries are generally due to denitrification. In young groundwater, the low DO and NO_3^- concentrations should show isotopic enrichment, unless the residual NO_3^- pool concentrations are too low to be measured. Denitrification below the A horizon is C-limited and the denitrification capacity of the profile is restricted by the bioavailability of the relict organic matter.

Spatial variability in the location of this relict C results in hotspots of denitrification and makes it difficult to assess the catchment as a whole unit.

8.1.2 Denitrification at the Waihora Well field

Evidence for denitrification under field conditions (other than observation of declining DO and NO_3^- concentrations), proved challenging at the Waihora well field. The primary hypothesis was that the relict organic matter found in the palaeosol and bottom of the Taupo ignimbrite layer would provide the necessary electrons to fuel heterotrophic denitrification. This was supported by the observation of oxidised groundwater above these layers, and reducing zones below. It was envisaged that the relatively small sub-catchment and close proximity of the wells (maximum distance apart was 90 m) would yield evidence of denitrification using $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ analysis. However, a wide range of isotopic source signatures were measured in the oxidised groundwater samples (Figure 3.22) and few reduced groundwater samples had sufficient NO_3^- remaining for successful isotopic analysis (Figure 3.23). In this situation, the isotopic analysis of NO_3^- proved unsuccessful for gaining an understanding of denitrification.

As the hydraulic conductivities estimated using slug test data were, in general, an order of magnitude greater than those measured at Toenepi, push-pull tests were unsuitable for this location because the tracer could not have been recovered from the groundwater. Thus, as a consequence of these findings, laboratory-based incubation experiments were conducted to evaluate the denitrification capacity (Chapter 5) and potential (Chapter 6) of the different layers encountered at the well field.

Of the three different profiles identified at the Waihora well field, the complete profile, with a thick palaeosol (PA and P) and remains of vegetation in the form of woody debris in the bottom of the Taupo ignimbrite (TIW) layer yielded the greatest total N fluxes ($^{15}N_2 + N_2O$) of samples from below the A horizon (Figure 5.4). Denitrification was related to the C content of the sample; however, it appeared that C in the A horizon samples was more bioavailable, as the A horizon samples produced larger total N gas fluxes (4.3 – 6.2 nmol N g^{-1} dry soil h^{-1}), compared to those samples from below the A horizon, which had similar amounts of total or extractable C (a maximum of 0.8 nmol N g^{-1} dry soil h^{-1} was measured) (Figure 5.5).

To investigate whether fluxes of a similar magnitude to the A horizons could be generated in these 'subsoil' samples when a readily available source of C was added, further experiments were performed (Chapter 6). Three different C additions were tested: two extracts of C from the A horizon using cold and hot water, and glucose.

Unfortunately, the bioavailability and microbial response to the cold water extract (CWE) could not be evaluated as a high concentration of NO_3^- was also extracted from the A horizon into solution and when the extract was applied to the samples, the high NO_3^- concentration resulted in an inhibition of denitrification. Total N gas fluxes were lower in the CWE treatment than the control (NoC) samples (Figure 6.6) and more N_2O was produced in these samples (Figure 6.4), meaning that both N_2O and NO_3^- reduction were inhibited. In contrast, the hot water extract (HWE) samples from the TIW, PA and P layers generated more than double the NoC total N gas fluxes (up to 5 nmol N g^{-1} dry soil h^{-1}) (Figure 6.6). When this extract was evaluated against glucose, no consistent treatment effect could be observed as the TI and TIW samples yielded greater total N fluxes for the glucose treatment while the PA layer showed a greater total N flux response to the HWE treatment.

When the denitrification capacity fluxes were adjusted to recharge temperature (11°C) using a Q_{10} function of 2.0 , the calculated rates of N removal in the TIW and P layers exceeded the Overseer®-estimated NO_3^- leaching rates (Chapter 5). This may well account for the low DO and NO_3^- concentrations observed *in situ* from wells screened in these materials.

Overall, research at this site has identified the challenges of obtaining evidence and quantifying denitrification *in situ* and highlights the importance of using multiple methods to gain an understanding of the process. Denitrification does occur and is associated with the relict organic material at the site, and therefore occurs in hotspots, making large scale assessment of denitrification capacity difficult.

8.2 Future research

This research has shown that denitrification can occur under *in situ* conditions in two Waikato catchments. At these two study sites, the presence of relict organic matter provided the necessary electrons for denitrification, and laboratory studies showed that the systems were C-limited; however, the identity and role of inorganic electron donors needs to be developed further. This would be beneficial for work in new catchments where the distribution and bioavailability of relict organic matter may be insignificant.

Future work could improve methodologies to overcome the difficulties of low NO_3^- concentrations, either by improved analysis techniques (e.g. anion exchange columns to concentrate the NO_3^-) or by sampling along a known groundwater flowpath to connect oxidised and reduced samples. A more

thorough understanding of groundwater ages would result in strategic sampling (e.g. only 'young' groundwater samples) and more conclusive interpretation of data.

The *in situ* methodologies used could be improved by increasing the tracer volume to enable longer incubation period between the push and pull phases, and longer sampling times, or installation of alternative instrumentation such as mesocosms, especially in sites where the high hydraulic conductivity of the aquifer prevented *in situ* measurements.

Future work could also involve devising methods of catchment scale or water management zone assessment of denitrification capacities to enable strategic land management to protect water quality.

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