Characterisation of Denitrification in the Subsurface Environment of the Manawatū Catchment, New Zealand

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ABSTRACT

A sound understanding of the quantity of nitrate lost from agricultural soils, as well as their transport and transformation in soil-water systems is essential for targeted and effective management and/or mitigation of their impacts on the quality of receiving waters. However, there is currently little known about the occurrence, variability, or factors affecting, nitrate attenuation by subsurface (below the root zone) denitrification in New Zealand, particularly in the Manawatū River catchment. This thesis developed and applied a combination of regional- and local-scale hydrogeochemical surveys and experiments, to gain an insight into the occurrence, variability, and hydrogeological features of subsurface denitrification in the Manawatū River catchment, particularly in the Tararua Groundwater Management Zone (GWMZ).

A regional survey and analysis of samples from 56 groundwater wells conducted in the Tararua GWMZ revealed mainly oxic groundwater with low denitrification potential in the southern part of the catchment (Mangatainoka sub-catchment), whereas mainly anoxic/reduced groundwaters with high potential to denitrify in the middle and northern parts (Upper Manawatū sub-catchments). Oxic groundwaters with enriched nitrate concentrations were generally correlated with coarse textured soil types and aquifer materials (e.g., well-drained soil, gravel rock type), allowing faster movement of percolating water and oxygen diffusion from surface to subsurface environments.

Local-scale laboratory incubations and in-field, push-pull test techniques were evaluated and optimised to measure and quantify denitrification in unsaturated (vadose) and saturated (shallow groundwater) parts of the subsurface environment. A novel incubation technique

using vacuum pouches was found to be more reliable than traditional Erlenmeyer flasks in determining denitrifying enzyme activity (DEA) in subsurface soils (>0.3 m depth) with low denitrification activity. A combination of 75 µg N g^{-1} dry soil and 400 µg C g^{-1} dry soil was also found to provide the optimum DEA in subsurface soils. In the evaluation of the push-pull test, denitrification rates estimated using the measurements of denitrification reactant (nitrate) were found to be significantly higher (6 to 60 times) as compared to the rates estimated using the measurements of denitrification product (nitrous oxide). The estimates of denitrification rates also differed depending on whether a zero-order or first-order kinetic model was assumed. However, either a zero-order or a first-order model appears to be valid to estimate the denitrification rate from push-pull test data.

The optimised laboratory incubation technique and in-field, push-pull test were applied at four sites with contrasting redox properties; Palmerston North, Pahiatua, Woodville, and Dannevirke. The incubation technique revealed that denitrification potential in terms of DEA is highest in the surface soil and generally decreased with soil depth. The push-pull test measured large denitrification rates of 0.04 to 1.07 mg N L^{-1} h^{-1} in the reduced groundwaters at depths of 4.5-7.5 m below ground level at two of the sites (Woodville and Palmerston North), whereas there were no clear indications of denitrification in the oxidised shallow groundwaters at the other two sites (Pahiatua and Dannevirke).

This new knowledge, information and techniques advance our scientific capability to assess and map subsurface denitrification potential for targeted and effective land use planning and water quality measures in the Manawatū catchment and other catchments across New Zealand's agricultural landscapes and worldwide.

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PUBLICATIONS AND PRESENTATIONS

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Rivas, A., Singh, R., Bishop, P., Horne, D., Roygard, J., Hedley, M.J., 2018. A novel acetylene inhibition incubation technique for accurate quantification of denitrifying enzyme activity in subsurface soils. *Soil Biology & Biochemistry* (submitted, under revision).

Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., Hedley, M.J., 2018. Quantification of denitrification rate in shallow groundwater using the single-well push-pull test technique. *Journal of Contaminant Hydrology* (submitted, under revision).

Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., Hedley, M.J., 2018. Contrasting Denitrification Characteristics in the Vadose Zone and Shallow Groundwater Beneath Temperate Pasture Lands and their Implications for Nutrient Management in Agricultural Landscapes. *Journal of Environmental Quality* (in preparation).

Published Conference Proceedings (presented by A. Rivas)

Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., Hedley, M., 2015. An assessment of the denitrification potential in shallow groundwaters of the Manawatu River catchment. In: *Moving farm systems to improved attenuation*. (Eds L.D. Currie and L.L Burkitt). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 28. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 15 pages.

Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., & Hedley, M., 2014. Characterization of denitrification in the subsurface environment of the Manawatu River catchment, New Zealand. In *Proceedings of the 21st Century Watershed Technology Conference and Workshop*, American Society of Agricultural and Biological Engineers (ASABE), The University of Waikato, Hamilton, New Zealand. DOI: 10.13031/wtcw.2014-029.

Rivas, A., Singh, R., Bishop, P., Horne, D., Roygard, J., Hedley, M., 2014. Measuring denitrification in the subsurface environment of Manawatu River catchment. In: *Nutrient management for the farm, catchment and community*. (Eds L.D. Currie and C L. Christensen). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 27. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 13 pages.

Published Conference Abstracts (presented by A. Rivas)

Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., Hedley, M., 2016. Variability in denitrification characteristics of the vadose and saturated zones in the Manawatu River catchment. In: *Integrated nutrient and water management for sustainable farming*. (Eds L. D. Currie and R. Singh). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 29. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand.

Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., Hedley, M., 2015. Variability in Hydrogeochemical Conditions in Shallow Groundwater in the Manawatu River Catchment and Implications for Denitrification Potential. In: *NZ Hydrological Society Annual Conference: From Data to Knowledge*. NZ Hydrological Society, Hamilton, New Zealand, pp. 163–164.

Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., Hedley, M., 2014. Indications of denitrification potential and occurrence in groundwater in the Manawatu catchment. In: *NZ Hydrological Society Annual Conference: Integration: 'The Final Frontier'*. NZ Hydrological Society, Marlborough, New Zealand, pp. 225.

Rivas, A., Singh, R., Horne, D., Bishop, P., Hedley, M., Roygard, J., 2013. Evaluation of Acetylene Inhibition Method to Measure Denitrification in Unsaturated Zone. In: *Joint Conference of the NZ Hydrological Society and the Meteorological Society of NZ: Water & Weather: Solutions for Health, Wealth & Environment*. NZ Hydrological Society, Palmerston North, New Zealand, pp. 183-184.

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Published Conference Proceedings (full paper):

Collins, S. B., Singh, R., **Rivas, A.**, Palmer, A., Horne, D., Roygard, J., Matthews, A., 2016. Assessment of nitrogen flow pathways and its potential attenuation in shallow groundwaters in the Lower Rangitikei catchment. In: *Integrated nutrient and water management for sustainable farming*. (Eds L. D. Currie and R. Singh). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 29. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 14 pages.

Singh, R., **Rivas, A.**, Espanto, P., Elwan, A., Horne, D., Roygard, J., Matthews, A., Clothier, B., 2014. Assessment of transport and transformation of nitrogen in the subsurface environment of Manawatu River Catchment – work in progress. In *Nutrient management for the farm, catchment and community*. L. D. Currie and C L. Christensen, eds. Occasional

Report No. 27. Palmerston North, New Zealand: Fertilizer and Lime Research Centre, Massey University.

Published Conference Abstracts:

Singh, R., Horne, D., Elwan, A., **Rivas, A.**, Roygard, J., Hedley, M. 2018. Influence of soil types and underlying geology on transport and transformation of nitrate in subsurface environment. *Soil science: beyond food and fuel. 21st World Congress of Soil Science*, 12-17 August 2018, Rio de Janeiro, Brazil. (submitted)

McGowan, P., Singh, R., **Rivas, A.**, Elwan, A., Palmer, A., Horne, D., Collins, S., Matthews, A., 2017. Redox characteristics of shallow groundwater in the Tararua Groundwater Management Zone. In: *New Zealand Hydrological Society Annual Conference: Filling the Knowledge Reservoir*. NZ Hydrological Society, Napier, New Zealand, pp. 139-140.

Singh, R., Horne, D., Elwan, A., **Rivas, A.**, Manderson, A., Roygard, J., and Hedley, M., 2017. Assessing and matching landuse with land suitability – the model development and landuse implications. In the proceedings of the international conference *'Innovative Solutions for Sustainable Nitrogen Management'*, June 26-30, Aarhus, Denmark. http://sustainablenconference.dnmark.org/?page_id=298

Collins, S., Singh, R., **Rivas, A.**, Palmer, A., Horne, D., Roygard, J. & Matthews, A., 2015. Transport and fate of nitrogen in the Lower Rangitikei Catchment. In: *NZ Hydrological Society Annual Conference: From Data to Knowledge*. NZ Hydrological Society, Hamilton, New Zealand.

Singh, R., **Rivas, A.**, Elwan, A., Heather, M., Horne, D.J., Burkitt, L., Morgenstern, U., Roygard, J., Matthews, A., Clothier, B., & Hedley, M. 2015. Research for catchment attenuation processes: advancing knowledge and tools to account for nitrogen transport and transformation in NZ agricultural catchments. In the proceedings of the international conference *'Catchment Science 2015'*, September 28-30th, 2015, Wexford, Ireland. http://www.teagasc.ie/agcatchments/cs2015/Presentations/Singh_R.pdf

Singh, R., **Rivas, A.**, Elwan, A., Horne, D.J., Burkitt, L., Roygard, J., Matthews, A., Clothier, B., & Hedley, M. 2015. Assessment of nitrogen attenuation in the subsurface environment of Manawatu River catchment, New Zealand. In the proceedings of the international conference *'Land Use and Water Quality 2015'*, September 21-24th, 2015, Vienna, Austria. http://web.natur.cuni.cz/luwq2015/download/oral/246_Singh%20et%20al._LuWQ2015_Manawatu%20Denitrification%20Case%20Study_Sept%202015.pdf

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Ag	silver
AI	acetylene inhibition
Al	alluvium
amsl	above mean sea level
ANOVA	Analysis of Variance
APHA	American Public Health Association
BD	bulk density
bgl	below ground level
Br^-	bromide
C	carbon
Ca^{2+}	calcium
CBE	charge balance error
Cl^-	chloride
CO_2	carbon dioxide
C_2H_2	acetylene
DAN	Dannevirke
DEA	denitrifying enzyme activity
DEM	digital elevation model
DIC	dissolved inorganic carbon
DNRA	dissimilatory nitrate reduction to ammonium
DO	dissolved oxygen
DOC	dissolved organic carbon
Eh	redox potential
EU	European Union
FeS_2	pyrite
Fe^{2+}	ferrous iron
FSL	Fundamental Soil Layer
g	gram
GC	gas chromatograph
Gr	gravel
GWMZ	Groundwater Management Zone
h	hour
ha	hectare
HCO_3^-	bicarbonate
HWC	hot water-extractable carbon
H_2O	water
H_2SO_4	sulphuric acid
ICP-OES	Inductively coupled plasma optical emission spectrometry
K^+	potassium
KBr	potassium bromide
kg	kilogram
kJ	kilojoule
km	kilometre
km^2	square kilometre
KNO_3	potassium nitrate
$K_2Cr_2O_7$	potassium dichromate
L	litre

Lo	loess
LSD	Least Significant Difference
m	metre
M	million
MAV	maximum allowable value
mg	milligram
mg L^{-1}	milligram per litre
Mg^{2+}	magnesium
mL	millilitre
mm	millimetre
Mn^{2+}	manganese
mol	mole
MRT	Mean Residence Time
mV	millivolts
m^3	cubic metre
N	nitrogen
Na^+	sodium
NH_4^+	ammonium
NO	nitric oxide
NO_2^-	nitrite
NO_3^-	nitrate
NPSFM	National Policy Statement for Freshwater Management
N_2	nitrogen gas
N_2O	nitrous oxide
OC	organic carbon
ORP	oxidation-reduction potential
O_2	oxygen
P	phosphorus
PAH	Pahiatua
PCA	Principal Components Analysis
PE	polyethylene
PNR	Palmerston North
ppb	parts per billion
ppm	parts per million
PVC	polyvinyl chloride
rpm	revolution per minute
R^2	coefficient of determination
SO_4^{2-}	sulphate
SPC	specific conductance
UK	United Kingdom
WDV	Woodville
WFPS	water-filled pore space
yr	year
°C	degree Celcius
µg	microgram
µL	microlitre
µm	micrometre

CHAPTER 1

INTRODUCTION

1.1 Background

The worldwide expansion and intensification of agriculture to meet the ever growing demand for food comes with environmental challenges, especially for the use and management of land and water resources. In enhancing production in cropped and grazed lands, the global consumption of nitrogen (N) fertiliser has increased from almost nil in the 1940s (Di and Cameron, 2002) to approximately 111.4 x 10^9 kg N yr^{-1} in 2013 (Food and Agriculture Organization of the United Nations (FAO), 2015). Unfortunately, the efficiency of N fertiliser use by crops, and the recycling efficiency of excretal N on grazed pastures can be low, resulting in N leaching and contamination of ground and surface waters (e.g., Liao et al., 2012; Clough et al., 1996). For instance, in certain land use types such as pastures grazed by dairy cows, urine has been found to contribute 70-90% of total N leached (Ledgard et al., 2009). In fact, the amount of N in urine patches has been found to be similar to application rates of 200–2000 kg N ha^{-1} (Selbie et al., 2015). Nitrogen leaching below the root zone is mainly in the form of nitrate (NO_3^-) due to its negative charge and water solubility. Depending on local conditions, leached NO_3^- accounts for 5-50% of the N applied to agricultural land (Liao et al., 2012).

Nitrate is a key contaminant in freshwater bodies worldwide. Large concentrations of NO_3^- have been observed in freshwaters in the; United States (Nolan et al., 1997), Europe (Strebel et al., 1989), Asia (Bouman et al., 2002; Suthar et al., 2009; Zhang et al., 2013) and also in

New Zealand (McLarin et al., 1999). Non-point sources, such as agriculture, have been identified as a major contributor to the high concentrations of NO_3^- in freshwater (Gu et al., 2013; Ledein et al., 2007). Recognition of the extent of the problem and its impacts on the environment and human health has led to the implementation of measures to minimise the impact of agricultural activities on water quality, examples of which include the EU Water Framework Directive (E.Union, 2000), the EU Nitrates Directive (E.Union, 1991) , and locally, the National Policy Statement on Freshwater Management (MfE, 2014).

Enriched concentrations of NO_3^- in freshwaters have undesirable consequences. In surface waters, the environmental consequences of high NO_3^- concentrations include eutrophication and fish poisoning (Di and Cameron, 2002; Martin et al., 1999; Puckett et al., 1999; Rivett et al., 2008). Moreover, drinking water which is highly contaminated with NO_3^- poses significant health risks such as methaemoglobinaemia (or blue baby syndrome) (Addiscott et al., 2004) and increased risk of colorectal cancer (Schullehner et al., 2018) in humans and asphyxiation in livestock (Martin et al., 1999). The remediation of nitrate-contaminated groundwater or surface water at the landscape scale is often not practicable as it entails large costs such as those associated with treatment systems and well replacement (Lewandowski et al., 2008).

Factors known to influence the amount of NO_3^- leached from the soil are numerous and include the; application of N fertilisers, N transformations and NO_3^- availability in the soil profile, plant N uptake, the quantity and pattern of drainage, and soil profile characteristics (including physical properties such as texture and permeability) (Paramasivam et al., 2002). Various studies have also identified landscape, climatic conditions and management practices (e.g. land use and tillage practices, water management, etc.) as important factors in the

transport of NO_3^- from their applied location (source) to water bodies (receptor) (Mastrocicco et al., 2011; Paramasivam et al., 2002). Nitrogen, once leached from agricultural soils, takes different flow pathways (mainly via subsurface drainage, subsurface flow and groundwater movement) to receiving surface water bodies. The cycling and transformation of N in the soil profile especially in the upper soil layer is reasonably well understood (Powlson, 1993), but there is limited information on its transport and transformation in the subsurface environment (i.e. below the root zone). Recent studies of subsurface denitrification used isotopes of NO_3^- to investigate the source and transformation processes occurring along subsurface pathways (Clague et al., 2015a; Kelley et al., 2013; Minet et al., 2012).

Nitrate leached into the subsurface environment may be removed from the system through different NO_3^- attenuation processes, which are influenced by climate and dilution. These include plant uptake (mostly as removal via phreatophytes in riparian areas), denitrification, dissimilatory NO_3^- reduction to ammonium (DNRA), and assimilation of NO_3^- into microbial biomass (Martin et al., 1999; Porubsky et al., 2011; Puckett et al., 2008; Puckett and Hughes, 2005; Rivett et al., 2008). Subsurface denitrification has been generally recognised as the most significant NO_3^- removal process (Anderson et al., 2014; Korom, 1992; Puckett and Cowdery, 2002; Puckett et al., 2008; Rivett et al., 2008; Starr and Gillham, 1993; van Cleemput, 1998). Significant denitrification occurs in areas of reducing conditions and high concentrations of labile organic matter, such as riparian zones, hyporheic zones, and aquifers that receive percolating water rich in dissolved organic carbon (DOC) (Puckett et al., 2008; Rivett et al., 2008). Several studies have identified the occurrence of NO_3^- attenuation in the subsurface from discrepancies observed between the quantity of NO_3^- leached from the soil profile and the NO_3^- in receiving surface waters (Clothier et al., 2007; Meixner et al., 2012; Pfeiffer et al., 2006; Stenger et al., 2008). On the other hand, recent findings show that

substantial amounts of N may also accumulate in the subsurface soils, such as in the Mississippi River Basin (van Meter et al., 2016) and in England and Wales (Ascott et al., 2016), hence the importance of subsurface soil investigations.

An integrative understanding of the denitrification process that occur in the subsurface as NO_3^- is transported from farms to receiving surface waters is important for a number of reasons. It provides information on the capability of the subsurface to reduce NO_3^- and indicates the vulnerability of the groundwater and surface waters to NO_3^- pollution. As such, it has the potential to become a key component in the management and mitigation of any adverse impacts of land use and management practices on receiving water quality and ecosystem health. Furthermore, an improved understanding of local denitrification mechanisms plays an important role in the development of future generations of catchment scale models to evaluate different management and mitigation scenarios.

1.2 Rationale of the Study

A sound understanding of the transport and effects of nitrate is a key component of managing the influence of agricultural production on receiving water quality. The N transformation processes in surface soils have been widely investigated and numerous studies have estimated and measured the amount of NO_3^- leached below the root zone in different soils (Clough et al., 1996; Di et al., 1998; Di and Cameron, 2002; Mantovi et al., 2006; Necpalova et al., 2012). In particular, denitrification in the surface soil (or root zone) has been widely studied (Clough et al., 1996; Deslippe et al., 2014; Hashimoto and Niimi, 2001; Jha et al., 2012; Luo et al., 1998; Ruz-Jerez et al., 1994). However, studies of denitrification in the subsurface, especially below the root zone, have been relatively scarce (Barkle et al., 2007; Castle et al.,

1998; Jahangir et al., 2013; Jahangir et al., 2012b; Kamewada, 2007; Murray et al., 2004; Vilain et al., 2012; Yeomans et al., 1992) with only few studies focusing on depths greater than 1m (Peterson et al., 2013; Thomas et al., 2012). As a consequence, there is limited understanding of the role of biogeochemical processes in determining the fate of NO_3^- in the vadose and saturated zones (Groffman et al., 2006; Khdyer and Cho, 1983). Furthermore, the extent of denitrification occurring in the subsurface varies widely depending on the different hydrogeological settings and biogeochemical conditions across landscapes (Clagnan et al., 2018; Jahangir et al., 2012b, 2012c, 2012a, 2010; Khalil and Richards, 2011; Mcaleer et al., 2017; Minet et al., 2012; Pfeiffer et al., 2006; Rivett et al., 2008; Stenger et al., 2008). It is, therefore, imperative that investigations of denitrification account for the spatial and temporal variations in the subsurface environment. Knowledge gained from these investigations would be useful in the application of the concept of Functional Land Management (Schulte et al., 2014; Sullivan et al., 2015), which is intended to enable policy to utilise the different functions of the soil resources to meet both expectations of agricultural productivity and environmental sustainability.

Considering the negative impacts of excessive NO_3^- on the receiving water environment and the beneficial role of attenuation, there is a need for an improved understanding of the transport, transformations and fate of NO_3^- in the subsurface environment. In view of the above background and rationale, the overarching research questions that this thesis addresses are as follows:

- What is the distribution of the potential for subsurface denitrification across agricultural landscapes?

- How do denitrification characteristics in the subsurface vadose and saturated zones vary over time and space?
- What hydrogeological factors influence denitrification, and its variation, in the subsurface environment?

1.3 Study Area

This lack of investigation of subsurface denitrification characteristics is particularly true for agricultural catchments across New Zealand (NZ). There are only a few regional studies of the potential of the subsurface environment to reduce NO_3^- in NZ landscapes (Rissmann, 2011). Limited site-specific studies of denitrification in the deeper vadose and saturated zones have been conducted elsewhere in NZ, e.g., Waikato region (Barkle et al., 2007; Clague et al., 2013; Stenger et al., 2008) and Canterbury region (Peterson et al., 2013; Thomas et al., 2012). There have been no detailed investigations of subsurface denitrification in the deeper vadose and saturated zones in the Manawatū-Wanganui Region, including the Manawatū River catchment.

Studies of denitrification potential in the subsurface environment of the Manawatū are required in order to gain an understanding of the capabilities of the subsurface to denitrify and subsequently decrease the vulnerability of a highly polluted groundwater and surface water system to NO_3^- contamination. Nitrogen concentrations exceeding the standard (i.e., 0.444 g soluble inorganic N m^{-3}) are routinely found in surface waters in the Manawatū catchment (Roygard and McArthur, 2008) and result in the growth of nuisance periphyton in many streams and rivers (McArthur and Clark, 2007). In terms of improving water quality, the focus is on land under intensive agricultural activities, such as pastoral farming, as the N

loading from these areas (non-point sources) is recognised as the main source (>95%) of high N concentration in surface waters (Ledein et al., 2007). The subsurface environment has a role in the NO_3^- loading to surface water given that leaching is the main loss pathway of nitrogen under grazed systems (Clothier et al., 2007).

Clothier et al. (2007) estimated that approximately only 50% of leached nitrogen from farms (dairy and sheep/beef farms) in the Upper Manawatū River (above Hopelands) contributes to the N loadings measured in the river. There is very limited information and knowledge of the occurrence, spatial variability, and biogeochemical processes and hydrogeological factors determining NO_3^- attenuation potential in subsurface environment of the catchment. This is, therefore, a knowledge gap that could be filled with denitrification investigations in the vadose and saturated zones in the catchment across time and space. This knowledge will be important for improving ground and surface water quality in many other catchments across New Zealand.

1.4 Research Objectives

Overall, this thesis aims to characterise denitrification in the subsurface environment of the Manawatū River catchment. The subsurface environment (below the root zone) is divided into two zones namely, the unsaturated (or vadose) zone and the saturated zone (shallow groundwater).

The main objectives of this thesis are as follows:

- *To identify the occurrence and potential for denitrification in groundwater in the Manawat*ū *catchment;*

- *To evaluate and establish reliable and practical methods for measurements of denitrification in the vadose and saturated zones;*
- *To quantify denitrification in the subsurface environment (vadose and saturated zones) at selected sites in the Manawatū catchment and their characteristics over different seasons; and*
- *To assess the various, site-specific hydrogeological factors that are responsible for the spatial and temporal variability in the subsurface denitrification characteristics.*

The above research objectives are addressed in the following chapters.

1.5 Thesis Structure

This thesis is structured into seven chapters and six appendices. The technical chapters were prepared in a manuscript style for submission to peer-reviewed journals. Thus, there is some repetition of introductory material and description of methods.

Chapter 1 (this chapter) sets the background and rationale of this research as well as listing the research objectives and describing the structure of this thesis.

Chapter 2 provides an extensive review of the literature on denitrification in the subsurface environment. It reviews different NO_3^- attenuation processes in the subsurface environment, environmental factors affecting denitrification, the variability in denitrification in the vadose and saturated zones, and the status of and need for denitrification studies in New Zealand. It further reviews various approaches and methods for measuring denitrification in soil-water systems.

Chapter 3 reports the results and findings of a groundwater survey conducted to determine the potential and occurrence of denitrification in groundwaters in the Manawatū catchment, with particular focus on the eastern side (Tararua Groundwater Management Zone). The results from this study informed the selection of field study sites for detailed investigation of subsurface denitrification in the vadose and saturated zones in the study catchment.

Chapter 4 develops and evaluates a novel technique for quantifying the denitrifying enzyme activity (DEA) in subsurface soil layers. The development of this new technique became necessary on the observation of the limitations of established methods for measuring DEA in subsoils.

Chapter 5 investigates the single-well, push-pull test technique for measuring the denitrification rate in shallow groundwater. It assesses the effects of the different analytical approaches and kinetic models used for accurate estimation of the denitrification rate in shallow groundwaters.

Chapter 6 investigates the spatial and temporal variability of subsurface denitrification characteristics and the controlling factors in the vadose and saturated zones at four selected sites representing areas with low or high potential for denitrification in the subsurface environment. This chapter builds on the information of variability in the denitrification characteristics in the study catchment (Chapter 3) and applies the techniques developed for measuring denitrification in the unsaturated (Chapter 4) and saturated (Chapter 5) zones.

Chapter 7 summarises and discusses the results from the previous chapters in the context of the research objectives of this study. It also recommends future research work to advance the limited understanding of subsurface denitrification across agricultural landscape, particularly in the Manawatū River catchment.

CHAPTER 2

DENITRIFICATION IN THE SUBSURFACE ENVIRONMENT – A REVIEW OF ITS PROCESSES, INFLUENCING FACTORS, MEASUREMENT APPROACHES, AND DENITRIFICATION STUDIES IN NEW ZEALAND

2.1 Introduction

This chapter aimed a review of literature on denitrification in the subsurface environment, including its role in nitrate transformation or 'attenuation' in both the vadose and saturated zones, and its implications for nitrate management to mitigate the impacts of land activities on receiving water quality. It reviewed the theoretical background on the denitrification process and the controlling factors responsible for its occurrence and variability in the subsurface environment. It also reviewed the existing approaches for measuring denitrification both in the vadose and saturated zones, including the advantages and disadvantages of different methods. Finally, this chapter studied and summarised the significance and status of denitrification studies in New Zealand and the identified knowledge gaps and research needs.

2.2 Nitrate and Its Attenuation in the Subsurface Environment

2.2.1 Nitrate in the subsurface environment

The presence of nitrogen (N) in soils is essential to support plant growth, particularly for growth of agricultural crops for food production. While usually naturally present in the soil in the form of organic matter, N is added to the agricultural soils through atmospheric

deposition (e.g., by rainfall), fixation (conversion of atmospheric N to organic N by lightning or biologically by legumes) and application of fertilisers (both in organic and inorganic forms). Due to the demand for increased food production, the global use of N fertiliser has increased from almost nil in the 1940s (Di and Cameron, 2002) to approximately 111.4 x 10^9 kg N in 2013 (Food and Agriculture Organization of the United Nations (FAO), 2015). Nitrogen present in the soil is transformed into different forms by microorganisms to harvest energy or acquire N to support growth. Microorganisms decompose organic N from manure, crop residue or organic matter to ammonium or ammonia (a process called mineralisation or ammonification), which is further converted to nitrate (NO_3^-, a process called nitrification) under aerobic conditions to obtain energy (Arp, 2009). Nitrate produced may be taken up by plants through their roots, or converted back by microorganism to organic N to become part of their cells and tissues (assimilation or immobilisation). Being negatively charged like soil particles, excess NO_3^- is easily leached with water flow through the soil profile (Appelo and Postma, 2005). The amount of NO_3^- in the soil depends on a number of factors namely, mineralisation and nitrification rates, plant N uptake, microbial immobilisation and nitrate movement by leaching and diffusion (Saggar et al., 2013). Leaching of NO_3^- to groundwater has been a global environmental concern brought about by extensive and intensive agricultural practices (Di et al., 1998; Di and Cameron, 2002; Necpalova et al., 2012). In particular NO_3^- leaching losses has been found to range from 6-162 kg N ha^{-1} yr^{-1} in grazed pasture systems in the UK and New Zealand (Di and Cameron, 2002).

Figure 2.1 The nitrogen cycle and its influence upon the water environment.
Source: Rivett et al., 2008

Figure 2.1 summarises the N transformation processes in the soil and the subsequent influence of N on the receiving water environment. The entry of NO_3^- into the subsurface environment is mainly through leaching to groundwater and, subsequently, transport to surface water systems. While NO_3^- may be present in the soil and water systems by natural processes, NO_3^--N concentrations greater than 0.25 mg L^{-1} in groundwater systems may indicate anthropogenic influence such as the application of fertilisers (Morgenstern and Daughney, 2012). On the other hand, the maximum allowable value for drinking water according to the 2005 Drinking-water Standards for New Zealand (DWSNZ) is 11.3 mg NO_3^--N L^{-1} (equivalent to 50 mg NO_3^- L^{-1}), whereas the long-term Irrigation Trigger Value defined by the Australia and New Zealand Environmnet Conservation Council (ANZECC) in

2000 to minimise N leaching into groundwater and surface water is 4.97 mg NO_3^--N L^{-1} (equivalent to 22 mg NO_3^- L^{-1}) (Daughney et al., 2009).

2.2.2 Nitrate attenuation processes in the subsurface environment

Nitrate percolating into the subsurface may be consumed or transformed into other forms by several processes. These processes, as discussed below, are driven by several factors that determine the dominant process in each setting or condition.

i. Plant uptake

Within the root zone, plant uptake is presumably the dominant and desired nitrate removal process (Liao et al., 2012), as it results in plant growth and crop production. Plant uptake is generally relatively limited from deeper depths (> 60 cm) of soil profile under grasslands due to the shallower root zone, but it could be significant in riparian areas depending on existing vegetation especially in the presence of phreatophytes with deep root systems (Rivett et al., 2008).

ii. Assimilation of nitrate into microbial biomass (immobilisation)

Microorganisms also uptake and consume NO_3^- to support their growth and could remove significant amounts of NO_3^- in the subsurface (Rivett et al., 2008). However, this NO_3^- removal process via assimilation and conversion to organic N by microbes is temporary as it can be expected that the organic N will be converted back to mineral N when the microorganisms die.

iii. Assimilatory nitrate reduction

Carried out by many types of bacteria, this process reduces NO_3^- into ammonium in soil-water systems. However, assimilatory NO_3^- reduction is dependent on high concentration of ammonium and organic N (Tiedje, 1994). In anaerobic environments where ammonium and organic N are typically low, the occurrence of this process is unlikely (Tiedje, 1994). Therefore, it appears that this assimilatory process is less significant in the subsurface environment where anaerobic conditions are more common than in the surface layer.

iv. Dissimilatory nitrate reduction to ammonium (DNRA)

Similar to the assimilatory nitrate reduction process, the DNRA process also reduces NO_3^- into ammonium in soil-water systems. As such, the importance of both processes, the assimilatory and dissimilatory reduction of NO_3^- to ammonium, is the retention of N in the system with NO_3^- being converted to another mineral N form for possible uptake by plants (Rütting et al., 2011). However, instead of being regulated by the amount of ammonium and organic N, DNRA is largely regulated by O_2 concentrations and occurs under low oxygen concentrations (Rütting et al., 2011; Tiedje, 1994). In addition, DNRA mainly occurs in presence of low *Eh* (redox potential), available NO_3^- and labile carbon (Thayalakumaran et al., 2008). Also, DNRA is expected to be a significant process in anoxic conditions where electron donors such as carbon are more abundant relative to NO_3^- (Storey et al., 2004; Tiedje, 1988). In such conditions, DNRA is the "more efficient pathway for removing electrons" compared to other processes (Storey et al., 2004) and "more electrons can be transferred per mole of nitrate" (Rütting et al., 2011). Rütting et al. (2011) specified the carbon: nitrate-N ratio of 12 as the threshold for the significant occurrence of DNRA which was based on a study on paddy soils in China and Australia. Groffman et al. (2006) reported that DNRA was found significant in sediments and wet tropical soils.

v. Denitrification

Nitrate in the subsurface environment can also be transformed and attenuated by a process called denitrification. Denitrification is a microbial-mediated transformation of NO_3^- to gaseous oxides (nitric oxide [NO] and nitrous oxide [N_2O]), with nitrogen gas (N_2) as the ultimate end-product (Hiscock et al., 1991; Knowles, 1982; Korom, 1992; Trudell et al., 1986). In this process, microorganisms derive energy via the "chemical potential that results from having nitrate (an electron acceptor) brought into contact with an electron donor (e.g. organic carbon)" (Böhlke, 2003). This NO_3^- transformation is described as follows (Appelo and Postma, 2005):

$$NO_{3\,(aq)}^- \rightarrow NO_{2\,(aq)}^- \rightarrow NO_{(enzyme\ complex)} \rightarrow N_2O_{(gas)} \rightarrow N_{2(gas)}$$

Denitrification is either heterotrophic or autotrophic depending on the source of electron donor. It is characterised as heterotrophic if electron donor is organic (particularly carbon), whereas autotrophic if electron donor is inorganic (e.g., reduced manganese, ferrous iron, and sulphides). The general formula for the heterotrophic denitrification reaction (Spalding and Parrott, 1994) is as follows:

$$5C + 4NO_3^- + 2H_2O \rightarrow 2N_2 + 4HCO_3^- + CO_2$$

In autotrophic denitrification, if pyrite (FeS_2) is the electron donor, the stoichiometry is as follows indicating that the process involves oxidation of both sulphur and ferrous iron (Appelo and Postma, 2005; Tesoriero et al., 2000):

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

$5Fe^{2+} + NO_3^- + 7H_2O \rightarrow 5FeOOH_{(goethite)} + 0.5N_2 + 9H^+$

In autotrophic denitrification, the overall reaction is as follows (Torrentó et al., 2010):

$15NO_3^- + 5FeS_2 + 10H_2O \rightarrow 7.5N_2 + 10SO_4^{2-} + 5Fe(OH)_3 + 5H^+$

As such, the oxidation of pyrite results to increases in sulphate (and Fe^{2+}, if not further oxidised).

In general terms, the conditions that regulate denitrification are similar with the DNRA, i.e. anaerobic or anoxic, low *Eh*, available NO_3^-, and labile carbon or other electron donor. However, except when carbon: nitrate-N ratio is high as mentioned above, denitrification is generally favoured over DNRA due to the higher potential free energy of the former (-2669 kJ mol^{-1} glucose, compared to -1796 kJ mol^{-1} glucose with DNRA; Rütting et al., 2011; Tiedje, 1994).

vi. Chemodenitrification

Chemodenitrification is another process that can reduce NO_3^- in the subsurface environment. Chemodenitrification is the non-biological reduction of NO_3^- that produces mainly NO, with N_2O and N_2 as minor products (Tiedje, 1994). In this reduction process, the most significant reaction is the nitrite destruction catalysed by acid (Tiedje, 1994). Thus, it may be expected in acidic soils with pH < 5.0 such as in acid forest soils, salting out of frozen soils, and wetting of dry soils (Tiedje, 1994).

Significance of different nitrate reduction processes

While the relative importance of each of the above described NO_3^- attenuation processes is not known (Martin et al., 1999), it can be inferred that conditions in the subsurface environment seem to favour denitrification than other NO_3^- reducing processes. Several studies have indicated this by observing the occurrence of denitrification but not of other processes like DNRA (Jahangir et al., 2012a; Stenger et al., 2008).

Compared to DNRA and assimilatory NO_3^- reduction to ammonium, denitrification may not be the desired N transformation process in the root zone as it converts NO_3^- into a gaseous product consequently not available to plants or crops. However, it becomes an essentially beneficial process once NO_3^- has leached below the root zone to prevent the degradation of the quality of groundwater and surface waters.

2.2.3 Factors affecting denitrification

Korom (1992) enumerated the four general requirements for denitrification to occur, i.e. the presence of N oxides as terminal electron acceptors, presence of bacteria possessing the metabolic capacity, availability of suitable electron donors, and anaerobic condition or restricted O_2 availability in soil-water systems. There are several other factors that influence the occurrence and extent of denitrification in unsaturated and saturated soils. These factors and the above described general requirements have been widely reviewed in the literature (Buss et al., 2005; Firestone, 1982; Hiscock et al., 1991; Knowles, 1982; Payne, 1981; Rivett et al., 2008; Saggar et al., 2013), and are generally illustrated in Figure 2.2.

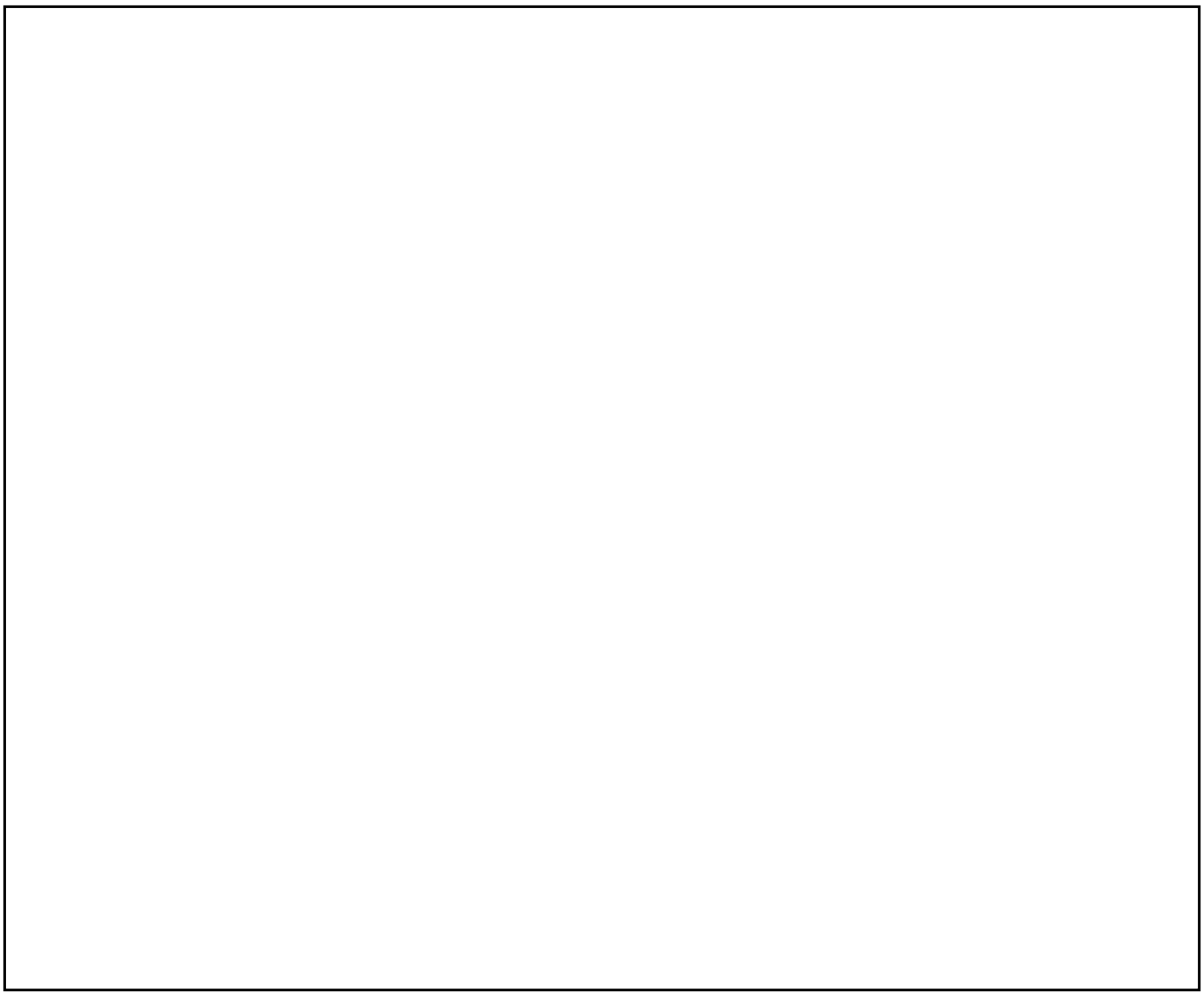


Figure 2.2 Factors affecting denitrification in soils.
Note: Proximal factors in red shading, distal factors in blue shading.
Source: Saggar et al., 2013

Saggar et al. (2013) categorised the factors of denitrification into proximal and distal regulators. Proximal regulators (NO_3^- concentrations, C availability, O_2 concentration, and temperature) are "those that immediately affect denitrifying communities leading to instantaneous changes in denitrification rates". On the other hand, distal regulators are those that "control the composition and diversity of denitrifying communities over larger spatial and temporal scales for a longer term and on a larger scale than proximal regulators". These include plant growth, management practices, soil texture, soil *pH*, and water availability. However, there is lack of comprehensive review of factors affecting denitrification with particular focus on the subsurface environment (both vadose and saturated zones). The

characteristics of proximal indicators and other factors that have direct influence on denitrification in the subsurface environment are described below.

i. Nitrate and other Nitrogen Oxides

One of the main factors controlling denitrification is the availability of nitrogen oxides (NO_x), especially NO_3^-, being a primary requirement for the process to occur (Saggar et al., 2013). The amount of available NO_3^- affects the rate of denitrification. This is demonstrated in positive correlations observed between denitrification rates and NO_3^- concentrations in freshwater and marine ecosystems (Seitzinger, 1988). At high NO_3^- concentration (>1 mg N L^{-1}), the denitrification reaction is considered of zero-order kinetics (Buss et al., 2005), meaning NO_3^- is not limiting the reaction; whereas at low NO_3^- concentration (<1 mg N L^{-1}) the denitrification reaction is considered of first-order kinetics (Knowles, 1982), meaning the rate of denitrification is dependent of the NO_3^- concentration. On the other hand, excessive amount of NO_3^- (e.g., >100 µg N g^{-1} soil) may inhibit denitrification activity in soil-water systems (Buss et al., 2005; Knowles, 1982; Luo et al., 1996). However, when the amount of carbon is much greater than the available NO_3^-, DNRA may become predominant process than denitrification in soil-water systems (see above discussions on DNRA).

Denitrification is not only affected by the amount but also by the diffusion of NO_3^- to denitrifying sites (Knowles, 1982). Luo et al. (1999) found that, especially in dry soils, denitrification in grazed pasture is primarily limited by the diffusion of NO_3^- to active microsites where denitrification occurs in soil-water systems.

ii. Electron Donor

There are several electron donors of denitrification in the subsurface environment that define whether the process is heterotrophic or autotrophic: organic carbon for the former, whereas reduced manganese, ferrous iron, and sulphides (in pyrite, glauconite, and other sulphur-containing iron silicate and aluminosilicate minerals) for the latter (Buss et al., 2005; Krantz and Powars, 2000; Rissmann, 2011; Rivett et al., 2008; Torrentó et al., 2010; Zhang et al., 2012, 2009). Compared with other electron donors, organic carbon (OC) compounds are deemed the most reactive substrate, whereas glauconite reacts more slowly than pyrite sulphides (Krantz and Powars, 2000), but both can be catalysed by the presence of Cu^{2+} (Eppinger and Walraevens, 1998; Korom, 1992). On the other hand, the role of pyrite in denitrification in unsaturated zone may be limited as it requires dissolution which could only be significant after 5 days to release sulphur (Krantz and Powars, 2000; Torrentó et al., 2010).

There are also different pools of OC with different levels of reactive characteristics with respect to the turnover of nutrients such as NO_3^- in agricultural soils (Hoyle et al., n.d.). In contrast to the resistant or recalcitrant pool of OC (such as protected humus and charcoal) which could take tens to thousands of years to turnover (Hoyle et al., n.d.), labile OC is the fraction with the most rapid turnover rate as it is degradable during microbial growth (Zou et al., 2005). Hence, labile OC is an important source of electron donor in the unsaturated zone given the relatively short retention time of NO_3^-. Soil labile OC is composed of the following: amino acids, microbial biomass, simple carbohydrates, and other simple organic compounds (Zou et al., 2005). Microbial biomass carbon, which comprised 1-3% of soil total OC, had been used as an index for soil labile OC (Zou et al., 2005). However, Zou et al. (2005) found poor positive correlation between them. The hot water-extractable carbon (HWC) has also been used to estimate labile OC (Ghani et al., 2003), especially in soils where limestone,

dolomite, calcite, or another-carbon bearing parent materials are not expected (Schumacher, 2002). The HWC pool is reasonable in size as it is composed of microbial biomass, soluble carbohydrates and amines, and constitutes 3-6% of the total OC in soils (Ghani et al., 2003). The HWC, therefore, could significantly account for the total labile OC in soils (Zou et al., 2005).

The extent of denitrification in soils partly depends on the type and availability of electron donors. While OC could be the main source of electron donor in the vadose and shallow groundwater zones due to proximity to the supply of organic matter inputs in soil, other electron donors like pyrite may be the significant contributor of electron donors in deeper groundwater (Bohlke et al., 2002; Green et al., 2008; Korom et al., 2012, 2005; Tesoriero et al., 2000). Generally, OC concentrations decrease with depth of subsurface profile (Cannavo et al., 2004; Saggar et al., 2013; Starr and Gillham, 1993). Although labile OC had been found in deeper groundwater (tens to hundreds of meters), it is deemed not ubiquitous (Starr and Gillham, 1993). Thus, unlike NO_3^- which is easily leached to the subsurface, the availability of OC limits the denitrification process in the subsurface soil and groundwater (Luo et al., 1998, 1996; Parkin and Meisinger, 1989; Starr and Gillham, 1993), except where buried organic matter is present such as in volcanic profiles (Clague et al., 2013).

A number of assessments of the contribution of electron donors on denitrification in the subsoil environment found relationships between denitrification and several forms of carbon. For instance, many studies found that the dissolved organic carbon (DOC) correlates strongly with denitrification activity in unsaturated and saturated zones (Kamewada, 2007; Paramasivam et al., 1999). But this may not always be true especially if organic carbon is not bioaccessible (Siemens et al., 2003; Zou et al., 2005). Moreover, the reactivity of DOC

depends on its molecular weight, with low-molecular-weight compounds (such as acetate) being more reactive biologically than those with high molecular weight (Buss et al., 2005). However, it appears that there is no consensus on the pools of DOC favourable for denitrification in surface environment. For instance, Castaldelli et al. (2013) found acetate to relate better with NO_3^- than DOC in the lower Po River flood plain in Italy. Other studies found that total soil OC have significant correlation with denitrification (Buss et al., 2005; Jahangir et al., 2012c). Even a positive linear relationship between total denitrification and total C was also observed (Jahangir et al., 2012c). On the other hand, there seems to be limited studies investigating the correlation between water-extractable carbon and denitrification in different soils (Corre et al., 1999; Peterson et al., 2013).

iii. Oxygen

The presence and concentration of oxygen in the subsurface environment highly influences the denitrification process. Compared with NO_3^-, oxygen is the preferred electron acceptor by microbial organisms due to the greater free energy available; -78.5 kJ/electron with O_2, compared with -72.3 kJ/electron with NO_3 (Korom, 1992; McMahon and Chapelle, 2008; Rivett et al., 2008). This, therefore, governs that denitrification is basically an anaerobic process. The status of oxygen in the soil depends on its diffusion rate, which in turn depends on several factors such as temperature, soil structure, and soil water content (Firestone, 1982; Mosier and Klemedtsson, 1994). Given that the diffusion rate of oxygen (O_2) in water is approximately 10,000 times slower than in air (Firestone, 1982; Mosier and Klemedtsson, 1994), the water content in the soil is an important factor of denitrification with increases in soil water content generally resulting in increases in denitrification activity (Firestone, 1982; Mosier and Klemedtsson, 1994; Tindall et al., 1995). Indeed, the effect of oxygen on denitrification in soils has been assessed based on the soil water content or water-filled pore

space (WFPS) (Jahangir et al., 2012c; Paramasivam et al., 1999). Anaerobic condition in soils are considered to occur when WFPS reaches 60 – 90 % due to observed significant increases in denitrification (Cannavo et al., 2004; Felber et al., 2012; Jahangir et al., 2012c; Paramasivam et al., 1999). Other parameters that have been used to describe the control of O_2 on denitrification are soil water potentials, partial pressures of O_2, O_2 concentrations in solution, percentage air-filled porosities, and redox potential (Firestone, 1982). Saggar et al. (2013) also emphasised the effect of soil compaction on denitrification due to its influence on the WFPS and diffusion of oxygen.

In the saturated zone where all soil pores are filled with water, the influence of oxygen on denitrification is determined through dissolved oxygen (DO) concentrations in groundwater. Denitrification has been observed in a wide range of DO concentrations, up to 4 mg L^{-1} with most measurements observed denitrification below 1-2 mg L^{-1} (Buss et al., 2005; Thayalakumaran et al., 2008), with 2 mg L^{-1} as the upper limit (Cheng et al., 2010; Rissmann, 2011; Rivett et al., 2008). However, 0.2 mg L^{-1} is considered the most ideal (Cheng et al., 2010; Hiscock et al., 1991; Seitzinger et al., 2006; Trudell et al., 1986) given that most denitrifying bacteria are inhibited at oxygen concentrations above 0.3 mg L^{-1} (Tiedje, 1988).

On the other hand, denitrification may still occur even in a generally aerobic environment, with denitrification observed at DO values more than 5 mg L^{-1} in lake systems (Cavari and Phelps, 1977). Apart from reasons related to anaerobic conditions in microsite or biofilms in sediments (Mengis et al., 1999; Parkin, 1987), this possibility could be due to the capability of several species of bacteria (Buss et al., 2005) and fungi (Cannavo et al., 2004) to denitrify at higher DO concentrations in soil-water systems. In such cases, N_2O could be expected as the main end-product instead of N_2, resulting in partial or incomplete denitrification.

However, the extent at which denitrification has been observed at aerobic conditions in the subsurface has been “rare to non-existent” (Buss et al., 2005).

iv. Temperature

Being a microbial-mediated process, denitrification is presumed to be affected by temperature as soil temperature regulates biological processes in the soil (Cannavo et al., 2004). Denitrification has been observed to occur normally in a wide range of temperature: 2-50 °C (Brady and Weil, 2002), and generally increases exponentially with increase in temperature (Heinen, 2006). While the range 25-35 °C is considered as optimum (Rivett et al., 2008), Knowles (1982) reported maximum rates in the range of 60-75 °C. Earlier studies did not observe the influence of different temperatures (5-30 °C) on the denitrification potential of the same soil incubated at aerobic conditions (Smid and Beauchamp, 1976). However, later studies showed the effects of temperature. For instance, Buss et al. (2005) reported lower denitrification found *in situ* (10 °C) than in the laboratory (25°C), and that increasing temperature by 5 °C could result in denitrification rate increased by ten-fold. While significantly lower, denitrification was still observed at temperatures between 0 and 5°C (Hiscock et al., 1991; Knowles, 1982).

Aside from direct effects on denitrification, temperature also affects denitrification activity with its indirect effects on substrate availability by influencing N mineralisation and nitrification (Saggar et al., 2013).

The effects of changing temperature on denitrification are evident in the observed temporal changes in denitrification in soils sown to perennial ryegrass in the Jealott's Hill Research Station, Berkshire, UK (Ryden, 1983). However, these effects of temperature on

denitrification may be more pronounced in surface soils than in the subsurface environment as variations in temperature have been observed to decrease with depth (Cannavo et al., 2004). This may also be true in groundwater where temperature is more stable (Rivett et al., 2008).

v. pH

Given that enzymes are responsible for heterotrophic denitrification, soil *pH* affects denitrification as the kinetics of enzymes is *pH* dependent (Ellis et al., 1998). Similar with heterotrophic denitrification, autotrophic denitrification (with Fe^{2+} as electron donor) is also regulated by *pH* (Buss et al., 2005). The preferred *pH* range by heterotrophic denitrifiers is between 5.5-8.0 (Rust et al., 2000). Strongly acidic environments ($pH < 5$) have been reported to inhibit denitrification (Jahangir et al., 2012a; Rivett et al., 2008); nevertheless, very low denitrification activity was still observed at $pH < 4.5$ (Buss et al., 2005; Torrentó et al., 2010), and very little or no denitrification at $pH < 3.5$ (Knowles, 1982; Saggar et al., 2013). One other hand, higher *pH* have also been reported to arrest denitrification (Heinen, 2006), with the *pH* of 8.3 considered as the acceptable upper limit (Rust et al., 2000). Several authors (Hiscock et al., 1991; Knowles, 1982) indicated optimum denitrification in the range 7.0-8.0, but Saggar et al. (2013), citing other researchers, stressed that an optimum *pH* "has little or no meaning unless qualified by specifying the particular aspect of denitrification". Interestingly, N_2 rather than N_2O was found to be the more important product of denitrification at $pH > 7$ indicating a complete denitrification (Šimek et al., 2002).

While spatial variation of denitrification may be expected with differences in soil *pH*, temporal variability due to *pH* seems unlikely as *pH* does not fluctuate much "under normal agricultural conditions except during periods after liming" (Heinen, 2006). Although soil *pH*

may increase as a result of denitrification due to the production of carbon dioxide and hydroxide (OH^-) (Jahangir et al., 2012c), this seems to be apparent only in situations wherein hydroxyl ion production exceeds that of the former as normally they combine to form bicarbonate (HCO_3^-) as a product of denitrification (Buss et al., 2005).

vi. Hydrogeological factors

Hydrogeological characteristics affect denitrification due to their influence on the proximal factors of denitrification described above. For example, soil hydraulic properties, which are dependent on soil texture and structure, could indirectly affect denitrification by regulating the movement and distribution of substrate (e.g., NO_3^-, OC) and gas (e.g., O_2) in the soil profile (Cannavo et al., 2004; Fenton et al., 2009; Kamewada, 2007; Korom et al., 2012; Paramasivam et al., 1999; Tindall et al., 1995). Vertical flow of water and contaminant transfer may also be impeded in stratified soils resulting in conditions conducive for denitrification (Haag and Kaupenjohann, 2001), such as longer residence time and subsequently longer exposure of microorganisms to sources of NO_3^- and OC. The residence time of substrate in the subsurface environment has been considered as an important factor of the potential of the subsurface to denitrify (Domagalski et al., 2008; Vidon and Hill, 2004). For instance, NO_3^- concentration in groundwater was found to be significantly related to saturated hydraulic conductivity (Fenton et al., 2009) and minimal denitrification has been found in aquifer types in Ireland in which transmissivity is high and travel times rapid (Orr, 2014).

Moreover, soil properties may also affect the product of denitrification. For instance, the residence time of N_2O may be increased in soils with higher bulk density by slowing the rate

of diffusion which could result in more complete transformation to N_2 (Jahangir et al., 2012c).

Soil texture, which determines properties such as hydraulic conductivity and drainage, has also been found to influence the denitrification characteristics not just of the vadose zone but also of the underlying groundwater. For instance, coarse-textured soils allow rapid percolation of water affecting the denitrification characteristics of groundwater and this is evident in high dissolved oxygen concentrations found beneath well-drained soils (Clague et al., 2015c).

Apart from hydraulic properties, soil and rock types may also influence denitrification in the subsurface environment due to the presence or absence of electron donor. For instance, denitrification has been found insignificant in loess materials due to lack of OC (Haag and Kaupenjohann, 2001). On the other hand, significant denitrification has been found in deeper soil layers due to the presence of relict organic matter in volcanic profile near Lake Taupo in the North Island of New Zealand (Clague et al., 2013).

vii. Other factors

One important factor of denitrification is the presence of organisms capable to denitrify. However, denitrifiers are considered ubiquitous in soils (Vilain et al., 2012) and widespread in aquifers (Starr and Gillham, 1993). Firestone (1982) listed the 23 genera of bacteria that have capacity to denitrify, but archae, eukarya, and fungi are also capable (Saggar et al., 2013). It has been observed, however, that heterotrophic bacteria decreased with depth (Cannavo et al., 2004; Jahangir et al., 2012c) and this could indicate variation in denitrification potential across the soil profile. Cannavo et al. (2004) found positive

correlation between denitrifier density and denitrification capacity in an experimental site in Comtat Venaissin in France.

Knowles (1982) listed several inhibitors of denitrification including acetylene, some pesticides and some sulphur compounds. Moreover, trace and heavy metals seem to have opposing effects on denitrification depending on their concentrations. Iron, copper and molybdenum have been reported as catalyst of autotrophic denitrification (Korom, 1992; Ottley et al., 1997; Rivett et al., 2008; Saggar et al., 2013). However, enriched concentrations of heavy metals (e.g., Cd, Cu, Zn, Pb) may inhibit denitrification as they become toxic to denitrifying bacteria, though it seems that inhibiting conditions occur only in an extremely polluted soil or groundwater environment (Rivett et al., 2008).

Redox potential is also known to affect denitrification, with a lower *Eh* value indicating a higher potential for denitrification. Spalding and Parrott (1994) noted the sharp contrast in nitrate concentrations found at *Eh* above and below 280 mV. Other authors indicated lower *Eh* values for significant denitrification to occur: < 225 mV (Thayalakumaran et al., 2008), < 150 or <100 mV (Jahangir et al., 2012a).

Land use or management practices could also influence denitrification through substrate loading and also on their effects on soil structure and texture. However, these effects could be more pronounced in the surface soil than in the subsurface soil environment. Furthermore, infiltrating water from rainfall or irrigation may enhance denitrification by supplying the soil profile with NO_3^- and OC and reducing oxygen content (Korom et al., 2012; Saggar et al., 2013).

2.3 Denitrification in the Vadose Zone

The vadose zone is generally understood as the unsaturated portion of the soil profile between the soil surface and the water table (Barkle et al., 2007; Holden and Fierer, 2005). Given that this study focuses on the subsurface, descriptions and assessments of the vadose zone will focus on the subsoil – the portion of the profile below the surface layer. While definitions on the soil surface layer may vary, this study considers the surface soil layer as the uppermost soil layer from the surface down to a depth of 30 cm, based on denitrification studies especially in grassland soils (Gusman and Marino, 1999; Murray et al., 2004; Thomas et al., 2012). As such, in this study, the vadose zone corresponds to the unsaturated portion of the subsurface environment.

2.3.1 Significance of denitrification in the vadose zone

There are relatively limited studies on subsurface soil denitrification as compared to surface soil denitrification measurements. However, in comparison with the surface soil, denitrification rates in the subsurface soil were found to be lower. For instance, while some researchers found no significant difference in denitrification potential (i.e., both NO_3^- and electron donor are not limiting) in the overall soil profile (Vilain et al., 2012; Yeomans et al., 1992), most studies found that denitrification potential in the subsurface layer is significantly lower than in the surface layer – even up to several orders of magnitude (Jahangir et al., 2012c; Jarvis and Hatch, 1994; Kamewada, 2007; Luo et al., 1998; Peterson et al., 2013). In terms of denitrification capacity (i.e., only NO_3^- is not limiting), there is an apparent consensus in findings that the capacity in subsurface soils to denitrify is much lower than in the surface soils (Barkle et al., 2007; Jahangir et al., 2012c; Kamewada, 2007; Luo et al., 1998; Vilain et al., 2012; Yeomans et al., 1992). Although DO concentration in the

subsurface soils could be lower than in the surface layers (Cannavo et al., 2004; Thomas et al., 2012), the inferior extent of denitrification in the subsurface could be due to the more favourable status of other denitrification factors in the surface soil. For instance, microorganisms responsible for denitrification have been found in higher abundance in the surface soils than in the subsurface (Cannavo et al., 2004; Firestone, 1982; Jahangir et al., 2012c; Paramasivam et al., 1999; Vilain et al., 2012; Yeomans et al., 1992). Abundance of microorganisms could even differ exponentially between the surface and subsurface layers (Parkin and Meisinger, 1989). Moreover, OC and NO_3^- has also been found to be more abundant in the surface soils (Jahangir et al., 2012c; Vilain et al., 2012), thus resulting in higher denitrification rates in surface soils.

Given the lower denitrification rate found in the subsurface soil, several researchers have argued that denitrification in the subsurface is not a significant mechanism of NO_3^- attenuation (Kamewada, 2007; Parkin and Meisinger, 1989). Several studies found that subsurface denitrification account for less than 5% of the total denitrification activity in the soil (Hashimoto and Niimi, 2001; Kamewada, 2007; Sotomayor and Rice, 1996). However, several studies observed otherwise that denitrification rates in subsurface soils could reduce a significant amount of NO_3^- before it reaches the groundwater (Castle et al., 1998; Elmi et al., 2005; Luo et al., 1998). While the denitrification rate could be low, the total denitrification in the unsaturated zone could still be significant given the substantial depth of the vadose zone (Groffman et al., 1999; Jarvis and Hatch, 1994). A N budget study indicated this as NO_3^- reaching groundwater was found lower than expected given that there was little evidence of surface soil denitrification (Castle et al., 1998). Jahangir et al. (2012b) also observed high denitrification in the subsoil in Wexford, Ireland, whereas Thomas et al. (2012) found that 5-10% of fertiliser applied in the surface and nitrogen present in the soil was denitrified in the

vadose zone in Canterbury, New Zealand. Moreover, Clague et al. (2013) measured denitrification at the Waihora Field Site in the volcanic subsurface environment in Taupo, New Zealand, particularly due to the presence of relict organic matter. Hence, in order to obtain an accurate estimation of NO_3^- losses and attenuation, investigation of the surface soils layer alone appears insufficient (Elmi et al., 2005) and more research efforts are required to measure and map denitrification potential of subsurface layers across agricultural landscapes.

Moreover, the contribution of the vadose zone is not limited to the extent of NO_3^- attenuation, but also to the end product of the denitrification process. Denitrification in the soil surface layer largely produces nitrous oxide (Vilain et al., 2012), which is a greenhouse gas, partly due to the relatively higher NO_3^- content in the soil which could inhibit complete reduction of N_2O to N_2. On the other hand, limited studies suggest that denitrification in the subsurface mainly produces N_2 (Castle et al., 1998; Elmi et al., 2005; Jahangir et al., 2012c; Vilain et al., 2012), which is a harmless atmospheric gas. The potential of the subsurface soil for a more complete denitrification process could be due to the significant difference in bacterial communities that consume N_2O between surface soils and subsoil (Vilain et al., 2012), higher bulk density and water-filled pore space (Jahangir et al., 2012c), or the fact that microbial reduction to N_2 is possible as N_2O diffuses slowly upward through the soil profile given limited DO and adequate sources of OC (Barkle et al., 2007; Castle et al., 1998; Elmi et al., 2005).

2.3.2 Variability of denitrification in the vadose zone

Effective management of NO_3^- losses and its potential attenuation across agricultural landscapes requires a sound understanding of spatial and temporal variability of

denitrification in the subsurface environment. There is considerable variability of denitrification in the vadose zone due to the complex variations and interactions of factors (e.g., oxygen, NO_3^-, carbon or other electron donor/s, and hydrogeological factors) (Saggar et al., 2013). This section explores the spatial variation of vadose zone denitrification with respect to hydrogeological settings, land use and management practices, as well as with respect to time.

2.3.2.1 Spatial variability

i. Morphological and hydrogeological settings

Several studies on surface soils suggest variations in denitrification across the landscape with higher denitrification found in lower topographical zones, which could be attributed to the accumulation of water content, NO_3^- and organic matter (Florinsky et al., 2004; Vilain et al., 2010). For instance, Oehler et al. (2007) found higher denitrification rates in riparian areas compared to hillslopes in an agricultural catchment in Brittany, France, although the rate at the latter is also significant. However, these morphological effects on denitrification appear to be more applicable to surface soils (Vilain et al., 2012).

In terms of depth, a decrease in denitrification rate has been generally observed with an increase in vadose profile depth (Cannavo et al., 2004; Jarvis and Hatch, 1994; Limmer and Steele, 1982; Luo et al., 1998; Parkin and Meisinger, 1989), but the trend is not continuous and far from uniform or consistent. In some areas, denitrification capacity has been found to decrease sharply in the upper horizons (at ~20 cm) and seem not to change significantly in deeper layers (Luo et al., 1998; Sotomayor and Rice, 1996), while in other areas the denitrification potential and/or capacity rates gradually decreased to a depth of approximately 1m before insignificant changes were observed (Jahangir et al., 2012c; Kamewada, 2007).

But higher denitrification rates were also measured in deeper depths of 3-4 m than in upper layers at the Waihora site in Lake Taupo catchment due to the presence of a palaeosol layer (Barkle et al., 2007).

This variation in denitrification with depth could also be due to the differences in soil properties, with gravelly layers having low capacity for nitrate attenuation (Thomas et al., 2012). Even in areas with similar soil types and land management practices (especially fertiliser application and irrigation), denitrification capacity could still vary at different depths. This is due to observed differences at even small and localised extents in biological and chemical factors, such as denitrifier population, amount of readily available carbon, *pH*, and presence of NO_3^-, or even physical factors such as porosity or permeability, temperature, and water table that affect the dynamics of transport and transformation of NO_3^- in soil-water systems (Firestone, 1982; Paramasivam et al., 1999; Tindall et al., 1995).

ii. Land use or land cover

Land use and land cover may influence the denitrification characteristics of the subsurface profile due to their effects on supply of carbon and NO_3^- substrates (Barkle et al., 2007) as well as on soil water content of subsurface layers (Hénault et al., 1998). Several studies have investigated denitrification in subsurface soil under forest (Hill et al., 2000), cropped lands (Jahangir et al., 2012c; Parkin and Meisinger, 1989), and grasslands (Jahangir et al., 2012c; Jarvis and Hatch, 1994; Luo et al., 1998). Subsurface denitrification rates under grazed grasslands could be significant compared to other land use types, and this is attributed to the ready supply of organic matter and NO_3^- from the OC and nitrogen in the excreta of grazing animals that could leach into the deeper layers (Barkle et al., 2007; Jarvis and Hatch, 1994). Sotomayor and Rice (1996) found that denitrification capacity in the subsurface of the prairie

site was much greater than in a cultivated site in eastern Kansas, USA. However, they also found that the denitrification potential was greater in the cultivated site as compared to the prairie site. Their findings underline that the potential availability of soluble OC under the prairie site (in contrast to the higher total OC in the vadose zone of the cultivated site) was responsible for higher subsurface denitrification capacity under the prairie land cover. Though greater compared to other land use types, quantitative figures of denitrification capacity in the subsurface of grazed pasture may be considerable (Luo et al., 1998), small (Jahangir et al., 2012c) or undetectable (Sotomayor and Rice, 1996). This underscores the need for further investigation of subsurface denitrification characteristics of grazed grasslands given the risks of considerable amounts of excess NO_3^- leaching (Jarvis and Hatch, 1994).

iii. Management practices

Management practices could influence subsurface denitrification in several ways. For example, ploughing may enhance the availability of OC to the soil profile (Luo et al., 1996), whereas irrigation could lead to the leaching of nutrients (Hénault et al., 1998) and subsequently influence denitrification in the subsurface. However, several studies did not observe the influence of tillage in subsurface denitrification finding no significant differences in DOC concentrations and denitrification among tillage systems in corn fields in Canada (Elmi et al., 2005) and the USA (Parkin and Meisinger, 1989). Moreover, while increased nitrogen inputs over a long period could alter the denitrification potential in the vadose zone (Sotomayor and Rice, 1996), Hashimoto and Niimi (2012) did not find enhanced denitrification potential in the subsurface with the application of livestock slurry, which provided both a source of N and DOC on an experimental site with forage crops in Okinawa, Japan.

2.3.2.2 Temporal variability

Actual denitrification rate in the subsurface is considered to change seasonally due to changes in the status of denitrification factors with respect to season. For instance, Cannavo et al., (2004) observed strong seasonal variations of activities of denitrifying microorganisms in the deeper portion of the vadose zone, whereas Vilain et al. (2012) stressed the probability of soluble C percolating deeper in the profile during wet periods affecting denitrification in the subsurface environment. However, changes in denitrification with season seem to be more apparent in the surface soil layer than in the subsurface (Sotomayor and Rice, 1996). Ruz-Jerez et al. (1994) found actual denitrification rates varied with season in the surface soil with high losses due to denitrification observed during winter in Palmerston North, New Zealand. But other studies investigating temporal changes in subsurface denitrification did not find marked differences at different sampling seasons (Jarvis and Hatch, 1994; Luo et al., 1998; Martin et al., 1999). Nevertheless, changes in denitrification activities within a short period, such as after a heavy rainfall, have been observed in the soils under pasture in Manawatū, New Zealand (Luo et al., 1998). Such changes in denitrification activities in the deeper layers could be due to the possible transport of soluble C and NO_3^- down the soil profile (Elmi et al., 2003; Luo et al., 1996). Therefore, it appears that temporal variations in subsurface soil denitrification largely depends on the changes in the status of the different factors (microorganisms, NO_3^- and soluble C supply, etc.) and one may not find significant differences across seasons when limited measurements were made at times when factors did not vary much. In order to capture temporal changes in subsurface denitrification, closer temporal-spaced measurements are needed, or denitrification rates are investigated in the laboratory for expected diverse conditions (e.g., different moisture contents, OC and NO_3^- concentrations, etc.).

2.3.3 Implications for research

It is clear from the review above that most of vadose zone denitrification studies have been mostly focused on surface layers than on subsurface environment. However, the existing research findings underscore the influence of the properties of the soil profile on denitrification (Kamewada, 2007) and emphasise that denitrification in the subsurface soil is driven by several factors "which may not predictably change with surface soil management" (Holden and Fierer, 2005). It also highlights that denitrification depends on the availability and abundance of a suite of proximal factors as described above. Thus, subsurface denitrification studies with respect to its spatial and temporal variability and influencing factors are important to assess and map subsurface NO_3^- attenuation potential across agricultural landscapes.

Also, apart from the indirect influence on denitrification of different soil structures (Tindall et al., 1995), specific rock and soil types contain minerals which could be electron donors for autotrophic denitrification and/or chemodenitrification. While compounds of OC are considered the most reactive electron donor (Krantz and Powars, 2000), other known donors of subsurface denitrification include sulphide minerals such as pyrite (Krantz and Powars, 2000; Rivett et al., 2008; Torrentó et al., 2010; Zhang et al., 2012, 2009) and certain iron silicates and aluminosilicates like glauconite (Krantz and Powars, 2000; Rissmann, 2011). Where these donors are present, denitrification may be multiple donor-driven (Rivett et al., 2008). Furthermore, donors other than carbon may become viable alternatives in systems with limited carbon depending on their structure and interaction with water (Krantz and Powars, 2000; Rivett et al., 2008; Torrentó et al., 2010). Therefore, there is a need for denitrification studies to look into the role of potential electron donors and not just a single

donor (Korom, 1992) to understand the denitrification characteristics of the subsurface environment.

Moreover, there appears to be lack of studies investigating the variation of subsurface denitrification with respect to hydrogeological settings (e.g., soil or rock types). As shown earlier, hydrogeological settings influence denitrification as the hydraulic properties (e.g., hydraulic conductivity) control flow and subsequently exposure between microorganisms and substrates, whereas some soil and rock types may or may not contain electron donor to support denitrification.

2.4 Denitrification in the Saturated Zone

2.4.1 Saturated zone or groundwater as a pathway of nitrate attenuation and/or contamination

With the increasing use of inorganic and organic fertilisers in agricultural lands, there are growing concerns with NO_3^- contamination of groundwater and subsequently the receiving surface waters. Indeed, there are increasing number of studies presenting evidence of groundwater contamination with NO_3^- especially in areas of extensive and intensive agriculture (Cheong et al., 2012; Zhu et al., 2003). However, groundwater systems do not merely act as a pathway to NO_3^- contamination of surface water but also of NO_3^- attenuation (Jahangir et al., 2012a; Stenger et al., 2009). Several studies have shown significant reduction of NO_3^- in groundwater systems (Fenton et al., 2009; Jahangir et al., 2013; Seitzinger et al., 2006; Singleton et al., 2007; Tesoriero et al., 2000).

Denitrification has been identified as the most significant NO_3^-e attenuation process in groundwater systems (Anderson et al., 2014; Rivett et al., 2008; Starr and Gillham, 1993). There could be substantial quantitative reduction of NO_3^- in groundwater systems, the extent of which depends on the availability of electron donors and other factors (Jahangir et al., 2012a; Korom et al., 2012). Interestingly, denitrification in groundwater entails "long-term improvement" of water quality given that the end products of denitrification are generally unreactive and unlikely to be "transformed back into nitrate" within the system (Starr and Gillham, 1993).

The extent of denitrification in groundwaters depends on the inputs to the groundwater system and also on the characteristics of the geological materials. In terms of inputs, areas with high labile organic matter tend to have significant denitrification (Rivett et al., 2008). As such relatively higher denitrification is observed in riparian zones, wherein organic matter could accumulate from surface vegetation and roots in the subsurface layers (Hill et al., 2000). The hydrogeological properties of the subsurface materials also contribute to the extent of denitrification by influencing the direction and rate of flow of nitrate-contaminated groundwater affecting its flow path and residence time (and thus, reaction time) (Haag and Kaupenjohann, 2001; Hiscock et al., 1991).

The role of groundwater in the attenuation of NO_3^- emphasizes the importance of denitrification studies in the saturated zone. Such studies would complement findings of surface and unsaturated zone denitrification studies to obtain comprehensive information on the capabilities of the whole subsurface systems to denitrify (Well et al., 2003; Yuan et al., 2012). Findings from saturated zone studies are significant components of the estimates of

denitrification capabilities of the subsurface environment given the relatively lower variation in redox conditions in groundwaters than in the unsaturated zones (Well et al., 2003).

2.4.2 Variability of denitrification in the saturated zone (i.e., groundwater)

2.4.2.1 Spatial variability

Denitrification rates have been found to vary across groundwater systems (Tesoriero et al., 2000) or even within a groundwater system (Green et al., 2008). As such, this variability in groundwater denitrification could be seen in a macro or smaller scale.

At the macro scale, denitrification rates appear to be higher in the upper portion of the saturated zone (shallow groundwater) and decreases with depth (Bragan et al., 1997; Mengis et al., 1999). Shallow groundwater has favourable conditions for denitrification, such as abundance of electron donors due to its proximity with the sources and supply of percolating OC (Bragan et al., 1997; Well et al., 2003). These conditions have implications also on the denitrification capacity of groundwater systems depending on the location of the water table. Those with shallow water tables (2-4 m) have been reported to show denitrification activity unlike those with deeper water tables (Starr and Gillham, 1993; Trudell et al., 1986). While this may indicate contamination threat to groundwater systems with deep water table, this may not be the case considering the significant depth of the vadose zone where NO_3^- may be attenuated during its transport from soil surface to the groundwater system. Moreover, deep groundwater also implies longer travel time to receiving surface water, and while the availability of OC may be limited, supply of sulphides as electron donor may be substantial (Well et al., 2003).

There are a number of factors, such as DO and hydrogeological characteristics, which could significantly affect denitrification in groundwaters. Although DO concentration could be relatively higher in shallow than in deep groundwater, this does not necessarily mean that denitrification is constrained in the shallow groundwater. Apart from the variability in overall DO concentration, denitrification can still occur even in oxic groundwater (DO > 2 mg L^{-1}) as long as electron donors are available since denitrification is known to be active in anoxic microsites (Mengis et al., 1999). This phenomenon is further discussed below under the smaller scale variability. Other sources of variation in the macro scale are the types of rocks, soils and vegetation cover. For instance, Well et al., (2003) reported that denitrification rates in groundwater are highest in organic soils compared to mineral soils, obviously due to the presence of OC as electron donor. Higher NO_3^- removal capacity had been found in groundwater under grazed grassland than under till farming system, which could be attributed to the greater amount of carbon leached in soils under grassland compared to arable land (Jahangir et al., 2010). Moreover, denitrification activities may not be extensive within the aquifer but only in narrow regions where there is a supply of an electron donor (Tesoriero et al., 2000; Vidon and Hill, 2004).

Several studies have also shown the influence of hydrogeological characteristics on denitrification in groundwater. For instance, where NO_3^- and carbon are both available, a negative correlation between denitrification activity and hydraulic conductivity has been found with higher denitrification activity have been found in groundwater with lower permeability (Sanchez-Perez et al., 2003). In a national scale study of effects of catchment characteristics on fate and transport of NO_3^- in groundwaters in Ireland, it was found that there is minimal denitrification where aquifers have high transmissivity and fast flow rates (Orr, 2014). Geologic materials can also influence denitrification in groundwater due to the

presence of OC as fossilized organic matter (Hiscock et al., 1991) or in volcanic profiles (Clague et al., 2013).

On variations in a smaller scale, groundwater may be oxygenated but denitrification may still occur within the system. This is because, at the smaller scale, denitrification activity does not necessarily happen in interstitial water but in anoxic microsites such as in biofilms around the geological particles comprising the groundwater system (Firestone, 1982; Sanchez-Perez et al., 2003). Thus, while residence time is a factor at the macro scale, it is the transport mechanism of nitrate-contaminated water from oxic interstices to anoxic biofilms that is more relevant at the smaller scale (Sanchez-Perez et al., 2003; Singleton et al., 2007).

2.4.2.2 Temporal variability

Several studies have indicated the possible changes in groundwater denitrification across different seasons which could be due to the changes in the availability of labile OC, for example, as a result of root turnover during autumn (Addy et al., 2002). For instance, highest groundwater denitrification rates were observed in spring and autumn, and lowest in summer, but seasonal pattern was not found to be dramatic (Bragan et al., 1997). On the other hand, Martin et al. (1999) reported that, while several studies indicated higher groundwater denitrification during winter months, other investigations focusing on riparian zones do not show similar pattern with increased rates observed in all other seasons (spring, fall and summer). Similarly, Anderson et al. (2014) observed distinct seasonal patterns in groundwater denitrification rate; highest in spring and summer at Cornell University's Animal Science Teaching and Research Center near Harford, New York. These observations emphasise the complexity of the contribution of factors to groundwater denitrification that could not be easily reflected in the changes in season and other changes that go along with it

(e.g., rainfall and percolation, etc.). Hence, this emphasises for further studies to investigate temporal variations in groundwater denitrification across diverse range of hydrogeologic and environmental conditions.

2.4.2.3 Dominant factors of denitrification in the saturated zone

As earlier outlined in this chapter, there are several factors that affect denitrification in the subsurface environment and their influence could vary depending on the type of environment. In groundwater, denitrification – being a microbial-mediated process – generally follows the succession of electron-accepting processes based on the energy generated in the process which is then available for microorganisms (McMahon and Chapelle, 2008). While DO is the most preferred electron acceptor, microorganisms then opt for NO_3^- as electron acceptor when DO becomes limited. Dissolved oxygen is expectedly lower in the saturated zone than in the vadose zone, this underlines the significance of the saturated zone to attenuate the presence of NO_3^-. Preceding discussions emphasise the importance of OC as electron donor to drive denitrification, and the role of other donors such as pyrite especially in carbon-limited environment. Moreover, as mentioned earlier, the hydrogeological properties of the groundwater system seem to influence denitrification in groundwater significantly. Several studies have indicated the strong relationship between denitrification and the permeability of the aquifer, which is dependent on the geology (Jahangir et al., 2012a). Where permeability or hydraulic conductivity is low, increased denitrification activity (and a more complete denitrification) was observed (Jahangir et al., 2012a; Sanchez-Perez et al., 2003). The capacity of the saturated zone to denitrify, therefore, seems to be largely dependent on the supply or availability of the electron donor and the permeability of the system.

2.4.3 Implications for research

The vulnerability of groundwater to NO_3^- contamination is dependent on the denitrification capacity of the unsaturated zone, whereas the vulnerability of surface water to NO_3^- contamination partly depends on the capacity of the groundwater system to denitrify. While shallow groundwater may have the greater capacity to denitrify compared to deep groundwater, it also poses threat to the receiving surface water due to its potential to discharge NO_3^- within a shorter time through lateral flow (Woodward et al., 2013). Hence, the importance of denitrification studies in shallow groundwater could not be overemphasised. However, there are limited studies of denitrification in shallow groundwaters to better understand its spatial and temporal variations and influencing factors. Therefore, local denitrification investigations to determine supply and role of electron donors and the hydrogeologic characteristics of the groundwater system will be helpful to understand the potential attenuation of NO_3^- in the subsurface environment.

2.5 Measuring Denitrification in the Subsurface Environment

2.5.1 Quantitative measures of denitrification

There are several forms of quantitative measurement of denitrification namely, total denitrification, actual or natural denitrification rate, denitrification capacity, denitrification potential, and denitrification enzyme activity. As each of these quantitative measures can be achieved in different ways, this section does not provide specific details on how these are measured. This section merely describes each measure and reviews the general conditions that are appropriate for these quantitative denitrification measures for subsurface environments including the vadose and saturated zones.

i. Total denitrification loss

Total denitrification loss is defined as the total amount of NO_3^- considered to have been denitrified in the soil-water system. In many cases, however, these values are obtained from nutrient balance and at an annual scale. Finer scale measurements would be more useful for aiding good management.

ii. Actual denitrification rate (natural denitrification rate)

This quantitative measure of denitrification is the rate of denitrification in natural conditions of the soil-water system. As such, for terrestrial investigations, the natural structure of the soil-water system need to be preserved otherwise it could result in the exposure of active microsites to oxygen or a new supply of electron donor (Tiedje et al., 1989). Moreover, no additional amount of substrates (N and/or C) is applied to measure actual denitrification rate of the system. A reliable measure of natural rates, however, may require large sample amount of the soil-water system. Parkin (1987) estimated that approximately 10 to 15 kg of soil was necessary to obtain a representative soil mass for estimating natural denitrification rates (using intact cores) in the soil profiles. This measure of denitrification is important as it reflects field conditions. Collection and analysis of disturbed samples may lead to over- or underestimation of the capacity of the system to denitrify, especially in subsurface soils, considering that substantial portion of denitrification occur in microsites (Parkin, 1987) which may not be properly reflected in measurements with disturbed soils.

iii. Denitrification capacity

Denitrification capacity may be defined as the ability of the system to reduce NO_3^- by utilising the existing electron donor, e.g. OC. Some authors use the term semi-potential denitrification rate in place of the denitrification capacity (Vilain et al., 2012). Measurements to determine denitrification capacity rate involves addition of NO_3^- into the sample/system

and incubation at anaerobic environment (Barkle et al., 2007; Yeomans et al., 1992). While it is generally preferred to preserve the natural structure of the soil-water sample, researchers have used different incubation temperature and moisture content (Sotomayor and Rice, 1996).

iv. Denitrification potential

Denitrification potential may be defined as the denitrification rate measured under anaerobic conditions where NO_3^- and electron donor (organic carbon) are not limiting (Qin et al., 2012). Denitrification potential measurements, therefore, are conducted at optimal conditions with the addition of NO_3^- and carbon substrates and incubation at anaerobic environment (Barkle et al., 2007; Heinen, 2006). However, apart from using different concentrations of substrates, investigators of denitrification potential have also used varying temperature and moisture content (Jahangir et al., 2012c; Jarvis and Hatch, 1994; Murray et al., 2004; Sotomayor and Rice, 1996; Yeomans et al., 1992). Also, there seems to be no consensus as whether this measure of denitrification potential should strictly be limited only to intact samples (i.e. intact soil structure or *in situ* measurements) or applicable for disturbed samples.

v. Denitrifying enzyme activity (DEA)

Denitrifying enzyme activity (DEA) measures "the maximum activity of the biomass of enzymes present in soil at the time of sampling" with "all limiting factors of denitrification removed (such as DO, nitrate, and carbon)" (Groffman et al., 1999). As such, all requirements for enzyme activity need to be optimised; accomplished by providing anaerobic condition (by saturation and removal of oxygen in the atmosphere), facilitating diffusion of substrates and gases (by agitating the sample), and no limitations on the availability of NO_3^- and electron donor (Tiedje et al., 1989). Given that only the activity of the present enzyme is

to be measured, DEA is generally measured in a short duration and it also involves the application of chloramphenicol to inhibit microbial growth and de novo synthesis of new enzymes (Groffman et al., 1999). Some researchers noted the possible inhibition effects of chloramphenicol which could result in underestimation of denitrification rate and recommended to keep the duration of incubation short (5h) (Drury et al., 2008). However, the onset of enzyme synthesis and growth had been found to be much shorter, within 2-3 hours of incubation (Smith and Tiedje, 1979; Tiedje, 1994; Tiedje et al., 1989). Drury et al., (2008) also referred to an investigation (Pell et al., 1996) which used 20 ppm chloramphenicol as the lowest concentration. Jha et al. (2011) also found lower rate for 20 ppm chloramphenicol but no significant difference in denitrification rates between treatments of 0 ppm and 10 ppm chloramphenicol. Hence, the use of chloramphenicol for DEA measurements seems warranted at 10 ppm concentration.

Several authors have used DEA interchangeably with denitrification potential (Groffman et al., 1999). However, considering that the use of chloramphenicol in DEA measurements blocks the subsequent influence of enzyme growth on denitrification, DEA values may not be strictly considered as denitrification potential (Limmer and Steele, 1982; Smith and Tiedje, 1979). Other researchers considered denitrification potential as basically the same as DEA except that there is no addition of chloramphenicol (McCarty et al., 2007).

2.5.2 Approaches for measuring denitrification

Given the challenges in quantifying denitrification in the natural environment due to the heterogeneity of the environment, variability of factors of denitrification, and the complexity in capturing and measuring relevant parameters, several approaches have been developed to measure denitrification in soil-water systems. A number of studies have reviewed different

approaches for measuring denitrification in terrestrial and aquatic environments (Knowles, 1982; Saggar et al., 2013; Tiedje et al., 1989). The most notable comprehensive review is the one done by Groffman et al. (2006). This review and other studies helped to identify a number of approaches that could be applied for measuring denitrification in different environments as follows: mass balance approach; *in situ* gradient of environmental tracers; acetylene inhibition method; ^{15}N tracer methods; direct N_2 quantification (coupled with N_2:Ar measurement for aquatic systems); stoichiometric approaches; stable isotope-based methods; and molecular approaches. A summary of the characteristics of these approaches is provided in Appendix A, which is largely based on the review by Groffman et al. (2006).

It can be observed that each type of approach has its share of advantages and disadvantages. The mass balance approach could only indicate the occurrence of denitrification but not the rate and/or the specific location where denitrification happens. While the gas-based methods (such as acetylene inhibition, ^{15}N tracer, direct N_2 quantification, etc.) are popular for denitrification investigations with soils or sediments, among the challenges in their application in aquatic environments including groundwater systems is degassing (movement of dissolved gases from groundwater to air) which could be a significant factor depending on incubation period, transverse dispersivities, and rate of molecular diffusion (Addy et al., 2002). It is, therefore, imperative that the choice of the appropriate method mainly depends on the objectives, capabilities and constraints of the study.

Within each type of general approaches to measuring denitrification, different specific methods or techniques may be appropriate for different systems. Hence, brief descriptions on specific methods are described below for vadose and saturated zones investigations.

2.5.3 Methods for measuring denitrification in the vadose zone

Measurements of denitrification in the vadose zone are generally conducted either *in situ* or in the laboratory. *In situ* studies are generally limited to mass balance or gradient methods by collecting pore water samples using suction plates/cups or ceramic cups (Hoogendoorn et al., 2011; Stenger et al., 2008). But such approaches may be unsatisfactory as they usually fail to account for different NO_3^- sources and fates in the profile aside from denitrification, e.g., nitrification (source/supply of nitrate), plant uptake, DNRA, etc. (Groffman et al., 1999; Tiedje et al., 1989). This is particularly challenging for pastoral systems where spatial variations are expectedly higher due to urinary and manure inputs by the grazing animals (Selbie et al., 2015). Moreover, *in situ* methods do not necessarily provide information on the denitrification rate unless travel time is accounted for. Denitrification rates could be determined through laboratory-based incubations. In laboratory-based incubations, *in situ* conditions are approximated by collecting intact core samples for incubation on the site or in the laboratory at ambient temperature and moisture content during the sample collection (Mosier and Klemedtsson, 1994; Tindall et al., 1995). Essentially, actual denitrification rates are measured if no substrates are added in the incubations. However, on site incubation may be more appropriate for surface soil samples than for subsoil samples given the accessibility issues in incubating samples at a depth in the soil profile (Jarvis and Hatch, 1994).

The most common approaches adopted for measuring denitrification rates for subsurface soil samples in the laboratory are the acetylene inhibition (AI) (Jarvis and Hatch, 1994; Luo et al., 1998; Oehler et al., 2007; Sotomayor and Rice, 1996; Well and Myrold, 2002) and ^{15}N-tracer methods (Barkle et al., 2007; Castle et al., 1998; Well and Myrold, 2002). Several studies adopted direct N_2 quantification with the use of gas-tight flow incubation systems (Jahangir et al., 2012c), but its adoption is not as widespread as the earlier methods. This may be

attributed to the difficulty in ensuring gas-tight flow system and the need to dedicate analytical instruments (e.g., gas chromatograph) for the experiment. The AI method has several advantages compared to stable isotopes (Tiedje et al., 1989). These include: the use of natural NO_3^- substrate, relatively low cost, ability to analyse large number of samples, its versatility that allows laboratory, field and remote site investigations. Concerns with incomplete diffusion of acetylene and N_2O in the AI method may be satisfactorily addressed by syringe pumping and the use of perforated PVC core liners (Groffman et al., 1999; Parkin, 1987; Tiedje et al., 1989).

Laboratory incubations with AI or ^{15}N-tracer methods are conducted for slurry and intact core soil samples. Slurries of usually composite and homogenised soils are mainly used for determining DEA, denitrification potential or capacity and effects of different treatments/amendments on denitrification (Luo et al., 1998, 1996). Intact core samples are used mainly for measurements of actual denitrification rate (Mosier and Klemedtsson, 1994; Ryden et al., 1987; Tiedje et al., 1989), although they are also used to determine the effects of treatments (such as for denitrification capacity and potential) (Jahangir et al., 2012c; Vilain et al., 2012). To minimise the effects of soil compaction on denitrification measurements, collection of intact cores need to ensure compaction of less than 5% (Mosier and Klemedtsson, 1994; Tiedje et al., 1989) or 10% (Ryden et al., 1987). An improved soil core incubation method has been developed and tested which addresses concerns on exposure and gas diffusion (Jarvis et al., 2001). However, aside from the constraints with the number of samples that can be run, such a method is more applicable for surface layer or upper horizons due to the quantity of required sample.

2.5.4 Methods for measuring denitrification below the water table (saturated zone or groundwater)

2.5.4.1 Laboratory incubation investigations

Laboratory-based investigations deal with the incubation of slurries of aquifer material (Clague et al., 2015b; Korom et al., 2012; Well et al., 2003), which may be collected during the installation of probes/piezometers (Korom et al., 2012; Tesoriero et al., 2000; Well et al., 2003) or from a nearby location from the same depth of investigation (Korom et al., 2005). Laboratory-based incubations may be designed to determine different measures of denitrification such as denitrification capacity or actual denitrification rates in the saturated zones (Clague et al., 2015b). However, there are several issues with slurry incubations in determining the denitrification characteristics of the groundwater systems, as follows:

- Small sample sizes may not adequately represent the study area (i.e., subsurface conditions) due to high variability of results from small sample sizes in a site (Addy et al., 2002; Bragan et al., 1997) and the required assumptions in extrapolating measurements to a larger scale (Bragan et al., 1997).
- Difficulty in preserving, simulating and reflecting *in situ* conditions including "structure, chemistry, hydrology and gas exchange" (Bragan et al., 1997; Well et al., 2003), and subsequent denitrification rates obtained may not adequately reflect actual conditions (Bragan et al., 1997).
- The exposure of subsurface material to the atmosphere may entail a longer recovery period (>1 yr) before the onset of denitrification (especially autotrophic denitrification, Korom et al., 2012).

Denitrification measurements obtained from laboratory incubations are, therefore, expected to differ from *in situ* measurements. In an investigation of denitrification in a sand and gravel aquifer in Cape Cod near Falmouth, MA, USA, the denitrification rates measured in laboratory incubations were found to be substantially higher than *in situ* rates (Smith et al., 1996). In contrast, Addy et al. (2002) found greater *in situ* rates in their investigation of groundwater denitrification in riparian zones in Rhode Island, though this could probably be traced to the difference in the time of sampling of subsurface material and the *in situ* investigation. Interestingly, Well et al. (2003) found that *in situ* and laboratory measurements were comparable (i.e., in good agreement), and stressed the applicability of laboratory measurements with slurries of subsurface material for large scale denitrification studies. Nevertheless, given the issues with the laboratory incubations, several authors emphasised that priority be given to *in situ* measurements of denitrification in saturated zones (Mengis et al., 1999).

2.5.4.2 In situ investigations

A review of on-site investigations of denitrification in the saturated zone suggests that they may be generally grouped into two categories namely, (i) flow path investigations and (ii) localised (discrete location) investigations. These are discussed as below:

i. Denitrification investigations following nitrate flow pathways

The rationale behind this type of investigation is that occurrence of denitrification could be deduced from monitoring changes in groundwater quality parameters related to denitrification along the flow path of groundwater. For instance, monitoring concentrations of NO_3^-, conservative tracers (bromide, chloride, etc.) and/or other parameters (e.g., bicarbonate, excess N_2) could indicate denitrification if NO_3^- has reduced more than what can be

accounted by dilution with tracers and this NO_3^- reduction is coupled with the increase of the products of denitrification N_2 and N_2O. This monitoring along the flow path could employ several approaches such as mass balance, acetylene inhibition, excess N_2, ^{15}N-labelling, and stable isotopes, among others.

Denitrification investigations along flow-paths have been conducted in several ways: installing and monitoring piezometers, piezometer nests, or multilevel wells along transects (Green et al., 2008; Stenger et al., 2008; Tesoriero et al., 2000; Vidon and Hill, 2004); a pair of injection and sampling wells/piezometers separated at a distance along the flow path (Pauwels et al., 1998; Smith et al., 1996); and groundwater wells selected along the determined groundwater flow direction (Mohamed et al., 2003; Spalding and Parrott, 1994). Appendix B provides brief descriptions and assessments of specific techniques used with particular attention on how denitrification rate can be determined along the flow path.

Measurements along flow-paths have generally been used to obtain indications of denitrification in subsurface environment. Based on these measurements accurate quantification of denitrification rates could face challenges. Amounts of NO_3^- denitrified calculated from mass balance approaches using tracers are, at best, rough estimates only of total denitrification considering the uncertainty in the direction of groundwater flow and NO_3^- transformation processes. Groundwater flow direction could be determined by modelling the groundwater flow (Green et al., 2008; Tesoriero et al., 2000). Also, determining denitrification rates requires measurements of groundwater travel time which may be determined with numerical groundwater flow models (Tesoriero et al., 2000) or breakthrough curves of tracers (Smith et al., 1996). However, the development and application of

numerical groundwater flow models require detailed hydrogeological field investigations to accurately simulate groundwater flow direction and travel times in the groundwater systems.

There are other challenges in conducting denitrification studies based on observations of flow pathway. One, it is important that sites are selected such that there are no other sources or sinks of the main analytes monitored, such as NO_3^- and tracers, so that measured values could be interpreted appropriately. Transect studies also present constraints in investigating isotopes of NO_3^- as "dispersion decreases the apparent N isotope fractionation" (Korom et al., 2012). On the other hand, flow path-based or transect-based methods are more applicable for slow denitrification rates which may be difficult to measure with localised investigations where reagents may be swept away by fast flowing groundwater before the denitrification reactions could be measured (Tesoriero et al., 2000).

ii. Localised or discrete denitrification investigations for saturated zone

Localised or discrete denitrification investigations for a specific site include push-pull injection wells or piezometers (Addy et al., 2002; Istok et al., 1997; Sanchez-Perez et al., 2003; Tesoriero et al., 2000; Well et al., 2003), piezometer nests composed of a cluster of an injection well and three sampling wells (Bragan et al., 1997); multilevel samplers (Korom et al., 2012); injection drive points (Toda et al., 2002; Trudell et al., 1986); and in situ microcosms and in situ mesocosms (Gillham et al., 1990; Korom et al., 2012, 2005; Starr and Gillham, 1993). Appendix B provides brief descriptions and assessments of specific techniques used for localised or discrete denitrification investigations for a specific site.

Techniques for localised investigations generally involve *in situ* incubation comprising two main steps: a) injection of solution with a conservative tracer (e.g., bromide), NO_3^- (^{15}N-labelled or unlabelled), and, in some cases, acetylene, and b) the recovery of the injected solution at a later time in the same or another piezometer or device nearby. Denitrification rate is computed based on the changes in the concentrations of the conservative tracer, NO_3^- and other parameters (gaseous products of denitrification) from samples taken at specific time interval/s. Except for piezometer nests wherein piezometers for injection and extraction of solution are separate, the test solution is injected and recovered from the same piezometer, drive point, microcosm, or mesocosm in all other methods (Korom et al., 2012; Trudell et al., 1986).

In situ localised investigations have several strong and weak points. In comparison with laboratory incubations, localised investigations sample a relatively larger volume of the subsurface environment and therefore measurements may be considered better representative of the natural subsurface environment (Well et al., 2003). Also, depending on the composition of solution injected, actual denitrification rate, denitrification capacity, or denitrification potential can be determined. On the other hand, applications may be limited for some specific hydrogeological settings. For example, the push-pull method is not practically suitable where hydraulic conductivity is either very low or high since injected solution may not be dispersed into the surrounding subsurface material or be easily swept by advection and lateral flow which has significant implications on the measurements (Addy et al., 2002; Istok et al., 1997). *In situ* microcosms and mesocosms techniques address this by isolating the subsurface material, yet it could also result to blocking of gas exchange and other hydrological processes (Bragan et al., 1997). *In situ* microcosms and mesocosms are suitable to investigate slow rates of denitrification but application could be limited for

shallow water table due to the labour requirement and cost involved with the installation, particularly for mesocosms.

2.5.5 Implications for research

This section reviewed various approaches or methods for quantifying denitrification measures in specific environments, i.e. the vadose or saturated zone. A diverse range of methods are available to quantify denitrification either in the laboratory or *in situ* in vadose and saturated zones of subsurface environment. Except for the denitrifying enzyme activity (DEA) which could only be done in the laboratory, all of the aforementioned measures of denitrification (capacity, actual, potential) may be obtained from *in situ* or laboratory investigations. The more recent techniques such as direct N_2 quantification and stable isotope approaches may offer better accuracy but also require more sophisticated or expensive equipment. The choice of methods to be used would largely depend on the research objectives and resources. Moreover, considering the different measures of denitrification, it would be essential to determine the type of denitrification rate measured (e.g., actual, capacity, potential) before meaningful comparisons of results from different studies could be done.

DEA has been widely applied to gain knowledge on the denitrification potential in soil-water systems. However, the method particularly adopting the acetylene inhibition technique vary in methodological details such as acetylene concentration, use of chloramphenicol, amount of substrates, amount of oxygen in headspace, etc. (Barkle et al., 2007; Drury et al., 2008; Jarvis and Hatch, 1994; Knowles, 1982; Murray et al., 2004; Paramasivam et al., 1999; Sotomayor and Rice, 1996; Vilain et al., 2012; Well and Myrold, 2002). Some aspects of the method (such as amount of substrates of NO_3^- and carbon, incubation temperature) have been

standardised with surface soils only (0-0.1m depth) (Luo et al., 1996). On the other hand, these methodological details, such as the amount of substrates, may not be applicable for subsurface soils (>0.3 m depth) with lower biological activity. There is lack of studies which have assessed the effects of these procedural differences on estimates of denitrification rates for subsurface soils (Murray et al., 2004).

There is also a need to look into how existing methods for quantifying denitrification in groundwater have been used and how results are interpreted. For example, the single-well, push-pull test technique is a practical and cost-effective method for quantifying denitrification rate in shallow groundwater and thus popular with researchers. However, analysis of data or observations from such technique to obtain estimates of denitrification rate varied significantly. Denitrification has been estimated either based on the decrease of the reactant (NO_3^-) or increase in the product (nitrous oxide, nitrogen gas) (Istok, 2013; Sanchez-Perez et al., 2003; Trudell et al., 1986; Well et al., 2003). Moreover, different kinetic models (zero- or first-order) have been used to estimate denitrification rate (Haggerty et al., 1998; Korom et al., 2012; Snodgrass and Kitanidis, 1998). Results from these different tracers (reactant or product) and kinetics models can be significantly different even if using the same push-pull test observations. However, there are limited studies that have done a thorough assessment of push-pull test observations. While a few studies have looked into differences using different tracers (reactant and product) (Gillham et al., 1990; Schürmann et al., 2003) or different kinetic models (Burbery et al., 2004; Korom et al., 2012), there are no known studies that investigated both.

2.6 Subsurface Denitrification Studies in New Zealand Environment

2.6.1 Importance of denitrification studies in New Zealand

New Zealand is largely an agriculture-based economy with majority of its land use under pastoral agriculture (Quinn et al., 2009). In recent decades, pastoral agriculture across New Zealand regions has intensified with an increase in dairy pasture from approximately 0.89 M ha to 1.41 M ha between 1997 to 2007, whereas sheep and beef farms decreased from approximately 10.99 M ha to 8.77 M ha (Quinn et al., 2009). Most recent estimates showed that dairy pasture increased to 1.75 M ha in 2016 with estimated total cows of approximately 5.0 million (LIC and DairyNZ, 2016).

This increase in agriculture intensification, the changes in land use and increase in fertiliser inputs are exerting more pressure on the environment considering that the "stocking rates on dairy farms are on average significantly higher than for sheep and beef farming on equivalent land" (Quinn et al., 2009). Consequently, river water quality monitoring data collected during 1989-2009 across the country showed degrading water quality trend especially in terms of nitrate-nitrogen (Ballantine and Davies-Colley, 2014). This declining trend was particularly observed in areas under pasture indicating the possible effect of the intensification of pastoral agriculture (Ballantine and Davies-Colley, 2014).

To address the growing concerns on the declining water quality, the New Zealand government released its National Policy Statement for Freshwater Management (NPSFM), initially in 2011 and then revised in 2014 (MfE, 2014). With the aim of instituting "quantitative enforceable limits on water quantity and quality by local authorities" (Duncan, 2014), the NPSFM emphasised the need to understand the sources of freshwater

contaminants when setting limits for nutrient loss from farm systems. Knowledge of the characteristics of the subsurface environment and their effects on subsurface denitrification is essential to the understanding of the potential to manage and mitigate NO_3^- contamination in agricultural catchments. Knowing the magnitude of subsurface denitrification potential and its variability will be helpful in setting non-uniform NO_3^- leaching limits for targeted and effective nitrate management solutions.

2.6.2 Subsurface denitrification studies in New Zealand

There has been an increasing interest in New Zealand in understanding the natural capacity of the subsurface environment to attenuate NO_3^-. A number of studies had been conducted from large scale to field or point scale denitrification measurements. For example, Rissmann (2011) mapped the distribution of groundwater denitrification potential in the Southland region aquifers based mainly on the presence of electron donors in the aquifer material. Further work resulted in the development of the Physiographic Regions in the Southland (Hughes et al., 2016) highlighting regions or areas with high or low denitrification potential. A study conducted by Singh et al. (2014) estimated the NO_3^- attenuation factor in the subsurface environment in the eastern part of the Manawatū River catchment by assessing the discrepancies in the estimates of NO_3^- leached below the root zone and NO_3^- load in rivers in sub-catchment scale. Recently, Close et al. (2016) conducted a study predicting the redox status of groundwater in the Waikato and Canterbury regions using linear discriminant analysis comprising spatial data of geology, topography and soil characteristics along with groundwater quality data. These regional and catchment scale studies generally revealed the spatial variability of denitrification characteristics. Combining flow and hydrochemistry data with hydrological modelling, Woodward et al. (2013) assessed the contributions and the potential for denitrification of different flow pathways to NO_3^- loadings in the stream of the

Toenepi catchment in Central North Island. They found that, while most of the NO_3^- in the outlet had been contributed by the fast groundwater flow, the NO_3^- concentration in the stream was significantly lower than the estimated NO_3^- concentration in the root zone leachate and near-surface flow, with denitrification accounting for 36% of the NO_3^- load reduced in recharging water (Woodward et al., 2013).

A number of studies have been conducted to quantify denitrification in soils but most of these were largely focused on surface soils (Appendix C). These studies have been conducted in the Manawatū (Deslippe et al., 2014; Luo et al., 2000, 1999, 1998; Ruz-Jerez et al., 1994), Waikato (Barkle et al., 2007; Clague et al., 2013; Clough et al., 1996; Stenger et al., 2008; Stevenson et al., 2011), and Canterbury regions (Peterson et al., 2013; Thomas et al., 2012). There seems to be an increasing number of studies in the recent years but there has generally been lack of investigation in the subsurface environment, especially in the Manawatū region in which the only known denitrification study on the subsurface below the root zone had been done by Luo et al. (1998), which was also limited to 0.40 m below ground surface.

There have also been recent investigations on groundwater denitrification in the country, but these had been largely done in the Waikato region (Burbery et al., 2013; Clague et al., 2013; Stenger et al., 2008; Zaman et al., 2008), with a few studies conducted in the Southland region (Burbery et al., 2013). There has been no investigation of groundwater denitrification in the Manawatū region, up until this research.

2.6.3 Knowledge gaps and research needs

This review of literature showed that subsurface denitrification may vary with different hydrogeological factors. However, there are a lack of investigations involving measurements of denitrification in the subsurface environment and its variability and relationship with respect to diverse hydrogeological settings. Moreover, there have been limited studies that investigate both the vadose and saturated zones in an integrated manner either in the international scene (e.g., Paramasivam et al., 1999; Grimaldi et al., 2011; Yuan et al., 2012) or locally (Clague et al., 2013). These kinds of studies are important to obtain comprehensive information on the capabilities of the subsurface systems to denitrify (Well et al., 2003; Yuan et al., 2012), yet none is known to have been conducted in the Manawatū River catchment.

These are gaps in our understanding of the denitrification characteristics of the subsurface environment of the Manawatū River catchment. Detailed investigations that include quantitative measurements of the denitrification characteristics of the subsurface environment are particularly relevant in the Manawatū catchment where estimated nitrogen loads in rivers have been found to vary across sub-catchments (Singh et al., 2014). Moreover, nitrogen concentrations exceeding the standard (i.e., 0.444 g soluble inorganic nitrogen m^{-3}) have been found in surface waters in the Manawatū catchment (Roygard and McArthur, 2008) which resulted in the growth of nuisance periphyton in many streams and rivers (McArthur and Clark, 2007).

2.7 Concluding Remarks

There is substantial international literature on the natural attenuation of NO_3^- in the subsurface environment. However, there has been little research in New Zaland of how this

phenomena reduces the impact of N leached from agriculture on groundwater or receiving surface waters. Thus, improved understanding and quantification of subsurface denitrification provides an opportunity for targeted and effective NO_3^- management solutions across agricultural landscapes. Subsurface denitrification has been found to vary spatially and temporally depending on the presence or absence of controlling factor(s). Several methods and approaches have been developed to quantify denitrification in the vadose and saturated zones, and their adoption generally depends on the research objectives and available resources. However, existing methods for measuring denitrification in the vadose zone, such as the acetylene inhibition technique for measuring DEA, had been standardised with surface soils and need to be optimised for subsurface soils with low denitrification activity. Moreover, the single-well, push-pull test has been widely used for quantifying denitrification rate in groundwater, yet studies differed in the analytical approaches and kinetic models used to estimate rates from push-pull test observations. While there has been increasing efforts to quantify denitrification, there is lack of investigations on the effects of hydrogeological factors on subsurface denitrification. In addition, there has been lack of integrated assessment of denitrification characteristics that investigate both the vadose and saturated zones of the subsurface environment. This lack of investigation of subsurface denitrification is particularly true in New Zealand, especially in the Manawatū region where there are concerns of the impact of intensive agriculture on groundwater and surface water quality. This research aimed to address some of these knowledge gaps to improve our understanding of the spatial and temporal variability of denitrification characteristics in subsurface environments and controlling factors. In particular, the specific objectives of this thesis include:

- To determine the spatial distribution of the potential for denitrification in groundwater in the Manawatū catchment and the hydrogeological characteristics responsible for the variability;

- To assess and standardise the acetylene inhibition (AI) technique for quantifying denitrification rates, in particular DEA, in subsurface soils;
- To evaluate the different approaches and kinetic models for estimating denitrification rates from single-well, push-pull tests in shallow groundwater;
- To investigate the denitrification characteristics in the vadose and saturated zones at selected sites with varying hydrogeologic settings across the Manawatū catchment, including,
    - the factors responsible for the variability, and
    - the implications on NO_3^- transport and transformation in the subsurface environment.

The improved understanding gained in this study is important to inform the quantifification, mapping and modelling of subsurface denitrification across agricultural landscapes, and therefore assist in identifying measures for optimal NO_3^- management that supports the balance between agricultural production and environmental sustainability.

CHAPTER 3

DENITRIFICATION POTENTIAL IN THE SUBSURFACE ENVIRONMENT IN THE MANAWATŪ RIVER CATCHMENT, NEW ZEALAND: INDICATIONS FROM OXIDATION-REDUCTION CONDITIONS, HYDROGEOLOGICAL FACTORS, AND IMPLICATIONS FOR NUTRIENT MANAGEMENT[1]

Abstract

A sound understanding of the effects of hydrogeological factors on loss, transport and transformation of farm nitrate is essential for predicting their impacts on ecosystem health of receiving waters. This study assessed the potential of groundwater to attenuate nitrate through denitrification, and the distribution of this potential across the Tararua Groundwater Management Zone (GWMZ) in the Manawatū River catchment, New Zealand. A number of methods were combined in an unprecedented manner to confirm findings and obtain supporting evidence for the features that determine the subsurface denitrification characteristics. The results showed that the denitrification characteristics of groundwater varied considerably in the Tararua GWMZ. The southern part of the Tararua GWMZ contained mainly oxic groundwater with low potential to denitrify, whereas the middle and northern parts of the Tararua GWMZ contained reduced groundwater with high denitrification potential. The hydrogeological features that influence denitrification potential in groundwater were identified as soil texture and drainage class, and the aquifer material or rock type. Low dissolved oxygen and nitrate concentrations were found in groundwater where the combinations of soil and rock types had poor drainage characteristics as opposed to higher concentrations in groundwater under well-drained soils and rocks (e.g. gravels).

[1] This chapter has already been published: Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., Hedley, M.J., 2017. Denitrification potential in the subsurface environment in the Manawatu River catchment, New Zealand: Indications from oxidation-reduction conditions, hydrogeological factors, and implications for nutrient management. *Journal of Environmental Management 197*, 476–489. doi:10.1016/j.jenvman.2017.04.015

Intensive pastoral farming over well-drained soils and rocks showed high nitrate concentration in groundwater. This spatial variability in denitrification potential of groundwater offers a targeted management of nitrate runoff and leaching from pastoral lands to limit their impacts on receiving surface waters.

Keywords: Agriculture intensification; Nitrate leaching; Groundwater; Redox conditions; Water quality; New Zealand.

3.1 Introduction

In enhancing production in cropped and grazed lands, the global use of nitrogen (N) fertilizer has increased from almost nil in the 1940s (Di and Cameron, 2002) to approximately 111.4 x 10^9 kg N in 2013 (Food and Agriculture Organization of the United Nations (FAO), 2015). Unfortunately, the efficiency of N fertilizer use by crops, and the recycling efficiency of excretal N on grazed pastures can be low due to losses to the atmosphere and leaching to ground and surface waters (e.g., Liao et al., 2012; Clough et al., 1996). An increase in nitrate (NO_3^-)concentrations in freshwater bodies often leads to undesirable environmental consequences such as eutrophication, algal blooms, and fish poisoning (Di and Cameron, 2002; Puckett et al., 1999). In order to minimise its effects on water quality of freshwater ecosystems, agricultural intensification must therefore be accompanied by strategies to mitigate N leaching from farms to receiving waters.

The amount of NO_3^- leached to groundwater and subsequently transported to surface waters depends on a number of physical, chemical and biological factors that influence NO_3^- flow pathways and attenuation processes (Haag and Kaupenjohann, 2001; Mastrocicco et al.,

2011; Paramasivam et al., 2002). These NO_3^- attenuation processes include plant uptake, assimilation into soil microbial biomass, dissimilatory nitrate reduction to ammonium, and denitrification (Martin et al., 1999; Porubsky et al., 2011; Puckett et al., 2008; Puckett and Hughes, 2005; Rivett et al., 2008). Denitrification – the microbial-mediated transformation of water soluble NO_3^- to gaseous N forms – has been identified as an important NO_3^- attenuation process in groundwater systems (Anderson et al., 2014; Rivett et al., 2008; Starr and Gillham, 1993). However, the capacity of groundwater to reduce NO_3^- by denitrification varies spatially (Jahangir et al., 2013) and this has implications for the management and mitigation of the impacts of leached NO_3^- on groundwater and surface water quality. This variability in the NO_3^- attenuation capability of groundwater systems depends on the characteristics of the contributing surface and subsurface environments. Areas with inputs of highly soluble organic matter from the surface environment to the groundwater system tend to have significant denitrification potential (Rivett et al., 2008). The hydrogeological properties of the subsurface materials also contribute to the extent of denitrification by influencing the direction, flow rate, and residence time (and thus, reaction time) of nitrate-contaminated groundwater in the subsurface environment (Haag and Kaupenjohann, 2001; Hiscock et al., 1991).

Knowledge of the characteristics of the subsurface environment and their effects on denitrification is essential to the understanding of the potential to manage and mitigate NO_3^- contamination in agricultural catchments. New Zealand's 'National Policy Statement for Freshwater Management 2014' emphasised the need to understand the sources of freshwater contaminants when setting limits for nutrient loss from farm systems. A sound understanding of denitrification potential in the subsurface environment is an important consideration when identifying measures to manage and mitigate the impacts of agricultural activities on

groundwater and surface water quality. Knowledge of the magnitude of the denitrification potential and its variability will be helpful in setting non-uniform NO_3^- leaching limits, for example “nitrate vulnerable zones” in the EU Nitrates Directive.

A number of studies have identified the variable nature of denitrification capacities in the subsurface environment in New Zealand and other places (Anderson et al., 2014; Clague et al., 2013; Jahangir et al., 2013; Peterson et al., 2013; Rissmann, 2011; Stenger et al., 2008). There is yet limited information and knowledge of the occurrence, spatial variability, and factors contributing to the denitrification potential in the subsurface environment, particularly in the Manawatū River catchment of New Zealand. Singh et al. (2014) conducted a preliminary investigation of potential nitrogen attenuation, by accounting and comparing estimates of average annual nitrogen leaching from farms and soluble inorganic nitrogen measured in streams and rivers, in the Tararua Groundwater Management Zone (GWMZ) in the eastern part of the Manawatū catchment. They found that the nitrogen attenuation capacity appears to vary among the sub-catchments within the catchment (Singh et al., 2014). But there is little known about the biogeochemical processes and hydrogeological factors determining NO_3^- attenuation potential in subsurface environment of the catchment.

The denitrification potential of groundwater and its variability was investigated in the Manawatū River Catchment, particularly in the Tararua GWMZ. The specific objectives were (1) to determine indicative extent of NO_3^- contamination of groundwater; (2) to characterise the denitrification potential of the catchment; and (3) to assess which hydrogeological characteristics are most responsible for the magnitude and variability in denitrification characteristics in the study area. A number of existing geographical information, field surveys, laboratory measurements and statistical (Principal Components Analysis and

Analysis of Variance) methods were combined to confirm findings and obtain supporting evidence for the features that determine the denitrification characteristics of subsurface environments. This advances our ability to map variable subsurface NO_3^- attenuation capacity and help inform improved management of the effects of agricultural production on water quality and freshwater ecosystems.

3.2 Methods and Materials

3.2.1 Study area description

This study focused on the Tararua GWMZ, comprising approximately 3,200 km^2 of the eastern parts of the Manawatū River catchment (approximately 6,000 km^2) located in lower North island of New Zealand (Figure 3.1a). Located to the east of the central ranges, the Ruahine and Tararua ranges, the Tararua GWMZ has the Manawatū Gorge as its only outlet. The Tararua GWMZ has a temperate climate with an average rainfall of 1509 mm yr^{-1} (Zarour, 2008). Pastoral farming dominates the land use in the Tararua GWMZ, with beef/sheep farming and dairy farming comprising approximately 64% and 15% of the land area, respectively. Natural cover comprises most of the remaining land, with native cover and exotic cover comprising 17% and 3% of the land area, respectively (Clark and Roygard, 2008).

The Tararua GWMZ is bordered by the Tararua and Ruahine ranges in the west and the Waewaepa and Puketoi ranges in the east (Lee and Begg, 2002). Known as the Pahiatua basin (Lee and Begg, 2002), the broad depression in between dominates the landform of the study area. The ridges and troughs formed by the basement (mainly greywacke) are filled by Tertiary sediments mainly comprised of marine mudstone and sandstone with thickness

varying from 0.03 m to 2.2 km below ground level (bgl) (Rawlinson and Begg, 2014). A thin layer of Quaternary sediments cover the Tertiary sediments mainly in the central portion of the Tararua GWMZ. The late Quaternary sediments, with thickness varying from 0.01 m to 228 m below ground level (bgl) (Rawlinson and Begg, 2014), have been considered to form the aquifers in the study area (Zarour, 2008). There are a variety of soils in the Tararua GWMZ with Matamau soil series (Typic Firm Brown Soils) comprising the largest proportion (16%). Other soil types especially those under dairy farms include Kopua (Typic Orthic Brown Soils), Dannevirke (Typic Allophanic Brown Soils) and Kairanga (Typic Orthic Gley Soils).

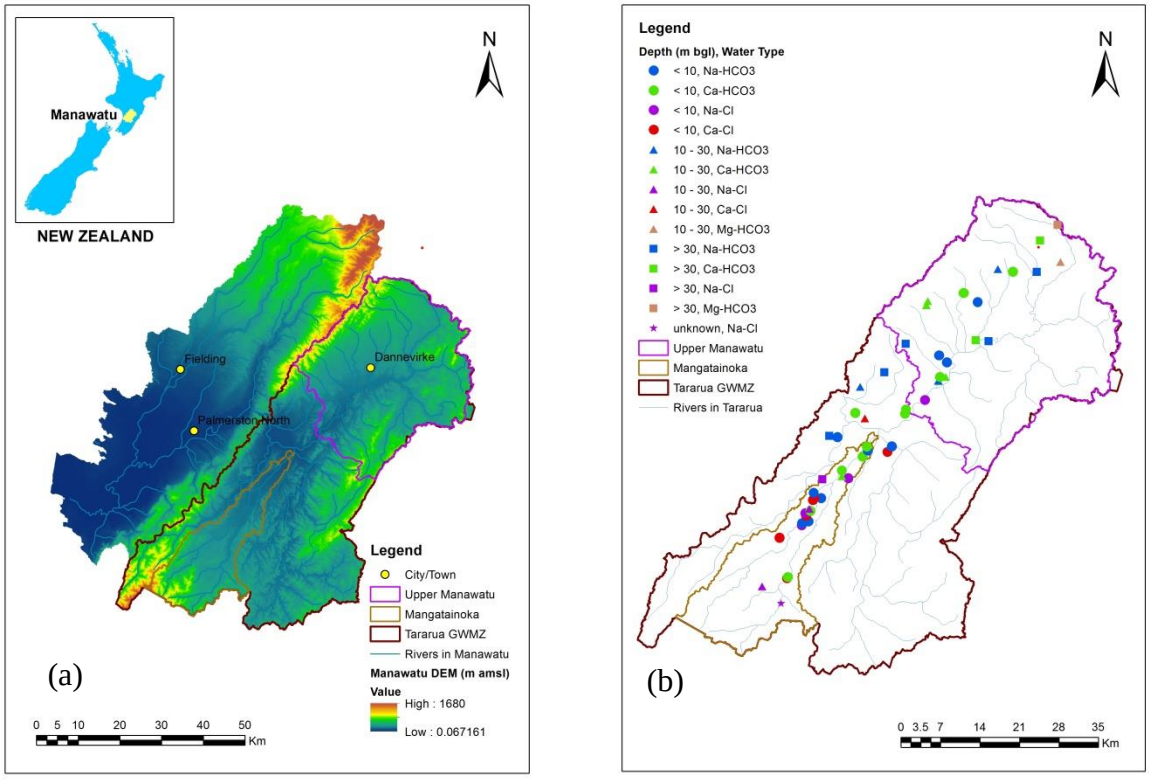


Figure 3.1 (a) The Manawatū River catchment and the Tararua GWMZ and its subcatchments and, (b) distribution of groundwater types based on groundwater survey in the Tararua GWMZ during February-March, 2014.

Recharge to groundwater in the Manawatū region is considered to be controlled predominantly by topography (Zarour, 2008). Bekesi (2001) noted that groundwater recharge in the Tararua GWMZ originates from infiltrated rainfall over the Tararua and Ruahine ranges as well as over the Puketoi Range in the eastern part. Recently, Pattle Delamore Partners Ltd. (2013a) estimated the rainfall infiltration recharge in the Tararua GWMZ at about 814 x 10^6 m^3 yr^{-1} (i.e. approximately 250 mm yr^{-1}). The potentiometric surface developed by Rawlinson and Begg (2014) indicates that groundwater flows in the direction that generally "mirrors that of the coincident surficial rivers".

3.2.2 Groundwater monitoring and quality trend

The Horizons Regional Council (the local government agency responsible for regional resource management) manually monitors groundwater levels in 138 wells monthly as well as 19 wells fitted with continuous water level recording transducers in the Manawatū River catchment. Twelve of the wells that are monitored monthly are located in the Tararua GWMZ (Pattle Delamore Partners Ltd., 2013a). Most of these monitoring wells are operational production wells (Pattle Delamore Partners Ltd., 2013b). Groundwater quality is also being monitored in 22 wells in the Manawatū catchment, four of which are located in the Tararua GWMZ. Groundwater quality is monitored mostly every seven months (Pattle Delamore Partners Ltd., 2013b); however this has recently been changed to quarterly to account for seasonal changes in water quality.

Daughney et al. (2009) assessed trends in groundwater quality in the Manawatū-Wanganui region from a total of 1522 wells (including mainly production wells and monitoring wells) from 1958 to 2008. Most (61%) of these wells, however, had only been sampled once. The median NO_3^- concentrations in the Tararua GWMZ for all samples collected between 1958

and 2008 was less than 50 mg NO_3^- L^{-1} (equivalent to 11.3 mg NO_3^--N L^{-1}), the maximum allowable value (MAV) according to New Zealand Drinking Water Standards (DWSNZ) of 2005, except for one site (Daughney et al., 2009). Recent figures from Pattle Delamore Partners Ltd. (2013b) on four monitoring sites showed that NO_3^- concentration since 2005 have not exceeded the MAV. However, recent data (2005-2015) obtained from the Horizons Regional Council showed observations as high as 9.8 mg NO_3^--N L^{-1} and as low as <0.002 mg nitrate-N L^{-1} in monitoring wells in the Tararua GWMZ. The existing groundwater quality observations from four wells only, however, are very limited to assess influences of land use and other hydrogeological factors on variations of NO_3^- and other hydrochemical variables in groundwater across the Tararua GWMZ.

3.2.3 Groundwater survey

In order to assess the hydrogeological characteristics that have the greatest influence on denitrification potential, a spatial survey of groundwater quality in 56 wells was conducted in the Tararua GWMZ over a period of 31 days between February – March 2014. These wells were selected from a list of 1,150 wells using the following data and procedure. Information on soil texture and drainage class was collated from Landcare Research's Fundamental Soil Layers (FSL), rock types and topography (25m DEM) from the NZ Land Resources Inventory (NZ LRIS), mean annual rainfall (1978-2007) from the National Institute of Water and Atmospheric Research (NIWA) database, and well information from the Horizons Regional Council database. The collected DEM (25 m) data, used as a proxy for topography and sediment deposition, was reclassified into three categories: < 100 m amsl, 100-200 m, and >200 m. The rainfall map was reclassified into four categories : <1100 mm, 1100-1300 mm, 1300-1800 mm, and >1800 mm. Well depth was categorised into three groups: shallow (<10 m bgl), medium (10-30 m), and deep (>30 m), according to Manawatū aquifer systems

(Taylor et al., 2001). The reclassified layers of the DEM, rock types, soil types, rainfall, and well information including depth were then overlaid to identify the corresponding values or descriptions of five variables for each well. Wells were then selected to cover the most common rock and soil types, wherever applicable, and to cover all the categories for DEM, rainfall and depths. In total around 100 wells were selected as potential wells for sampling, but ultimately only 56 wells were sampled as it was not possible to collect groundwater samples from other wells due to the configuration of the existing pumping system, limited access, and/or the well had dried up during the sampling period. The depth of the wells sampled varied from 2.6 to 135 m bgl with shallow (<10 m bgl) wells comprising 55% (31 wells), medium (10-30 m) wells comprising 29% (16 wells), and the deep (>30 m) wells comprising 16% (9 wells) of total 56 wells sampled (Figure 3.1b).

3.2.4 Groundwater sample collection and analytical methods

Selected wells were sampled as per national groundwater sampling protocols (Daughney et al., 2006). Whenever applicable, wells were purged with at least three well volumes until values for temperature, pH, dissolved oxygen (DO), and electrical conductivity (Daughney et al., 2006) as measured in the field with a YSI Professional Plus multiparameter water quality probe had stabilised. In some cases purging three well volumes was not practical due to the large amount of water involved. These wells were used daily for dairy farm operations indicating that water was not standing in the well for long periods. Here, on-site parameters were monitored every 10-20 minutes until values had stabilised. This approach is justified as Daughney et al. (2007) found that the stabilisation of field parameters is a more appropriate assessment of the adequacy of purging than the total volume of water used, though they stressed that one criterion is not as effective as both together.

Once the measured field parameters stabilised, groundwater samples were collected in a syringe fitted with a 0.45 µm syringe-tip filter. Separate 50 ml PE bottles were filled with field-filtered water for nutrients, anions, and dissolved metals (cations) analyses. Field-filtered samples for dissolved organic carbon (DOC) analysis were collected in 200 ml amber glass bottles, whereas unfiltered samples for alkalinity (bicarbonate) analysis were collected in 500 ml PE bottles. Samples were transported in chilly bins with a bag of ice or frozen pads (Daughney et al., 2006) for analysis within 24 hrs. Anions including chloride, bromide, nitrate, sulphate, and nitrite were analysed by the Ion Chromatography (USEPA 300.0 modified); ammoniacal-N was analysed by flow injection Autoanalyser (Method 4500 APHA 22nd Edition (Rice et al., 2012)); cations or dissolved metals were analysed by ICP-OES (Method 3120 B modified, APHA 22nd Edition); DOC by Dissolved Non-purgeable Organic Carbon by (Method 5310B,APHA 22nd Edition); dissolved inorganic carbon (DIC) by Method 5310B (APHA 22nd Edition); and bicarbonate calculated following Method 4500-CO_2 (APHA 22nd Edition) from pH (measured on site) and alkalinity determined following Method 2320 B (APHA 22nd Edition). The oxidation-reduction potential (ORP) measured in-field with an Ag/AgCl reference electrode were converted to *Eh* (i.e., with respect to standard hydrogen electrode) by adding 200 mV to the measured values (Rice et al., 2012).

3.2.5 Data processing for statistical analysis

The quality of groundwater data was first assessed through determination of charge balance error (CBE) (Freeze and Cherry, 1979) considering Na^+, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Cl^-, HCO_3^-, SO_4^{2-}, and NO_3^- (Appelo and Postma, 2005). Concentrations of Fe^{2+} and Mn^{2+} were derived from dissolved iron and manganese concentrations (Jurgens et al., 2009; Stenger et al., 2008). For samples filtered on site with 0.45µm filter and collected into a container with nitric acid as preservative, analysis of dissolved iron and manganese concentrations were considered

often accurate estimates of Fe^{2+} and Mn^{2+}, especially for a range of pH usually found in groundwater (Jurgens et al., 2009; Stenger et al., 2008). Results below the detection limit were assigned a value of $1/\sqrt{2}$ of the detection limit (D) which is applicable for slightly skewed and log-transformed data (Finkelstein and Verma, 2001; Helsel, 2010). Except for one sample, all of the data had CBE of less than the acceptable ±10% (Gueler et al., 2002; Guggenmos et al., 2011), with more than 82% of samples having CBE of less than 5% (recommended by Freeze and Cherry, 1979). The use of data with even up to 15% CBE is not unusual (e.g., Tidswell et al., 2012); hence, all 56 sample data were used in further analysis.

Afterwards, the data for each parameter was assessed for normality using the Shapiro-Wilk test (Helsel and Hirsch, 2002), the Kolmogorov-Smirnov test (Daughney and Reeves, 2005), as well as Fisher's measure of skewness (±1.95 is significant; Ghasemi and Zahediasl, 2012), and the data was considered to be normally distributed if at least one of the tests was satisfied. When required, data were transformed until normal distribution was obtained. Most of the parameters had a log-normal distribution (e.g., electrical conductivity, pH, ammonium-N, nitrite-N, bicarbonate, bromide, chloride, calcium, ferrous iron, manganese, magnesium, potassium, silica, sodium, dissolved organic carbon, sulphate), while other parameters such as dissolved oxygen (exponent=1/2), and depth, nitrate-N and sulphate (exponent=1/4) were power-transformed to achieve normal distribution. The transformed data were then analysed using IBM SPSS Statistics 22.0 which determines the correlations between groundwater quality parameters and their differences among different hydrogeological factors.

3.2.6 Classification of redox conditions and determination of denitrification potential of groundwater

The method of McMahon and Chapelle (2008) (Table 3.1) was used for distinguishing between groundwater redox processes and indications of nitrate-reducing conditions. The redox processes were inferred by comparing measured concentrations of relevant groundwater quality parameters with the suggested threshold values given in Table 3.1.

Table 3.1 Threshold concentrations used for identifying redox process in groundwater (modified from McMahon and Chapelle, 2008).

Redox category	**Redox process**	**Criteria for inferring process from water quality data**				
		DO (mg L^{-1})	**NO_3^--N** (mg L^{-1})	**Mn^{2+}** (mg L^{-1})	**Fe^{2+}** (mg L^{-1})	**SO_4^{2-}** (mg L^{-1})
Oxic	O_2 reduction	≥1.0	-	<0.05	<0.1	-
Suboxic		<1.0	<0.5	<0.05	<0.1	
Anoxic	NO_3^- reduction	<1.0	≥0.5	<0.05	<0.1	
	Mn(IV) reduction	<1.0	<0.5	≥0.05	<0.1	
	Fe(III)/SO_4^{2-} reduction	<1.0	<0.5	-	≥0.1	≥0.5
	Methanogenesis	<1.0	<0.5	-	≥0.1	<0.5

Source: Modified from McMahon and Chapelle, 2008. Mixed redox processes are identified when criteria for more than one redox process are met. Instead of the DO threshold of 0.5 mg L^{-1}, this study used 1 mg L^{-1} based on site-specific information as recommended by Jurgens et al. (2009) with DO vs NO_3^--N plot showing very low NO_3^--N found at DO < 1 mg L^{-1}.

Further, the denitrification potential of groundwater was determined based on the redox indicator species and electron donors parameters (Jahangir et al., 2012a; McMahon and Chapelle, 2008; Rivett et al., 2008; Thayalakumaran et al., 2008). This study used mainly DO and *Eh* as indicators of redox status, whereas DOC and Fe^{2+} were considered as electron donors for the denitrification process (Thayalakumaran et al., 2008). Groundwater was classified as under NO_3^- reducing conditions if DO < 1 mg L^{-1} and *Eh* < 150 mV, and under non-reducing conditions if DO ≥ 1 mg L^{-1} and *Eh* ≥ 150 mV (Jahangir et al., 2012b; Rivett et al., 2008; Thayalakumaran et al., 2008). As for electron donors, the threshold concentration

value of DOC or Fe^{2+} > 1 mg L^{-1} was used to indicate the presence of electron donors significant enough to support denitrification (Rivett et al., 2008; Thayalakumaran et al., 2008).

3.2.7 Assessment of effects of hydrochemical processes and hydrogeological factors on denitrification potential

Principal Component Analysis (PCA), a multivariate statistical tool (see Johnson and Wichern, 2007; Matiatos et al., 2014), was used to identify the main processes responsible for the hydrochemistry in groundwater in the study area. It was further used to determine hydrogeological characteristics that influence such processes, with a particular focus on redox processes and denitrification potential. In groundwater studies, variables that are highly correlated to a certain principal component when interpreted together are considered to "represent a process influencing the data" (Kim et al., 2009; Matiatos et al., 2014; Nolan, 1999; Uddameri et al., 2014). As such, the main processes responsible for the hydrochemistry of groundwater could be identified by assessing the relationships of the variables with high loadings in respective principal components. Moreover, aside from the loadings of variables for each principal component, the scores of each groundwater sample for each component can be computed and, therefore, the hydrogeochemical processes responsible for the hydrochemistry of the specific sample can be identified.

In this study, all relevant groundwater quality parameters measured independently were included in the analysis. The strength of the association of variables to the principal components was classified according to the absolute value of their loadings, suggesting strong (>0.75), moderate (0.5-0.75), and weak (0.3-0.5) associations (Liu et al., 2003; Matiatos et al., 2014; Uddameri et al., 2014). The relevance of each component/process to

each sample as well as the relationship between processes representing the principal components and hydrogeological properties were assessed with principal component scatter plots (similar with Matiatos et al., 2014; Nolan, 1999). Moreover, an interpolation map was prepared based on the scores of wells/samples for the redox process (Kim et al., 2009; Lambrakis et al., 2004) to gain insight of the areas where redox processes seem significant.

Statistically significant differences in selected hydrochemical parameters among different hydrogeological settings were further assessed using *t*-test or ANOVA, depending on the number of factors considered. Box-whisker plots were further used to assess and visualise differences in hydrochemistry, particularly those related to redox conditions, among different combinations of land use, soil texture, drainage class and rock types in the study area.

3.3 Results and Discussion

3.3.1 Groundwater hydrochemistry and distribution of nitrate concentrations and other redox-related parameters

Table 3.2 summarises descriptive statistics of 26 chemical and physical parameters measured for 56 groundwater samples in the Tararua GWMZ, except for well depth and depth to water level measured in 55 and 35 wells, respectively (see notes under Table 3.2). The complete well and groundwater quality data are provided as supplementary materials (Appendix D.1).

Figure 3.1b shows five types of groundwater across the Tararua GWMZ, where mainly Na-HCO_3 and Ca-HCO_3 types found in the Upper Manawatū sub-sub-catchment, and chloride type waters (Na-Cl or Ca-Cl) mostly observed in the Mangatainoka sub-catchment. There was no clear evidence of influence of depth on groundwater types especially for shallower

wells (Figure 3.1b). However, the chloride concentrations were generally lower but HCO_3^- concentrations higher at deeper wells (>30 m) (data not shown). The increase in HCO_3^- concentration with depth (r = 0.408, p<0.01; Appendix D.2) could be explained by carbonate mineral dissolution (Appelo and Postma, 2005) and/or redox processes such as heterotrophic denitrification with HCO_3^- as the product (Rivett et al., 2008). These hydrochemical processes are discussed further below (sub-section 3.3.3).

The measured nitrate-N concentrations varied from below detection limit (<0.002 mg L^{-1}) to 15.83 mg N L^{-1}, with mean and median values of 1.71 mg N L^{-1} and 0.39 mg N L^{-1}, respectively (Table 3.2). This suggests NO_3^- contamination of groundwater in the Tararua GWMZ was not extensive and spatially variable with greater NO_3^--N concentrations observed in the Mangatainoka sub-catchment (Figure 3.2a). Several wells with NO_3^--N concentrations > 2.26 mg N L^{-1} were concentrated in the middle and outlet parts of the Mangatainoka sub-catchment (Figure 3.2a). In the Upper Manawatū, most of the wells had NO_3^--N concentrations <1.13 mg N L^{-1} except four wells recording NO_3^--N concentrations between 4.65 and 15.83 mg N L^{-1}. Only, two locations in the Upper Manawatū recorded NO_3^--N concentrations greater than the maximum allowable value of 11.3 mg nitrate-N L^{-1} for drinking water standards (Daughney et al., 2009).

Table 3.2 Summary statistics of groundwater quality parameters measured for groundwater samples collected in the Tararua GWMZ during February-March, 2014.

Parameter	Unit	Mean	Median	Mode	Std. Deviation	Range	Minimum	Maximum	Skewness	Kurtosis
Well depth	m	18.67	8.77	4.70	26.52	132.40	2.60	135.00	3.141	10.414
Depth to water	m	4.60	3.75	1.26	3.79	22.02	1.26	23.28	3.763	17.822
Temperature	°C	14.94	14.70	13.90	1.80	10.30	10.90	21.20	0.878	2.302
DO	mg L^{-1}	3.04	2.05	0.21	3.03	8.98	0.07	9.05	0.642	-1.021
DOC	mg L^{-1}	1.00	0.66	0.30	1.24	8.64	0.11	8.75	4.704	27.992
DIC	mg L^{-1}	21.32	15.68	2.06	21.14	118.24	2.06	120.30	2.726	9.625
SPC	µS cm^{-1}	239.83	181.00	73.50	212.19	1244.10	63.90	1308.00	3.126	12.557
pH		6.15	6.02	5.81	0.67	3.32	5.03	8.35	0.918	1.530
ORP (Eh)	mV	295.43	338.10	27.40	135.72	526.90	27.40	554.30	-0.243	-0.765
HCO_3^-	mg L^{-1}	88.54	51.84	6.10	108.63	583.57	6.10	589.67	2.805	9.500
Br^-	mg L^{-1}	0.11	0.10	0.10	0.07	0.34	0.02	0.36	1.836	4.002
Cl^-	mg L^{-1}	22.33	15.83	5.69	17.45	72.30	5.69	77.99	1.700	2.256
B	mg L^{-1}	0.069	0.017	0.013	0.208	1.446	0.004	1.450	5.798	36.894
Ca^{2+}	mg L^{-1}	15.64	10.53	0.263	15.70	84.80	0.26	85.06	2.810	9.608
Fe^{2+}	mg L^{-1}	0.4164	0.0145	0.0035	1.1690	6.1865	0.0035	6.1900	3.979	16.565
Mg^+	mg L^{-1}	4.96	3.61	10.10	5.22	35.24	0.03	35.27	3.833	20.378
Mn^{2+}	mg L^{-1}	0.2000	0.0150	0.00354	0.3240	1.3425	0.0035	1.34	1.831	2.709
K^+	mg L^{-1}	2.97	2.12	0.46	3.24	21.18	0.46	21.64	4.276	21.774
SiO_2	mg L^{-1}	24.72	19.40	9.09	17.72	74.82	6.46	81.28	1.323	1.294
Na^+	mg L^{-1}	26.13	13.95	4.85	36.90	200.52	4.85	205.36	3.366	12.214
NO_3^--N	mg L^{-1}	1.711	0.386	0.001	2.944	15.826	0.001	15.827	2.904	10.463
NO_2^--N	mg L^{-1}	0.0089	0.0017	0.0014	0.0377	0.2824	0.0014	0.2838	7.330	54.399
NH_4^+-N	mg L^{-1}	0.345	0.012	0.007	1.148	7.367	0.007	7.374	5.009	27.489
SO_4^{2-}	mg L^{-1}	13.791	9.660	0.004	21.751	160.881	0.004	160.885	5.853	39.329
Total Alkalinity	mg L^{-1}	73.04	42.50	8.00	90.48	481.00	5.00	486.00	2.827	9.530

Note: No. of observations: 56, except for well depth (55) and depth to water level (35). Values below detection limit have been adjusted by multiplying with ($1/\sqrt{2}$). As such, some minimum values shown are lower than their respective detection limits mentioned in the text. DO – dissolved oxygen, DOC – dissolved organic carbon, DIC – dissolved inorganic carbon, SPC – specific conductance or electrical conductivity, ORP – oxidation-reduction potential, B - boron

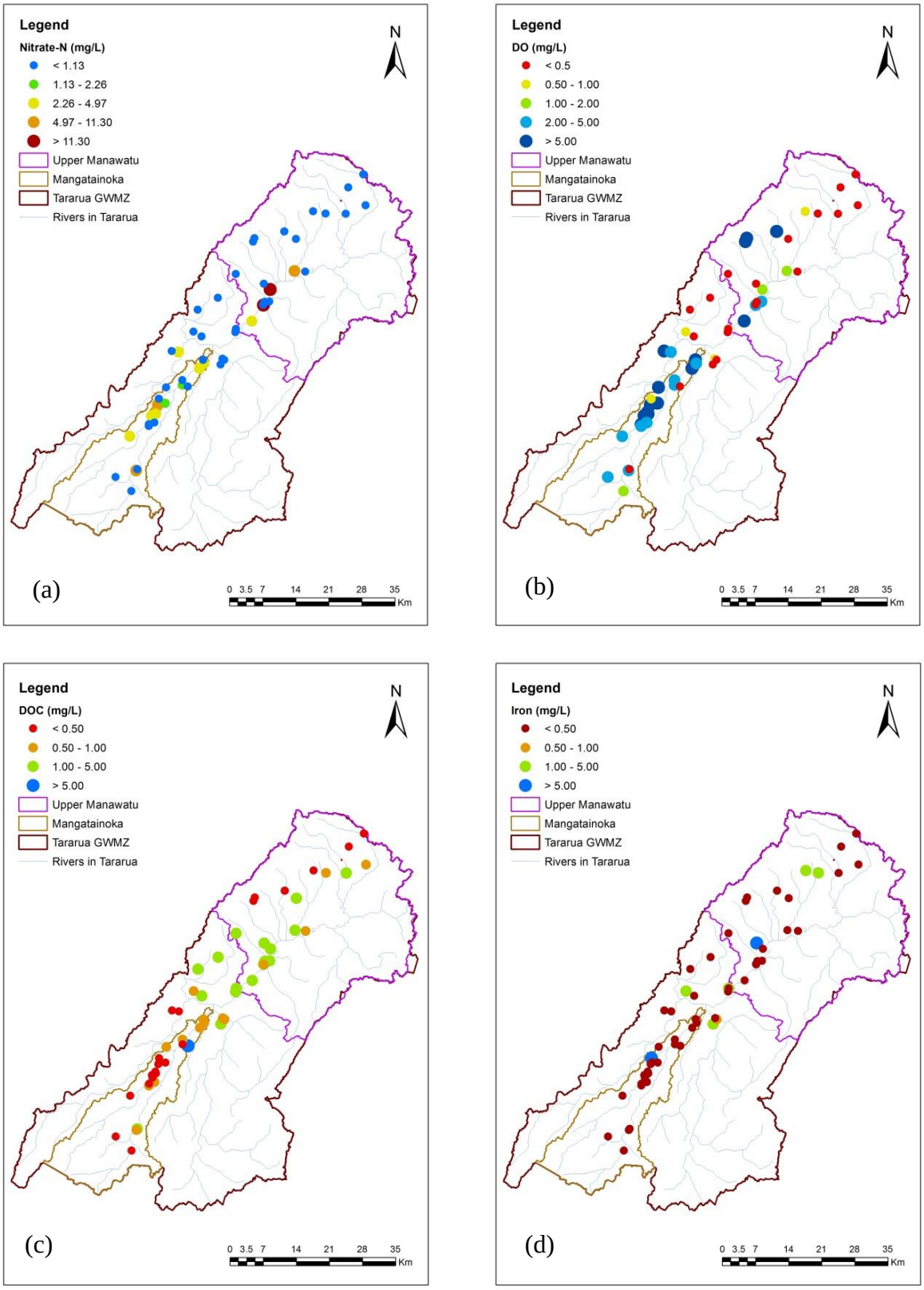


Figure 3.2 Distribution of (a) nitrate-N (mg L^{-1}), (b) dissolved oxygen (mg L^{-1}), (c) dissolved organic carbon (mg L^{-1}) and (d) ferrous iron (mg L^{-1}), in the Tararua GWMZ during February-March, 2014.

The measured dissolved oxygen (DO) concentrations also varied widely from 0.07 to 9.05 mg L^{-1}. As shown in Figure 3.2b, most of the wells in the Mangatainoka sub-catchment had DO concentrations >2 mg L^{-1}, the upper limit considered for denitrification to occur (Rivett et al., 2008). On the other hand, most of the wells in the Upper Manawatū had DO <2 mg L^{-1}, indicating denitrification potential in upper parts of the catchment. The measured DO concentrations did not seem to have any significant relationship with well depth or depth to the water table (WL) (Appendix D.2), indicating other factors aside from groundwater depth might be responsible for low DO concentrations in shallow groundwater. However, the negative correlation between DO and silica (SiO_2) (r = -0.625, p<0.01; Appendix D.2) indicates lower DO concenrations in older groundwater (e.g., Landon et al., 2011). A positive relationship between DO and NO_3^--N (r = 0.618, p<0.01) indicates greater NO_3^- concentrations in oxidised groundwaters with high DO concentrations (Appendix D.2).

Figure 3.2c&d shows the distribution of potential electron donors for the denitrification process namely, dissolved organic carbon (DOC) and ferrous iron (Fe^{2+}). The DOC concentrations were found to be in the range of 0.1-8.75 mg L^{-1}, but most were <3 mg L^{-1}. One well which had the highest DOC concentration (8.75 mg L^{-1}) was located close to a drainage canal where surface runoff may have contaminated the groundwater. Generally lower DOC concentrations were found in the Mangatainoka sub-catchment and relatively high concentrations were found in the middle and upper parts of the Tararua GWMZ. The Fe^{2+} concentrations were also low with most locations having <0.5 mg L^{-1}, while around 10 wells have Fe^{2+} concentrations >0.5 mg L^{-1}. Two wells recorded Fe^{2+} concentrations >5 mg L^{-1}. The DOC and NO_3^--N had a negative but weak correlation (Appendix D.2) as found by other authors (Minamikawa et al., 2015). A moderate negative correlation (r = -0.63; p<0.01)

existed between Fe^{2+} and NO_3^--N indicating the low presence of NO_3^--N in reduced groundwaters (e.g., Landon et al., 2011).

3.3.2 Denitrification characteristics of groundwater

3.3.2.1 Redox processes

Using the measured redox related groundwater quality parameters and threshold concentrations (Table 3.1), the redox processes and their distribution were identified as shown in Figure 3.3a. In general, most of the wells sampled (54%) were assessed to be oxic groundwater (30 out of 56 wells), whereas 38% (21 wells) and 7% (4 wells) were assessed as anoxic or mixed anoxic, and suboxic groundwaters, respectively. One well was assessed with mixed oxic-anoxic groundwater. It is apparent that oxic groundwater was mostly found in the Mangatainoka sub-catchment, and in the lower portion of the Upper Manawatū sub-catchment. Anoxic groundwater was found mainly in the upper portion of the Upper Manawatū sub-catchment as well as in the middle of the Tararua GWMZ.

The anoxic and mixed anoxic groundwaters were further classified as NO_3^- reduction, manganese reduction, iron/sulphate reduction, and methanogenesis. The NO_3^- reduction process was identified in two wells, as mixed NO_3^- reduction-iron/sulphate reduction and NO_3^- reduction-manganese reduction. Most of anoxic wells (13 of 21 wells; 62%) were classified as iron/sulphate reduction. Manganese reduction was found in five wells, and methanogenesis in one well (Figure 3.3a). This indicates that groundwaters in 38% (21 out of 56) of wells were highly reduced where reduction of NO_3^- can be expected (Korom, 1992; Rivett et al., 2008). Further, denitrification appears to be the dominant NO_3^- reduction process. Dissimilatory nitrate reduction to ammonium (DNRA) could be possible but appears a less significant process. Of the 56 samples, only 11 samples exceeded both the

concentration of 0.15 mg NH_4^+-N L^{-1} (Stenger et al., 2008) and the C:NO_3^--N ratio of 12 (Rütting et al., 2011) for the possibility of DNRA occurrence. A repeat groundwater survey and assessment of 28 wells in March 2015 (data not shown) showed that the redox conditions in general remained similar over the two groundwater surveys conducted in similar climatic conditions (summer) (Appendix D.3).

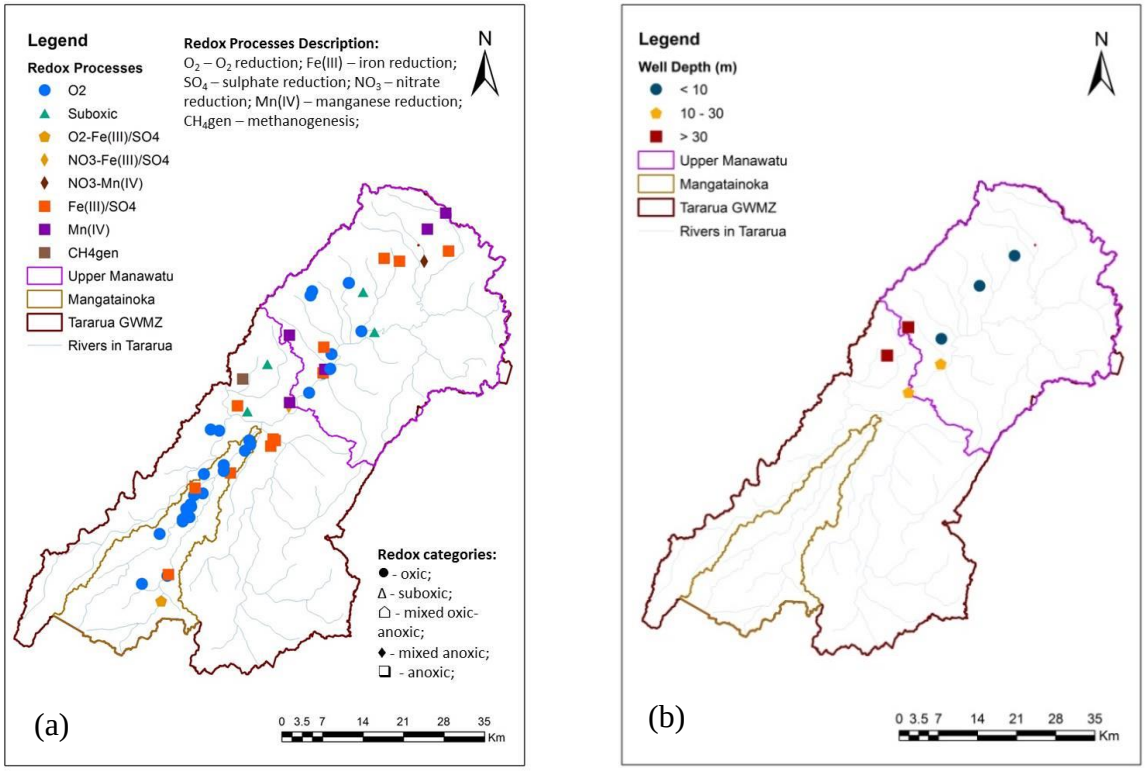


Figure 3.3 Distribution of (a) ambient redox processes, and (b) wells with high denitrification potential in groundwater in the Tararua GWMZ during February-March, 2014.

3.3.2.2 Denitrification potential of groundwater

This study further assessed denitrification potential, as the process requires the presence of electron donors (Rivett et al., 2008). Figure 3.3b shows the location of wells where groundwater was found to have the potential to denitrify, or reduce NO_3^-, based on the criteria described earlier (section 3.2.6). It is apparent that such wells are located in the middle and upper parts of the Tararua GWMZ, and none were found in the Mangatainoka

sub-catchment. These findings confirm that, in general terms, the areas with reducing groundwater conditions (in the Upper Manawatū sub-catchment) had the potential to reduce NO_3^- in the subsurface environment in the study area.

Figure 3.2 suggests that most parts of the Mangatainoka sub-catchment had low denitrification potential, given the high DO (Figure 3.2b) but low DOC and Fe^{2+} concentrations (Figure 3.2c&d). This is confirmed by the corresponding higher concentrations of NO_3^--N in the sub-catchment (Figure 3.2a). Most of the wells with higher NO_3^--N concentrations in the range of 2.26 – 11.3 mg N L^{-1} (equivalent to 10-50 mg nitrate L^{-1}) were located in the Mangatainoka sub-catchment (Figure 3.2a). In contrast, most of the wells in the Upper Manawatū sub-catchment and in the middle of the Tararua GWMZ drew groundwater with reducing conditions and potential to denitrify.

3.3.3 Hydrochemical processes affecting groundwater chemistry

The PCA identified five components with eigenvalues greater than 1 (Table 3.3). The first three components accounted for 64.9% of the variance of the original variables. The low amount of variance (approx. 10%) explained by each of the remaining two components may just represent the noise in the data rather than a hydrochemical process (Nolan, 1999). Thus, this study focused on the first three components as they explain the major variance in the data.

Component 1 accounted for almost a quarter of the total variance in the data (23.5%). Parameters with strong loadings (>0.75) in this component include chloride, bromide, and sodium, while those with moderate loadings (0.50-0.75) include boron, DOC, DIC, potassium and specific conductance (Table 3.3). Based on these parameters, there are four possible

processes affecting groundwater quality that could represent this component, namely: 1**)** anthropogenic activities (Flury and Papritz, 1993; Kim et al., 2009), 2) volcanic activity (Alcalá and Custodio, 2008; Cakin et al., 2012), 3) extensive interaction between groundwater and greywacke (Morgenstern et al., 2007; Schofield, 1960), and 4) deposition of seaborne salts (Alcalá and Custodio, 2008; Flury and Papritz, 1993). Further investigation is required to attribute with certainty the process being represented by Component 1, but this is outside the scope of this study being focused on assessment of denitrification potential in the subsurface environment.

Table 3.3 Loadings (rotated) from Principal Components Analysis of groundwater quality data from wells in the Tararua GWMZ during February-March, 2014

	Component				
Parameter	1	2	3	4	5
pH	.191	.307	.854	.072	-.206
Dissolved inorganic carbon (DIC)	.531	.158	.768	.071	.125
HCO_3^-	.458	.431	.666	.219	-.011
Specific conductance	.630	.020	.621	.119	.162
NH_4^+-N	.362	.576	.599	-.051	-.083
NO_2^--N	-.214	.057	.708	.005	.514
Fe^{2+}	.149	.900	-.062	.079	.003
Mn^{2+}	.058	.875	.057	.335	.084
DO	-.384	-.777	-.314	-.140	-.034
NO_3^--N	.018	-.727	-.316	.080	.448
ORP	-.152	-.731	-.467	-.149	.276
SiO_2	.281	.705	.192	-.037	.086
Cl	.904	.172	.042	.192	.006
Br	.851	.197	-.124	.229	.062
Na^+	.796	.257	.476	-.034	.014
Boron	.746	.152	.451	.132	-.112
Dissolved organic carbon (DOC)	.606	.225	.234	.088	.180
Ca^{2+}	.173	.090	.117	.931	.176
Mg^+	.208	.202	.056	.889	.113
SO_4^{2-}	.125	-.111	-.058	.325	.798
K^+	.602	.071	.262	.028	.596
Eigenvalues	4.9	4.7	4.1	2.1	1.7
Percentage of total variance	23.5	22.2	19.3	10.1	8.2
Cumulative percentage of variance	23.5	45.6	64.9	75.0	83.3

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.

Component 2 accounted for over a fifth of the total variance (22.2%) and includes iron, manganese, and DO with strong loadings (>0.75), as well as nitrate-N, ORP (*Eh*), ammonium-N and silica with moderate loadings (0.50-0.75) (Table 3.3). This component represents the redox related processes in groundwater (Nolan, 1999). DO was inversely related to iron and manganese and positively related to NO_3^--N, which is consistent with the biochemical succession of redox processes and sequential production of final products (McMahon and Chapelle, 2008). The strong positive relationship of this component with Fe^{2+} and Mn^{2+} and negative relationship with DO, NO_3^--N and ORP (*Eh*) indicates that the groundwater identified with this component is highly reduced (Table 3.3). The redox processes represented by this component were, therefore, specific to groundwater in reducing conditions. The moderate positive loading of silica indicates these reduced groundwaters were older compared to other areas in the Tararua GWMZ. The negative relationship between NO_3^--N and DOC (Table 3.3, Appendix D.2) further indicates the role of DOC as electron donor in the reduction processes.

Component 3 accounted for almost a fifth of the total variance (19.3%), and includes *pH* and dissolved inorganic carbon (DIC) with strong loadings (>0.75), and bicarbonate, specific conductance, ammonium-N and nitrite-N with moderate loadings (0.50-0.75) (Table 3.3). DIC accumulation in groundwater indicates bacterial activity to oxidise organic matter, or dissolved carbonate minerals (Nolan, 1999). Likewise, high bicarbonate and total alkalinity indicate interactions with carbonate minerals in the aquifer (Uddameri et al., 2014). The positive relationship of *pH* with this component supports the measurements of increased alkalinity in the groundwater (Nolan, 1999). Thus, this component may represent the dissolution of carbonate minerals considering the extensive presence of mudstone and sandstone in the study area.

Distribution of redox processes in reduced groundwater in the Tararua GWMZ

The spline interpolation tool in ArcGIS 10.3 was used to estimate the scores for Component 2 in other areas not sampled (Figure 3.4). With symbols for redox categories as identified by the method developed by McMahon and Chapelle (2008) superimposed on the plot of Component 2 scores, it is very clear that areas with high scores for Component 2 matched with areas identified independently as anoxic groundwater (reducing conditions). Figure 3.5 shows that wells with anoxic conditions related positively to Component 2 (wells on the right or positive side of the plot), while wells with oxic conditions did not.

Figures 3.4 and 3.5 support the identification and labelling of PCA Component 2 as redox processes in groundwater with reducing conditions. The reduced groundwater in the vicinity of Dannevirke (Figure 3.4) in the Upper Manawatū sub-catchment (Figure 3.1a) coincides with the location of layers of carbonaceous silt and lignite (rich in organic matter) found in the Mangatarata Formation of the geology in the Dannevirke area, in some cases within 20 m of the surface with more at depth (Krieger, 1992).

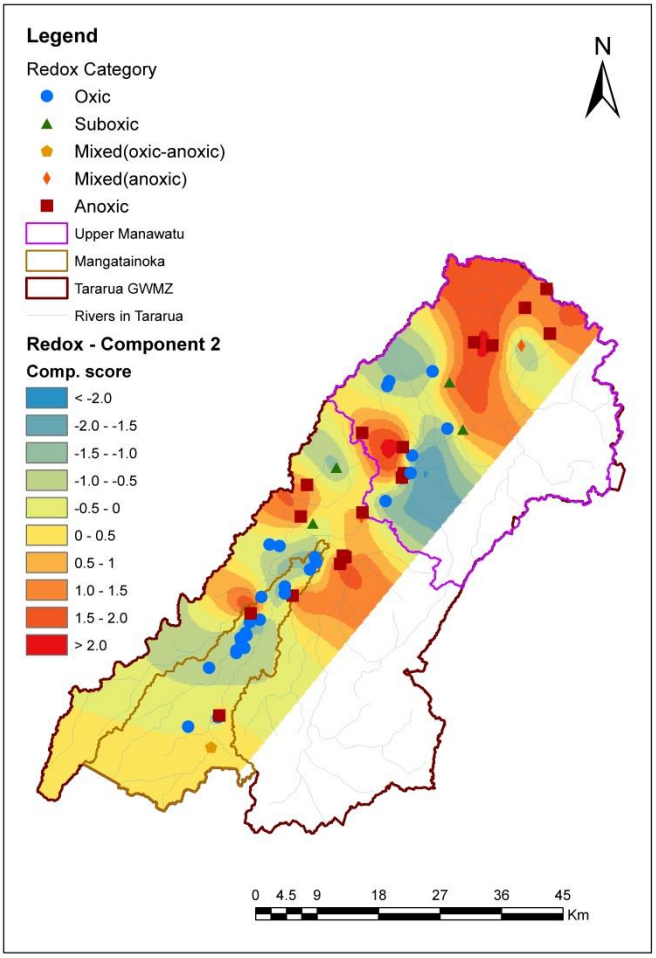


Figure 3.4 Estimated spatial distribution of contours with iso-factor scores for PCA Component 2 (redox processes in reducing conditions) for groundwater in the Tararua GWMZ based on 56 samples collected in February-March, 2014.

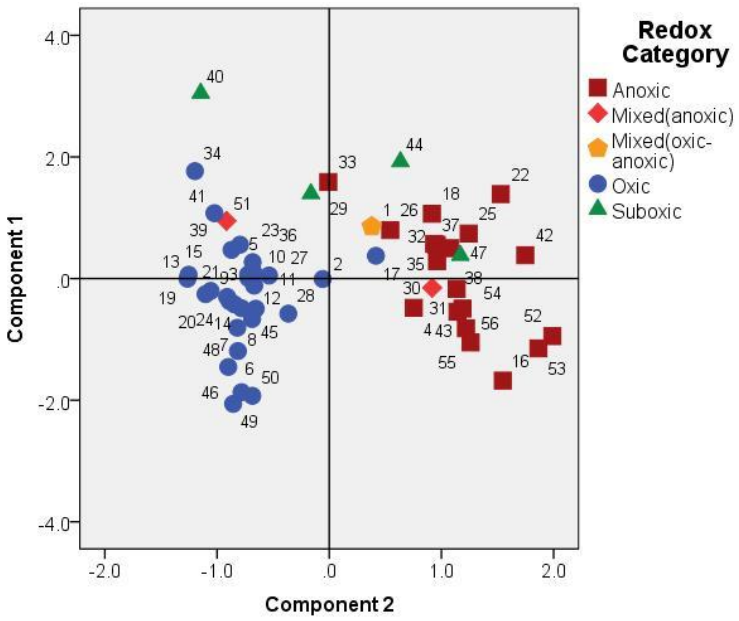


Figure 3.5 Scatter plot of PCA Components 1 and 2 (redox processes in reducing conditions), with respect to identified redox status for groundwater in the Tararua GWMZ based on 56 samples collected in February-March, 2014. Labels in the plot are well numbers.

3.3.4 Anthropogenic and hydrogeological factors affecting hydrochemistry and denitrification potential in groundwater

3.3.4.1 Land use

This study found that land use did not have a consistent relationship with the principal components identified in the sampled groundwater in the Tararua GWMZ. With land use as the classification category, the scatter plots of Component 1 and 2 scores of each well showed the wells to be evenly distributed across all quadrants (Figure 3.6a), indicating no relationship with the identified hydrochemical processes. This is consistent with other studies indicating that there are other factors aside from land use that affect the variability in groundwater quality (e.g., Kulabako et al., 2007). The differences in observed concentrations among redox sensitive parameters with respect to dairy and beef/sheep land use types are provided as supplementary material (Appendix D.4). It is apparent that no significant differences in the redox and other selected parameters exists between the two land use types except for NO_3^-N

at a significance level of $p<0.10$. The mean concentration of NO_3^--N in groundwater under dairy farms was found to be higher (2.24±0.70 mg L^{-1}) compared with the beef/sheep farms (1.18±0.34 mg L^{-1}). Estimates of leaching rate in the Tararua GWMZ confirmed this with the average N leaching rate for sheep and/or beef farms (16 kg N ha^{-1} yr^{-1}) was much lower than the estimated leaching rate for dairy farms (33.9 kg N ha^{-1} yr^{-1}) (Roygard and Clark, 2012). Interestingly, the wide range of NO_3^--N concentrations in groundwater under dairy (from 0.001 to 15.83 mg N L^{-1}) suggests that land use was not the only variable responsible for NO_3^--N concentration situation in groundwater.

3.3.4.2 Soil texture or drainage class

Hydrogeological factors influencing the movement and hydrochemistry of water percolating to groundwater, such as the permeability of the vadose zone, may also affect the quality of groundwater (e.g., McLay et al., 2001; Orr, 2014). This study assessed the relationship of soil texture and drainage classes to redox processes, as well as specific redox related parameters in the study area (Table 3.4). The Fundamental Soil Layer (FSL) in the Tararua GWMZ was regrouped based on their soil texture into three soil texture classes namely, fine, medium and coarse textured soils. The fine soil texture class included; heavy silt loam, silt loam & clay loam, and silt loam. The medium soil texture included; sandy loam & silty loam, and silt loam/sandy loam. The coarse soil texture included; sand, sand & stony gravel, and stony loam. The FSL has five soil drainage classifications as follows: 1 – very poor; 2 – poor; 3 – imperfect; 4 – moderately well; 5 – well.

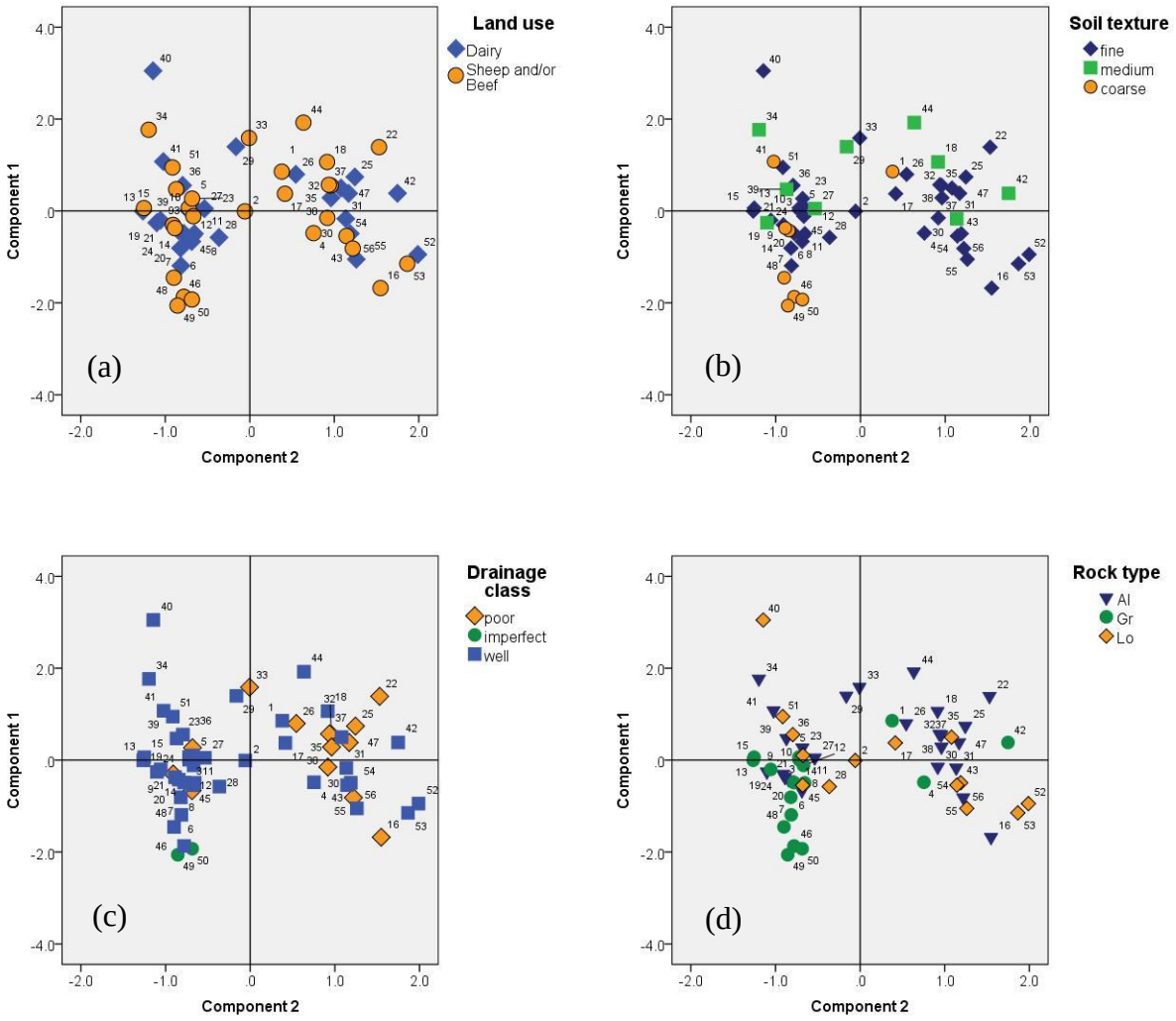


Figure 3.6 Scatter plot of PCA Components 1 and 2 (redox processes in reducing conditions), with respect to (a) land use, (b) soil texture, (c) soil drainage class, and (d) top rock types for groundwater in the Tararua GWMZ based on 56 samples collected in February-March, 2014. Labels in the plot are well numbers.

Figure 3.6b&c show the relationship of each groundwater sample with PCA Components 1 and 2 with respect to different soil texture and drainage class. No clear relationship could be observed between the Component 2 (redox processes in reducing conditions) and the fine and medium soil texture, but a negative relationship with the coarse soil texture class (Figure 3.6b). With respect to soil drainage class, wells under poorly drained soils seemed to be more positively related to the Component 2 (Figure 3.6c). These results suggest that soils with poor drainage influence the redox status in groundwater in the study area (Clague et al., 2015c).

Table 3.4 Redox sensitive parameters in groundwater with respect to different soil texture and rock types in the Tararua GWMZ during February-March, 2014.

Parameter	Unit	**Soil texture** (mean±SE mean)			Sig.	**Rock types** (mean±SE mean)			Sig.
		Fine (n=39)	Medium (n=9)	Coarse (n=8)		Alluvium (n=25)	Gravel (n=17)	Loess (n=14)	
DO	**mg L^{-1}**	**2.66±0.46^{b}**	**2.26±0.89^{b}**	**5.76±1.08^{a}**	**0.022**	**1.86±0.44^{b}**	**5.81±0.73^{a}**	**1.79±0.56^{b}**	**<0.001**
ORP (Eh)	mV	275.59±23.93	316.04±34.99	368.96±15.87	0.185	**263.41±24.62^{b}**	**385.06±26.40^{a}**	**243.79^{a}±37.82^{b}**	**0.003**
NH_4-N	mg L^{-1}	0.465±0.218	0.112±0.067	0.025±0.017	0.222	**0.271±0.160^{ab}**	**0.041±0.019^{b}**	**0.848±0.534^{a}**	**0.044**
HCO_3^-	mg L^{-1}	98.47±19.50	86.96±28.34	41.92±10.70	0.459	**94.10±14.67^{a}**	**29.27±7.16^{b}**	**150.59±46.39^{a}**	**<0.001**
Fe^{2+}	mg L^{-1}	0.4121±0.1769	0.7743±0.6054	0.0347±0.0309	0.113	0.5312±0.2613	0.3599±0.3237	0.2799±0.1663	0.106
Mn^{2+}	**mg L^{-1}**	**0.2337±0.0548^{a}**	**0.2036±0.1187^{ab}**	**0.0311±0.0276^{b}**	**0.047**	0.2585±0.0781	0.0693±0.0368	0.2541±0.0855	0.051
DOC	mg L^{-1}	0.90±0.11	1.84±0.88	0.53±0.14	0.144	**1.32±0.33^{a}**	**0.51±0.11^{b}**	**1.00±0.24^{ab}**	**0.007**
NO_3-N	mg L^{-1}	1.711±0.401	1.267±0.551	2.210±1.946	0.979	1.435±0.652	2.093±0.535	1.740±0.873	0.183
NO_2-N	mg L^{-1}	0.0113±0.0072	0.0022±0.0006	0.0046±0.0012	0.368	0.0028±0.0006	0.0048±0.0010	0.0248±0.0200	0.069
SO_4^{2-}	mg L^{-1}	14.790±4.049	13.650±2.560	9.077±4.299	0.530	13.615±1.965	7.482±0.910	21.765±10.973	0.541

Soil texture: (1) fine – silt loam and clay loam, heavy silt loam, silt loam; (2) medium – sandy loam and silt loam, silt loam/sandy loam; (3) coarse – sand, sand and stony gravel, stony loam; ANOVA was performed using the transformed values of parameters. Different letters denote significant differences according to LSD and Tukey HSD. DO – dissolved oxygen, DOC – dissolved organic carbon, ORP – oxidation-reduction potential. Errors are standard errors of mean. Sig. – significance level.

Table 3.4 provides some explanation of the negative relationship between coarse soil texture and Component 2. Significant differences in DO and Mn^{2+} concentrations were found among different soil texture with the highest DO concentrations (p=0.022) and the lowest Mn^{2+} concentrations (p=0.047) under coarse soil texture. This is further supported by the observed largest ORP (*Eh*) and lowest Fe^{2+} concentrations for coarse textured soils, although differences between soil texture were not significant. Clague et al. (2015c) also observed a similar situation in the Waikato region of New Zealand, in which oxidised shallow groundwater was found beneath well-drained soils.

3.3.4.3 Aquifer material: rock types

The relationship of aquifer material to redox processes was further assessed, as well as specific redox related parameters in the study area (Table 3.4). The wells sampled in this study were found to be tapping three rock types namely, alluvium (Al), gravel (Gr), and loess (Lo) based on the NZ LRIS. We found no clear relationship between the Component 2 and Al and Lo (Figure 3.6d), but Gr rock type was negatively related with Component 2, i.e. the redox processes in reducing conditions. The rock types showed significant differences with respect to concentrations of DO, bicarbonate, ammonium, DOC, and ORP (*Eh*) (Table 3.4). Highest DO was found in Gr rock type with a mean of 5.81 mg L^{-1}, and highest ORP (*Eh*) at 385.06 mV (Table 3.4). These values indicate oxidised conditions in groundwater and that groundwater in these areas was relatively young or newly recharged (based on silica concentrations, data not shown). The lowest concentration of Mn^{2+} was also found in Gr rock type indicating that reduced groundwater is not prevalent in this aquifer material. DOC was also found to be lowest in gravels while Fe^{2+} concentration ranges from below detection limit (0.005) to 5.530 mg L^{-1} (mean 0.36 mg L^{-1}), indicating that except in a few locations, there

was generally low concentrations of electron donors to lower DO concentrations in Gr aquifer material. In such aquifer conditions, if NO_3^- is leached from soil profile above, it is likely that NO_3^- will be transported to the receiving surface waters.

3.3.4.4 Subsurface environment: combination of selected land use and hydrogeological factors

Different combinations of selected land use, soil drainage class and underlying rock types were assessed for their influence on groundwater quality. Statistically significant differences (at least at the $p<0.05$ level) were found in redox related parameters, except nitrite-N and sulphate, for the different combinations (data not shown). The measured DO concentrations were greatest in the following rock type + soil drainage class combinations: Gr+well and Gr+imperfect (Figure 3.7a). This suggests that DO was generally higher when the texture of the subsurface environment facilitates faster water percolation or groundwater movement resulting in shorter residence times for redox processes to operate (Orr, 2014). This is reflected in the NO_3^--N concentrations, where except for some outliers, highest concentrations were found in Gr+well combination (Figure 3.7b).

Figure 3.8 shows the difference in NO_3^--N and DOC concentrations among combinations of different land use + rock type + soil drainage class. Except for some outliers, NO_3^--N concentrations were highest under the combination of Dairy+Gr+well and Dairy+Al+well combinations, whereas NO_3^--N concentrations were low under the Dairy+Al+poor combination (Figure 3.8a). Generally low NO_3^--N concentrations were found in combinations involving Sheep/beef land use type. While the data presented here may be limited, it is apparent that relatively high NO_3^- concentrations may be expected in groundwater under dairy farming if the underlying subsurface environment facilitates faster groundwater

recharge and water movement. Figure 3.8b shows that land use had little influence on DOC concentrations, but DOC appeared to be consistently higher in the Al rock type (Table 3.4). The higher DOC concentration in alluvium rock types could be due to the presence of silt which is rich in organic matter (e.g., Kim et al., 2009). Further assessment is needed to determine the possible influence of the Al rock type on denitrification properties.

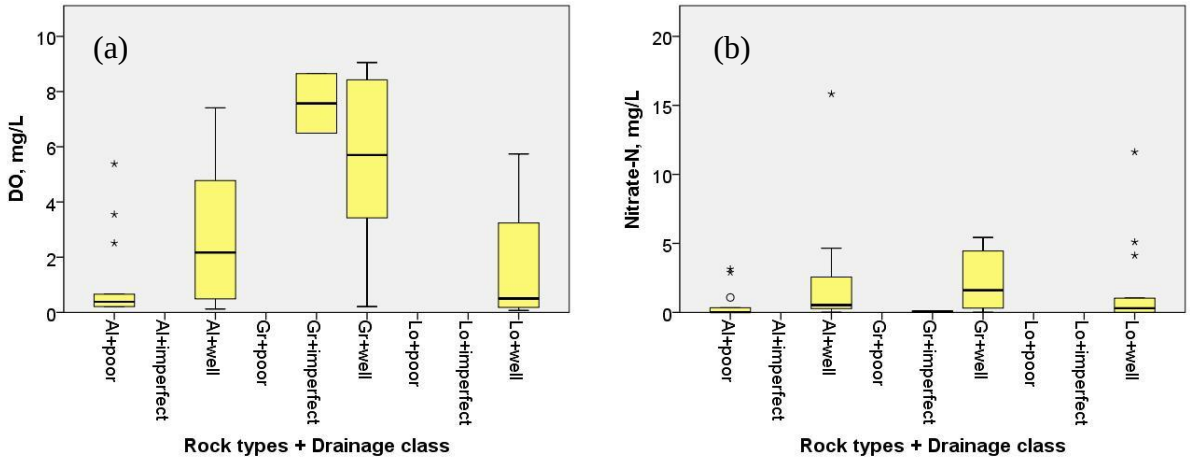


Figure 3.7 (a) DO and (b) nitrate-N concentrations in groundwater under different combinations of rock type+drainage class in the Tararua GWMZ during February-March, 2014. The absence of box plot for some combinations indicates that no sample fell into such category. Rock types: Al – alluvium, Gr – gravel, Lo – loess.

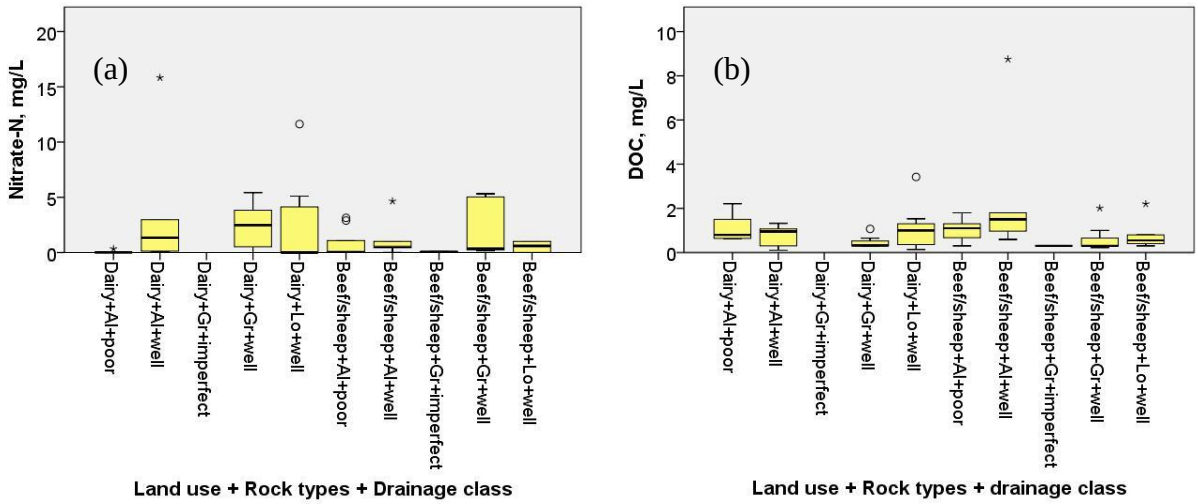


Figure 3.8 (a) Nitrate-N and (b) dissolved organic carbon (DOC) concentrations in groundwater under different combinations of land use+rock type+drainage class in the Tararua GWMZ during February-March, 2014. The absence of box plot for some combinations indicates that no sample fell into such category. Rock types: Al – alluvium, Gr – gravel, Lo – loess.

3.3.5 Implications of subsurface denitrification characteristics on nitrogen loadings

The results of this study show that the denitrification characteristics of groundwater varied spatially across the Tararua GWMZ. The groundwater in the Upper Manawatū and in the middle of the Tararua GWMZ had the potential to reduce NO_3^-. On the other hand, groundwaters in the Mangatainoka were generally under oxic conditions with low potential for denitrification to occur.

Table 3.5 Estimated average N leaching and river loading rates in the Upper Manawatū and Mangatainoka sub-catchments in the Tararua GWMZ.

Sub-catchment	**Area, ha**	**Ave. SIN at outlet[a], mg L^{-1}**	**Leaching rate[b], kg N ha^{-1} yr^{-1}**	**River load[c], kg SIN ha^{-1} yr^{-1}**
U. Manawatū	**126,188**	0.82	17	6
Dairy	20,155			
Sheep/beef	88,903			
Mangatainoka	**40,128**	0.93	19	13
Dairy	14,036			
Sheep/beef	17,526			

Source: Elwan et al., 2015; A. Elwan (personal communication, 2015)

Notes: [a]Sub-catchment outlets: Manawatū at Hopelands (Upper Manawatū), Mangatainoka at Pahiatua Bridge (Mangatainoka); Soluble inorganic nitrogen (SIN) is average of available monthly data from January 1990 to December 2014 from the Horizons Regional Council.

[b]Leaching rates (kg N ha^{-1} yr^{-1}) for different land use types were obtained from Roygard and Clark (2012) - sheep/beef (16), cropping (50.5), horticulture (80), exotic cover (4.0) and native cover (2.4), built-up areas (3), dairy (33.9)

[c]River load was computed from non-point sources only, i.e. excluding point sources (0.4 – 3% of total river load); based on SIN concentrations and corresponding flow rates from January 1990 to December 2014.

Contrasting subsurface denitrification characteristics between the Upper Manawatū and Mangatainoka sub-catchments stimulated a comparison of nitrogen leached from agricultural lands and soluble inorganic nitrogen (nitrate-N+nitrite-N+ammonium-N) measured in the surface waters near the river outlet of the two sub-catchments. Table 3.5 presents the estimates of average N leaching rate (kg N ha^{-1} yr^{-1}) and the average river N load (kg N ha^{-1} yr^{-1}) for the Upper Manawatū and Mangatainoka sub-catchments. Comparable average

leaching rates (17 and 19 kg N ha^{-1} yr^{-1}) were found for the sub-catchments despite the different extent of land use types. However, the estimated average river N loads (computed from soluble inorganic nitrogen concentration and flow rate at the specified sub-catchment outlets) were significantly different. Higher river N load was found in the Mangatainoka sub-catchment (13 kg N ha^{-1} yr^{-1}**)** than in the Upper Manawatū (6 kg N ha^{-1} yr^{-1}). It has to be noted that this assessment did not consider travel time of water from farms to rivers and additional investigations are needed to determine whether denitrification is occurring in the soil, groundwater, riparian, and hyporheic zones. Nevertheless, the results presented here provide supporting evidence of the effects of contrasting subsurface denitrification characteristics on surface water quality.

3.4 Conclusions

The denitrification potential varies in the subsurface environment (below the root zone) in the Tararua Groundwater Management Zone (GWMZ) of the Manawatū River catchment. Oxic groundwater conditions with low denitrification potential were observed in the southern part (Mangatainoka sub-catchment) of the Tararua GWMZ, whereas anoxic/reduced groundwater with high potential to denitrify was found in some parts of the middle and northern areas of the catchment. An analysis of different hydrogeological factors indicates that soil texture and aquifer materials that allow faster movement of percolating water or groundwater (well-drained soil, gravel rock type) lead to enriched dissolved oxygen concentrations in groundwater. This creates an oxidised groundwater environment unsuitable for subsurface denitrification. Further analysis is required to determine how the subsurface environment influences the presence of electron donors such as DOC and Fe^{2+} in groundwater. There were

no significant differences in Fe^{2+} concentrations among different soil texture or rock types. On the other hand, higher DOC concentrations were found in aquifers with alluvium material.

Land use could influence the load of NO_3^- entering the subsurface environment: there is likely to be more NO_3^- in groundwater under dairy than under sheep and beef farms. However, NO_3^- concentrations in groundwater are further influenced by the redox processes occurring in the subsurface environment, as shown by differences found in the measured NO_3^- concentrations in different areas under the same land use such as dairy. Critical areas of concern are particularly those with well-drained soil and aquifer with fast moving water underlying land with high nitrogen loading rates. Thus, NO_3^- management interventions need to be prioritised in vulnerable areas being underlain by well-drained soil and aquifers with fast moving groundwater where NO_3^- loadings are high. At the sub-catchment scale, the significance of variable redox processes in the subsurface environment was evident in the significantly different nitrogen loads to the river from the two sub-catchments despite the similar nitrogen leaching rates. Further investigation and characterisation of the capabilities (and variabilities) of the subsurface is suggested, particularly the groundwater system, to reduce or 'attenuate' NO_3^- leached from agricultural land use. This will inform targeted measures to manage and mitigate the impacts of agricultural activities on groundwater and surface water quality and freshwater ecosystems.

CHAPTER 4

A NOVEL ACETYLENE INHIBITION INCUBATION TECHNIQUE FOR THE ACCURATE QUANTIFICATION OF DENITRIFYING ENZYME ACTIVITY IN SUBSURFACE SOILS

Abstract

Quantitative information on denitrification in the subsurface environment is essential in the design of appropriate agricultural nitrate management measures to mitigate the contamination of freshwater ecosystems. The acetylene inhibition (AI) method has been widely used for quantifying the denitrifying enzyme activity (DEA) in soils, which is the potential of the soil to reduce nitrate to gaseous forms of nitrogen. However, the application of an AI method varies greatly in methodological details, and the existing techniques developed and standardised with surface soils (<0.3 m depth) may not be suitable for low DEA activity subsoils. The applicability of a vacuum pouch technique in DEA assays was assessed in comparison with the well-established technique with the Erlenmeyer flasks. Also, different combinations of substrate amounts (nitrate-N and glucose-C) were assessed to determine the optimum concentrations for DEA assays, particularly for subsurface soils (>0.3 m depth). A total of 264 DEA assays were conducted for fresh soil samples collected from 0 to 2 m below ground level (bgl) for two soil types (Manawatū fine sandy loam and Rangitikei silt loam) at the Massey University Experimental Farm No. 1, Palmerston North, New Zealand. Study results suggest that the use of either the vacuum pouch or flask is appropriate for DEA measurements in surface soils (0 to 0.3 m bgl), but the vacuum pouch technique appears more accurate and practical for subsurface soils (0.3 to 2.0 m bgl). The vacuum pouch technique gave a better representation of the headspace composition resulting in lesser variations and more certainty in the DEA values measured for subsurface soils. A combination of 75 µg N

and 400 µg C g^{-1} dry soil was also found to provide the optimum DEA in subsurface soils, as compared to the recommended 50 µg N and 300 µg C g^{-1} dry surface soils. Further assessment and evaluation of the vacuum pouch technique is recommended with other soil types as a practical alternative technique for DEA measurements in subsurface soils.

Keywords: Agriculture; Water Quality; Nitrate Attenuation; Denitrification Assays; Nutrient Cycling; Manawatū, New Zealand

4.1 Introduction

Expansion and intensification of agriculture to meet the growing demand for food is leading to greater inputs of fertilisers (nitrogen (N) and phosphorus (P)) to agricultural soils worldwide. The global consumption of N fertiliser has increased from almost nil in 1940s (Di and Cameron, 2002) to approximately 111.4 x 10^9 kg N in 2013 (Food and Agriculture Organization of the United Nations (FAO), 2015). The nutrients applied, if not managed properly, may leak from agricultural soils and contaminate receiving surface water and groundwater bodies (Di and Cameron, 2002). The impact of excessive N leached mostly in the form of nitrate (NO_3^-) from agricultural soils, however, strongly depends on its flow pathways and potential attenuation or reduction of NO_3^- in the subsurface environment (Haag and Kaupenjohann, 2001; Orr, 2014; Stenger et al., 2008). Subsurface denitrification, a microbially mediated transformation of NO_3^- to gaseous forms of N, such as nitrous oxide (N_2O) and dinitrogen (N_2), attenuates NO_3^- leaching to groundwater and surface waters (Castle et al., 1998; Knowles, 1982). Quantification of denitrification in the subsurface soil or deeper vadose zone is essential to better understand the capability of the subsurface environment to reduce NO_3^- before it contaminates receiving groundwaters and surface

waters. However, there have been limited studies quantifying denitrification in the subsurface soils compared with surface soil investigations (Castle et al., 1998; Elmi et al., 2005; Jahangir et al., 2012c; Luo et al., 1998). The observed variability in the denitrification characteristics of subsurface soils (Jarvis and Hatch, 1994; Luo et al., 1998; Sotomayor and Rice, 1996) and the recent findings showing that substantial amount of N may accumulate in the subsurface soils, such as in the Mississippi River Basin (van Meter et al., 2016), underline the importance of subsurface soil investigations. Information on the capability of the subsurface environment to remove NO_3^- via denitrification could help to identify the most critical areas for site-specific and targeted management and mitigation measures to reduce NO_3^- contamination of freshwater bodies in agricultural landscapes (Singh et al., 2017). Quantitative information on subsurface denitrification would also assist in the design and evaluation of different *in situ* remediation measures (Schroth and Istok, 2006).

Groffman et al. (2006) provided a comprehensive summary of methods for measuring denitrification in the terrestrial environment. These include mass balance approaches, *in situ* gradient of environmental tracers, acetylene inhibition method, stable isotope or ^{15}N tracer methods, direct N_2 quantification, stoichiometric approaches, and molecular approaches. The acetylene inhibition (AI) method has been one of the most commonly used methods to quantify denitrification in terrestrial environments (Luo et al., 1996; Yoshinari et al., 1977; Yu et al., 2012). This method uses acetylene in denitrification assays to inhibit the reduction of N_2O to N_2 gas (Yoshinari et al., 1977), enabling an easy and cost-effective measurement of N_2O as the end product of the denitrification process. The AI method has several advantages compared to stable isotope-based methods (Tiedje et al., 1989); e.g. the use of a natural NO_3^- substrate, relatively low cost, ability to analyse large number of samples, and its versatility that allows laboratory, field and remote site investigations. However, there are several

concerns raised with the AI method, such as the possibility of enhanced denitrification with acetylene as the carbon source particularly if carbon is limited in the denitrification assay (Tiedje et al., 1989; Yeomans and Beauchamp, 1982), incomplete diffusion of acetylene into the soil sample (Felber et al., 2012; Groffman et al., 1999), and incomplete inhibition of N_2O reduction (Groffman et al., 1999; Knowles, 1982; Qin et al., 2012), although other studies observed complete inhibition of N_2O reduction (Well et al., 2001). On the other hand, the simplicity of the AI method still makes it a popular method considering the complexity and cost involved in the more sophisticated methods such as the stable isotope-based methods (Groffman et al., 1999; Well and Myrold, 2002). It has been reasonably accurate in the measurement of denitrification rates as several studies observed no significant difference in the rates measured between the AI and ^{15}N tracer methods (Mosier et al., 1986; Parkin et al., 1985; Raymond et al., 1992). Moreover, the temporal and spatial variability of isotopic signatures and measurement uncertainties, particularly in low NO_3^- natural environments, poses challenges in quantifying denitrification rates with stable isotope-based methods (Clague et al., 2015a). The continued use of the AI method in recent denitrification studies supports its popularity and usefulness (Bellini et al., 2013; Hayakawa et al., 2012; Jha et al., 2017; Yu et al., 2012, 2008).

The most commonly used incubation technique in denitrification assays is the use of flasks, such as Erlenmeyer flasks (Jha et al., 2017; Luo et al., 1996; Vilain et al., 2012) or Wheaton bottles (Marton et al., 2012; Mcphillips et al., 2015). However, in earlier denitrification assays with subsurface soils (>0.3 m depth), challenges were encountered in obtaining significant concentration of denitrification products (N_2O) from assays with 125 mL Erlenmeyer flasks (Rivas et al., 2013). It was hypothesised that this was due to the low amount of soil used in the flask and the small headspace that gas samples could be taken

from. This is important considering that much lower biological activity may be expected in subsurface soils compared to surface soils (Taylor et al., 2002). Thus, in this study the applicability of another incubation technique, particularly the use of vacuum pouches was assessed to address the abovementioned constraints with the Erlenmeyer flasks. While the use of Erlenmeyer flasks or Wheaton (serum) bottles is very common, gas-tight plastic bags or vacuum pouches have also been used in incubations for denitrification studies (Bishop et al., 2014; Klemedtsson et al., 1987; Twining et al., 2007). With vacuum pouches, a large amount of soil (40 g) could be used and larger gas sample volumes (25 mL) taken from incubations to obtain N_2O concentrations much higher than the detection limit of the Gas Chromatograph (see also Bishop et al., 2014). On the other hand, there are some constraints to use larger amount of soil and gas samples in flask incubations, such as the limited flask volume, a negative pressure generated inside the flasks when extracting a larger gas sample, and increased internal pressure in the flasks during the denitrification process which could lead to erroneous calculations of concentrations as it is assumed that the internal pressure is at atmospheric pressure (Bishop et al., 2014). It remains to be seen if similar accuracy could be obtained by using bigger flasks in assays, which may enable the use of larger amount of soil samples and extraction of larger gas volumes. Smaller glass vials may also be used to store smaller undiluted gas sample volumes taken from flasks incubations, but likely inability to repeat sample analysis and subsequently the loss of data if for some reason there were some issues with gas analysis. Nevertheless, the use of vacuum pouches in incubations has other advantages over Erlenmeyer flasks and Wheaton bottles, including: simpler steps with fewer syringes and needles, and much larger number of samples can be processed at once with the rotary shaker.

Moreover, there have been observed differences in procedural details on how the AI method was applied to quantify denitrification in agricultural soils. Denitrification studies using the AI method varied significantly in their methodological details (Hayakawa et al., 2012; Jarvis and Hatch, 1994; Oehler et al., 2007). The main methodological differences include variations in the used amounts of substrates as sources of NO_3^- and electron donor (such as DOC) (Jarvis and Hatch, 1994; Luo et al., 1996; Murray et al., 2004; Paramasivam et al., 1999; Sotomayor and Rice, 1996; Yeomans and Beauchamp, 1982), pre-incubation of samples (Barkle et al., 2007; Drury et al., 2008), acetylene concentration (Jarvis and Hatch, 1994; Knowles, 1982; Ryden et al., 1987; Singh et al., 1989; Sotomayor and Rice, 1996; Well and Myrold, 2002), and amount of oxygen in the headspace (Jahangir et al., 2012c; Luo et al., 1998; Vilain et al., 2012; Well and Myrold, 2002), amongst others. While some studies, e.g. Luo et al., (1996) have standardised the amounts of substrates as sources of NO_3^- and electron donor for New Zealand surface soils (0 – 0.1 m), these may not be applicable for subsurface soils (>0.3 m soil depth) due to the lower biological activity. There are limited studies which have assessed effects of the AI method's procedural differences on estimates of denitrification rates, particularly in subsurface soils (>0.3 m depth) (e.g., Murray et al., 2004).

This study aimed at assessing and standardising the AI technique for quantifying denitrification rates in subsurface soils. It focused on assessing the applicability of a new incubation technique and the different concentrations of NO_3^- and DOC on measurements of DEA in topsoil (<0.3 m bgl) and subsurface soil (>0.3 m bgl). Specifically, the study aimed to (i) evaluate the applicability and performance of the vacuum pouch technique for DEA measurements of subsurface soils; and (ii) determine optimum NO_3^- and DOC concentrations for DEA measurements in the composite subsoil samples. The results of these assessments are intended to contribute to a more standardised AI technique for the measurement of

denitrification in subsurface soils (>0.3 m depth) for improved accuracy in assessing the capability of the vadose zone to reduce NO_3^- before it contaminates receiving groundwaters and surface waters.

4.2 Materials and Methods

4.2.1 Soil sample collection

Soil samples were collected from 0 to approximately 2 m below ground level (bgl) at two locations with different soil types (Manawatū fine sandy loam [Weathered Fluvial Recent Soil] and Rangitikei silt loam [Typic Fluvial Recent Soil]**)** in an experimental study site at Massey University No. 1 Dairy Farm in Palmerston North (New Zealand). Table 4.1 summarizes the physical and chemical characteristics of the soil profiles sampled. The soil depth classification was based on the observed soil layers or horizons (Boone et al., 1999; Crepin & Johnson, 1993) to account for changes in denitrification with depth. The larger depth for lower soil layers was based on the decreasing difference in soil horizons and denitrification characteristics (Boone et al., 1999; Petersen & Calvin, 1986). According to Massey University's atlas of soil types (http://atlas.massey.ac.nz/soils), Manawatū fine sandy loam soils are productive soils with medium texture and moderately well drained, whereas the Rangitikei silt loam soils are highly productive soils underlain by sand and are well to excessively drained (Killick, 2013).

Composite samples for each soil type were collected by percussion corer (6.7 cm inside diameter, 5-10 cm sections) from at least three bores (Jarvis and Hatch, 1994; Kamewada, 2007; Sotomayor and Rice, 1996). Soil samples from the location with the Rangitikei silt loam were collected on 27 June 2013 from 3 holes which were less than 2 m apart. At this

location, the soil samples were collected up to 2 m bgl, and four soil layers were identified based on the soil colour and texture: 0-0.3 m, 0.3-0.6 m, 0.6-1.0 m, and 1.0-2.0 m (Table 4.1). Soil samples from the location with the Manawatū fine sandy loam soil type were collected on 14 November 2014 from 6 holes in a triangular location of not more than 3 m apart. At this location, the soil samples were collected up to 2.1 m bgl, and four soil layers were identified: 0-0.3 m, 0.3-0.6 m, 0.6-1.2 m, and 1.2-2.1 m (Table 4.1). The collected soil samples from each soil layer were homogenised in a basin, sieved while still moist with a 2 mm screen and stored at 4°C until analysis (Drury et al., 2008; Sotomayor and Rice, 1996; Yeomans and Beauchamp, 1982). High soil water contents made sieving impossible, and so soil samples were air-dried for 24 hours at room temperature (Cannavo et al., 2004; Luo et al., 1996). In DEA assays, to which C sources were added (such as glucose), air-drying of soils up to 4 days was not found to significantly affect denitrification compared to fresh soils (Luo et al., 1996).

While the Rangitikei silt loam soil samples were collected in June 2013, the denitrification assays with flasks were conducted in July 2013 whereas assays with vacuum pouches were conducted in October 2013, a delay of nearly four months due to some constraints with the method development. Luo et al. (1996) found a significant decrease of 26-33% in DEA when incubation is conducted two to eight weeks after sampling compared to analysis within 5 days. While DEA values between the two sites could not be directly compared due to this reason, comparison of DEA values within site is still valid as the analyses were conducted either simultaneously (for same depth) or within two weeks (between soil layers), particularly for the Manawatū fine sandy loam soil samples. These soil samples were collected in November 2014 and DEA analysis were conducted in December 2014 for the four different soil layers.

Table 4.1 Physical and chemical properties of soils collected at Massey Dairy No. 1 farm, Palmerston North, New Zealand.

Soil depth, m bgl	Soil pH	Soil texture				Bulk density[2], g cm^{-3}	Porosity[2]	Nitrate-N (mean±std dev) µg g^{-1}	DOC (mean±std dev) µg g^{-1}
		% Clay	% Silt	% Sand	Texture class[1]				
Manawatū fine sandy loam (collected on 14 November 2014)									
0-0.3	5.62	2.61	66.56	30.83	Silt loam	1.30	0.42	7.01±0.32	31.36±2.71
0.3-0.6	5.74	0.63	79.24	20.14	Silt loam	1.38	0.42	1.36±0.09	33.92±3.20
0.6-1.2	6.34	0.07	30.25	69.68	Sandy loam	-	-	0.42±0.04	20.99±2.92
1.2-2.1	6.51	0	16.23	83.77	Loamy fine sand	-	-	0.66±0.03	17.88±2.10
Rangitikei silt loam (collected on 27 June 2013)									
0-0.3	5.25	1.88	84.44	13.68	Silt	1.26	52.84	10.70±0.79	18.90±1.62
0.3-0.6	5.80	3.56	90.25	6.19	Silt	1.24	56.50	4.92±0.20	14.06±1.68
0.6-1.0	6.17	1.41	96.47	2.12	Silt	-	-	4.24±0.13	19.60±0.29
1.0-2.0	6.25	1.13	93.89	4.98	Silt	-	-	3.56±0.19	26.40±3.41

Notes: 1) Soil texture classes based on particle size analysis of soil samples and USDA NRCS soil texture calculator based on the soil textural triangle (https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/?cid=nrcs142p2_054167), 2) bulk density and porosity values from Espanto (2015) for the Manawatū fine sandy loam soil and Killick (2013) for the Rangitikei silt loam soil for samples collected in the same paddock.

4.2.2 Denitrifying enzyme activity (DEA) measurements

In this study, two different incubation techniques were applied and the effects of different concentrations of NO_3^- and DOC on measurements of DEA were assessed in surface (<0.3 m bgl) and subsurface soils (>0.3 m bgl) in the collected soil samples. DEA measures "the maximum activity of the biomass of denitrifying enzymes present in soil at the time of sampling" with "all limiting factors of denitrification removed (such as oxygen (O_2), nitrate, and carbon)" (Groffman et al., 1999). As such, all requirements for the enzyme activity need to be optimised; accomplished by providing anaerobic condition (by saturation and removal of oxygen in the headspace), facilitating diffusion of substrates and gases (by agitating the sample), and no limitations on the availability of NO_3^- and electron donors such as DOC (Tiedje et al., 1989). Given that only the activity of the present enzyme is to be measured, DEA is generally measured over a short duration and it also involves the application of chloramphenicol to inhibit the enzyme growth (Groffman et al., 1999).

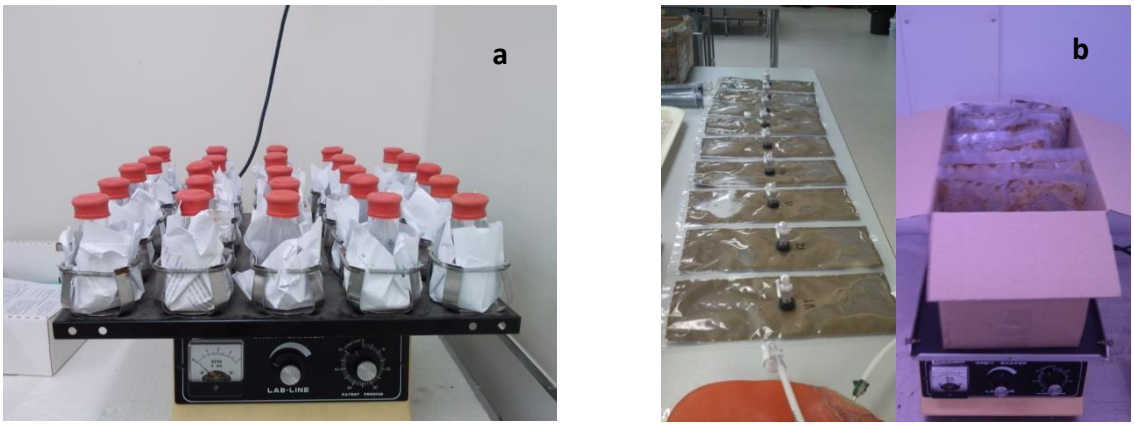


Figure 4.1 Laboratory incubation techniques used to measure denitrification enzyme activity (DEA): (a) Erlenmeyer flask, and (b) Vacuum pouches.

Two incubation techniques (the Erlenmeyer flasks and vacuum pouches; Figure 4.1) and different amounts of substrate in DEA solution were used to determine the optimum

concentration of NO_3^--N and organic C for the DEA assays, i.e. the concentrations needed to obtain the highest DEA per unit amount of soil. The following concentrations of NO_3^--N and organic C (in the form of glucose-C, a commonly used source of organic C in denitrification assays, Bowman and Focht, 1974; Luo et al., 1996; Srinandan et al., 2012) were evaluated based on several studies: 50, 75, and 100 µg nitrate-N g^{-1} dry soil (based on different concentrations used by Barkle et al., 2007; Luo et al., 1996; Murray et al., 2004; Paramasivam et al., 1999; Singh et al., 1989; Yeomans and Beauchamp, 1982); and 200, 300, and 400 µg glucose-C g^{-1} dry soil (based on different concentrations used by Jarvis and Hatch, 1994; Luo et al., 1996; Murray et al., 2004; Paramasivam et al., 1999; Sotomayor and Rice, 1996; Yeomans and Beauchamp, 1982). The DEA solution was prepared with deionised water by adding the required amount of KNO_3^- and glucose both of analytical grade as sources of NO_3^--N and organic C, respectively for the different treatments. Moreover, chloramphenicol was added into the DEA solution with a resulting concentration of 10 ppm to inhibit microbial growth and de novo synthesis of new enzymes (Jha et al., 2012, 2011; Kamewada, 2007; Paramasivam et al., 1999). Some researchers noted the possible inhibition effects of chloramphenicol which could result to underestimation of DEA and recommended to keep the duration of incubation short (5 h) without adding chloramphenicol (Drury et al., 2008). However, the onset of enzyme synthesis and growth had been found to be much shorter, within 2-3 hours of incubation (Smith and Tiedje, 1979; Tiedje et al., 1989). Moreover, Drury et al., (2008) referred to an investigation by Pell et al., (1996) which used 20 ppm chloramphenicol as the lowest concentration. Jha et al., (2011) also found lower rate with 20 ppm chloramphenicol added but no significant difference in denitrification rates between treatments with ≤10 ppm chloramphenicol. Hence, the use of chloramphenicol for DEA measurements seems warranted at 10 ppm concentration.

4.2.2.1 DEA measurements using Erlenmeyer flasks incubation technique

Erlenmeyer flasks are commonly used as an incubation technique for DEA measurements in soils (Kamewada, 2007; Luo et al., 1996). Erlenmeyer flasks (125 mL) were also used to measure DEA of fresh Rangitikei silt loam soil samples in five replicates (10 g dry equivalent, sieved <2 mm) (Fig. 4.1a). The samples were placed with 10 mL of DEA solution into the flasks fitted with suba-seals (Kamewada, 2007; Luo et al., 1996). In case of the Manawatū fine sandy loam, soil samples in three replicates (20 g dry equivalent, sieved <2 mm) were placed with 20 mL of DEA solution into the flasks. While the amounts of soil sample and DEA solution were different for the two tests, the proportions between the soil amount and the DEA solution was maintained at 1:1 by weight, assuming the solution has a similar weight of water with only very small amount of solute added. The resulting headspace volumes in the flasks were approximately 125 mL for the Rangitikei silt loam soil assays and a headspace: soil ratio of 12.5 mL per g. For the Manawatū fine sandy loam soil assays the headspace volumes were 111-115 mL, which varied depending on the soil moisture content, and an average headspace: soil ratio of 5.7 mL per g.

Anaerobic conditions were created by removing air from the flasks and flushing them with N_2 three times with syringe and hypodermic needles on the suba-seals (Jha et al., 2012). Then an equivalent of 10% of the headspace of N_2 was removed and replaced with purified acetylene to prevent the conversion of N_2O to N_2 during the denitrification process (Beauchamp and Bergstrom, 1993; Drury et al., 2008; Luo et al., 1998). To remove impurities in the acetylene like acetone, acetylene was first purified by passing through two H_2SO_4 (sulphuric acid) traps (Paramasivam et al., 1999) followed by another trap of distilled water (Castle et al., 1998; Mosier and Klemedtsson, 1994). The Erlenmeyer flasks were incubated in the dark at 20°C on a rotary shaker at 160 rpm for six hours to ensure access of microorganisms to substrate

given that most microorganisms and denitrifiers are attached to soil particles (Reddy et al., 1978; Smith and Duff, 1988). Gas samples (5 mL) were collected at 0, 2, 4, and 6 hrs from the start of incubation using a 10 mL syringe. Extracting larger amount of samples (e.g., 10 mL) was previously observed to result in negative pressure in the flask as indicated by the retraction of the syringe handle when released. The gas samples were combined with 20 mL of N_2 and placed in 12 mL pre-evacuated glass vials (Labco Exetainers). The space left by the sample taken from the Erlenmeyer flasks was replaced by 5 mL of a mix of acetylene and N_2 (with the same proportion as the headspace) prepared in a rubber bladder. This was based on a preliminary study that measured significantly lower DEA rates when replacement gas was composed of N_2 only than with a mixture of acetylene and N_2, except when the initial acetylene amount in the headspace was much higher than 10% (Rivas et al., 2013).

4.2.2.2 DEA measurements using vacuum pouches incubation technique

A second set of DEA measurements was carried out using vacuum pouches (100x285mm, gauge 70 µm Cas-Pak). The collected soil samples in three replicates (40 g dry equivalent, sieved <2 mm) were placed into the vacuum pouches fitted in the middle with a luer-lock valve (secured by a rubber fitting sealed with F2 contact adhesive) (Fig. 4.1b), as an entry port for the addition of headspace gases and DEA solution.

The vacuum pouches were sealed and air was removed by a syringe and flushed with N_2 once. Given the flexible nature of the pouch, there was no need to do this multiple times like in the assays with flasks to achieve satisfactory removal of oxygen and to keep the assays anaerobic; thus resulting in fewer steps in conducting the experiments. The solution-headspace ratio in the flask was replicated in the vacuum pouches, particularly for the assays with the Manawatū fine sandy loam soil, and corresponding amount of purified acetylene

(C_2H_2) (10% of headspace; Beauchamp and Bergstrom, 1993; Groffman et al., 1999) was added via a syringe. This replication of headspace: solution ratio was not specifically done for the Rangitikei silt loam soil assays, which were done much earlier. The resulting headspace volume in the vacuum pouch was 180 mL for the Rangitikei silt loam soil assays (headspace: soil ratio of 4.5 mL per g) and 222-231 mL for the Manawatū fine sandy loam soil assays, also varied depending on the soil moisture content (average headspace: soil ratio of 5.7 mL per g). Thereafter, 40 mL of DEA solution was added from a dispenser to the pouch via plastic tubing (maintaining 1:1 ratio by weight between soil sample and solution) and then an appropriate amount of N_2 was added via syringe to complete the headspace. It has to be noted that for the Manawatū fine sandy loam soils the flask and vacuum pouch assays had a similar headspace: soil ratio at 5.7 mL per g.

Together with the assay in flasks, the vacuum pouch samples were placed upright on a cardboard box and incubated in the dark at 20 °C on a rotary shaker at 160 rpm to ensure good mixing of soil samples and DEA solution. Bishop et al. (2014) found discrepancies in N_2O production depending on the vacuum pouch orientation during shaking (horizontal or vertical), but the comparable results in assays between flasks and vacuum pouches in vertical position for surface soils (Table 4.2 and 4.3) strongly support the correctness of the orientation adopted. Similar to the flasks, gas samples of larger volumes (25 mL) were collected from the pouches at 0, 2, 4 and 6 hours from the start of the incubation and placed in pre-evacuated 12 mL glass vials (Labco Exetainers). There was no need to replace the amount of gas taken from the assays given that the pouches are flexible and atmospheric pressure is maintained in the assays. Again as a consequence, there were fewer steps in the experiments compared to the assay with flasks. With larger gas sample volumes taken from the incubations, a better representation of the headspace composition was expected and

subsequently more accurate measurement of N_2O produced in the assays compared with the incubations with flasks especially with subsurface soils. The use of short duration incubation (e.g., Luo et al., 1996) is justified by the more rapid denitrification with the use of glucose as source/type of organic carbon (Bowman and Focht, 1974).

4.2.3 Analytical methods and quantification of DEA

The nitrate-N in soil water extracts from the collected soil samples were analysed by continuous flow analysis (Technicon® AutoAnalyzer II). The DOC in the soil water extracts was determined generally following the potassium dichromate wet oxidation and titration (method 5220B) (Rice et al., 2012). Soil extraction for the two soil types involved an additional hot water extraction step (Ghani et al., 2003) conducted for the Rangitikei silt loam soil samples, whereas this step was not done for the Manawatū fine sandy loam as was later deemed not necessary and given the large amount of DOC added in DEA assays. Thus, the DOC values for the two soil types could not be directly compared. Nevertheless, the DOC values obtained still provide useful information on background DOC in soils and for comparing soils at different depths within a certain soil type. The collected gas samples during the denitrification incubations were analysed for N_2O with a Shimadzu Gas Chromatograph (GC) 17 A (Japan) which has a ^{63}Ni-electron capture detector, with detection limit of 0.05 ppm N_2O. The GC readings (analysis conducted at Landcare Research, Palmerston North) of 500 ppb standards varied by up to 62 ppb (i.e. from 413 ppb to 475 ppb).

In quantifying the DEA, the amount of N_2O generated in each assay was computed from the gas samples collected from the headspace at 0, 2, 4, and 6 hours of the incubation. First, the

total amount of N_2O (V_{N2O}) generated in each assay was computed as follows to include the amount of N_2O dissolved in the solution in addition to the amount in the headspace (Groffman et al., 1999; Hill et al., 2000; Luo et al., 1996):

$$V_{N2O}(\mu L) = C_g \left(V_g + [V_l * \beta]\right) * \frac{1}{1000} \quad \text{Eq. 1}$$

where; C_g is the concentration (volumetric) of N_2O in the gas phase (µL N_2O/L); V_g is the volume of the gas phase, i.e. the headspace volume (mL); V_l is the volume of liquid phase, i.e. the solution volume (mL); and β is the Bunsen absorption coefficient (0.632 at 20 °C; Groffman et al., 1999; Mosier and Klemedtsson, 1994; Tiedje, 1982).

The equivalent mass of N_2O (M_{N2O}) was then computed using the ideal gas law with gas density of N_2O (ρ_{N2O}) at 20 °C being $1.83*10^{-6}$ g N_2O/µL (Luo et al., 1996; Jha et al., 2011). The equivalent mass of N_2O-N ($M_{N2O\text{-}N}$) was then computed and the mass of N_2O-N was then converted into the amount of N_2O-N per kg of dry soil in the assay.

For a more accurate determination of DEA in the Erlenmeyer flasks, it is important to note that some N_2O has been removed from the headspace after every gas sampling. In order to account for all the N_2O-N produced in each assay, the cumulative N_2O mass produced during the incubation was computed by calculating the corrected N_2O mass produced considering the constant volume of incubation assay, as follows:

$$M_T(n) = M_A(n) + \sum_1^{n-1} M_S\,(n) \quad \text{Eq. 2}$$

Where, $M_T(n)$ is the corrected total N_2O mass at time t_n (µg); $M_A(n)$ is the computed actual N_2O mass at time t_n (µg); $M_S(n)$ is the computed N_2O mass removed in the previous gas samplings t_{n-1} (µg) (Eq. 3 below). Thus, for example, the total N_2O mass at time t_2 is equal to the N_2O mass determined from the gas sampling at time t_2, plus the N_2O mass removed by the previous gas samplings at time t_1. The N_2O mass removed in a gas sampling (M_S) was computed as follows:

$$M_S = (C_g * V_S) * \rho_{N2O} * 1000 \qquad \text{Eq. 3}$$

Where, C_g is the concentration (volumetric) of N_2O in the gas phase (µL N_2O/L); V_S is the volume of the gas sample taken from the incubation flask (mL); ρ_{N2O} is the gas density of N_2O as above.

The corrected cumulative mass of N_2O (M_{N2O}) was converted to the equivalent mass of N_2O-N (M_{N2O-N}) and the DEA was then determined from the plot of cumulative N_2O-N mass (M_{N2O-N}) accumulated up to the sampling time. It was observed that the amount of N_2O-N at the last sampling (6^{th} hour) determined with corrected N_2O-N mass was 5-12% higher than with the actual (uncorrected) N_2O-N mass measured.

Statistical analysis

The measured DEA values were further subjected to statistical analysis to compare the results of the two incubation techniques (the flasks and vacuum pouches) and evaluate the effects of different substrate treatments on DEA values in the composite surface and subsurface soil samples. The accuracy of the results of the vacuum pouch technique was validated by

comparing with the results of the flask technique for surface soil samples, considering that incubation with flasks has been well established for surface soils. The DEA values were log transformed to obtain a normal distribution before conducting statistical analysis (*t*-test and ANOVA) using the SAS software.

4.3 Results and Discussion

4.3.1 Comparison of DEA measured with different incubation techniques

Tables 4.2 and 4.3 show the mean and standard deviation of DEA values measured for different soil depths of the Manawatū fine sandy loam (Table 4.2) and the Rangitikei silt loam soil sites (Table 4.3), respectively. The measured DEA values were analysed for comparing the different incubation techniques, i.e. the Erlenmeyer flasks and the vacuum pouches, with significant difference (at the $p < 0.05$ level) between the incubation techniques indicated by bold numbers in Tables 4.2 and 4.3.

For the Manawatū fine sandy loam soil (Table 4.2), no significant difference was found in the DEA measurements between the vacuum pouch and the Erlenmeyer flask incubations for the surface soil layer (0-0.3 m bgl), except for the Treatment 4 (75 µg N g^{-1} and 200 µg C g^{-1}) in which DEA in the vacuum pouch incubation was significantly higher for no obvious reasons. There were also no significant differences in the DEA measurements between the vacuum pouch and the Erlenmeyer flask incubations for the Rangitikei silt loam surface soil layer (0-0.3 m bgl) (Table 4.3). The DEA values (14,940 - 18,750 µg N_2O-N kg^{-1} soil d^{-1}) in the surface soil (0-0.3 m bgl) in the Manawatū fine sandy loam site (Table 4.2) were comparable to the DEA values obtained for a similar Manawatū fine sandy loam soil (approx. 19,000 - 20,000 µg N_2O-N kg^{-1} soil d^{-1}) obtained by Luo et al. (1996). The DEA values (660 – 1,002

μg N_2O-N kg^{-1} soil d^{-1}) in the surface soil (0-0.3 m bgl) of the Rangitikei silt loam site (Table 4.3) were in the range of the DEA values (300 - 5,000 μg N_2O-N kg^{-1} soil d^{-1}) measured from soils of similar texture (silt loam and sandy loam soils) and depths (0.1-0.4 m bgl) in other areas in the Manawatū region (Luo et al., 1998). These results indicate the applicability of the vacuum pouch technique to measure DEA of soils, given that DEA measurement using the flasks technique has been well established and standardised for surface soils (Luo et al., 1996). Interestingly, the DEA measurements for the surface soil layer have apparently less variation in case of the vacuum pouch incubations as compared to the flasks incubations based on standard deviations (Tables 4.2 and 4.3) and coefficient of variations (Appendices D.5 and D.6). These results suggest that either the vacuum pouch or flask incubation technique could be used for measuring DEA in surface soil samples (<0.3 m depth), without any significant difference in the results obtained.

However, there were observed significant differences between the two incubation techniques for the measured DEA values in the subsoil (>0.3 m bgl) samples. For the Manawatū fine sandy loam soil samples (Table 4.2), the DEA values for the soil depth 0.3-0.6 m bgl obtained using the vacuum pouch technique were significantly higher (4 to 28 times) than those obtained with the flask technique, particularly for the substrate treatments 1, 6, 7 and 8. Similar results were observed for the 1.0-2.0 m bgl subsoil layer in the Rangitikei silt loam samples where the pouch technique resulted in about 2 to 11 times higher DEA values as compared to the flasks technique (Table 4.3). It has to be also noted, however, that the DEA values from the flask technique with the Rangitikei silt loam subsurface soils may not be reliable due to very low N_2O-N concentrations (20 - 87 ppb only, with most gas samples having N_2O concentrations <50 ppb) and within the detection error of the Gas Chromatograph (GC) (62 ppb). This low N_2O concentration in the gas samples was avoided

with the use of the vacuum pouches with lowest concentrations recorded greater than 300 ppb for the same set of soil samples providing more certainty in the measurements. The DEA values obtained with the vacuum pouches for the subsurface layer in the Rangitikei silt loam site (15.9 - 54.1 µg N_2O-N kg^{-1} dry soil d^{-1}; 1.0 - 2.0 m bgl; Table 4.3) were within the range of DEA values (5 - 100 µg N_2O-N kg^{-1} dry soil d^{-1}) found in subsoils (1.2 - 2.35 m bgl) of the Canterbury plains (Peterson et al., 2013).

In general, Tables 4.2 and 4.3 showed that either of the incubation techniques (the Erlenmeyer flasks or the vacuum pouches) could be used for the measurement of DEA for surface soils (<0.3 m bgl) where N_2O production is expected to be higher. However, the use of vacuum pouch technique appears more accurate and practical for denitrification assays of subsurface (>0.3 m bgl) soil samples wherein N_2O production is expected to be lower. The larger gas sample volumes obtained from incubations using vacuum pouches gave a better representation of the headspace composition resulting into lower variations and more certainty in the DEA values obtained by the vacuum pouch incubations (Tables 4.2 and 4.3; Appendices D.5 and D.6).

While this study had tested the vacuum pouch technique in comparison with the flasks with only two soil types, the technique appears robust given the consistency in terms of similar results obtained compared to using flasks for DEA in surface soils, in which results using the flask technique have been widely accepted. Nevertheless, further investigations with other soil types would help establish the vacuum pouch technique as a reliable and practical technique for DEA measurements in subsurface soils.

4.3.2 Effect of different substrate amounts on DEA measurements

The measured DEA values for both, the Manawatū fine sandy loam and Rangitikei silt loam soil layers, were further analysed for assessing effects of different substrate treatments to determine the optimum concentration of nitrate-N and organic C for the DEA assays in subsurface soils (Tables 4.2 and 4.3).

4.3.2.1 Optimum nitrate-N concentration

The variation of nitrate-N from 50, 75, and 100 µg nitrate-N g^{-1} dry soil slightly affected the DEA values in surface and subsurface soils (Tables 4.2 and 4.3). As shown in Table 4.2, for the Manawatū fine sandy loam soil samples, the DEA values were measured slightly lower for most of the denitrification assays conducted with added 100 µg N g^{-1} dry soil. This agrees with other studies that higher N (e.g. > 100 N µg N g^{-1} dry soil) added in incubation may inhibit denitrification (Luo et al., 1996). This inhibiting effect of 100 µg N g^{-1} dry soil was also observed in the subsurface samples (1.0-2.0 m depth) of the Rangitikei silt loam soil samples, but not clearly observed in the surface samples (<0.3 m depth) (Table 4.3). The use of 100 µg N g^{-1} dry soil or higher is therefore not recommended for obtaining highest possible DEA for subsoil samples (>0.3 m depth) that may even have lower background nitrate-N concentrations (Table 4.1).

Table 4.2 Denitrifying enzyme activity (DEA) values (μg N_2O-N kg^{-1} dry soil day^{-1}, mean±stdev) for the Manawatū sandy loam soil (sampled in November 2014) at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand.

Soil depth	Incubation technique	Substrate (N and C concentrations) treatments								
		50 μg N g^{-1}			75 μg N g^{-1}			100 μg N g^{-1}		
		200 μg C g^{-1}	300 μg C g^{-1}	400 μg C g^{-1}	200 μg C g^{-1}	300 μg C g^{-1}	400 μg C g^{-1}	200 μg C g^{-1}	300 μg C g^{-1}	400 μg C g^{-1}
		1	2	3	4	5	6	7	8	9
0-0.3 m	Vacuum Pouch	18029.4^{abc}	18428.8^{ab}	18685.4^{a}	**18748.8^{a}**	18213.8^{abc}	18425.2^{ab}	17202.4^{bc}	16978.3^{c}	16955.7^{c}
		±529.8	±389.8	±848.4	**±309.4**	±353.0	±987.6	±1308.4	±881.5	±648.0
	Flask	18593.8^{a}	17059.5^{ab}	18541.9^{a}	**17053.3^{ab}**	17215.1^{ab}	17867.8^{ab}	14936.5^{c}	15694.2^{bc}	16501.3^{abc}
		±1990.9	±1783.3	±343.7	**±967.6**	±1247.0	±1012.7	±1103.1	±932.6	±761.3
0.3-0.6 m	Vacuum Pouch	**43.8^{abc}**	30.1^{cd}	50.6^{ab}	44. 8^{abc}	23.4^{d}	**61.1^{a}**	**35.1^{abcd}**	**28.5^{bdc}**	33.8^{abcd}
		± 3.6	± 26.0	± 5.2	± 11.3	± 11.5	**± 10.3**	**± 10.7**	**± 1.3**	± 16.5
	Flask	**1.5^{b}**	14.4^{a}	16.3^{a}	15.9^{ab}	21.5^{a}	**14.8^{a}**	**8.2^{ab}**	**3.4^{ab}**	19.0^{a}
		± 0.7	± 15.2	± 20.7	± 20.3	± 17.4	**± 17.2**	**± 4.4**	**± 0.6**	± 25.7
0.6-1.2 m	Vacuum Pouch*	**0.4^{bcd}**	**1.1^{abcd}**	2.7^{a}	**1.6^{abc}**	**0.8^{bcd}**	1.7^{abc}	**1.4^{abc}**	**1.1^{abc}**	**0.3^{d}**
			± 0.3	± 1.4		**± 0.4**	± 1.9	**±0.5**	**± 0.4**	**± 0.0**
	Flask**	**5.9**	**4.9**	6.9	**6.1**	**8.2**	6.2	**10.6**	**6.3**	**6.1**
		± 0.8	**± 1.0**	± 2.3	**± 1.3**	**± 0.7**	± 3.4	**±6.8**	**± 1.3**	**± 2.6**
1.2-2.1 m	Vacuum Pouch	**3.5^{abcd}**	5.6^{ab}	6.0^{ab}	**3.5^{abcd}**	**4.0^{abc}**	3.2^{abcd}	3.6^{abcd}	2.0^{d}	**2.2^{cd}**
		± 1.2	± 3.4	± 2.1	**± 0.7**	**± 1.2**	± 0.7	± 1.7	± 1.5	**± 0.8**
	Flask	**9.6^{abc}**	6.0^{cd}	6.8^{bcd}	**14.5^{a}**	**13.3^{ab}**	6.1^{cd}	7.4^{bcd}	5.6^{cd}	**4.8^{d}**
		± 2.3	± 1.1	± 1.1	**± 7.9**	**± 8.0**	± 2.0	± 4.2	± 1.3	**± 0.5**

Note: Significance differences at the $p < 0.05$ level between the incubation techniques (pouch and flask) for specific substrate treatments and soil depths are indicated by **bold numbers**; significant differences at the $p < 0.05$ level across different substrate treatments for each incubation technique and soil depth are indicated by different superscripts. Significant differences were determined by Least Significant Difference (LSD) in SAS.

* Values with missing standard deviation values indicate lack of replicates due to the inability to approximate an acceptable regression line on the N_2O-N amounts accumulated in the replicate assays.

** No significant difference in DEA was found among the treatments.

Table 4.3 Denitrifying enzyme activity (DEA) values (µg N_2O-N kg^{-1} dry soil day^{-1}, mean±stdev) for the Rangitikei silt loam soil (sampled in June 2013) at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand.

Depth	Incubation technique	Treatments		
		50 µg N, 300 µg C	75 µg N, 300 µg C	100 µg N, 300 µg C
		2	5	8
0-0.3 m	Vacuum Pouch	660.77^{b} ± 81.6	809.2^{a} ± 87.8	814.3^{a} ± 17.7
	Flask*	844.1 ± 225.4	990.5 ±397.4	1001.9 ± 308.9
1.0-2.0 m	Vacuum Pouch	**15.9^{b} ± 5.0**	**54.1^{a} ± 15.6**	**18.4^{b} ± 9.2**
	Flask	**7.1^{a} ± 1.5**	**4.7^{b} ± 0.9**	**5.9^{ab} ± 0.6**

Note: Significance differences at the $p < 0.05$ level between the incubation techniques (pouch and flask) are indicated by **bold numbers**; significant differences among treatments is indicated by different superscripts. Significant differences were determined by LSD in SAS.
* No significant difference in DEA was found among the treatments.

With surface soil samples of either the Manawatū fine sandy loam or Rangitikei silt loam soil type, there is no clear evidence of a single N treatment that provided significantly highest DEA values (Tables 4.2 and 4.3). For the Manawatū fine sandy loam soil, the addition of either 50 or 75 µg N g^{-1} dry soil did provide the highest DEA for surface soils (<0.3 m depth) (Table 4.2). These results seem to support the findings of Luo et al. (1996) which showed 50 µg NO_3-N g^{-1} dry soil as giving the optimum DEA values for surface soils (0 to 0.1 m depth). On the other hand, the addition of either 75 or 100 µg N g^{-1} dry soil did provide the highest DEA with the Rangitikei silt loam surface soil samples (Table 4.3). The use of 75 µg N g^{-1} dry soil also provided higher DEA values for most of the subsurface soil layers for both soil types (Tables 4.2 and 4.3). For soil layer 0.3-0.6 m bgl of the Manawatū fine sandy loam, the highest mean DEA value was measured with treatment 6 (i.e. 75 µg N and 400 µg C g^{-1} dry soil) and treatment 5 (i.e. 75 µg N and 300 µg C g^{-1} dry soil) using the vacuum pouches and flasks incubations, respectively (Table 4.2). Further, using one carbon treatment alone with the Rangitikei silt loam soil, significantly highest DEA was observed for subsurface soil layer (1.0-2.0 m bgl) with addition of 75 µg N g^{-1} dry soil in assays with the vacuum pouches (Table 4.3). Hence, the use of 75 µg N g^{-1} dry soil appeared to be the optimum concentration for the DEA assays in both surface and subsurface soils (Tables 4.2 and 4.3).

4.3.2.2 Optimum C concentration

The assessment for the optimum C concentration to measure DEA was done only with the Manawatū fine sandy loam soil assays (Table 4.2). As shown in Table 4.2, different amounts of C (200, 300, 400 µg C g^{-1} dry soil) did not seem to significantly affect the DEA measurements in the Manawatū fine sandy loam surface soil samples (0-0.3 m bgl) for different amounts of N added, regardless of the incubation technique used. However, in at least half of both vacuum pouch and flask analyses, the highest DEA for a specific N amount added was observed when 400 µg C g^{-1} dry soil was added in the assays of surface soils. The DEA measurements further down the soil profile seem to support the use of 400 µg C g^{-1} dry soil as optimum to measure DEA. In most cases with the vacuum pouch technique, which was inferred to be more reliable than the flask technique for subsurface soil assays, the highest mean DEA was observed with the addition of 400 µg C g^{-1} dry soil in soil layers 0.3-0.6 m bgl and 0.6-1.2 m bgl (Table 4.2), particularly in treatments with 50 or 75 µg N g^{-1} dry soil. This suggests that adding more carbon into the incubation (up to 400 µg C g^{-1} dry soil) generally tends to result in higher DEA measurements, with no indications of negative effects, in subsurface soils.

4.3.2.3 Optimum combination of N and C concentrations

The discussions above have suggested the optimum amounts of N or C to be added in denitrification assays measuring DEA (Tables 4.2 and 4.3), but considering the substrates N and C independently may not be appropriate due to the possible combined effect of the two substrates. For instance, adding much higher C in assays may not necessarily result in highest DEA as high glucose concentrations may retard denitrification (Bowman and Focht, 1974). It may be more appropriate to consider the C/N ratio in determining the most appropriate

combination of substrate as the C/N ratio is "a measure of the electron donor to acceptor ratio in biological denitrification, influencing the denitrification rate and accumulation of nitrite and ammonium" (Yang et al., 2012). Several studies assessed the effect of different C/N ratios on denitrification and found that highest the C/N did not always result in the highest denitrification observed (Her and Huang, 1995; Khanitchaidecha, 2010; Mees et al., 2014; Yang et al., 2012). Bowman and Focht (1974) argued that "the C/N ratio necessary to effect maximal denitrification rates will vary depending on the electron donors per mole of carbon substrate". Though the optimum values of 75 µg N and 400 µg C g^{-1} dry soil were identified independently, this is also the combination that consistently provided the highest DEA in the subsurface soils (the vacuum pouch technique), and significantly highest for the subsurface layer 0.3-0.6 m bgl in the Manawatū fine sandy loam soils (Table 4.2). This combination of N and C substrate has a C/N ratio of 5.33, which is within the range of optimum values from 2.5 to 20 found by other studies and particularly close to the C/N ratio of 6 (Mees et al., 2014; Yang et al., 2012).

It has to be noted, though, that the DEA values obtained in this study with the vacuum pouch technique represent the denitrification potential of the soils and not the actual denitrification occurring in the soil. Nevertheless, these values are very useful for comparing denitrification potential among sites and among depths. The vacuum pouch technique also has the potential for use in determining actual denitrification rate, but this remains to be seen and is subject of future work.

4.4 Conclusions

Accurate measures of denitrification in surface and subsurface soils is key to better understand and map the capability of the vadose zone to attenuate leached NO_3^- from agricultural soils. This study assessed the applicability of the commonly used acetylene inhibition (AI) method using two incubation techniques (the Erlenmeyer flasks and the vacuum pouches) and different substrate treatments to determine optimum concentrations of N and C additions to measure denitrification enzyme activity (DEA), particularly for subsurface soils (>0.3 m bgl). A total of 264 DEA assays were conducted for fresh soil samples collected from 0 to 2 m bgl for two soil types (Manawatū fine sandy loam and Rangitikei silt loam) at the Massey University Experimental Farm No. 1, Palmerston North, New Zealand.

Results of the study confirmed the applicability and reliability of incubations conducted in vacuum pouches in measuring DEA in soils, as indicated by the comparable DEA values obtained from the surface soils by the commonly used Erlenmeyer flasks incubations. This study found that the vacuum pouch method provided reliable DEA values for subsurface (>0.3 m bgl) soil samples. The vacuum pouches method allowed use of a large amount of soil (≥40 g) sample and a larger gas sample (25 mL) from the incubations. The larger gas sample volumes gave a better representation of the headspace composition resulting in less variations and more certainty in the DEA values obtained by the vacuum pouch incubations. It also included simpler steps with less use of syringes and needles and allows a larger number of samples to be processed at once with the rotary shaker, making it a reliable and practical method to measure DEA in subsurface soils. Further assessment and evaluation of the

vaccum pouch technique with other soil types is recommended in order to fully evaluate the potential of this method for the measurement of DEA.

It was also found that using the recommended combination of DEA substrate (50 µg N and 300 µg C g^{-1} dry soil; Drury et al., 2008; Luo et al., 1996) may not necessarily result in optimum DEA for subsurface soils. Results of the study showed that a combination of 75 µg N and 400 µg C g^{-1} dry soil generally gave higher DEA values compared to other amounts assessed (50, 75, and 100 µg N g^{-1} dry soil; 200, 300 and 400 µg C g^{-1} dry soil). Using and further testing the vacuum pouches incubations with a combination of 75 µg N and 400 µg C g^{-1} dry soil is suggested to measure DEA of different subsurface soil types (>0.3 m bgl). This will help develop a more standardised measurement of denitrification in subsurface soils to accurately assess the capability of the vadose zone to attenuate or reduce leached NO_3^- from agricultural soils, before it contaminates groundwater and surface waters. This information is needed to better guide the selection of appropriate nitrate management measures to limit water contamination from agricultural fields.

CHAPTER 5

QUANTIFICATION OF DENITRIFICATION RATE IN SHALLOW GROUNDWATER USING THE SINGLE-WELL, PUSH-PULL TEST TECHNIQUE

Abstract

Denitrification has been identified as a significant nitrate attenuation process in groundwater systems. Hence, accurate quantification of denitrification rates is consequently important for the better understanding and assessment of nitrate contamination of groundwater systems. There are, however, few studies that have investigated the differences in denitrification rates estimated using different analytical approaches or assuming different kinetic reaction models. In this study, observations from commonly used single-well, push-pull tests were assessed to quantify denitrification rates in shallow groundwater at two sites in the Manawatū River catchment, Lower North Island of New Zealand using different analytical approaches and kinetic models. Denitrification rates estimated using the measurements of denitrification reactant (nitrate reduction) were 0.42-1.07 mg N L^{-1} h^{-1} and 0.05-0.12 mg N L^{-1} h^{-1} at the Palmerston North (PNR) and Woodville (WDV) sites, respectively, using the most common zero-order kinetic models. Using first-order kinetic models, the denitrification rates were 0.03-0.09 h^{-1} and 0.002-0.012 h^{-1} at the PNR and WDV sites, respectively. These rates are significantly higher (6 to 60 times) than the rates estimated using the measurements of denitrification product (nitrous oxide production). When all nitrogen species could not be measured during a push-pull test so as to determine all possible nitrate reduction processes, the denitrification rate quantified based on the reactant may provide representative value of denitrification characteristics of shallow groundwater. While estimates of denitrification rates

also differed depending on the kinetic model used, either a zero-order or a first-order model may both appear to be valid to analyse and estimate denitrification rate from push-pull tests. This discrepancy in estimates of denitrification rates using either reactant or product and using zero- or first-order kinetics models may have implications in the simulation of nitrate transport and transformation in groundwater systems. This necessitates further research and analysis for appropriate measurements and representation of spatial and temporal variability in denitrification characteristics of the shallow groundwater system.

Keywords: Agriculture, Water Quality, Nitrate Attenuation, Denitrification

5.1 Introduction

Agricultural intensification and expansion has driven the increase in the use of fertiliser to support food production (Di and Cameron, 2002; Food and Agriculture Organization of the United Nations (FAO), 2015). If not used properly, excess nitrogen (N) in agricultural lands could eventually leach and contaminate groundwater and subsequently receiving surface waters resulting in the degradation of water quality such as eutrophication and fish poisoning (Di and Cameron, 2002; Puckett et al., 1999). Groundwater systems, however, do not merely act as a pathway for nitrate (NO_3^-) transport and contamination of surface water, but could also transform NO_3^- via biogeochemical processes such as denitrification attenuating the NO_3^- load to surface waters (Jahangir et al., 2012b; Starr and Gillham, 1993; Stenger et al., 2008). A number of studies have shown significant attenuation of NO_3^- in groundwater systems (e.g., Jahangir et al., 2013; Seitzinger et al., 2006; Singleton et al., 2007; Tesoriero et al., 2000). Jahangir et al. (2013) found that denitrification in groundwater accounted for the 24% of N inputs to the land in a grassland site in southeastern Ireland. Thus, a sound

understanding of the transport and transformation of NO_3^- in groundwater systems is essential to manage and mitigate any negative impacts of agriculturally-derived NO_3^- on the quality of receiving surface water bodies.

Denitrification has been identified as a significant NO_3^- attenuation process in groundwater systems (Jahangir et al., 2013; Starr and Gillham, 1993). Several methods have been developed to assess denitrification processes and estimate *in situ* denitrification rates in shallow groundwater, e.g. single-well, push-pull test (Istok, 2013; Istok et al., 1997), well cluster method (Bragan et al., 1997; Woodward et al., 2009), *in situ* microcosms (Gillham et al., 1990; Mengis et al., 1999; Starr and Gillham, 1993), *in situ* mesocosms (Korom et al., 2012, 2005), and multilevel wells (Korom et al., 2012). The single-well push-pull test, however, is the most commonly used method allowing cost-effective and less time consuming measurement of *in situ* denitrification rates in groundwater (e.g., Addy et al., 2002; Istok, 1997; Jahangir et al., 2013; Sánchez-Pérez et al., 2003; Tesoriero et al., 2000; Well et al., 2003). The applicability of push-pull tests in quantifying *in situ* denitrification rate hinges on assumptions that (a) the injection solution containing the reactant and conservative tracer is well mixed, (b) reactants and tracers have identical retardation factors and transport properties, (c) reaction rates are homogeneous, and (d) that there is negligible regional groundwater flow (Burbery, 2004; Haggerty et al., 1998; Istok, 2013; Snodgrass and Kitanidis, 1998).

The quantification of denitrification rates could also be affected by the choice of an analytical approach and kinetic model to analyse the push-pull tests data. There have been different analytical approach developed to estimate denitrification rates using field data obtained from single-well, push-pull tests (Korom et al., 2012; Sanchez-Perez et al., 2003; Well et al.,

2003). These methods depend on the parameter used in the analysis namely, denitrification reactant, product, or both reactant and product (Istok et al., 1997). The first method using the denitrification reactant, i.e. NO_3^-, is the simplest as the rate can be obtained by analysing changes in concentrations of the reactant (NO_3^-) and the conservative tracer such as bromide (Br) (Istok et al., 1997). The other two methods using the denitrification product such as nitrous oxide (N_2O) or/and dinitrogen (N_2) need additional treatment or the use of stable isotopes to estimate the denitrification rate. For instance, some studies (e.g., Sánchez-Pérez et al., 2003; Well et al., 2003; Woodward et al., 2009) added acetylene to inhibit the reduction of the intermediate product N_2O to N_2, enabling estimation of denitrification rate by analysing concentrations of N_2O measured during the test. Other studies (e.g., Schürmann et al., 2003) used isotopically labelled nitrate ($^{15}NO_3^-$), thus enabling them to determine the amount of N_2O and N_2 derived from the reactant (NO_3^-).

Groundwater denitrification rates can be estimated from decrease in reactants and/or increase in products (Istok et al., 1997). However, denitrification studies usually estimate denitrification rate analysing only one parameter, either the reactant (e.g., Korom et al., 2012; Toda et al., 2002; Trudell et al., 1986) or the product (e.g., Sánchez-Pérez et al., 2003). The use of acetylene or isotope enrichment in push-pull tests enables the estimation of denitrification rate from both the reactant and products of the denitrification process (e.g., Addy et al., 2002; Schürmann et al., 2003). However, there are limited studies that have estimated denitrification rates based on the reactant and product, and assessed their accuracy and implications on quantification of NO_3^- attenuation in groundwater systems. Moreover, different kinetic models, i.e. zero-order or first-order, have been assumed by several studies in quantifying denitrification rates in groundwater using the push-pull test (e.g., Burbery et al., 2004; Korom et al., 2012; Tesoriero and Puckett, 2011). Enzyme kinetics would be zero-

order if conditions are not limited by the reactant concentration (i.e. at high concentrations), or first-order when conditions are limited by the reactant concentration (i.e. at low concentrations) (Tesoriero and Puckett, 2011). The kinetics of denitrification process depends on several factors, e.g., available carbon, temperature, pH, dissolved oxygen, redox potential, NO_3^- concentration, and the presence and activity of denitrifiers (Reddy et al., 1978; Bekins et al., 1998). Existing denitrification studies generally used either zero-order or first-order kinetics model and did not specify which kinetic model is more appropriate for the analysis (Burbery et al., 2004; Schroth et al., 2001; Tesoriero and Puckett, 2011). Currently, there is no clear objective guidance on which model is more appropriate to analyse the push-pull tests data. It is, therefore, imperative to assess which kinetic model should be used with a specific set of data of a push-pull test.

The main objectives of this study were to evaluate the use of different tracers (denitrification reactant or product) and kinetic models to estimate denitrification rates from single-well, push-pull test data in shallow groundwater aquifers (e.g., <10 m below ground level). In particular, this study compared denitrification rates estimated using the single-well, push-pull test with acetylene inhibition and analysed the denitrification rates obtained based on the denitrification reactant (NO_3^-) and product (N_2O), as well as following zero-order and first-order kinetic reaction models. No other studies are known to have investigated the differences in denitrification rate estimates in this manner. This is important considering that the choice of an analytical approach and/or kinetic model could affect estimates of denitrification rates of shallow groundwater in a certain location. Moreover, the influence of the addition of acetylene in quantifying the denitrification rate in push-pull tests was also assessed. Several authors argued the possibility of enhanced denitrification with acetylene as the carbon source in denitrification assays particularly if carbon is limited (Tiedje et al., 1989; Yeomans and

Beauchamp, 1982). The comparison of results from push-pull tests with or without added acetylene is expected to provide useful information on the effect of acetylene, as well as could provide indications whether denitrification is partial/incomplete releasing N_2O or complete releasing N_2 as the final product (Saggar et al., 2013). The study advances our knowledge and understanding of whether different denitrification rate estimation methods (reactant- or product-based, zero- or first-order kinetic models derived) using single-well push-pull test data provide significantly different denitrification rates, which may have implications on the denitrification characteristics of the groundwater system.

5.2 Methods and Materials

5.2.1 Study area and experimental site

Field experiments were conducted at the study sites in the Manawatū River catchment, located in the lower North Island of New Zealand (Figure 5.1). The catchment covers approximately 6000 km^2 with pastoral farming dominating the land use: sheep and/or beef farms accounting for 58% and dairy farms 17%, whereas native cover comprises 17%, exotic cover 4%, and cropping a very small percentage (<1%) (Clark and Roygard, 2008). The landform is dominated by the axial mountain ranges (Ruahine and Tararua ranges) traversing the middle of the catchment on a north-south direction with elevation reaching 1600 m above sea level (Bekesi and Mcconchie, 2002). East of the ranges, the broad depression known as the Pahiatua basin is filled with Quaternary sediments forming the aquifers in this part of the catchment (Zarour, 2008). In the west, these deposits are overlain by dunes brought inland from the coast (Bekesi and Mcconchie, 2002).

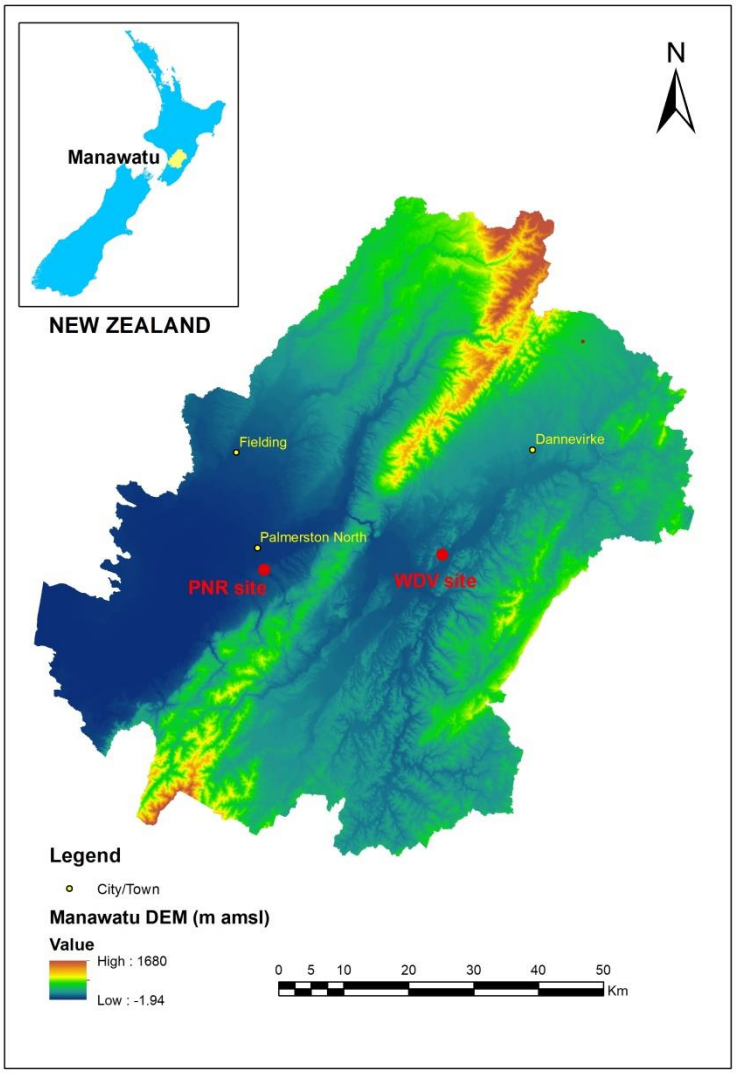


Figure 5.1 The Manawatū River catchment showing the location of the study sites: (1) Massey University's No. 1 Dairy Farm in Palmerston North (PNR site), (2) sheep and beef farm near Woodvile (WDV site).

The Manawatū river and some of its tributaries have been found with higher concentrations of soluble inorganic nitrogen, even exceeding 0.444 g N m^{-3} set as water quality standard for the river (Roygard and McArthur, 2008). A major portion (> 95%) of this nitrogen load is estimated to originate from diffuse sources, mainly farming in the catchment (Ledein et al., 2007). The catchment has been selected to gain a better understanding of the capabilities of subsurface environment to transport and transform or denitrify NO_3^- and subsequently the vulnerability of groundwater and surface water to NO_3^- contamination. Rivas et al. (2017)

found variable denitrification potential in groundwaters of the eastern parts of the catchment. However, direct measurements and analysis of denitrification processes in the catchment have been lacking.

One experimental site (the PNR site) was established at the Massey University No. 1 Dairy Farm in Palmerston North (Figure 5.1) to investigate the ability of the push-pull test to quantify denitrification in shallow groundwater in the catchment. This site is characterised as Manawatū fine sandy loam (Weathered Fluvial Recent Soil) underlain by gravel starting at approximately 4.5 m below ground level (bgl). The WDV site was established in the eastern part of the Manawatū catchment near Woodville. The soil at the site is characterised as Kairanga silt loam and clay loam (Recent/Orthic Gley Soil), and is underlain by gravel at approximately 3 m bgl. The sites are yet to be characterised in detail, but the absence of geological details should not be critical to conducting push-pull tests (e.g., Burbery et al., 2004), considering that denitrification rates were estimated using a simplified analytical method that does not require estimates of aquifer physical properties.

5.2.2 Piezometer installation

Two shallow groundwater piezometers were installed at the PNR site at depths of 6.5 m and 7.5 m bgl at the study site, whereas three piezometers were installed at the WDV site at screened depths of 4.5-5.0, 5.5-6.0, and 7.0-7.5 m bgl. The piezometers were made of polyvinyl chloride (PVC) pipes with internal diameter of 28 mm and perforated (5 mm diameter) at the bottom 0.50 m. The perforated portion was covered with 250 µm mesh nylon screen (Rasiah et al., 2003; Zhu et al., 2003) attached with silicon caulking (Well et al., 2003). The piezometers were installed by direct push-probe percussion with a double tube system (Lapham et al., 1997). Depending on the formation of materials surrounding the

borehole, sand and gravel may collapse and fill the lower part of the bore (functioning as a primary filter pack) and held the piezometer in place (Lapham et al., 1997; Puckett and Cowdery, 2002; Well et al., 2003). Wherever applicable, a secondary filter pack of < 0.60 mm sand (quartz material; Lapham et al., 1997) of 0.30 m length was placed above the primary filter pack to prevent the annular seal from entering the primary filter pack and the screened portion of the piezometer. The piezometers at the PNR site were installed in October 2013, whereas at the WDV site were installed in September 2014 and were developed by pumping with a peristaltic pump before the first push-pull test were conducted.

5.2.3 The single-well push-pull tests and analytical methods

Three push-pull tests were conducted at the Palmerston North (PNR) test site on the piezometer screened at 6.0 - 6.5 m bgl, whereas two push-pull tests at the Woodville (WDV) site on the piezometer screened at 5.5 – 6.0 m bgl (Table 5.1). A push-pull pre-test (e.g. Addy et al., 2002; Istok, 2013; Mcphillips et al., 2015; Pauwels et al., 1998) was conducted in July 2013 on another piezometer on the same farm of the PNR site screened at 5.5 to 6.0 m bgl, to determine the recovery fraction of the conservative tracer and the appropriate volume of groundwater to be extracted for the preparation of the test solution. The first push-pull test at the PNR site was conducted in October 2013, using 40 L of test solution with added acetylene to measure the product, N_2O (Sanchez-Perez et al., 2003; Well et al., 2003) (Table 5.1). The increase in test solution injection volume from 40 L in October 2013 to 100 L in May and July 2014 at the PNR site and at both tests at the WDV site (Table 5.1) was intended to "minimise the effect of the slug fringe" (Snodgrass and Kitanidis, 1998) and to further decrease the "bias from convective and diffusive mixing processes" (Well et al., 2003). At the PNR site, the push-pull test in May 2014 was also conducted with use of acetylene (Sanchez-Perez et al., 2003; Well et al., 2003) and the test in July 2014 was

conducted without acetylene (Istok et al., 1997; Tesoriero et al., 2000). These were used to assess the effect of acetylene on quantification of the denitrification rates (Table 5.1). Acetylene was used at both tests at the WDV site.

Before each push-pull test, the piezometer was purged with at least three well volumes until selected in-field groundwater parameters (temperature, pH, electrical conductivity, and dissolved oxygen) were stable to ensure the collection of representative groundwater (Daughney et al., 2006). Groundwater samples for determination of background concentrations were first collected in triplicates, and then an additional 40-100 L of groundwater was extracted into 20 L collapsible or flexible polyethylene bags (Coleman Water Carrier, made of heavy duty polyethylene consisting of 50% polyethylene and 50% ethylene-vinyl acetate). Flexible bags or containers to collect and prepare test solution have been used in other studies (e.g., Baker and Vervier, 2004; Well et al., 2003), given their advantages such as small quantities of solutes required, operational convenience since they collapse during injection instead of requiring pressured gas to fill the void left by the water pumped into the piezometer, and 'the entire system is closed' minimising losses of solute or exposure to the atmosphere (Istok, 2013).

Table 5.1 Parameters for the push-pull tests conducted at the two stusy sites in the Manawatū catchment, New Zealand.

Item	Unit	Massey No. 1 Dairy Farm (PNR site)			Woodville Sheep & Beef Farm (WDV site)	
		October 2013	**May 2014**	**July 2014**	**February 2015**	**August 2015**
Br^- (test solution)*	mg L^{-1}	11.06±0.57	12.88±2.60	10.01±0.30	10.59±0.36	10.99±0.16
NO_3^--N (test solution)*	mg L^{-1}	11.23±0.53	12.66±2.20	9.55±0.28	10.73±0.35	11.08±0.15
Test solution volume	L	40	100	100	100	100
Test duration	minutes	225	420	420	420	360
Acetylene used		Yes	Yes	No	Yes	Yes

*Including background concentrations (±stdev); background concentrations for Br^- and NO_3^--N are very low (<0.10 mg L^{-1}) during the tests.

The extracted groundwater was stored overnight in a temperature-controlled room with the temperature set similar to the groundwater temperature upon collection, approximately 15 °C (except for the October 2013 test, when the test was completed on the same day due to the smaller volume of the test solution). While other studies stored collected groundwater at 4 °C (Addy et al., 2002; Mcphillips et al., 2015; Mehnert et al., 2006), the background water temperature was used in the study to approximate *in situ* conditions. The low temperature set for overnight storage of groundwater sample could affect the activities of denitrifying microorganisms not just during storage, but also during the test, especially for short duration tests. Several incubation studies even suggested pre-incubation of samples for 24 hours to allow for temperature equilibration or acclimatising soil microbes (Barkle et al., 2007; Drury et al., 2008). The test solution was prepared on the site just before injection by adding bromide (KBr) and NO_3^- (KNO_3) to a small container (120 mL x 2) of groundwater which was then transferred back into the test solution containers using a syringe. Acetylene, purified by passing through two traps of concentrated sulphuric acid and one trap of distilled water (Castle et al., 1998), was also added by syringe into the extracted groundwater. The target concentrations (excluding the background concentrations) in the test solution were approximately as follows: 10 mg L^{-1} Br^-, 10 mg L^{-1} NO_3-N, and 50 ml purified acetylene L^{-1} (Mosier and Klemedtsson, 1994; Paramasivam et al., 1999; Well et al., 2003). The use of 10 mg NO_3-N L^{-1} was based on the maximum allowable limit for drinking water (e.g., Burbery, 2004; Mehnert et al., 2006) and close to the estimated average NO_3-N concentrations leaching from dairy farms in New Zealand considering rainfall amounts in the study area (10.8 mg N L^{-1}; Ledgard et al., 2000; Pattle Delamore Partners Ltd., 2013b; Singh et al., 2014). The test solution bags were then vigorously shaken to dissolve the substrate and acetylene bubbles and mix the solution properly (Well et al., 2003). Duplicate samples

(approximately 50 mL each) from each test solution bag were collected and filtered (0.45 µm) on the site, and frozen at approximately -20 °C until needed for further analysis (e.g. Barns, 2010; Stenger et al., 2008). It has to be noted that since NO_3^--N was added in the test solution, the estimated denitrification rates from the push-pull tests may be more appropriately interpreted as denitrification capacity instead of actual denitrification rate.

The test solution was injected into the piezometer using the injection system by reversing the peristaltic pump (Tesoriero et al., 2000). At completion of the injection of the test solution, extraction of groundwater was commenced and samples were collected periodically (see Addy et al., 2002; Burbery, 2004; Burbery et al., 2013; Istok et al., 1997; Istok, 2013; Schroth et al., 1998; Schürmann et al., 2003; Snodgrass and Kitanidis, 1998; Toda et al., 2002). Groundwater samples were collected from time 0 (right after completion of the test solution injection) (Baker and Vervier, 2004; Istok et al., 1997), and at 15, 30, 60, 90, 120 minutes later, and then hourly up to four or seven hours later (e.g., Baker and Vervier, 2004; Mehnert et al., 2006; Sánchez-Pérez et al., 2003). The piezometer was purged prior to each sampling by pumping out approximately 2 L of water to ensure collection of groundwater samples from the aquifer formation surrounding the piezometer (Istok, 2013). Following purging, two sets of groundwater samples were collected at each sampling time. First, triplicate of approximately 50 mL samples were field-filtered with a 0.45 µm syringe filter and collected in polyethylene bottles for the measurement of reactant (NO_3^-) and conservative (bromide) tracer. Second, duplicate samples of 120 mL (in October 2013 and May 2014 tests at the PNR site) or 180 mL (in July 2014 test at the PNR site and at both tests at the WDV site) were collected for the extraction and analysis of dissolved nitrous oxide (N_2O) gas. Groundwater samples for dissolved gas were collected by syringe through a t-type Luer-lock mini valve to avoid sample exposure to air (Addy et al., 2002; Well et al., 2003) and transferred into

evacuated vacuum pouches (100x285mm, gauge 70 µm Cas-Pak) for further analysis. The collected samples were transported in a chilly bin with ice (Daughney et al., 2006). The collected samples for hydrochemical analysis were frozen until analysis (e.g. Barns, 2010; Stenger et al., 2008); whereas, the collected samples for N_2O measurements were kept chilled at 4°C until the gas extraction, which was done within 24 hours of groundwater collection. To extract dissolved N_2O gas from the collected water samples, the phase equilibrium headspace extraction method (Addy et al., 2002; Lemon and Lemon, 1981) was adapted in which 50 mL (in October 2013 and May 2014 test samples from the PNR site) or 60 mL (in July 2014 test samples from the PNR site and both tests at the WDV site) of N_2 were added to create a headspace in the collection pouches, which were then placed on a shaker for 1.5 hours at 200 rpm under 20 °C. After shaking, 25 mL of gas samples were removed from each pouch and placed into 12 mL glass vials (Labco Exetainer) for analysis by a gas chromatograph. The use of a larger groundwater sample for dissolved gas extraction, i.e. 180 mL in all the other tests compared to 120 mL in October 2013 and May 2014 tests at the PNR site, was presumed to increase the accuracy of dissolved N_2O measurements.

The collected groundwater samples were analysed for NO_3^--N and Br^- concentrations by ion chromatography (Lachat Instruments IC5000 Ion Chromatograph), ammonium-N (NH_4^+-N) by continuous flow analysis (Technicon® AutoAnalyzer II). Dissolved organic carbon (DOC) concentrations in groundwater samples at the PNR site were determined by potassium dichromate wet oxidation and titration (method 5220B, (Rice et al., 2012), but with some adjustments: using 10 mL sample, 20 mL H_2SO_4 with 5 g Ag_2SO_4 L^{-1}, and 10 mL 0.025N $K_2Cr_2O_7$ in digestion, and 0.01N ferrous ammonium sulphate for titration), whereas DOC concentrations in samples at the WDV site were determined by measuring absorbance with a spectrophotometer (wavelength = 660 nm). The collected gas samples were analysed for N_2O

with a Shimadzu Gas Chromatograph (GC) 17 A (Japan) which has a ^{63}Ni-electron capture detector.

5.2.4 Quantification of denitrification rate

The tracer concentrations from samples were first analysed and corrected for dilution and/or advection to measure changes in the reactant (i.e. NO_3^--N) or product (i.e. N_2O-N) during the tests. The measured NO_3^--N concentrations were corrected by multiplying with a dilution factor (DF) calculated as the ratio of the average initial concentration of the conservative tracer Br^- in the test solution and the concentration of the conservative tracer Br^-at respective samplings. The measured concentrations of N_2O were first corrected for the amount of N_2O dissolved in groundwater during the phase equilibrium headspace extraction, similar to the procedure used in denitrification assays with soil samples in solution (Hill et al., 2000; Sanchez-Perez et al., 2003; Well et al., 2003). Thereafter, the amount of N_2O-N produced per sampling was corrected for dilution based on the concentration of Br^- tracer (Sanchez-Perez et al., 2003; Well et al., 2003). The corrected N_2O-N concentration was computed in the same manner as described above for NO_3^--N concentrations.

The dilution corrected NO_3^--N and N_2O-N concentration measurements were used to quantify denitrification rate by analysing the decrease in the reactant (NO_3^--N) and/or the increase in the product (N_2O-N) (Istok, 2013; Istok et al., 1997). Further, denitrification rate was quantified assuming different kinetic models, i.e. the zero-order or first-order kinetics.

A review of existing studies suggests a number of models developed and used to quantify denitrification rate based on the reactant (NO_3^--N) or the product (N_2O-N) (Table 5.2).

Assuming zero-order kinetics, Toda et al. (2002) developed a simple model to compute the denitrification rate based on the initial and final concentrations of NO_3^--N (Eq. 1), after corrected for dispersion based on the concentration of the conservative tracer Br^- (Table 5.2). Alternatively, several studies adopted a model based on best-fit slope of regression line of dilution-corrected concentration of NO_3^--N (C_{dc}) against time to quantify zero-order denitrification rate (Baker and Vervier, 2004; Istok, 2013; Korom et al., 2012; Tesoriero et al., 2000). Baker and Vervier (2004) also applied this model to quantify the zero-order denitrification rate as the slope of regression line of dilution-corrected N_2O-N concentration. Trudell et al. (1986) fitted a curve to describe the data under the assumption that the "slope of the line tangent to the curve at any point represents the nitrate loss at that point". In this case, different denitrification rates could be quantified depending on which part of the curve a tangent line is drawn. Trudell et al. (1986) used the rate towards the end (highest rate) as reasonable estimate of denitrification rate in natural conditions – considering the increase in denitrifier population during the test. Sánchez-Pérez et al. (2003) applied the Trudell et al. (1986) model to determine the N_2O-N production rate as the maximum slope in the ascending part of the tangent to the curve approximating the data. Well et al. (2003) developed a method for estimating zero-order denitrification rate accounting for dilution, considering that production and dilution of N_2O are concurrent (Eqs. 7-9) (Table 5.2). Denitrification rate was estimated on a time step basis according to sampling intervals. Given that different rates would be obtained in each sampling interval, Well et al. (2003) suggested to consider the maximum concentration gradient in calculating denitrification rates, similar to the proposal of Trudell et al. (1986). This interval "represents the denitrification rate during an intermediate phase where microbial adaptation is complete and diffusive loss is still insignificant" (Well et al., 2003).

Istok et al. (1997) and Mehnert et al. (2006) quantified zero-order reaction rates for reactants based on the mass of reactant recovered and the mean residence time for the test solution in the aquifer (Eq. 2) (Table 5.2). Istok et al. (1997) also applied this model to quantify the zero-order rate based on N_2O-N concentration measured during the test. This model, however, is applicable only to push-pull data from continuous pumping. This could not be applied to the test results as this study adopted periodical sampling where only a small portion of the injected solution was recovered. Snodgrass and Kitanidis (1998) came up with an analytical model (Eq. 3) (Table 5.2) for the quantification of a zero-order rate from transport equations considering a well-mixed slug and short injection time compared with reactive solute degradation. This method differs from other methods above in accounting for the effect of dilution as, instead of directly applying a dilution factor to the measured concentration to obtain the dilution-corrected reactant concentrations, Snodgrass and Kitanidis (1998) estimated the transformed or dilution-corrected concentration by adding the amount lost due to dilution to the measured concentration (see Eq.3).

Table 5.2 Summary of different models for estimating zero-order (r) or first-order (k) denitrification rate from single-well push-pull test data.

Tracer	Kinetic model	Approach and/or Formula	References
Reactant	Zero-order	$r = \frac{C_{dc}^{i} - C_{dc}^{f}}{T}$ (Eq. 1)	Toda et al., 2002
		Rate (r) based on best-fit slope of regression line of C_{dc} against time	Baker and Vervier, 2004; Istok, 2013; Korom et al., 2012; Tesoriero et al., 2000
		Rate (r) based on the slope of tangent line to the regression curve approximating C_{dc} plotted against time	Trudell et al., 1986
		$r = \frac{M_{r.inj} - M_{r.ext}}{V_{inj} \times RT_{ave}}$ (Eq. 2)	Istok et al., 1997; Mehnert et al., 2006
		Rate (r) based on the slope of the transformation of the reactive solute concentration, $C_r(t)$, plotted against time $C_r(t) = C_r^0\left(\frac{C_r^m(t)}{C_r^0} - \frac{C_t^m(t)}{C_t^0} + 1\right)$ (Eq. 3)	Snodgrass and Kitanidis, 1998
	First-order	$\ln\left(\frac{C_r^*(t)}{C_t^*(t)}\right) = \ln\left[\frac{\left(1 - e^{-kt_{inj}}\right)}{kt_{inj}}\right] - kt$ (Eq. 4)	Haggerty et al., 1998
		$\ln\left(\frac{C_r^*(t)}{C_t^*(t)}\right) = \ln\frac{C_{dc}}{C_r^0} = -kt$ (Eq. 5)	Korom et al., 2005, 2012
		$\ln\left(\frac{C_r^m(t)}{C_t^m(t)}\right) = \ln\left(\frac{C_r^0}{C_t^0}\right) - kt$ (Eq. 6)	Snodgrass and Kitanidis, 1998
Product	Zero-order	Rate (r) based on best-fit slope of regression line of C_{dc} against time	Baker and Vervier, 2004

Tracer	Kinetic model	Approach and/or Formula	References
		Rate (r) based on the slope of tangent line to the regression curve approximating C_{dc} plotted against time	Sánchez-Pérez et al., 2003; Trudell et al., 1986
		$r = \frac{C_{dc}(t) - C_p(t-1)}{dt}$ (Eq. 7) $C_{dc}(t) = \frac{C_p(t) - C_p^0 * [1 - F_{dil}]}{F_{dil}}$ (Eq. 8) $F_{dil} = \frac{C_t^*(t)}{C_t^*(t-1)}$ (Eq. 9)	Well et al., 2003
		$r = \frac{M_{p.ext}}{V_{inj} \times RT_{ave}}$ (Eq. 10)	Istok et al., 1997
	First-order	Adapted Eq. 4, replacing the left-side term with an expression for products as below: $\frac{C_r^*}{C_t^*} = 1 - \frac{C_p^*}{C_t^*}$ (Eq. 11) $C_p^* = \frac{C_p}{C_r^0}$ (Eq. 12) $C_p = C_r^0(1 - e^{-kt})$ (Eq. 13)	Schürmann et al., 2003

Note: C_{dc}^i is the initial dilution-corrected concentration of the reactant; C_{dc}^f is the final dilution-corrected concentration of the reactant; T is the duration of the extraction or 'pull' phase; C_{dc} is the dilution-corrected concentration of either the reactant (nitrate) or product (e.g., nitrous oxide); $M_{r.inj}$ is the mass of the injected reactant; $M_{r.ext}$ is the mass of the extracted reactant; $M_{p.ext}$ is the mass of the product formed; V_{inj} is the test solution injection volume; RT_{ave} is the mean residence time for the test solution; C_r^0 and C_t^0 are the injection solution concentrations of the reactive and conservative tracer, respectively; $C_r^m(t)$ and $C_t^m(t)$ are the measured concentrations of the reactive and conservative tracer, respectively, during the 'pull' phase at time *t*; $C_r^*(t)$ and $C_t^*(t)$ are relative concentrations (ratio of concentration measured at time *t* and concentration of the injected solution) of the reactant and the conservative tracer, respectively; t_{inj} is the duration of injecting the test solution; C_p is the concentration of the denitrification product; C_p^0 is the background concentration of the denitrification product; F_{dil} is a dilution factor.

Several studies have quantified the denitrification rate based on first-order kinetics using push-pull test data, generally when the substrate in question (in this case, NO_3^--N) is considered as the limiting substance (Haggerty et al., 1998; Korom et al., 2012; Snodgrass and Kitanidis, 1998) (Table 5.2). Schroth and Istok (2005) compared three different models for determining first-order rate coefficients for single-well push-pull tests based on different mixing models; namely, well mixed, plug flow, and variably mixed. They found that all models were robust for estimating first-order rates from push-pull data, as long as the time to complete injection is much less than the extraction phase like in the push-pull tests of this study. The variably mixed model, however, requires numerical simulation to compute for the weighted mean residence time. The simplest method for obtaining first-order rate is by plotting the natural logarithm of substrate concentration versus time and determining the slope of the best fit regression line (Bekins et al., 1998). But this could not be directly applied in the push-pull tests data due to the effect of dilution on NO_3-N concentrations. Haggerty et al. (1998) quantified the first-order rate (k), the only unknown quantity in Eq. 4 (Table 5.2), by plotting the natural logarithm of the ratio of the relative concentrations of the reactant (NO_3-N) and tracer (Br^-) against time and fitting Eq. 4 to the plot using least-squares non-linear regression in MS Excel. This model of Haggerty et al. (1998) takes into account the reactions during injection time (Burbery, 2004; Istok, 2013) and considering the test solution as well-mixed and the retardation factors for both the reactant and tracer are the same. The reduction in reactive tracer during injection is represented by the y-intercept at time t=0 of the pull phase. Several authors (Korom et al., 2005, 2012) used a more simplified version of the model of Haggerty et al. (1998), with the y-intercept set to zero (Eq. 5). Snodgrass and Kitanidis (1998) developed a similar model to estimate the first-order reaction rate, but derived the first-order rate from the slope of a line fitted to the plot of natural logarithm of the

ratio of the measured reactant (NO_3^--N) and tracer (Br^-) concentrations against time (not relative concentrations) (Eq. 6). Given that the y-intercept is set at the natural logarithm of the injection solution concentration, the model "does not account for any reaction during the finite injection time" (Burbery, 2004).

Considering the estimation of first-order kinetic rates based on product, Schürmann et al. (2003) adopted the method of Haggerty et al. (1998) with a defined relationship between the reactants and products (Eq. 13). The left side term of Eq. 4 was replaced accordingly with an expression for products (Table 5.2) (see Schürmann et al., 2003 for details).

While, as discussed above (Table 5.2), there are several available models for estimating denitrification rates, this study assessed only the most common models used for estimating denitrification rate using either reactants or products Particular attention was made to ensure that this assessment included models that were applicable for both the reactant and product. For instance, for estimates assuming zero-order kinetics, the model of Trudell et al. (1986) based on the reactant and the model of Sánchez-Pérez et al. (2003) based on the product were included in the assessment (Table 5.2). Both models estimate denitrification rate based on the slope of the line tangent to the regression curve of the data. For estimates assuming first-order kinetics, the model of Haggerty et al. (1998) for the reactant (Eq. 4), and the model of Schürmann et al. (2003) for the product (Eqs. 11-13) were both included, as the latter is a modification of the former for product-based estimates (Table 5.2).

5.3 Results and Discussion

5.3.1 Push-pull test results

Table 5.3 summarizes the background characteristics of shallow groundwater measured during the push-pull tests at the two study sites. At the PNR site, the measured background groundwater characteristics were similar during the three tests except for sulphate (SO_4^{2-}) concentration in July 2014 test (Table 5.3), which could indicate anthropogenic sources (e.g. fertilisers) due to recharge in the winter season (June-August). The background concentrations of NO_3^--N and NO_2^--N were measured at very low concentrations (<0.05 mg L^{-1}). Low concentrations of dissolved oxygen (DO) indicate the presence of reduced groundwater at the site. The oxidation reduction potential or ORP (in terms of *Eh*) measured with an Ag/AgCl reference electrode was a bit higher in May 2014 (243.7 mV) and July 2014 (253.5 mV) tests which could be due to groundwater recharge during the late autumn (May) and winter seasons (June-August), but suggested reduced groundwater conditions (225 mV; Thayalakumaran et al., 2008). Coupled with significant amounts of DOC values (Table 5.3), this suggests a high potential for denitrification to occur at the study site. This is supported by measured low concentrations of NO_3^- (<0.05 mg N L^{-1}), despite the study site being located on a dairy farm with sandy loam soil. At the WDV site, reduced groundwater condition suitable for denitrification to occur is indicated by the low DO concentrations (0.22-0.37 mg L^{-1}) and ORP (*Eh* of 68.7-94.5). This is also reflected in very low NO_3^- concentrations (≤ 0.02 mg L^{-1}). However, the lower DOC content (1.24-1.72 mg L^{-1}) may indicate lower potential for denitrification to occur compared to the PNR site.

Figures 5.2 and 5.3 show the measurements of push-pull tests conducted on three occasions at the PNR site and on two occasions at the WDV site. At the PNR site, all three push-pull tests measured a quick decrease in NO_3^--N and Br^- concentrations from about 10 mg L^{-1} (at t=0) to

less than 2 mg L^{-1} within 7 hours (at *t*=7). Faster decrease in concentrations were observed in the October 2013 experiment and could be attributed to the smaller volume of test solution used (40 L). A larger deviation in the measured concentrations (shown by the error bars) was apparent in the early stages of the pull phase, with much lower deviation in the later stages (Figure 5.2). This was also observed at the WDV site (Figure 5.3) and could be due to mixing of injected test solution with the resident groundwater around the test piezometer. The measured concentrations of the tracers consistently showed a decreasing of NO_3^--N /Br^- ratio at the two sites (although smaller decrease at the WDV site), indicating that NO_3^--N decreased more than Br^- during the tests. This suggests NO_3^- attenuation processes such as denitrification occurring in the shallow groundwater during the tests. This is also supported by the observed trend of increasing N_2O-N production during the tests (Figure 5.2 a2, b2, c2; Figure 5.3 a2, b2). It should be noted that these N_2O-N values shown in Figures 5.2 and 5.3 have not yet been corrected for diffusion and/or advection transport that might have diluted N_2O-N produced during the tests.

Table 5.3 Background characteristics of shallow groundwater measured during the assessment of push-pull tests conducted at the two study sites in the Manawatū catchment, New Zealand.

Parameter	Unit	Massey No. 1 Dairy Farm (PNR site)			Woodville Sheep & Beef Farm (WDV site)	
		October 2013	May 2014	July 2014	February 2015	August 2015
Depth to water (bgl)	m	4.29	4.65	4.71	3.96	3.05
Temperature	°C	14.6	15.7	15.1	14.4	13.9
Dissolved oxygen	mg L^{-1}	0.40	0.45	0.44	0.22	0.37
SPC	µS cm^{-1}	234.6	228.9	255.9	415.0	424.7
pH		6.25	5.89	5.82	6.30	6.31
ORP (Eh)	mV	188.9	243.7	253.5	94.5	68.7
Br^-	mg L^{-1}	0.099	0.099	0.080	0.08	0.15
NO_3^--N	mg L^{-1}	0.049	<0.01	<0.01	<0.01	0.02
NO_2^--N	mg L^{-1}	<0.003	<0.003	<0.003	<0.003	<0.003
DOC	mg L^{-1}	3.55	3.81	n/a	1.24	1.72
Cl^-	mg L^{-1}	19.10	22.16	20.51	29.98	36.19
SO_4^{2-}	mg L^{-1}	5.57	6.17	22.38	25.04	3.39

Note: Except for field measured parameters (temperature, DO, pH, SPC, ORP), values presented are averages of three replicates. bgl – below ground level, DO – dissolved oxygen, DOC – dissolved organic carbon, SPC – specific conductance or electrical conductivity, ORP – oxidation-reduction potential.

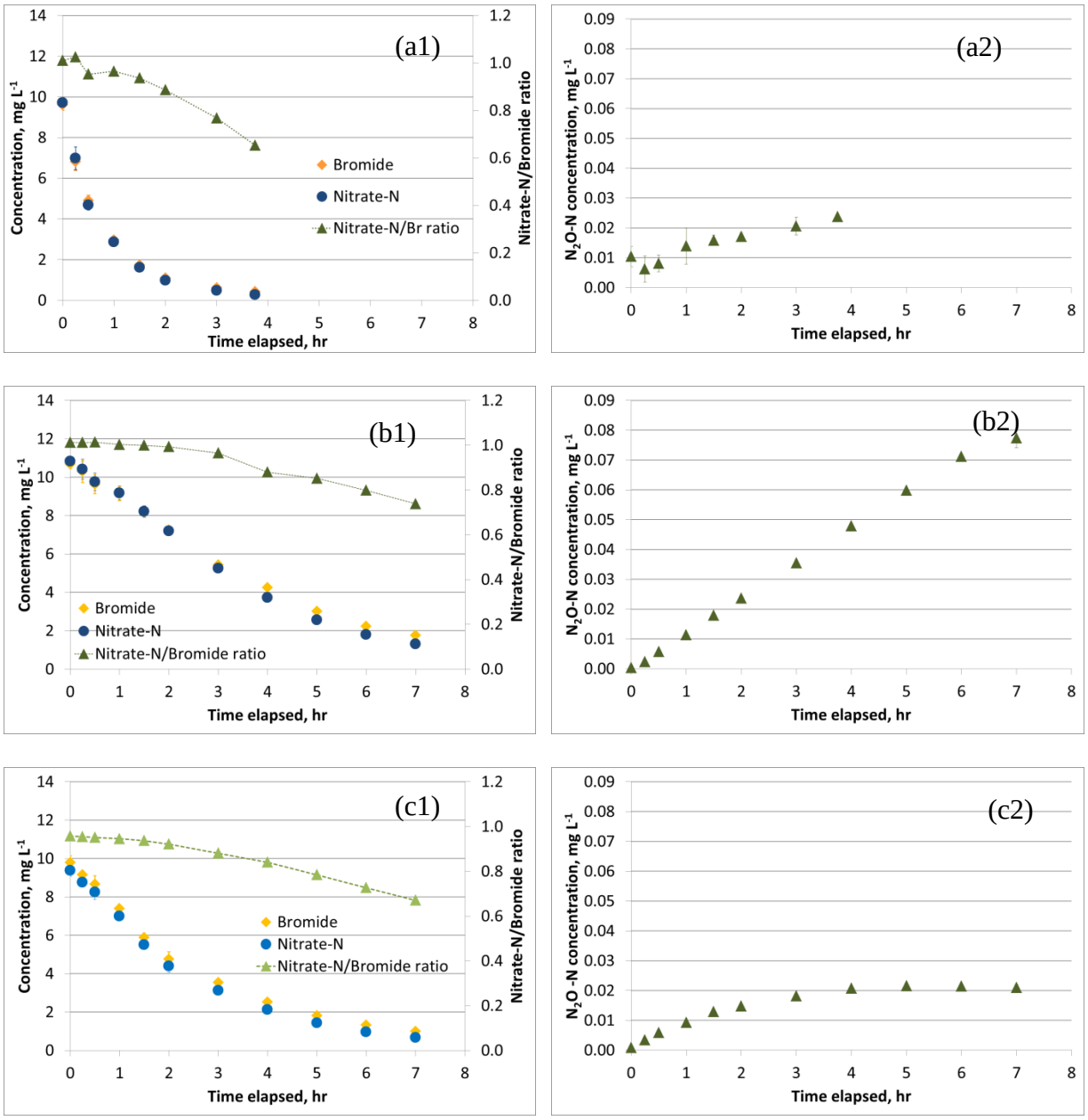


Figure 5.2 Concentrations of (1) nitrate-N and bromide, and (2) nitrous oxide-N during the push-pull test conducted in (a) October 2013, (b) May 2014, and (c) July 2014 at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand (PNR site). Error bars represent standard deviations of the sampling replicates. Volume of test solution – 40 L (Oct 2013) and 100 L (May 2014 and July 2014).

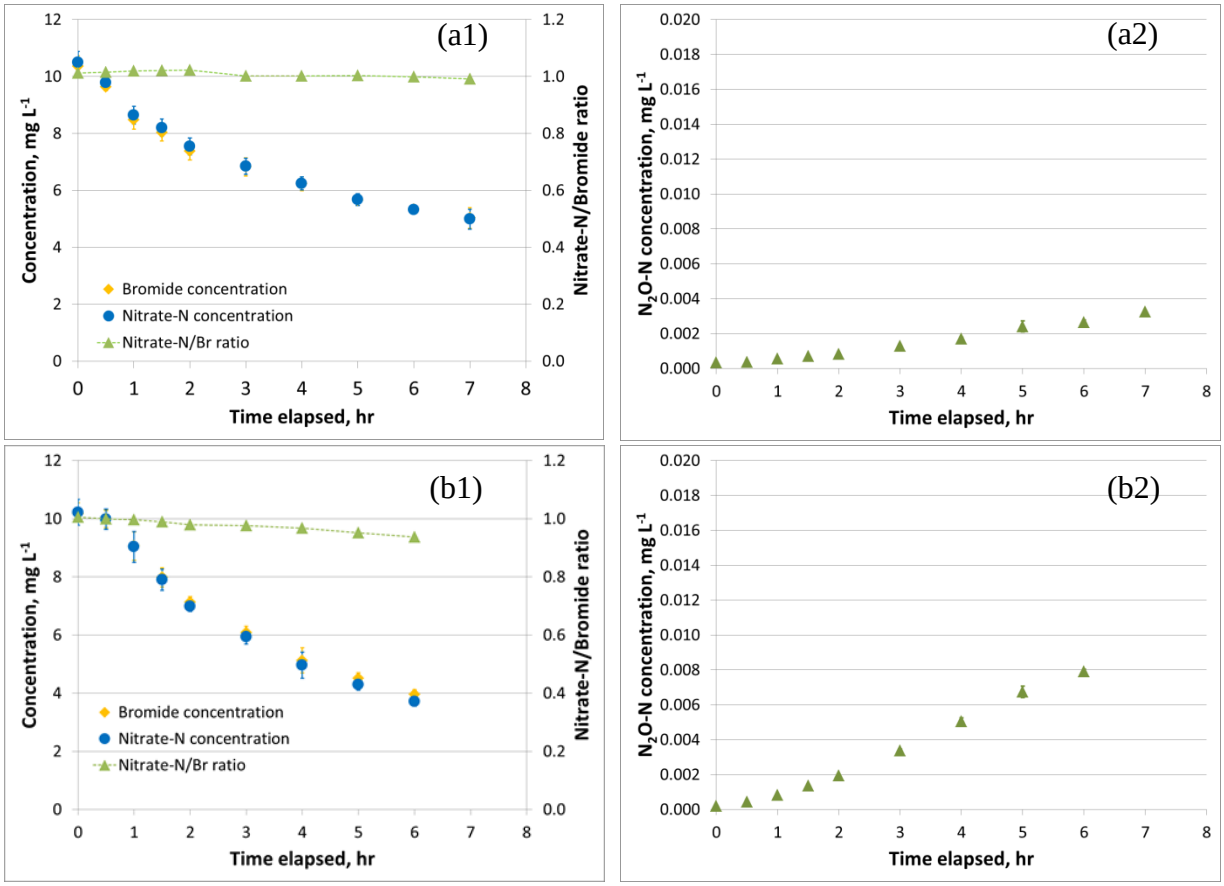


Figure 5.3 Concentrations of (1) nitrate-N and bromide, and (2) nitrous oxide-N during the push-pull test conducted in (a) February 2015 and (b) August 2015 at a sheep and beef farm near Woodville, New Zealand (WDV site). Error bars represent standard deviations of the sampling replicates. Volume of test solution 100 L.

Figure 5.4 shows the dilution-corrected concentrations of NO_3^--N, NO_2^--N and N_2O-N observed during the May 2014 test at the PNR site. Nitrite-nitrogen (NO_2^--N) is an intermediate product of denitrification (Appelo and Postma, 2005; Rivett et al., 2008). As more NO_3^--N was lost, as shown by decreasing dilution-corrected NO_3^--N concentration, more NO_2^--N and N_2O-N were produced. The background concentrations of NO_2^--N were found to be below the detection limit (<0.003 mg N L^{-1}) in all tests (Table 5.3), but significant amounts were subsequently observed during the May 2014 and July 2014 tests (up to 0.207 and 0.090 mg N L^{-1}, respectively) at the PNR site. This further confirms the

occurrence of denitrification, with NO_2^--N being produced as an intermediate product of the process (Appelo and Postma, 2005; Rivett et al., 2008). The concentrations of N_2O-N, deemed as the terminal product of denitrification process with the use of acetylene, increased during the duration of the tests confirming the occurrence of denitrification at the test site.

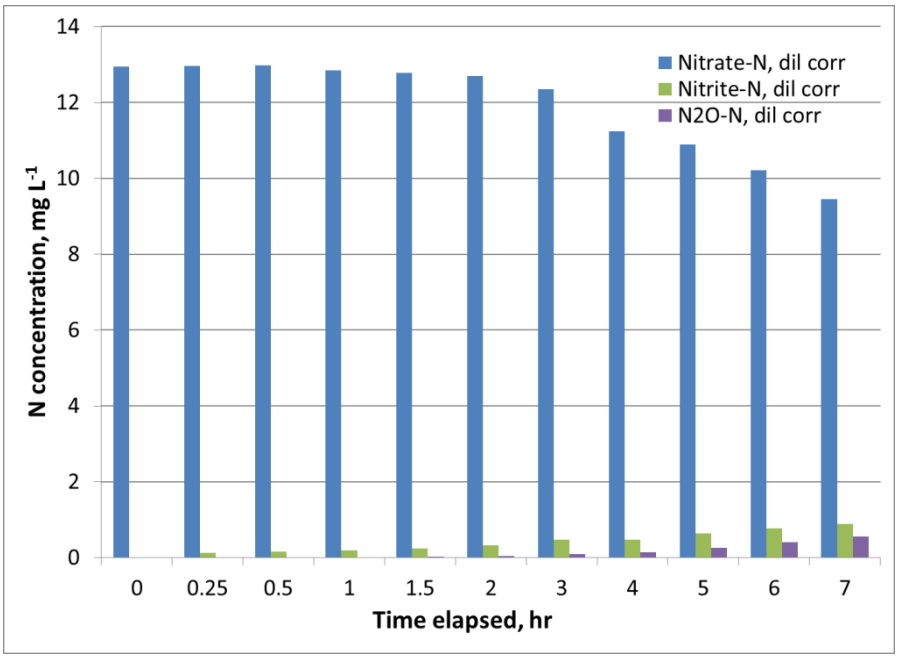


Figure 5.4 Concentrations of different forms of nitrogen in groundwater samples collected during the push-pull test conducted in the May 2014 at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand (PNR site).

Effect of acetylene on nitrous oxide concentrations during push-pull tests

Figure 5.5 highlights the effects of acetylene on N_2O-N concentrations produced during the May and July 2014 tests at the PNR site. A linear trend on increase in N_2O-N concentrations was observed in the May 2014 test (with acetylene) but not in the July 2014 test (without acetylene). In the July 2014 test, the measured N_2O-N concentrations showed an initial increase (up to the 4^{th} hour) and then plateaued afterwards. This put in contrast to a steadily rise of N_2O-N concentrations measured during the May 2014 test (with acetylene) and indicates the possibility of N_2O conversion to N_2 in denitrification process during the July

2014 test. Although N_2 measurements are needed to confirm this, the apparent conversion of N_2O to N_2 in the July 2104 test suggests the potential of complete denitrification in shallow groundwater as observed in other studies (e.g., Jahangir et al., 2012a). Such potential for complete subsurface denitrification underlines the significance of NO_3^- attenuation in groundwater systems in the "long-term improvement" of water quality as dinitrogen gas (N_2), the end product of denitrification, is unlikely to be converted back to NO_3^- within the system (Starr and Gillham, 1993).

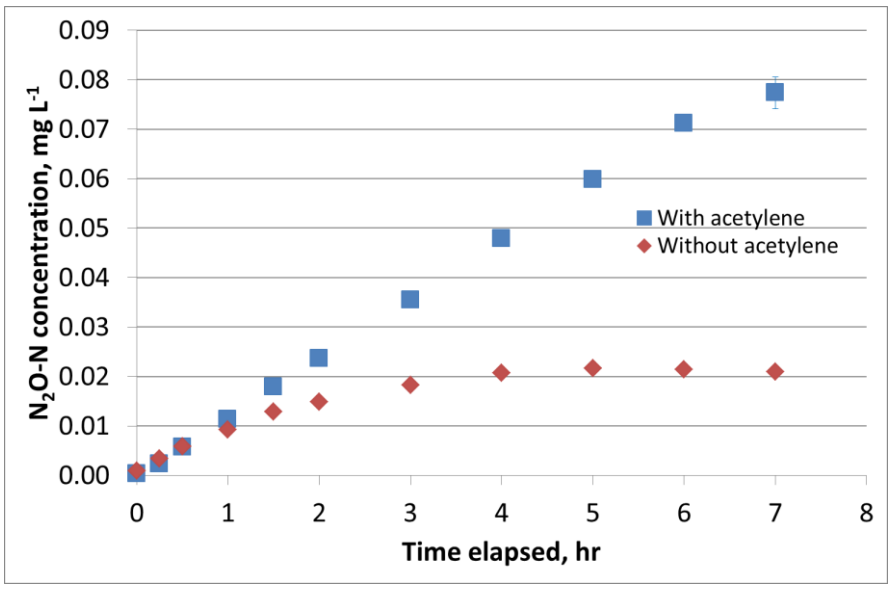


Figure 5.5 Nitrous oxide concentrations (uncorrected for dilution) during the push-pull tests conducted in May 2014 (with acetylene) and July 2014 (without acetylene) at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand. Error bars represent standard deviations of the sampling replicates. The volume of test solution for both tests was 100 L.

5.3.2 Zero-order denitrification rates

For comparison purposes, the zero-order denitrification rate was calculated using the most common methods. Using the concentrations of the reactant, the zero-order rate was estimated using: a regression line of dilution corrected NO_3^--N or Zero-order 1 model (Baker and Vervier, 2004; Istok, 2013; Korom et al., 2012; Tesoriero et al., 2000); a tangent line to the curve approximating dilution corrected NO_3^--N or Zero-order 2 model (Trudell et al., 1986);

and the method by Snodgrass and Kitanidis (1998) or Zero-order 3 model. Using the concentrations of the product (N_2O), the method of Sánchez-Pérez et al. (2003) (Zero-order 4 model) was used to estimate zero-order rate.

Figures 5.6 and 5.7 show the plots for estimating zero-order rate through linear regression of dilution-corrected NO_3^--N concentrations at the PNR and WDV sites, respectively. Following Trudell et al. (1986), Figures 5.8 and 5.9 show the fitted curve on the dilution-corrected NO_3^--N concentrations to estimate zero-order denitrification rates for the push-pull tests conducted at the PNR and WDV sites, respectively. The slope, or the zero-order rate, of the curve at each sampling interval was determined from the derivative of the fitted curve, resulting in variable denitrification rates depending on the sampling interval chosen. In a similar manner, Figures 5.10 and 5.11 show the fitted curve on the dilution-corrected N_2O-N data to estimate the zero-order rate following Sánchez-Pérez et al. (2003) for the test conducted at the PNR and WDV sites, respectively. Tables 5.4 and 5.5 summarise the zero-order denitrification rates quantified based on the reactant (NO_3^--N) or product (N_2O-N), respectively, during the tests. The average (mean) rate of the Zero-order 2 model was estimated from the average of rates computed from the slopes at sample times and in between, minimum (min) rate was obtained from the tangent line with the least slope, maximum (max) rate from the tangent line with the largest slope (at the last sampling time), and the max interval rates from the slope of the tangent line drawn in the middle of the sampling interval with maximum variation or gradient (at the last sampling interval). These rates showed that the estimated Zero-order 2 denitrification rates (Tables 5.4 and 5.5) vary depending on which point of the curve the tangent line is drawn (Figures 5.8 and 5.9). Figures 5.12 and 5.13 show the plots of transformed NO_3^--N concentrations at the PNR and WDV sites, respectively, following the

method of Snodgrass and Kitanidis (1998) or Zero-order model 3, with the denitrification rates being the slopes of the regression lines.

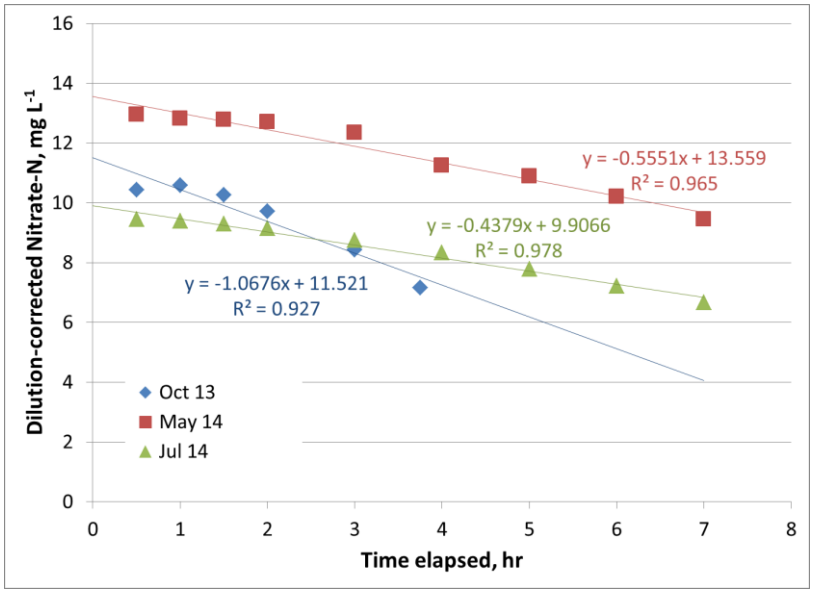


Figure 5.6 Estimating zero-order denitrification rate based on linear regression of dilution-corrected nitrate-N concentrations for the push-pull tests conducted in October 2013, May 2014, and July 2014 at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand (PNR site).

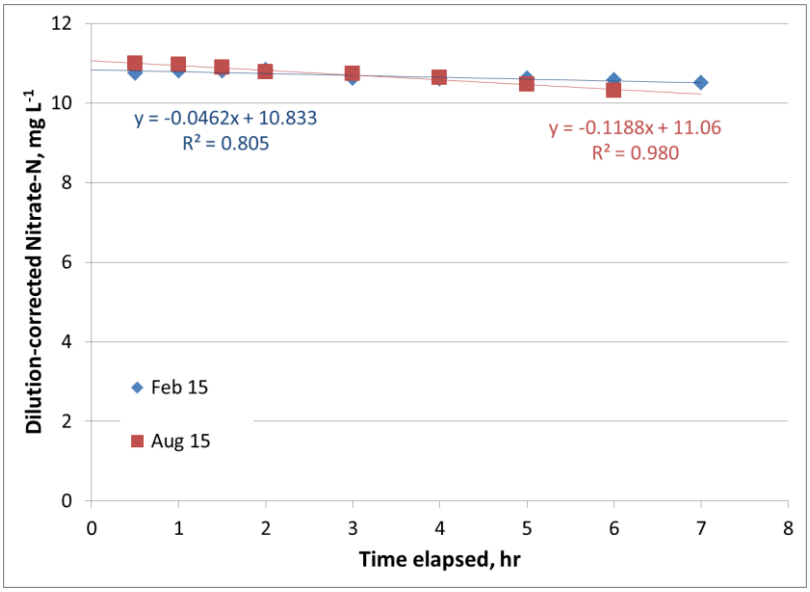


Figure 5.7 Estimating zero-order denitrification rate based on linear regression of dilution-corrected nitrate-N concentrations for the push-pull tests conducted in February 2015 and August 2015 at a sheep and beef farm near Woodville, New Zealand (WDV site).

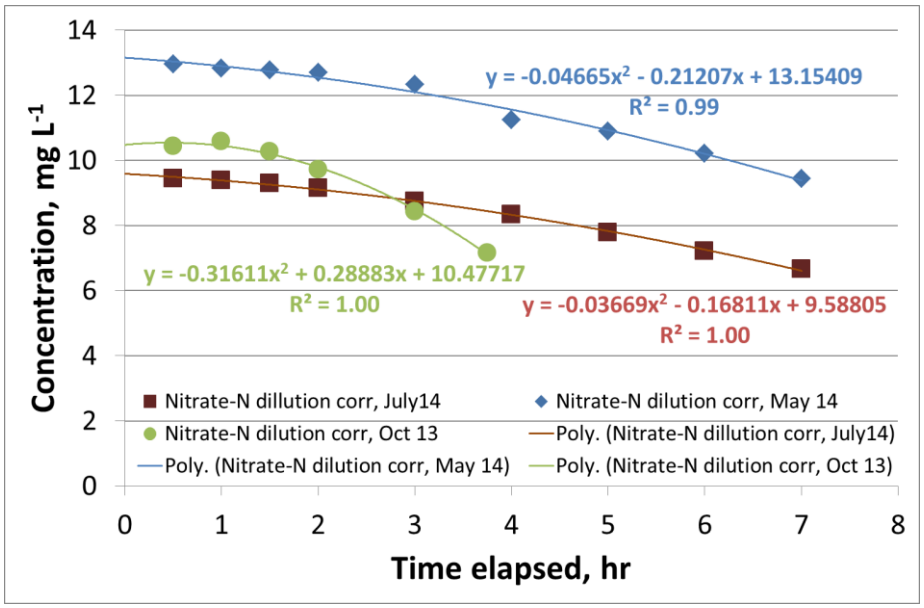


Figure 5.8 Estimating zero-order denitrification rate from a curve fitted on the dilution-corrected nitrate-N concentrations (Trudell et al., 1986) during the push-pull test conducted in October 2013, May 2014, and July 2014 at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand (PNR site).

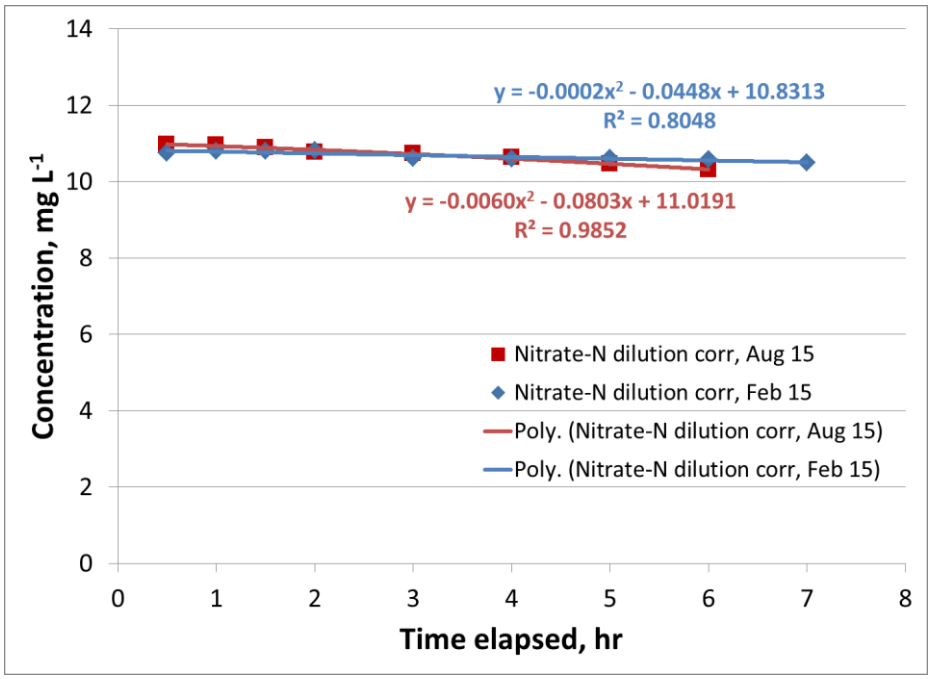


Figure 5.9 Estimating zero-order denitrification rate from a curve fitted on the dilution-corrected nitrate-N concentrations (Trudell et al., 1986) during the push-pull test conducted in February 2015 and August 2015 at a sheep and beef farm near Woodville, New Zealand (WDV site).

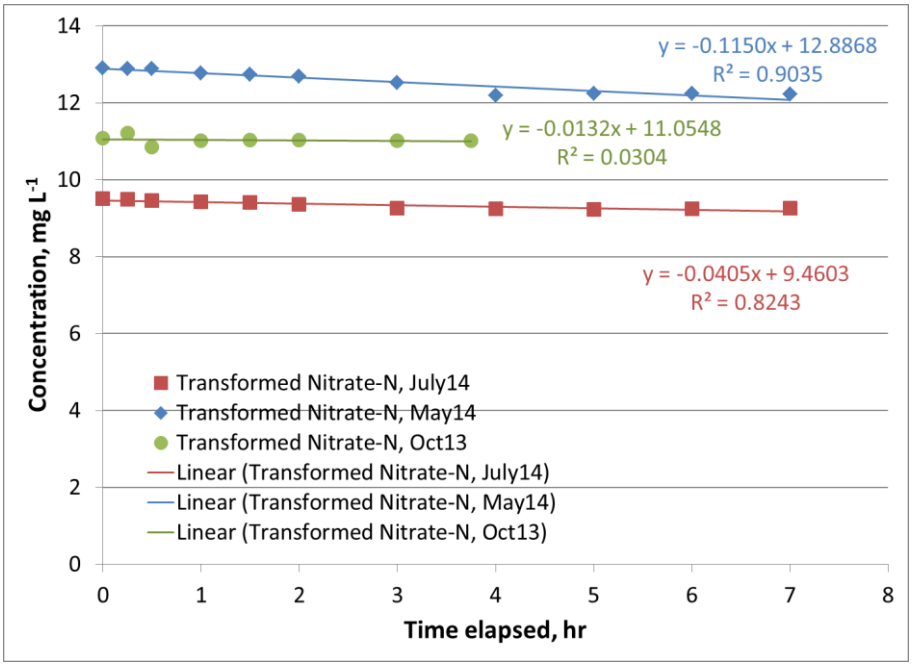


Figure 5.10 Estimating zero-order denitrification rate based on Snodgrass and Kitanidis (1998) for the push-pull tests conducted in October 2013, May 2014, and July 2014 at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand (PNR site).

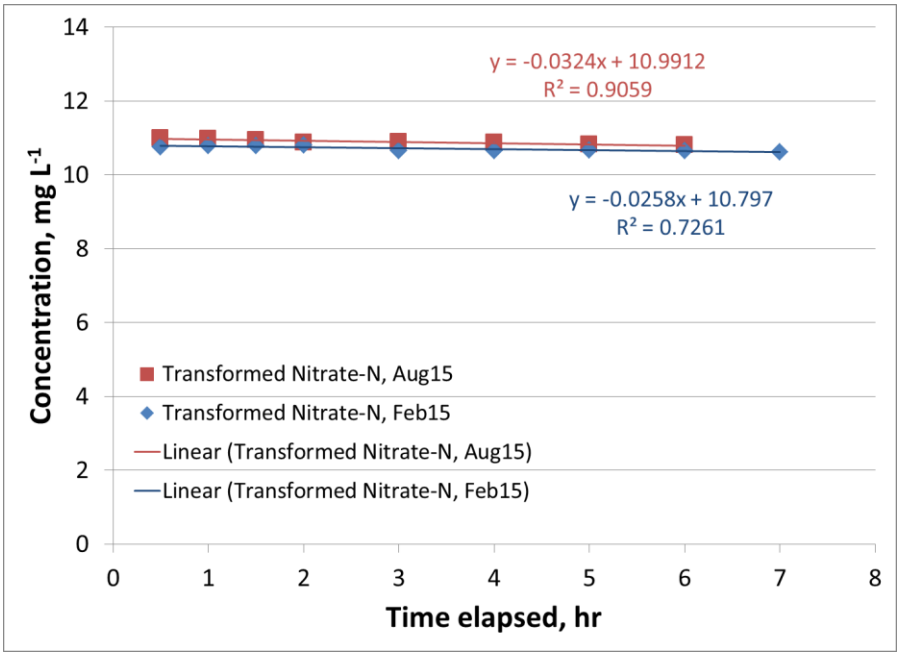


Figure 5.11 Estimating zero-order denitrification rate based on Snodgrass and Kitanidis (1998) for the push-pull tests conducted in February and August 2015 at a sheep and beef farm near Woodville, New Zealand (WDV site).

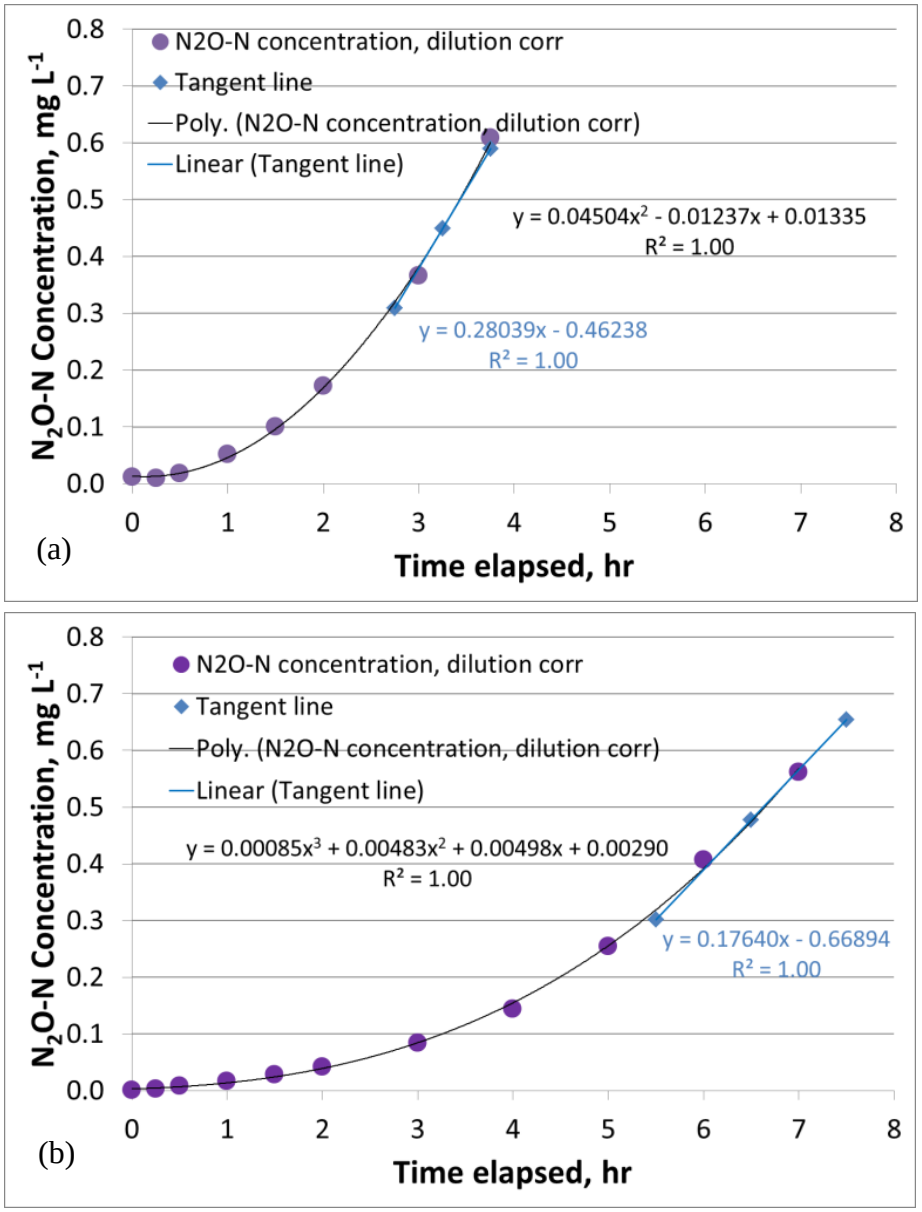


Figure 5.12 Estimating zero-order denitrification rate from a curve fitted on the dilution-corrected nitrous oxide-N concentrations (Sanchez-Perez et al., 2003) for the push-pull tests conducted in (a) October 2013 and (b) May 2014 at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand (PNR site).

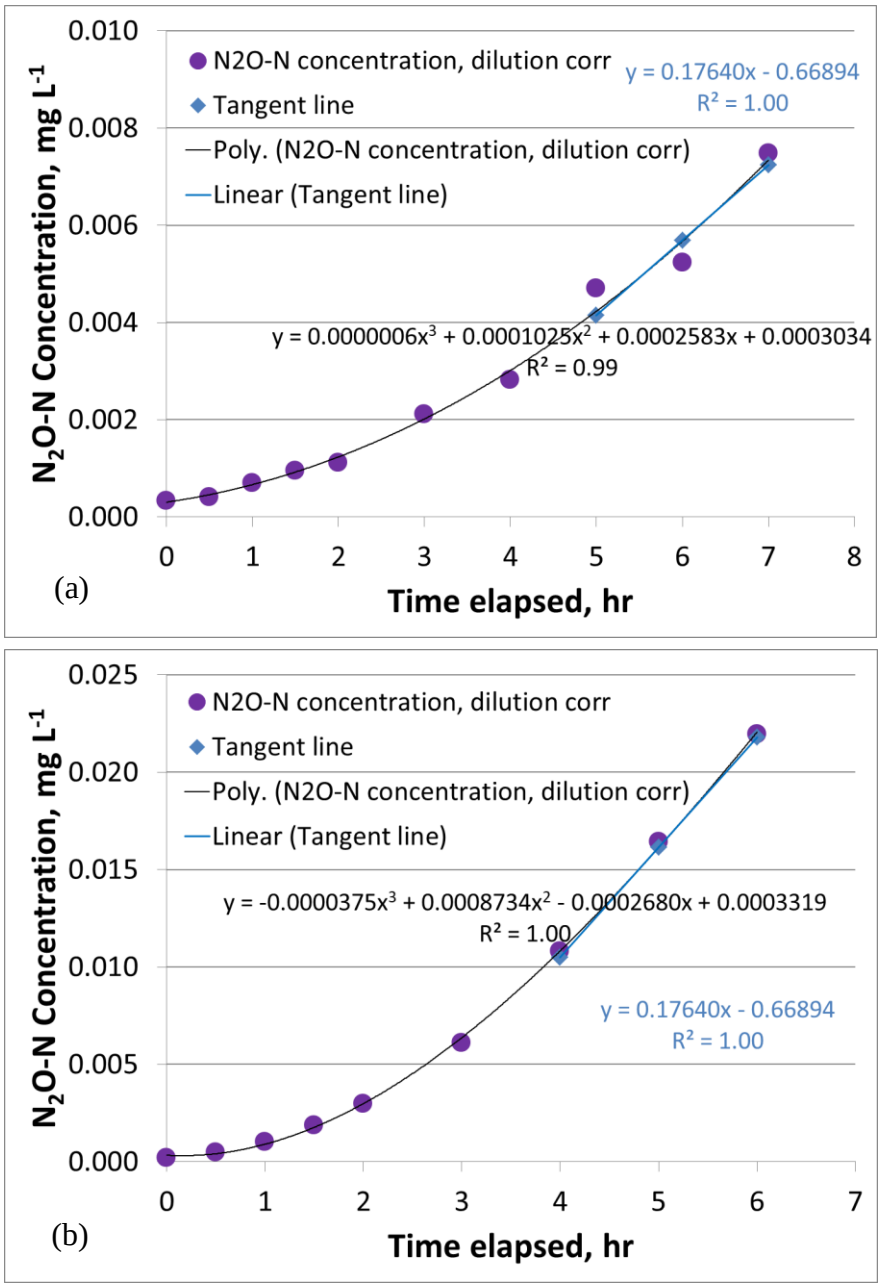


Figure 5.13 Estimating zero-order denitrification rate from a curve fitted on the dilution-corrected nitrous oxide-N concentrations (Sanchez-Perez et al., 2003) for the push-pull tests conducted in (a) February 2015 and (b) August 2015 at a sheep and beef farm near Woodville, New Zealand (WDV site).

Table 5.4 Estimates of denitrification rate by different models based on zero-order and first order-kinetics using denitrification reactant (nitrate-nitrogen) measured during the push-pull tests conducted at the two study sites in the Manawatū catchment, New Zealand.

Push-pull test	**Estimated denitrification rate**					
	Zero-order 1 (several references)*	**Zero-order 2** (Trudell et al., 1986)	**Zero-order 3** (Snodgrass and Kitanidis, 1998)	**First-order 1** (Haggerty et al., 1998)	**First-order 2** (Snodgrass and Kitanidis, 1998)	**First-order 3** (Korom et al., 2005; 2012)
PNR site						
October 2013	-1.067 mg N L^{-1} h^{-1}	Mean: -0.949 mg N L^{-1} h^{-1} Min (0.5 hr): -0.027 Max (3.75 hr): -2.082 Interval (3.25 hr): -1.770	-0.013 mg N L^{-1} h^{-1}	-0.085 h^{-1}	-0.098 h^{-1}	-0.098 h^{-1}
May 2014	-0.555 mg N L^{-1} h^{-1}	Mean: -0.523 mg N L^{-1} h^{-1} Min (0.5 hr): -0.259 Max (7.0 hr): -0.865 Interval (6.5 hr): -0.819	-0.115 mg N L^{-1} h^{-1}	-0.027 h^{-1}	-0.032 h^{-1}	-0.032 h^{-1}
July 2014	-0.438 mg N L^{-1} h^{-1}	Mean: -0.413 mg N L^{-1} h^{-1} Min (0.5 hr): -0.205 Max (7.0 hr): -0.682 Interval (6.5 hr): -0.645	-0.040 mg N L^{-1} h^{-1}	-0.034 h^{-1}	-0.043 h^{-1}	-0.043 h^{-1}
WDV site						
February 2015	-0.046 mg N L^{-1} h^{-1}	Mean: -0.046 mg N L^{-1} h^{-1} Min (0.5 hr): -0.045 Max (7.0 hr): -0.048 Interval (6.5 hr): -0.047	-0.026 mg N L^{-1} h^{-1}	-0.002 h^{-1}	-0.002 h^{-1}	-0.002 h^{-1}
August 2015	-0.119 mg N L^{-1} h^{-1}	Mean: -0.115 mg N L^{-1} h^{-1} Min (0.5 hr): -0.086 Max (6.0 hr): -0.152 Interval (5.5 hr): -0.146	-0.032 mg N L^{-1} h^{-1}	-0.010 h^{-1}	-0.012 h^{-1}	-0.012 h^{-1}

* References: Baker and Vervier, 2004; Istok, 2013; Korom et al., 2012; Tesoriero et al., 2000

Table 5.5 Estimates of denitrification rate by different models based on zero-order and first order-kinetics using denitrification product (nitrous oxide-nitrogen) measured during the push-pull tests conducted at the Palmerston North (PNR) and Woodville (WDV) sites in the Manawatū catchment, New Zealand.

Push-pull test site	**Estimated denitrification rate**	
	Zero-order 4 (Sanchez-Perez et al., 2003)	**First-order 4** (Schurmann et al., 2003)
PNR site		
October 2013	Ave: 0.1692 mg N L^{-1} h^{-1} Max interval: 0.2827	0.0090 h^{-1}
May 2014	Ave: 0.0741 mg N L^{-1} h^{-1} Max interval: 0.1764	0.0041 h^{-1}
WDV site		
February 2015	Ave: 0.0009 mg N L^{-1} h^{-1} Max interval: 0.0017	0.0001 h^{-1}
August 2015	Ave: 0.0030 mg N L^{-1} h^{-1} Max interval: 0.0059	0.0002 h^{-1}

Note: No denitrification rate was estimated based on the denitrification product (nitrous oxide-nitrogen) for the July 2014 test at the PNR site as acetylene was not used in the push-pull test.

The zero-oder rates based on the concentrations of the reactants estimated from the Zero-order 1 model or linear regression methods (Baker and Vervier, 2004; Istok, 2013; Korom et al., 2012; Tesoriero et al., 2000) were in the range of 0.44-1.07 and 0.05-0.12 mg N L^{-1} h^{-1} at the PNR and WDV sites, respectively (Table 5.4). A similar range of denitrifrication rates were obtained using the Zero-order 2 model following Trudell et al. (1986) with mean zero-order rates of 0.41-0.95 and 0.05-0.12 mg N L^{-1} h^{-1} at the PNR and WDV sites, respectively (Table 5.4). These zero-order rates are also comparable to the reported zero-order rates in the literature using the push-pull test technique (0.01-1.12 mg N L^{-1} h^{-1}) (Addy et al., 2002; Istok et al., 1997; Starr and Gillham, 1993; Toda et al., 2002; Trudell et al., 1986; Well et al., 2003). On the other hand, lower zero-order rates were obtained using the Zero-order 3 model following Snodgrass and Kitanidis (1998) with ranges of 0.01-0.12 and 0.026-0.032 mg N L^{-1} h^{-1} at the PNR and WDV sites, respectively (Table 5.4). Moreover, the rates obtained by the Zero-order 3 model do not seem to follow the trend as estimated by the other models as shown above. For instance, with the first two models, Zero-order 1 and Zero-order 2

denitrification rates were found to be highest in the October 2013 test, then in the May 2014 test, and lowest in the July 2014 test at the PNR site. However, the Zero-order 3 estimates based on Snodgrass and Kitanidis (1998) was highest in the May 2014 test, followed by the July 2014 test and the October 2013 test. It is apparent that the way the dilution was accounted for in the calculation of denitrification rates, as with the Zero-order 1 and 2 vs Zero-order 3, has implications for the rates obtained.

In considering results of Zero-order 1 and Zero-order 2 models, which have comparable results, the denitrification rates estimated based on the reactant (NO_3^--N) was observed higher in the October 2013 test and lower in the May and July 2014 tests at the PNR site (Table 5.4). The apparent decrease in denitrification rates in the May 2014 and July 2014 tests is not unique considering the different time for the tests as this has also been observed in other studies (e.g., Korom et al., 2012). Possible factors for this decrease in denitrification rates in May 2014/July 2014 tests may include increase an ORP and decrease in *pH* (Table 5.3). At the WDV site, denitrification rate in February 2015 was lower than in August 2015 (Table 5.4). However, this difference may not be of particular importance considering the low denitrification rate and lack of difference in *pH*, DO and DOC in groundwater. Nevertheless, the greater rate in August 2015 could be due to acclimitisation of denitrifiers brought by the earlier test in February 2015, considering that NO_3^- leaching to groundwater at the WDV site could be low due to the fine-textured soil in the vadose zone and this seems to be reflected in the low background groundwater NO_3^- concentrations (< 0.03 mg N L^{-1}). For instance, several studies (e.g., Eschenbach et al., 2015) showed that conducting preliminary push-pull tests results in preconditioning and stimulation of the denitrifiers and therefore in much greater denitrification rates (30-65 times) than those measured without such preconditioning.

Using denitrification product for estimating zero-order rates, in particular following Sánchez-Pérez et al. (2003), the denitrification rates obtained at the PNR site were 0.169 and 0.074 mg N L^{-1} h^{-1} in October 2103 and May 2014, respectively, whereas at the WDV site the rates were lower at 0.001 and 0.003 mg N L^{-1} h^{-1} in February and August 2015, respectively (Table 5.5). These rates are generally comparable to the zero-order rates of 0.006-1.97 mg N L^{-1} h^{-1} estimated by other studies using the denitrification product (Sanchez-Perez et al., 2003). Note that a zero-order denitrification rate based on the product (N_2O-N) measurements could not be estimated for the July 2014 test at the PNR site as acetylene was not used in this test and thus N_2O-N could not be considered as the terminal product of the denitrification process (Table 5.1). Interestingly, the zero-order rates based on the reactant were many times higher (6 to 50 times) than the rates estimated based on the product (Tables 5.4 and 5.5). The discrepancies in these estimates are discussed later in section 5.3.4 (below) comparing denitrification rates obtained using the reactant (NO_3^--N) and product (N_2O-N).

5.3.3 First-order denitrification rates

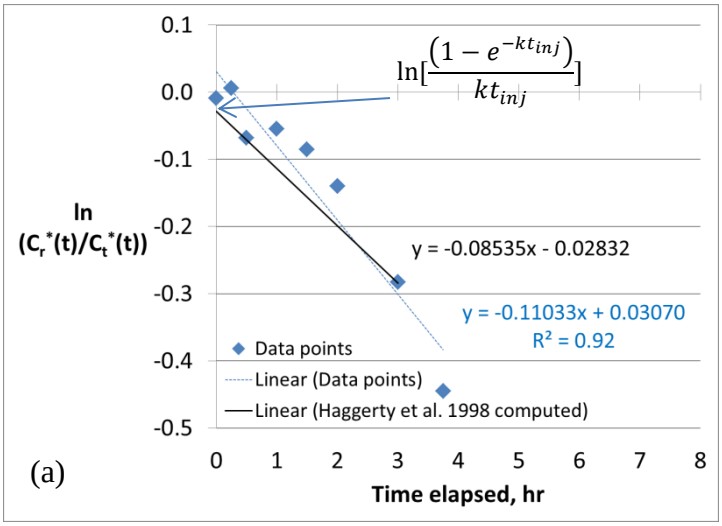
Three models were applied to estimate the first-order denitrification rates using the reactant namely, First-order model 1 (Haggerty et al., 1998), First-order model 2 (Snodgrass and Kitanidis, 1998), and First-order model 3 (Korom et al., 2012, 2005). Following Haggerty et al. (1998), Figures 5.14 and 5.15 show the plots for estimates of first-order denitrification rate based on the reactant during the tests at the PNR and WDV site, respectively. In Haggerty et al. (1998), forcing y to a certain value at time t=0 assumes that a similar reduction rate is in effect to reduce NO_3^- during injection and thus less NO_3^- was expected at the end of injection. This consequently results in the plot line starting (at t=0) a bit lower than the sample data (Figures 5.14 and 5.15). Plots for estimating first-order denitrification rate following Snodgrass and Kitanidis (1998) are shown in Figures 5.16 and 5.17 for the PNR and WDV

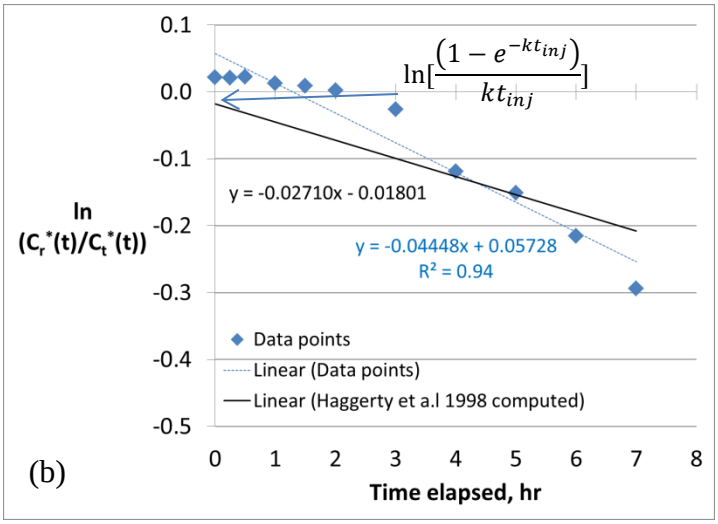
sites, respectively. Moreover, the plots following Korom et al. (2012, 2005) are provided in Figures 5.18 and 5.19 for the two sites, respectively.

Table 5.4 summarises the first-order denitrification rates quantified by the above-mentioned models based on the reactant. The estimated first-order denitrification rates are generally comparable among the three models for each of the push-pull test conducted. First-order rates were in the range 0.027-0.098 h^{-1} and 0.002-0.012 h^{-1} at the PNR and WDV sites, respectively. The rates obtained at the WDV site were in the range of observed first-order rates in a few similar studies conducted elsewhere (0.001-0.02 h^{-1}) (Burbery et al., 2013; Korom et al., 2012), whereas the rates at the PNR site were greater. However, it is difficult to assess the first-order rates obtained in this study in terms of the different groundwater environments given the limited studies that quantify denitrification in shallow groundwater. The main difference between the model of Haggerty et al. (1998) and that of Snodgrass and Kitanidis (1998) and Korom et al. (2012, 2005) is that the latter models do not assume NO_3^- reduction during injection. These latter models may not be appropriate for push-pull tests with long injection duration in which NO_3^- reduction may be significant but suitable for short injection duration such as in this study, as supported by the comparable estimates of the first-order rates by the models (Table 5.4).

Similar to the zero-order denitrification rates, the first-order denitrification rates were also larger (0.09 h^{-1}) during the October 2013 test as compared to the July 2014 and May 2014 tests (0.03-0.04 h^{-1}) at the PNR site (Table 5.4). Moreover, a lower denitrification rate was observed at the WDV site in February 2015 (0.002 h^{-1}) compared to August 2015 (0.010-0.012 h^{-1}) (Table 5.4).

The model of Schürmann et al. (2003) was applied to quantify first-order denitrification rates based on measurements of product (N_2O-N) during the push-pull tests. The estimated first-order denitrification rates were also measured higher (0.009 h^{-1}) during the October 2013 test as compared to the July 2014 tests (0.004 h^{-1}) at the PNR site (Table 5.5). These rates are comparable to the results (0.0079-0.01 h^{-1}) from limited studies using the same model (Schürmann et al., 2003). Lower rates were obtained at the WDV site (0.0001-0.0002 h^{-1}). Nevertheless, a greater rate was also estimated for the February 2015 test compared to the August 2015 test (Table 5.5). Similar to the results using zero-order models, the first-order denitrification rates estimated using the reactant were multiple times (8 to 11 times at the PNR site; 20 to 60 times at the WDV site) higher than the first-order estimates based on the product (Tables 5.4 and 5.5).





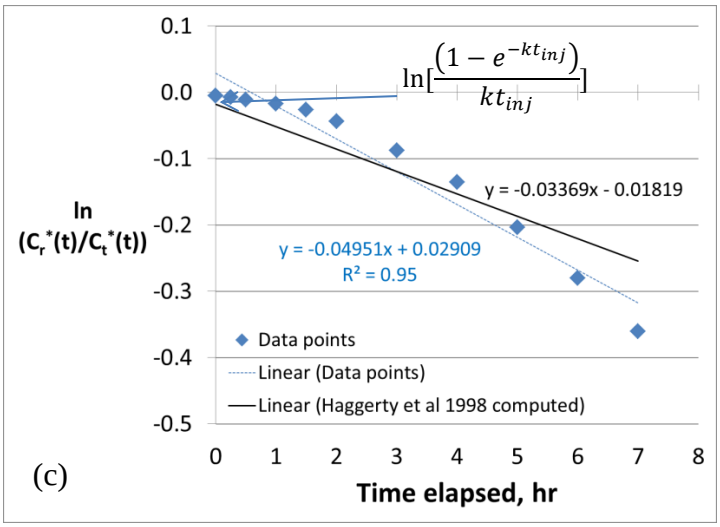


Figure 5.14 Estimating first-order denitrification rate based on Haggerty et al. (1998) using the nitrate-N concentrations measured during the push-pull tests conducted in (a) October 2013, (b) May 2014, and (c) July 2014 at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand (PNR site). The data points shown are average values of triplicates.

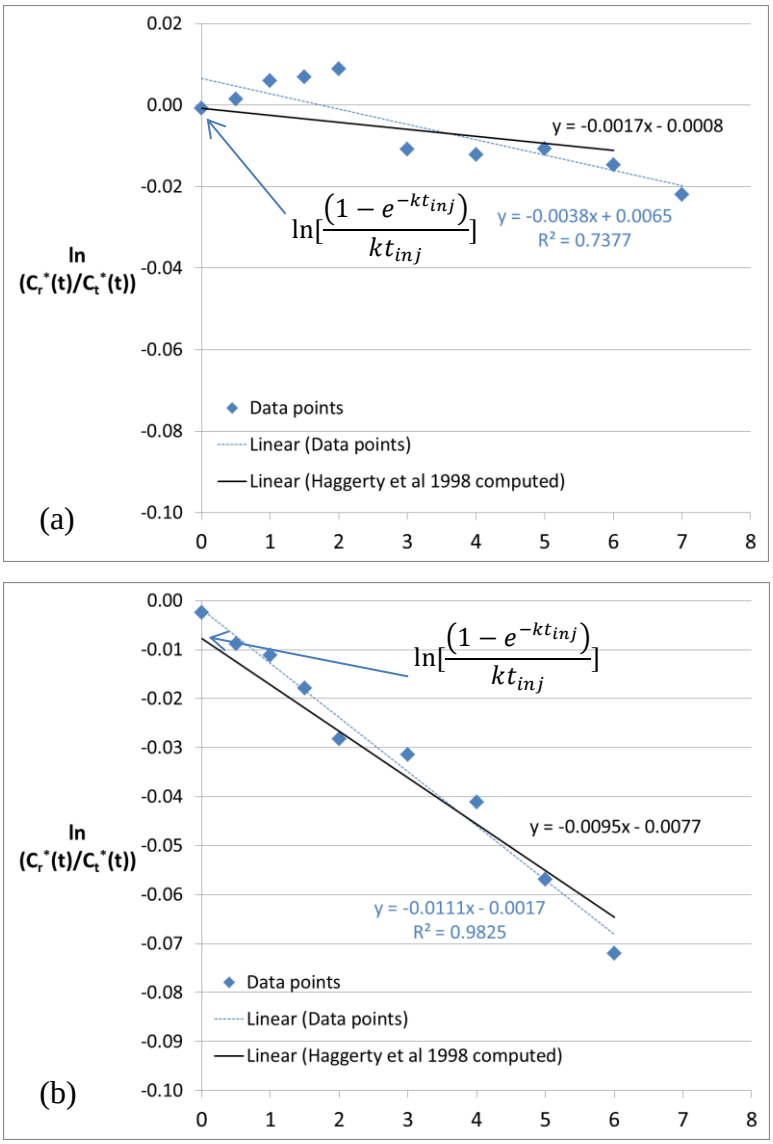


Figure 5.15 Estimating first-order denitrification rate based on Haggerty et al. (1998) using the nitrate-N concentrations measured during the push-pull tests conducted in (a) February 2015 and (b) August 2015 at a sheep and beef farm near Woodville, New Zealand (WDV site). The data points shown are average values of triplicates.

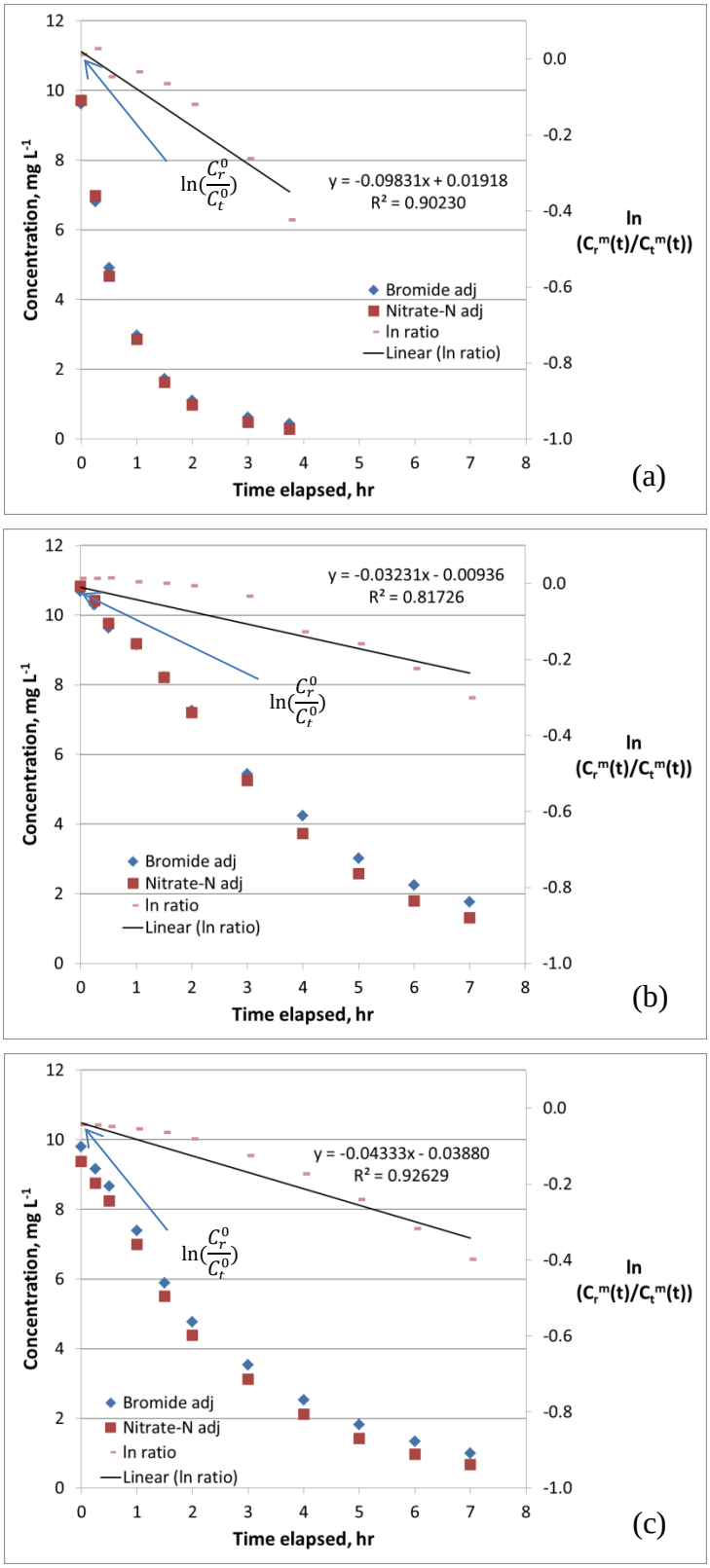


Figure 5.16 Estimating first-order denitrification rate based on Snodgrass and Kitanidis (1998) for the push-pull tests conducted in (a) October 2013, (b) May 2014, and (c) July 2014 at Massey No. 1 Dairy Farm, Palmerston North, New Zealand (PNR site).

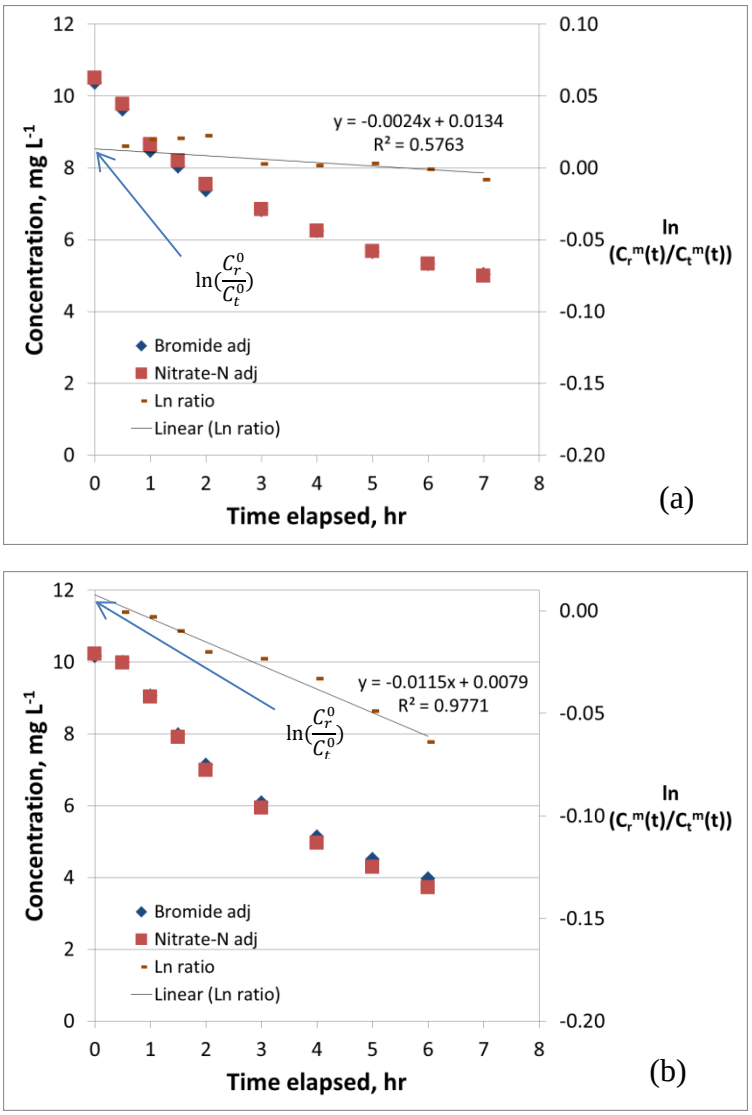


Figure 5.17 Estimating first-order denitrification rate based on Snodgrass and Kitanidis (1998) for the push-pull tests conducted in (a) February 2015, and (b) August 2015 at a sheep and beef farm near Woodville, New Zealand (WDV site).

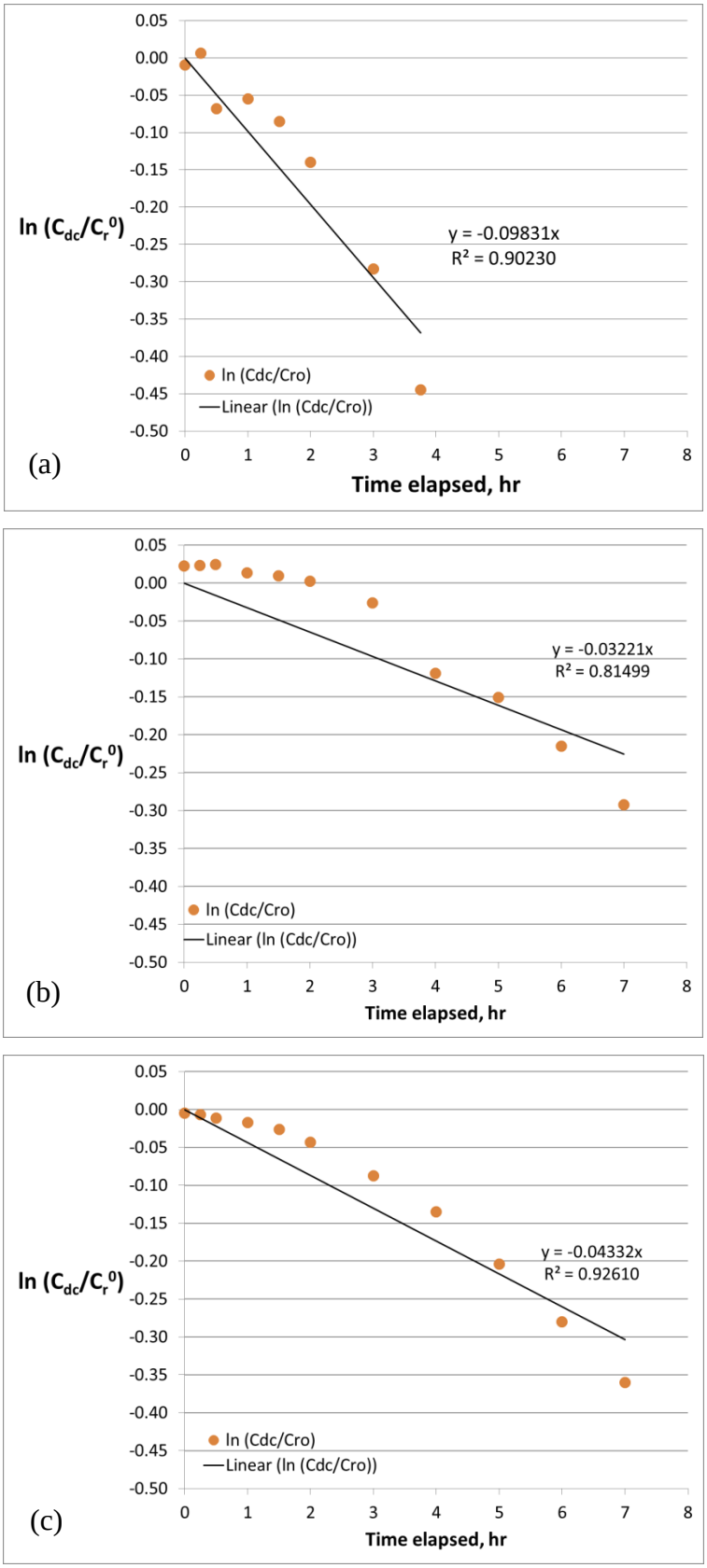


Figure 5.18 Estimating first-order denitrification rate based on Korom et al. (2005, 2012) for the push-pull tests conducted in (a) October 2013, (b) May 2014, and (c) July 2014 at Massey No. 1 Dairy Farm, Palmerston North, New Zealand (PNR site).

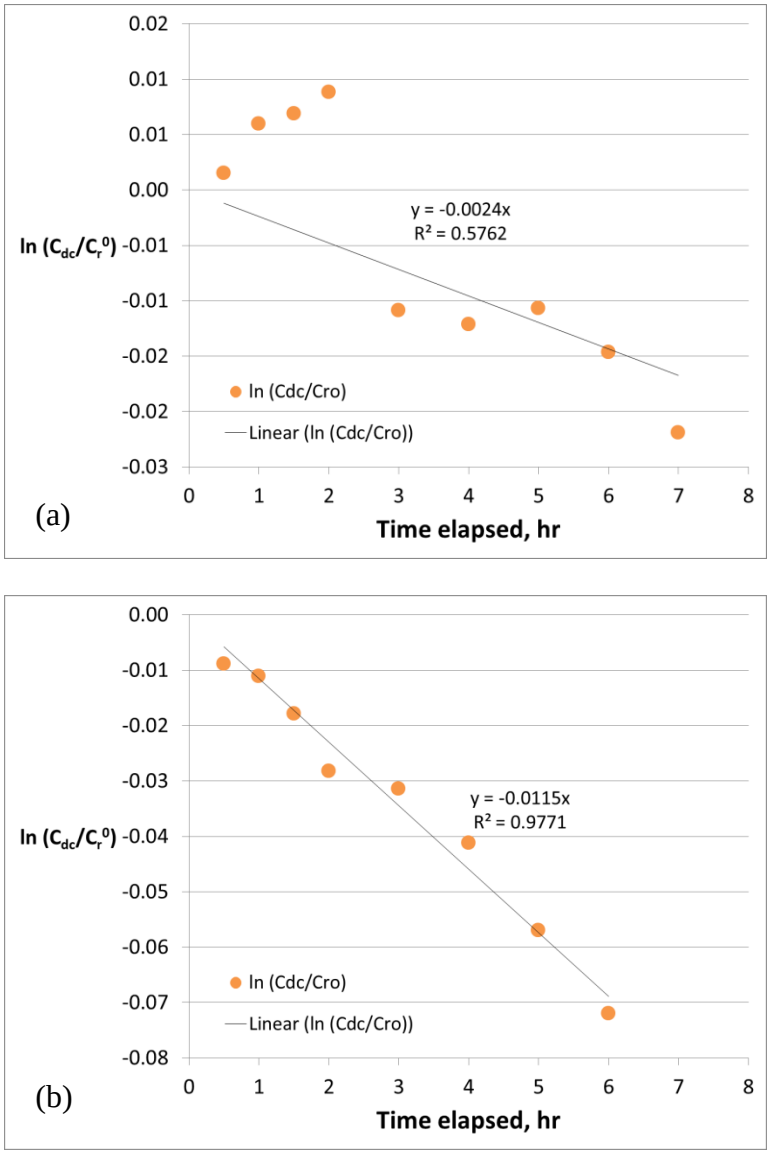


Figure 5.19 Estimating first-order denitrification rate based on Korom et al. (2005, 2012) for the push-pull tests conducted in (a) February 2015 and (b) August 2015 at a sheep and beef farm near Woodville, New Zealand (WDV site).

5.3.4 Comparison of denitrification rates obtained using denitrification reactant (NO_3^--N) and product (N_2O-N)

Tables 5.4 and 5.5 show that denitrification rate estimates can vary depending on which tracer is measured and used (e.g. the reactant (NO_3^--N) or product (N_2O-N)). The denitrification rates estimated based on the NO_3^--N reduction were much higher (6 to 60 times) than the rates estimated based on the N_2O-N production during the push-pull tests. This discrepancy in rate estimation has also been observed in soil denitrification assays (Yu et al., 2008). Figure 5.4 shows the dilution-corrected concentrations of NO_3^--N, NO_2^--N and N_2O-N observed during the May 2014 test at the PNR site. In this test, from the fourth hour of the 'pull' phase, the sum of NO_2^--N and N_2O-N accounted for approximately 40% of the NO_3-N reduced. While ammonium-N concentrations were not measured in samples from the push-pull test conducted in May 2014, measurements obtained for the October 2013 test at the same site showed very low ammonium-N concentrations (<0.08 mg N L^{-1}; mostly <0.05 mg N L^{-1}). Thus, it is unlikely that dissimilatory NO_3^- reduction to ammonium (DNRA) was a significant process for the reduction of NO_3^--N in shallow groundwater at the study site. The amounts of NO_2^--N, N_2O-N and ammonium-N concentrations combined in the samples could not account for the total NO_3^--N lost during the test. It is therefore possible that, apart from denitrification and DNRA, there may be other processes involved such as accumulation of microbial biomass and/or incomplete inhibition of N_2O conversion to N_2 by the acetylene used during the tests (Starr and Gillham, 1993).

Few studies have investigated estimates of denitrification rates using both NO_3^- reduction and N_2O production. While Gillham et al. (1990) found an increase in N_2O-N corresponding to NO_3^--N lost, other studies did not provide conclusive explanations of the discrepancies

between the rates obtained based on NO_3^--N reduction and N_2O-N production (e.g. Istok et al., 1997; Starr and Gillham, 1993). Schürmann et al. (2003) measured first-order denitrification rates (based on Haggerty et al., 1998) from reactants and products (N_2+N_2O) using ^{15}N-labelled NO_3^- in push-pull tests. They also measured nitrite and ammonium concentrations, and conducted a mass balance based on recovery (relative) values and accounted for the portion of NO_3^- lost from products. They attributed unaccounted NO_3^- losses to other processes, such as abiotic NO_3^- consumption, DNRA (based on NH_4^+ increase; although this minor process was unlikely to affect the mass balance) and assimilatory NO_3^- reduction (from ^{15}N recovered in suspended biomass; although this was not quantified) based on ^{15}N isotope analyses. It seems, therefore, that without measuring all the other species of N in both water and sediment samples during the push-pull test (e.g., NO_3^--N, nitrite-N, ammonium-N, organic-N, N_2O-N, NO-N, N_2-N), the denitrification rate estimates remain indicative and not absolute.

Nevertheless, given the complexity in ascertaining the complete inhibition of nitrous oxide reduction (Starr and Gillham, 1993) and uncertainties in capturing all the nitrous oxide produced *in-situ* in push-pull tests adopting the acetylene inhibition method, the estimated denitrification rate based on the reactant (NO_3^--N) may provide a representative value of the denitrification characteristics of shallow groundwater. This is reasonable given that the limited number of studies that assessed possible processes influencing the reduction of nitrate do not indicate significant contribution from other NO_3^- attenuation processes (Schürmann et al., 2003; Starr and Gillham, 1993).

5.3.5 Comparison of denitrification rates obtained by zero-order and first-order kinetic models

This study applied different kinetic models to estimate zero- and first-order denitrification rates analysing the observed changes in reactant (NO_3^--N) and product (N_2O-N) during the push-pull tests (Tables 5.4 and 5.5). Denitrification rates are typically considered to be dependent on the substrate (NO_3^-) at low concentrations, approximating first-order kinetics, and independent on substrate at higher concentrations, approximating zero-order kinetics (Tesoriero and Puckett, 2011). While first-order rate estimates may be adequate to describe the reduction processes in low concentrations (Burbery and Wang, 2010), studies have identified varying threshold concentrations where the transition from zero-order to first-order kinetics was observed to occur (Bowman and Focht, 1974; Knowles, 1982; Starr and Gillham, 1993; Yu et al., 2008). For instance, Tchobanoglous et al. (2014) stated that "the effect of the NO_3^- concentration on substrate utilisation rate is only at concentrations below 0.10 to 0.20 mg of NO_3 L^{-1}", much lower than the threshold of 1.0 to 1.5 mg of NO_3 L^{-1} observed by Bowman and Focht (1974).

The differing threshold concentrations for the transition between zero-order and first-order kinetics could be due to the fact that other factors influence the denitrification process. These include the availability of an electron donor and suitable denitrifier populations (Bekins et al., 1998; Bowman and Focht, 1974), not to mention the required low concentration of oxygen or anoxic conditions. These site specific factors (denitrifier population, electron donor, etc.) may be addressed by the use of a half-saturation constant as a location-specific parameter assuming no-growth Monod or Michaelis-Menten kinetics. "The half-saturation constant is the concentration of substrate at which the transformation rate is half the maximum value" (Bekins et al., 1998). First-order kinetics is considered in play when substrate concentration is

much less than the half-saturation constant (Bekins et al., 1998). Bekins et al. (1998) and Counotte and Prins (1979) presented ways to estimate the half-saturation constant by assuming constant microbial biomass, justified considering the insignificant change observed in the microbial population even after prolonged exposure (Bekins et al., 1998).

This study attempted to estimate the half-saturation constant for the push-pull tests following the method of Counotte and Prins (1979), using the time-series NO_3^--N concentration data observed during the May 2014 push-pull test at the PNR site. A high half-saturation constant of 4.0 mg N L^{-1} was obtained using NO_3^--N concentrations uncorrected for dilution, whereas a negative value using dilution-corrected NO_3^--N concentrations measured during the test. There are several reasons that could point out that the methods for estimating half-saturation constant (Counotte and Prins, 1979; Bekins et al. 1998) may not be directly applicable for the push-pull test data collected from an open system. The methods of Bekins et al. (1998) and Counotte and Prins (1979) for estimating half-saturation constant are basically based on closed-system incubation in which the decrease in substrate concentration was solely due to microbial activities. On the other hand, the NO_3^--N concentration data of the push-pull test are significantly influenced by the dilution of NO_3^--N in groundwater during the test. The use of NO_3^--N concentrations uncorrected for dilution would imply that the decrease in concentrations were due only to microbial activity and the effect of dilution being disregarded, hence would result to an erroneous half-saturation constant. On another hand, using the dilution-corrected NO_3^- concentrations would mean using high NO_3^--N concentrations in the estimation of a half-saturation constant. This misrepresents the actual conditions (low NO_3^- concentration at later stages of the test) that influence the microbial activity, and subsequently the estimation of half-saturation constant. Moreover, the increasing instantaneous zero-order denitrification rate with time observed in this study following

Trudell et al. (1986) indicates higher denitrification rates measured at the later stage of all the tests when NO_3^- concentrations have already gone down (Tables 5.4 and 5.5; Figs. 5.8, 5.9, 5.12 and 5.13). This is contrary to the Michaelis-Menten or Monod model in which reaction rate is expected to be higher at higher substrate concentration. This could be another reason that a reasonable half-saturation constant could not be estimated with the push-pull test data. However, developing a method to estimate half-saturation constant in cases where dilution plays a big part like in a push-pull test is beyond the scope of this study.

Several authors have used other assessment methods such as residual some of squares (RSS) (Liao et al., 2012) and coefficient of determination (R^2) (Korom et al., 2012, 2005) to determine which model is more appropriate for their data. These performance statistics were determined for the denitrification rates estimated using the reactant only (Table 5.6), considering the outcome of comparison with results using the denitrification product (section 5.3.4). However, no metrics could be estimated for the zero-order rates that were estimated using tangent lines to the curve using Trudell et al., (1986) (Zero-order 2 model) as the rates vary depending on the location of the tangent line. While zero-order models have larger RSS values than the first-order models (Table 5.6), this difference is immaterial and could not be used to compare between the two types of kinetic models because RSS is unit dependent and the units for zero-order and first-order models are not similar. Generally, comparable R^2 were obtained for all the models assessed, except for odd values in a couple of instances (Zero-order 3, PNR site, Oct 13 [R^2=0.03]; First-order 1, WDV site, Feb 15 [R^2= 0.329]). Disregarding these two R^2 values, Zero-order 1 model gave R^2 values of 0.93-0.98 and 0.80-0.98 at the PNR and WDV sites, respectively; Zero-order 3 model gave R^2 of 0.82-0.90 and 0.73-0.91, respectively; First-order 1 model gave R^2 of 0.73-0.85 and 0.94, respectively; First-order 2 and 3 models gave similar R^2 of 0.82-0.93 and 0.58-0.98, respectively. Thus, it

appears that, based on these metrics alone, the use of either zero-order or first-order kinetics appears reasonable to estimate the denitrification rate for using the push-pull test data. These established methods for estimating denitrification rates with zero-order or first-order kinetic models use different terms to account for the effect of dilution on NO_3^- concentrations in estimating the NO_3^- reduction rate (Table 5.2). Methods used to estimate zero-order rates used a dilution factor which is a ratio of tracer (e.g., bromide) concentration of the injection solution to the tracer concentration at time of measurement (Sanchez-Perez et al., 2003; Trudell et al., 1986). On the other hand, established methods for estimating first-order rates by Haggerty et al. (1998) used the ratio of the relative concentrations of NO_3^- (ratio of measured concentration with the injection solution concentration) and tracer (e.g., bromide). As a result of this difference in representing the 'y' component of the regression line, both zero-order or first-order kinetic models may yield satisfactory R^2 results for both kinetic models, as has been observed in other studies (Korom et al., 2012; Tesoriero and Puckett, 2011). However, use of either zero-order or first-order denitrification rates may have impact on the simulation of NO_3^- transport and transformation in groundwater systems. Quantification of the effects of the denitrification rates based on either reactant or product, and zero-order or first-order kinetics (Tables 5.4 and 5.5), such as in simulating NO_3^- delivery from groundwater to receiving surface water, is beyond the scope of this study and a subject of further study.

Table 5.6 Comparison of different models assuming zero-order or first-order kinetics for the push-pull tests conducted at Massey No. 1 Dairy Farm, Palmerston North (PNR site) and at a sheep and beef farm near Woodville (WDV site).

Approach/Method	Residual Sum of Squares, RSS					Coefficient of Determination, R^2				
	PNR site			WDV site		PNR site			WDV site	
	Oct 13	May 14	Jul 14	Feb 15	Aug 15	Oct 13	May 14	Jul 14	Feb 15	Aug 15
Reactant-based models										
Zero-order 1	0.6786	0.4753	0.1837	0.0221	0.0080	0.927	0.965	0.978	0.805	0.980
Zero-order 2*	-	-	-		-	-	-	-		
Zero-order 3	0.0704	0.0839	0.0207	0.0106	0.0060	0.030	0.903	0.824	0.726	0.906
First-order 1	0.0248	0.0341	0.0224	0.0005	0.0002	0.851	0.728	0.853	0.329	0.944
First-order 2	0.0163	0.0229	0.0113	0.0012	0.0127	0.902	0.817	0.926	0.576	0.977
First-order 3	0.0163	0.0229	0.0113	0.0004	0.0001	0.902	0.815	0.926	0.576	0.977

Note: *No performance statistics computed as the estimated rates were variable based on the tangent of the curve approximating the data; similarly with rates estimated using the denitrification product.

5.4 Conclusions

The push-pull tests conducted at two study sites quantified the denitrification rate in shallow groundwater. The use of acetylene in the push-pull tests reinforced the evidence of denitrification. Also, a comparison of N_2O-N concentrations measured during the push-pull test with acetylene (May 2014 test at the PNR site) and without acetylene (July 2014 test at the PNR site) indicates the potential of partial or complete denitrification in shallow groundwater as observed in the study.

The denitrification rates estimated based on the measurements of denitrification reactant (NO_3^- reduction) were quantified much higher (6 to 60 times) compared to the rates estimated based on the measurements of denitrification product (nitrous oxide production). These differences may be attributed to other processes such as dissimilatory NO_3^- reduction to ammonium (DNRA), assimilatory NO_3^- reduction, and/or incomplete inhibition of nitrous oxide reduction during the tests. It seems, therefore, that without measuring all the other species of N in both water and sediment samples during the push-pull test (e.g., NO_3^--N, nitrite-N, ammonium-N, organic-N, N_2O-N, NO-N, N_2-N), the denitrification rate estimates remain indicative and not absolute. Nevertheless, given that previous studies that attempted to measure the other species of N during a test found that other processes are not significant compared to the reduction by the denitrification process, the denitrification rate quantified on the basis of the reactant (NO_3^- reduction) may provide representative value of denitrification characteristics of shallow groundwater.

This study attempted to resolve the question on which kinetic model (zero- or first-order) should be used in estimating denitrification rates but the results were short of providing definitive answers. Statistical comparison of data from two study sites showed that either a zero-order or a first-order model may be valid for estimating the denitrification. This may have significant implications in the representation of spatial and temporal variability in denitrification characteristics of the shallow groundwater system including in simulation models. Having a robust estimate of different denitrification rates estimated by zero-order or first-order kinetics could inform appropriate scale of denitrification rate measurements and simulation of NO_3^- transport and transformation processes in shallow groundwater systems.

CHAPTER 6

CONTRASTING DENITRIFICATION CHARACTERISTICS IN THE VADOSE ZONE AND SHALLOW GROUNDWATER BENEATH TEMPERATE PASTURE LANDS AND THEIR IMPLICATIONS FOR NITRATE MANAGEMENT IN AGRICULTURAL LANDSCAPES

Abstract

A sound understanding of nitrogen loss from agricultural soils, and its transport and transformation in soil-water systems is essential for targeted and effective management and/or mitigation of their impacts on the quality of receiving freshwaters across agricultural landscapes. This study investigated the denitrification characteristics in the vadose zone and shallow groundwater at four sites under pastoral farming in the Manawatū River catchment, located in the lower part of North Island, New Zealand. The denitrification potential of the vadose zone was determined by the laboratory incubation assays measuring the denitrifying enzyme activity (DEA) in soil samples collected from different soil horizons (up to 2.1 m below ground surface), whereas denitrification rates in shallow groundwaters were measured *in situ* by single-well, push-pull tests conducted in piezometers installed at multiple depths at each site. Results of this study strongly suggest that different hydrogeologic settings influence spatial variability of denitrification characteristics affecting transport and transformation of nitrate in the subsurface environment. Where the vadose zone is thin and composed of coarse-textured soils, percolation of nitrate was evident in observed high nitrate concentrations in oxic and young shallow groundwaters, but low nitrate concentrations and reduced older shallow groundwater was found underneath the fine textured soils and/or a thick vadose zone. These contrasting denitrification characteristics in subsurface environment suggest that the ultimate delivery of nitrate losses from agricultural soils depends on its

attenuation in the vadose zone and in shallow groundwater where highly oxic groundwater offer limited reduction potential but further nitrate reduction may be expected in reduced groundwater conditions.

Keywords: Agriculture; Water quality; Nitrate attenuation; Push-pull test; Acetylene inhibition; Nitrate management; Manawatū catchment; New Zealand

6.1 Introduction

The growing concern on the impact of intensive agricultural activities on the quality of groundwater and surface water has led countries to implement policies to protect freshwater bodies across agricultural landscapes. Examples include the implementation of the EU Water Framework and Nitrates Directives (E.Union, 1991) and the New Zealand's National Policy Statement for Freshwater Management 2014 (MfE, 2014). Such policies aim to minimise the environmental impact of anthropogenic activities including intensive agricultural farming by implementing a variety of nutrient management actions. Efforts to lessen the impact of diffuse nitrogen (N) discharge to waters focus on identifying and reducing N losses from the root zone of farms (Monaghan et al., 2007; Wu and Ma, 2015). These efforts can be more effectively targeted, when there is a sound understanding not just of the N losses from farm root zone but also its transport and transformation processes along their flow pathways from farms to receiving freshwaters. Nitrate (NO_3^-), a key nutrient implicated in contamination of groundwaters and surface waters, could be attenuated by biogeochemical processes such as denitrification in the subsurface environment (Knowles, 1982; Rivett et al., 2008). Hence, a sound understanding of the NO_3^- attenuation characteristics in the subsurface environment is

an important consideration when identifying specific and effective measures to manage and mitigate the impacts of agricultural activities on groundwater and surface water quality.

The cycling and leaching of NO_3^- from surface soils is well studied (Jahangir et al., 2012c; Jarvis and Hatch, 1994; Kamewada, 2007; Luo et al., 1998; Peterson et al., 2013), and there is a growing interest in further measurements of denitrification potential in the subsurface environment to better understand and quantify transport and transformation of NO_3^- before it reaches ground and surface waters (Castle et al., 1998; Elmi et al., 2005; Luo et al., 1998). Jahangir et al. (2012c) found that approximately 10% of N inputs can be denitrified in a specific layer of the subsoil (0.45-0.55 m below ground level (bgl)) in Wexford, Ireland. Thomas et al. (2012) found that 5-10% of fertiliser applied and N present in the soil profile (0.6 – 1.0 m bgl) was denitrified in the vadose zone composed of Pahau silt loam soil (Mottled Argillic Pallic) in Canterbury region, New Zealand. Several studies have also shown significant reduction of NO_3^- in groundwater systems (Seitzinger et al., 2006; Singleton et al., 2007; Tesoriero et al., 2000), some quite shallow (Clague et al., 2013). For instance, Jahangir et al. (2013) found in an investigation in a grassland site in southeastern Ireland that denitrification in groundwater accounted for 24% of N inputs to the land. However, the denitrification characteristics of the subsurface environment vary across different landscapes (Oehler et al., 2007; Seitzinger et al., 2006).

Limited studies have investigated both the vadose and saturated zones in an integrated manner to characterise and quantify the potential for denitrification in the subsurface environment across agricultural landscapes (e.g., Paramasivam et al., 1999; Grimaldi et al., 2011; Yuan et al., 2012). Most of existing denitrification studies have been focused on either the vadose (Cannavo et al., 2004; Castle et al., 1998; Jahangir et al., 2012c; Kamewada,

2007) or the saturated zone (Bragan et al., 1997; Jahangir et al., 2014, 2013; Toda et al., 2002). Investigating a single zone results in incomplete information on the fate of NO_3^- that could leach to groundwater.

This lack of detailed and integrated investigation of the vadose zone and shallow groundwater is particularly true for New Zealand's agricultural landscape, where several field studies have focused on investigating denitrification either in the vadose (Barkle et al., 2007; Deslippe et al., 2014; Luo et al., 1998; Peterson et al., 2013) or the saturated zone (Clague et al., 2015b; Collins et al., 2017; Stenger et al., 2008), and rarely (e.g., Clague et al., 2013) focused on both the vadose and saturated zones. Other studies have been limited to general assessment of NO_3^- attenuation at catchment scales and lack detailed measurements and characterisation of denitrification in the subsurface environment (Rivas et al., 2017; Singh et al., 2014). For instance, a recent study provided evidence of the variable groundwater redox potential in the Manawatū catchment in the lower North Island of New Zealand (Rivas et al., 2017; Chapter 3), but no quantitative measurements of denitrification have been conducted for both the vadose zone and groundwater in the catchment. Detailed investigations that include quantitative measurements of the denitrification characteristics in the subsurface environment is particularly relevant in the Manawatū catchment wherein the estimated N loads in rivers have been found to vary across sub-catchments (Elwan et al., 2015).

This study aims to advance our limited knowledge and capability to assess effects of different hydrogeologic settings on transport and attenuation of NO_3^- in the subsurface environment. This study investigates the denitrification characteristics in the vadose and saturated zones at four selected sites under different hydrogeologic settings across the Manawatū River catchment. In particular, this study aimed to i) quantify denitrification both in the vadose and

saturated zones, ii) determine the spatial and temporal variability in denitrification across sites and within sites, iii) investigate the factors responsible for variability in subsurface denitrification, and iv) determine the implications on NO_3^- transport and transformation in the subsurface environment.

6.2 Materials and Methods

6.2.1 Study area and selection of study sites

The study area, the Manawatū River catchment, is located in the lower part of North Island, New Zealand covering an area of approximately 6,000 km^2 (Figure 6.1). It has a temperate climate favourable for pastoral farming. Land cover in the catchment is dominated by pastoral farming covering 75%, with sheep and/or beef farming accounting for 57.7% and dairy farming for 17.3%, whereas native and exotic cover account for just over 21% of the catchment (Clark and Roygard, 2008). Cropping and horticulture covers only approximately 1% of the catchment (Clark and Roygard, 2008). Pastoral farming and intensive land uses are major sources of NO_3^-, one of the key nutrients implicated in contamination of groundwater and surface waters in the catchment (Ledein et al., 2007).

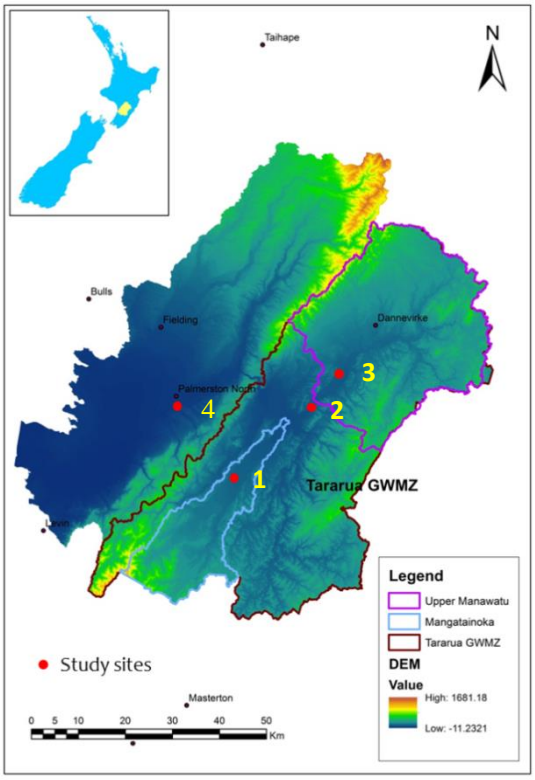


Figure 6.1 Locations of the study sites at 1) Pahiatua, 2) Woodville, 3) Dannevirke, 4) Palmerston North in the Manawatū River catchment, New Zealand.

The landform of the catchment is dominated by the axial ranges (Tararua and Ruahine ranges) traversing the middle of catchment in a southwest to northeast direction and dividing the catchment into two with the east portion covering approximately 3200 km^2 with the rest of the area in the west portion of the catchment. Elevation ranges from sea level to approximately 1700 m amsl in the axial ranges. The axial ranges are comprised of low permeability greywacke basement rocks (Rawlinson and Begg, 2014), and this rock type covers approximately 17% of the study area. Other major rock types include alluvium (11%), sandstone (9%), loess over gravel (7%), loess (6%), and mudstone (6%). The geology of the north portion of the western side of the ranges is mainly comprised of marine terrace deposits and marginal marine and terrestrial deposits of variable composition, whereas the southern portion is dominated by more recent terrestrial deposits (Pattle Delamore Partners Ltd., 2013a). In the eastern side, the broad depression known as the Pahiatua basin is filled with

Tertiary sediments mainly comprised of marine mudstone and sandstone (Rawlinson and Begg, 2014). A thin layer of late Quaternary sediments is considered to form the aquifers in the area (Zarour, 2008). More description on the eastern side of the catchment is provided by Rivas et al. (2017) and references therein. The redox status of groundwater in the catchment was found to vary widely with reduced groundwater found both in the eastern and western parts of the catchment (Daughney et al., 2009; Rivas et al., 2017).

Four sites were selected (Figure 6.1) to measure and characterise denitrification in vadose (unsaturated) and shallow groundwater (saturated) zones of the subsurface environment in the Manawatū catchment. The selection of study sites was based on findings of earlier studies which identified areas as having high or low denitrification potential based on groundwater surveys conducted in the area (Rivas et al., 2017; Singh et al., 2014). Two of the selected sites were identified as highly reducing shallow groundwaters (Palmerston North and Woodville sites), and other two sites as oxic or mixed (oxic-anoxic) shallow groundwaters (Pahiatua and Dannevirke sites). Table 6.1 provides the general characteristics of these sites with respect to land use, soil types, underlying rock type and depth to groundwaters. The Pahiatua, Dannevirke and Palmerston North sites were located under intensive dairy farming, while the Woodville site was under sheep and beef farming. Rock types at the Pahiatua and Dannevirke sites are comprised of loess over gravel with relatively thinner vadose zones (1.43-3.38 m below ground level or bgl). On the other hand, rock type at the Palmerston North and Woodville sites was alluvium with thicker vadose zones (3.02-4.65 m bgl).

Table 6.1 Characteristics of the study sites in the Manawatū River catchment.

Characteristics	Site 1 Pahiatua	Site 2 Woodville	Site 3 Dannevirke	Site 4 Palmerston North
Land use*	Dairy	Beef/sheep	Dairy	Dairy
Soil type	Kopua stony silt loam	Kairanga silt loam and clay loam	Takapau silt loam	Manawatū fine sandy loam
Rock type	Loess over gravel	Alluvium	Loess over gravel	Alluvium
Depth of vadose profile investigated, m bgl	0-0.75	0-1.95	0-1.0	0-2.1
Depth to groundwater level, m bgl	2.50-3.38	3.02-3.96	1.43-3.12	4.29-4.65

Information on soil series and type from farm survey, where available, or from the New Zealand Fundamental Soil Layer and Rock type from the New Zealand Land Resource Inventory based on the location of the study sites (https://soils.landcareresearch.co.nz/soil-data/the-lris-portal/). *Land use at the study sites had been for at least five years by the time the study was conducted. bgl – below ground level

6.2.2 Installation of piezometers

At each of the four study sites (Figure 6.1), two to three piezometers were installed with screens at different depths, ranging from 4 to 7.5 m bgl (Table 6.2). The piezometers were made of PVC pipes with internal diameter of 28 mm (32 mm external diameter) and perforated at the bottom 0.50 m except the 20 mm margin at the bottom. Perforation entailed drilling 5 mm diameter holes along eight columns (on offset) at approximately 10 mm intervals along the bottom of PVC. This was to ensure that water flow into the piezometer was not restricted by the pore size being less than the porosity of the surrounding formation (Lapham et al., 1997). The perforated portion was covered with a 250 µm mesh nylon screen (Rasiah et al., 2003; Zhu et al., 2003) attached with silicon caulking (Well et al., 2003). The piezometers were installed using direct push-probe percussion with a double tube system (Lapham et al., 1997). The double tube system allows for backfilling and sealing the well (Lapham et al., 1997). Depending on the formation of materials surrounding the borehole,

sand and gravel may collapse and fill the lower part of the bore (functioning as a primary filter pack) supporting the piezometer in place (Lapham et al., 1997; Puckett and Cowdery, 2002; Well et al., 2003). Wherever applicable, a secondary filter pack of < 0.60 mm sand (quartz material; Lapham et al., 1997) of 0.30 m length was placed above the primary filter pack to prevent the annular seal (bentonite pellets; Puckett and Cowdery, 2002) from entering the primary filter pack and the screened portion of the well/piezometer. Another advantage of the double tube system is the minimal disturbance of subsurface geochemistry and permitting screens to be emplaced without exposure to overlying materials (Lapham et al., 1997). The piezometers were developed by pumping with a peristaltic pump and several episodes of groundwater sampling before push-pull tests were conducted.

6.2.3 Hydrogeologic characteristics of the vadose and saturated zones of the study sites

This study collated existing information and conducted in-field measurements to define/measure hydrogeologic characteristics of the vadose and saturated zones at the study sites (Tables 6.2 and 6.3). At each site, soil cores were collected and sectioned based on the sediment colour and texture to define different soil horizons (Table 6.2). The depths of soil profiles sampled were constrained by the ability to extract soil samples using a percussion corer. Where the soil profile contains reasonable amount of gravel or stones, such as at the Pahiatua (PAH) and Dannevirke (DAN) sites, the percussion corer could only get down to <1 m below ground level (bgl). At other sites, the percussion corer was able to reach approximately 2 m bgl at the Woodville (WDV) and Palmerston North (PNR) sites wherein the soil profile is composed of finer sediments. Overall, the soil cores were collected from two soil layers (up to 0.75 m bgl) at the PAH site, four soil layers (up to 1 m bgl) at the DAN site, four soil layers (up to 2.10 m bgl) at PNR site, and five soil layers (up to 1.95 m bgl) at

the WDV site (Table 6.3). The collected soil core samples were analysed to characterise relevant physical and chemical attributes of the vadose zone (Table 6.3). Soil texture (proportions of sand, silt, clay) was determined by particle size analysis (Sheldrick and Wang, 1993) using a laser scattering particle size distribution analyser Horiba Partica LA-950V2. Among the four sites, soils at the WDV site were mostly fine textured, whereas significant amount of gravel were found even in shallow depths (< 0.3 m bgl) at the PAH and DAN sites (Table 6.3).

The average depth to groundwater varied from 2.14 to 4.47 m bgl at the study sites (Table 6.2). The hydraulic conductivity of saturated zone was estimated following the Hvorslev Piezometer Test (Hiscock, 2005; Hvorslev, 1951; Mengis et al., 1999) measured by effecting and measuring a sudden change in the water level (Bouwer, 1989) in the shallow piezometers installed at the study sites. The hydraulic conductivity was estimated relatively higher in the shallowest piezometer (at 5.41 m day^{-1} in PAH-A, 4.0 - 4.5 m bgl), but very low in the deeper piezometers (at 0.17 – 0.63 m day^{-1} in PAH-B and PAH-C, 5.0 – 6.5 m bgl) at the Pahiatua site. Low hydraulic conductivity (at 0.004 – 0.13 m day^{-1}) was also measured in the three piezometers (4.0-7.5 m bgl) at the Dannevirke site (Table 6.2). These hydraulic conductivity measurements indicate semi-pervious, poor aquifer formation (Bear, 1972) at the Pahiatua and Dannevirke sites. The hydraulic conductivity, however, could not be determined for WDV and PNR sites using the Hvorslev Piezometer Test (Hvorslev, 1951) by effecting a sudden change in the water level in the piezometer as the water level did not even change at the highest pumping rate (approximately 3.5 L per minute) with the peristaltic pump. This suggests relatively high conductivity in the alluvium formation, with gravel at the screened portion of piezometers, at the WDV and PNR sites (Tables 6.1 & 6.2). Based on the pumping

rate of the pump, the hydraulic conductivity the WDV and PNR sites was assumed to be $\geq 10^{-2}$ cm s^{-1} (8.6 m day^{-1}), classified as a pervious aquifer (Bear, 1972).

The piezometers were also sampled in August 2015 and analysed for estimates of shallow groundwater age, determined in terms of mean residence time (MRT) in years using Exponential Piston Flow Model (Morgenstern et al., 2017; Morgenstern and Daughney, 2012) (Table 6.2). Where the aquifer materials surrounding the screened portions of the piezometers were deemed similar, such as at the PAH, WDV and PNR sites, a representative groundwater sample from only the middle piezometer was collected and analysed for the groundwater age. The groundwater age in MRT was determined based on concentrations of tritium, deuterium, ^{18}O, and hydrochemistry, measured and interpreted by the Tritium and Water Dating Laboratory at GNS Science located in Lower Hutt, Wellington (New Zealand) (Morgenstern et al., 2017; Morgenstern and Daughney, 2012).

Table 6.2 Physical characteristics of piezometers used for push-pull tests conducted to quantify the denitrification rate at four sites in the Manawatū River catchment, New Zealand.

Parameter	Unit	Pahiatua (PAH) site			Woodville (WDV) site			Dannevirke (DAN) site			Palmerston North (PNR) site
		PAH-A	PAH-B	PAH-C	WDV-A	WDV-B	WDV-C	DAN-A	DAN-B	DAN-C	PNR
Screened depth (bgl)	m	4.0-4.5	5.0-5.5	6.0-6.5	4.5-5.0	5.5-6.0	7.0-7.5	4.0-4.5	5.5-6.0	7.0-7.5	6.0-6.5
Depth to water table (bgl, average)	m	3.03	2.89	2.89	3.60	3.50	3.38	2.14	2.15	2.42	4.47
Hydraulic conductivity*	m day^{-1}	5.41	0.17	0.63	≥ 8.6	≥ 8.6	≥ 8.6	0.05	0.004	0.13	≥ 8.6
Aquifer classification**		Poor aquifer; semi-pervious	Poor aquifer; semi-pervious	Poor aquifer; semi-pervious	Good aquifer	Good aquifer	Good aquifer	Poor aquifer; semi-pervious	Poor aquifer; impervious	Poor aquifer; semi-pervious	Good aquifer
Groundwater age (MRT)**	yr	-	0.2	-	-	20	-	0.2	-	39	7

* Hydraulic conductivity, K, estimated using the Hvorslev test (Hvorslev, 1951; Hiscock, 2005; Mengis et al., 1999); K could not be determined for WDV and PNR sites with the method used (abrupt pumping) due to much higher conductivity in the gravel formation (K can be reasonably assumed as ≥ 10^{-2} cm s^{-1} (≥ 8.6 m day^{-1}); good aquifer, pervious according to Bear, 1972).

** Aquifer classification based on Bear (1972) using estimates of hydraulic conductivity for different sites.

** Mean residence time (groundwater age in years) measured for selected groundwater samples collected in August 2015 (Morgenstern and Daughney, 2012). Only representative piezometers (middle depth) sampled when there is good reason to believe that the properties are similar among piezometers at each site (e.g., PAH, WDV).

Table 6.3 Physical and chemical characteristics of the soil profile (vadose zone) of the four study sites in the Manawatū River catchment.

Characteristics	Pahiatua (PAH) site		Woodville (WDV) site						Dannevirke (DAN) site				Palmerston North (PNR) site			
Soil layer	a	b	a	b	c	d	e	f	a	b	c	d	a	b	c	d
Layer depth, m	0-0.29	0.29-0.75	0-0.18	0.18-0.40	0.40-1.00	1.00-1.60	1.60-1.85	1.85-1.95	0-0.29	0.29-0.40	0.40-0.70	0.70-1.00	0-0.30	0.30-0.60	0.60-1.20	1.20-2.10
Gravel, %[a]	0	*	0	0	0	0	0	0	18.11	70.04	75.10	76.28	0	0	0	39.53
Sand, %	31.12	20.03	19.27	7.59	7.54	10.30	37.65	24.45	31.95	44.57	68.01	68.99	30.83	20.14	69.68	82.71
Silt, %	57.58	79.08	75.07	87.79	90.76	87.23	62.04	74.74	54.59	44.67	30.17	28.86	66.56	79.24	30.25	17.17
Clay, %	11.30	0.88	5.66	4.63	1.70	2.47	0.30	0.81	13.57	10.76	1.82	2.15	2.61	0.63	0.07	0.12
Soil Texture[b]	Silt loam	Silt loam	Silt loam	Silt	Silt	Silt	Silt	Silt loam	Silt loam	Loam	Sandy loam	Sandy loam	Silt loam	Silt loam	Sandy loam	Loamy fine sand
Summer/Autumn																
pH	5.00	5.86	5.77	5.67	5.70	6.08	6.20	6.30	6.13	5.59	5.69	5.98				
BD, g cm^{-3}	1.05	1.22	1.25	1.21	1.53	1.64	1.61	1.31	1.13	-	1.63	1.65				
Nitrate-N, µg g^{-1}	133.33	1.64	9.34	1.51	0.69	0.65	0.52	0.92	6.53	2.08	1.72	0.51				
Soil-water Nitrate-N[c], mg L^{-1}	397.99	5.38	62.71	10.81	4.09	5.96	6.70	18.48	43.23	15.67	12.29	4.76				
Ammonium-N, µg g^{-1}	2.03	0.99	2.44	1.36	1.83	1.68	1.06	2.31	3.05	0.71	0.30	0.22				
Winter/Spring																
pH	5.40	5.87	5.35	5.58	5.69	6.10	6.19	6.28	5.80	5.60	5.79	6.00	5.62	5.74	6.34	6.51
BD, g cm^{-3}	1.04	1.09	1.24	1.54	1.40	1.60	1.62	-	1.16	-	1.71	1.81	1.30	1.38	-	-
DOC, µg g^{-1}	17.50	10.48	21.39	29.71	8.12	6.50	7.14	19.36	41.99	57.70	56.43	27.16	31.36	33.92	20.99	17.88
Nitrate-N, µg g^{-1}	78.19	6.08	78.37	8.10	3.60	0.73	0.52	1.29	73.26	14.05	3.76	1.45	7.01	1.36	0.42	0.66
Soil-water Nitrate-N[c], mg L^{-1}	144.79	12.41	223.91	62.28	27.70	4.53	3.71	6.16	244.20	56.19	18.81	12.11	21.96	7.17	3.85	7.01
Ammonium-N, µg g^{-1}	3.91	2.69	2.42	1.62	0.51	0.41	1.63	1.48	2.42	0.96	0.79	1.45	<0.01	<0.01	<0.01	<0.01

[a] Proportion of gravel, when present, in the soil layer; * With some gravel but the exact proportion was not determined. [b] Soil texture classes based on particle size analysis of soil samples (excluding gravel when present) and USDA NRCS soil texture calculator based on the soil textural triangle (https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/?cid=nrcs142p2_054167). [c] Soil-water nitrate-N determined from amount of nitrate-N and water content in the soil used for extraction.

6.2.4 Quantification of denitrification potential in the vadose zone by measuring denitrifying enzyme activity (DEA)

The denitrification potential of the vadose zone was determined by measuring the denitrifying enzyme activity (DEA) of different soil layers at the study sites. In this study, at least two soil cores were collected, using a percussion corer, at each site to obtain representative soil samples from different soil depths for DEA measurements. The number and depths of different soil layers at each study site were based on the soil horizons identified during the site characterisation (Table 6.3) (Boone et al., 1999; Crepin and Johnson, 1993), and also considering changes in denitrification potential with soil depth. The larger interval for lower soil depths was based on a generally observed decreasing difference in characteristics in deeper soil layers (Boone et al., 1999; Petersen and Calvin, 1986).

To determine the temporal variability of DEA in the vadose zone layers, soil cores were collected (as described above) during the dry (summer/autumn) and wet (winter/spring) seasons at the study sites. The soil cores at the PAH, DAN, and WDV were collected and analysed during February 2015 and October 2015 corresponding to the dry (summer/autumn) and wet (winter/spring) seasons, respectively. The soil cores at the PNR sites, however, were collected only once in November 2014 corresponding to the wet (winter/spring) season.

The collected composite samples were then homogenised, sieved (<2 mm), and stored at 4 °C until further analysis, which was done up to approximately three weeks from the sample collection. The amount of dissolved organic carbon (DOC), NO_3^--N and ammonium-N in soil samples (sieved, <2mm) were determined within a few days of the DEA experiment. Soil

DOC was determined following the procedure for the extraction of water soluble carbon from soil by Ghani et al. (2003). Using the same set of fresh soil samples used for DEA analysis (sieved < 2mm), three replicates of 10 g of soil and 25 mL of deionised water from each soil sample were placed into polypropylene tubes, shaken for 1 hour on a rotary shaker, and centrifuged at 4900 rpm for 10 minutes. The soil extractants were then filtered with the Whatman no. 42 filter paper (Maynard and Kalra, 1993) and stored in freezer until further analysis (Jones and Willett, 2006). The filtrates prepared earlier were later centrifuged at 5000 rpm for 2 hours and the supernatant was gently decanted and stored below 4 °C until analysis. The DOC concentrations of the supernatant were determined by measuring absorbance with a spectrophotometer (wavelength = 660 nm), except for the PNR site samples in which DOC in the soil water extracts was determined earlier using the potassium dichromate wet oxidation and titration (method 5220B) (Rice et al., 2012). The amounts of soil mineral N (NO_3^--N, ammonium-N) was determined following the standard procedures for soil N analysis (Blakemore et al., 1987; Maynard and Kalra, 1993). Three replicates of fresh soil samples (3 g, sieved < 2 mm) from each soil sample were weighed into polypropylene tubes and 30 mL of 2M KCL was added. The samples were shaken on a rotary shaker for 1 hour, and centrifuged at 4900 rpm for 10 minutes. The soil extractants were filtered with Whatman no. 42 filter paper (Maynard and Kalra, 1993) and stored in a freezer until analysis. Nitrate-N and ammonium-N in filtrates for the DEA analysis were analysed by continuous flow analysis (Technicon® AutoAnalyzer II).

Soil *pH* was determined following Blakemore et al. (1987). Two replicates of each air-dried soil samples (10 g, sieved < 2 mm) and 25 mL of deionised water were added into a 100 mL beaker and stirred vigorously for several seconds. The solutions were left to settle overnight and *pH* was determined with a *pH* meter. Soil bulk density at different soil depths were

obtained by measuring the oven-dried soil weight and volume (Culley, 1993) from sections of the soil in the percussion corer, except for the PNR site for which the values were taken from Espanto (2015). Some soil compaction might have been induced by the percussion corer especially in the subsoil. However, the measured length of soil cores taken each time (approx. 1 m), indicated bulk density could be reasonably estimated from the soil section weight and volume.

The procedure for measuring DEA in surface and subsurface soils has been optimised and described in Chapter 4 (Rivas et al., 2018a). Rivas et al. (2018a) found that soil incubation assays using the vacuum pouches resulted in a higher accuracy of DEA measurements, especially for the subsurface soils with low denitrification activity. In brief, fresh soil samples (40 g dry equivalent, sieved <2 mm) in four replicates (except for PNR site with triplicates) were placed into the vacuum pouches (100x285mm, gauge 70 µm Cas-Pak). Purified acetylene (C_2H_2) (20 mL) (Castle et al., 1998), then the DEA solution (40 mL) containing sources of organic carbon (glucose-C; 400 µg C g^{-1}) and NO_3^- (KNO_3-N; 75 µg N g^{-1}), as well as chloramphenicol (10 ppm), and N_2 gas (180 mL) were added to complete the headspace (via the luer-lock valve attached to the middle of the vacuum pouches), resulting in the concentration of acetylene at 10% of headspace (Beauchamp and Bergstrom, 1993; Groffman et al., 1999). The vacuum pouch samples were then placed upright on a cardboard box and incubated for 6 h in the dark at 20 °C on a rotary shaker at 160 rpm to ensure well mixing of the soil and solution. A gas sample of 25 mL was collected from each pouch at 0, 2, 4 and 6 h from the start of incubation and placed in 12 mL glass vials (Labco Exetainers). The collected gas samples from DEA assays were analysed for N_2O with a Shimadzu Gas Chromatograph (GC) 17 A (Japan) which has a ^{63}Ni-electron capture detector and detection limit of 0.05 ppm N_2O.

In quantifying the DEA, the amount of N_2O generated in each assay was computed from the amount of N_2O in the headspace and the amount of N_2O dissolved in the solution at each sampling time (Groffman et al., 1999; Hill et al., 2000; Luo et al., 1996). The DEA was then determined from the plot of total N_2O-N mass accumulated up to the sampling time (Luo et al., 1996; Rivas et al., 2018a). The DEA values were log transformed to approximate a normal distribution. The DEA values obtained were subjected to the independent *t* test or ANOVA, performed in IBM SPSS 23, to compare DEA across the study sites, at different soil depths within the sites, and between different seasons.

6.2.5 Quantification of denitrification rate in the saturated zone by using the single-well, push-pull test technique

The single-well, push-pull test is one of the most commonly used methods for measuring *in situ* denitrification rate in groundwaters (Istok, 2013; Istok et al., 1997; Sanchez-Perez et al., 2003). Rivas et al. (2018b; Chapter 5) applied and evaluated the push-pull test and its associated calculation models to quantify denitrification rate in shallow groundwaters. They found that denitrification rates can be quantified with reasonable accuracy using a zero-order or first-order kinetic reaction model using the changes in denitrification reactant (NO_3^--N) concentrations measured during the test, particularly when NO_3^--N concentrations are greater than 1.5 mg L^{-1} (Bowman and Focht, 1974). In this study, single well push-pull tests were conducted on all piezometers at the study sites, except the middle piezometer (DAN-B, 5.5 – 6.0 m bgl) at the DAN site due to the very low hydraulic conductivity (Table 6.2). Two sets of push-pull tests were conducted, first during the dry (summer/autumn) and second during the wet (winter/spring) seasons at the study sites (Tables 6.4 and 6.5). The push-pull tests at the PAH, DAN, and WDV sites were conducted during January - May 2015 and August –

September 2015 corresponding to the dry (summer/autumn) (Table 6.4) and wet (winter/spring) (Table 6.5) seasons, respectively. The push-pull tests at the PNR site were conducted in May 2014 and October 2013 corresponding to the dry (summer/autumn) (Table 6.4) and wet (winter/spring) (Table 6.5) seasons, respectively. During each test season, push-pull tests were conducted sequentially from the deepest to the shallowest piezometer at each site to minimise the possible influence of tracer additions on succeeding tests at each site.

Rivas et al. (2018b; Chapter 5) optimised the push-pull test procedure to measure denitrification in shallow groundwaters at PNR site in the Manawatū catchment. In brief, the push-pull test consisted of the sequential extraction of groundwater, preparation, and injection of test solution with the extracted groundwater (Istok, 2013). When the selected in-field groundwater parameters (temperature, *pH*, EC, and DO) were stabilised during the piezometer purging, duplicate samples of groundwater were collected in PE bottles (50 mL) and subsequently stored in freezer for the determination of background concentrations (Stenger et al., 2008). Groundwater for the preparation of injection solution was then collected into 20 L collapsible or flexible PE bags (Coleman Water Carrier, made of heavy duty polyethylene with 50% PE and 50% ethylene-vinyl acetate) (Rivas et al., 2018b). The amount of groundwater extracted for the injection solution varied from 10-100 L depending on the hydraulic conductivity of the study site (Tables 6.4 & 6.5). The collected groundwater were then stored overnight in a temperature-controlled room with temperature set similarly to the background temperature of groundwater.

Table 6.4 Shallow groundwater conditions, push-pull test parameters and measured denitrification rates in Summer/Autumn season at the four sites in the Manawatū River catchment, New Zealand.

Parameter	Unit	Pahiatua (PAH) site			Woodville (WDV) site			Dannevirke (DAN) site		Palmerston North (PNR) site
Piezometer*		PAH-A	PAH-B	PAH-C	WDV-A	WDV-B	WDV-C	DAN-A	DAN-C	PNR
Date of test		25/3/15	3/3/15	19/2/15	6/3/15	5/2/15	30/1/15	8/5/15	13/3/15	14/05/14
Shallow groundwater conditions**										
Depth to water (bgl)	m	3.38	3.27	3.28	3.95	3.96	3.73	2.85	3.12	4.65
Temperature	°C	15.2	19.2	14.4	15.4	14.4	16.2	16.9	17.2	15.7
DO	mg L^{-1}	8.54	7.05	8.26	0.25	0.22	0.12	2.78	0.45	0.45
SPC	µS cm^{-1}	148.6	146.4	146.8	353.7	415.0	562.2	369.9	349.0	228.9
pH		4.78	5.07	4.90	5.92	6.30	6.86	5.95	6.29	5.89
ORP (Eh)	mV	393.8	349.9	426.3	108.3	94.5	71.6	284.0	139.0	243.7
Br^-	mg L^{-1}	0.06	0.09	0.07	0.11	0.08	0.10	0.11	0.14	0.10
NO_3-N	mg L^{-1}	6.53	5.96	6.53	0.02	<0.01	<0.01	10.72	<0.01	<0.01
NO_2-N	mg L^{-1}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
N_2O-N	µg L^{-1}	7.86	8.73	7.80	0.04	0.07	0.12	33.47	0.08	n/a
NH_4^+-N**	mg L^{-1}	<0.01	<0.01	<0.01	0.08	0.11	0.04	0.11	<0.01	n/a
DOC	mg L^{-1}	0.89	1.05	2.54	1.58	1.24	0.49	1.96	1.38	3.81
Push-pull test parameters**										
Test volume	L	60	20	60	100	100	100	10	20	100
Test duration	h	5	4.5	5	7	7	7	4	4	7
Br^-	mg L^{-1}	11.01	9.32	11.55	10.65	10.59	10.54	9.63	10.43	12.88
NO_3-N	mg L^{-1}	11.89	9.57	11.88	10.40	10.73	10.57	9.96	10.63	12.66
Denitrification rate	mg N L^{-1} h^{-1}	-	-	-	0.18	0.04	0.06	-	0.04	0.56

*Note: PAH – Pahiatua; WDV – Woodville; DAN – Dannevirke; A, B, C refer to the different piezometers based on depth with A as the shallowest and C as the deepest piezometer (see Table 2). Push-pull test could not be conducted at DAN-B due to very low hydraulic conductivity.

**Note: bgl – below ground level, DO – dissolved oxygen, DOC – dissolved organic carbon, SPC – specific conductance or electrical conductivity, ORP (Eh) – oxidation-reduction potential. Except for in-field measured parameters (temperature, DO, pH, SPC, ORP), and DOC and N_2O-N (duplicate samples), the values presented for shallow groundwater background concentrations are averages of three replicates. The push-pull tests solution concentrations are average of 2 replicates per 20 L of the solution. NH_4^+-N concentrations from the samples collected in the same month as the push-pull test.

Table 6.5 Shallow groundwater conditions, push-pull test parameters, and denitrification rates measured in Winter/Spring at the four sites in the Manawatū River catchment, New Zealand.

Parameter	Unit	Pahiatua (PAH) site			Woodville (WDV) site			Dannevirke (DAN) site		Palmerston North (PNR) site
Piezometer*		PAH-A	PAH-B	PAH-C	WDV-A	WDV-B	WDV-C	DAN-A	DAN-C	PNR
Date of test		15/9/15	1/9/15	18/8/15	8/9/15	21/8/15	14/8/15	18/9/15	11/9/15	31/10/13
Shallow groundwater conditions**										
Depth to water (bgl)	m	2.68	2.51	2.50	3.26	3.05	3.02	1.43	1.71	4.29
Temperature	°C	12.7	12.4	13.0	13.5	13.9	13.9	13.4	14.3	14.6
DO	mg L^{-1}	8.83	9.69	9.59	0.19	0.37	0.07	5.23	0.24	0.40
SPC	µS cm^{-1}	143.0	136.0	143.1	352.2	424.7	512.3	391.9	337.6	234.6
pH		5.55	5.03	5.26	6.13	6.31	6.45	5.83	6.20	6.25
ORP (Eh)	mV	306.0	366.7	356.2	176.4	68.7	81.8	249.0	226.8	188.9
Br^-	mg L^{-1}	0.03	0.03	0.07	0.10	0.15	0.09	0.13	0.22	0.10
NO_3-N	mg L^{-1}	5.86	5.51	5.16	<0.01	0.02	<0.01	14.16	<0.01	0.05
NO_2-N	mg L^{-1}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
N_2O-N	µg L^{-1}	5.13	5.14	4.85	0.19	0.06	0.33	46.93	14.60	n/a
NH_4^+-N**	mg L^{-1}	<0.01	<0.01	<0.01	0.04	0.12	0.21	<0.01	<0.01	n/a
DOC	mg L^{-1}	0.56	1.61	0.37	0.91	1.72	1.10	3.15	1.14	3.55
Push-pull test parameters**										
Test volume	L	80	40	80	100	100	100	20	40	40
Test duration	h	6	5	6	7	6	6	4	5	3.75
Br^-	mg L^{-1}	10.84	11.14	9.97	10.39	10.99	10.67	9.44	10.06	11.06
NO_3^--N	mg L^{-1}	11.63	12.11	10.04	10.25	11.08	10.63	9.67	10.00	11.23
Denitrification rate	mg N L^{-1} h^{-1}	-	-	-	0.15	0.12	0.08	-	-	1.07

*Note: PAH – Pahiatua; WDV – Woodville; DAN – Dannevirke; A, B, C refer to the different piezometers based on depth with A as the shallowest and C as the deepest piezometer (see Table 2). Push-pull test could not be conducted at DAN-B due to very low hydraulic conductivity.

**Note: bgl – below ground level, DO – dissolved oxygen, DOC – dissolved organic carbon, SPC – specific conductance or electrical conductivity, ORP (Eh) – oxidation-reduction potential. Except for in-field measured parameters (temperature, DO, pH, SPC, ORP), and DOC and N_2O-N (duplicate samples), the values presented for shallow groundwater background concentrations are averages of three replicates. The push-pull tests solution concentrations are average of 2 replicates per 20 L of the solution. NH_4^+-N concentrations from the samples collected in the same month as the push-pull test.

On the day of conducting the push-pull test, the test solution was prepared at the site by diluting sources of the tracer bromide (KBr) and NO_3^- (KNO_3) into a small container (120 mL x 2) of the extracted groundwater and injected back into the test solution container using a syringe. This was followed by adding acetylene, purified by passing the gas through two traps of concentrated H_2SO_4 and one trap of distilled water (Castle et al., 1998), into the extracted groundwater. The resulting target concentrations (excluding the background concentrations) in the test solution were approximately 10 mg L^{-1} Br^-, 10 mg L^{-1} NO_3-N, and 50 ml purified acetylene L^{-1} (Tables 6.4 & 6.5). The test solution bags were then vigorously shaken to dissolve the substrate and acetylene bubbles and mix the solution properly (Well et al., 2003). A set of duplicate samples (approximately 50 mL each) from each test solution bag were then collected and filtered (0.45 µm) on the site, and frozen at approximately -20 °C until further analysis (Barns, 2010). The test solution was then injected back into the piezometer using the injection system by reversing the direction of the flow of the peristaltic pump.

At completion of the injection of test solution, groundwater extraction commenced and groundwater samples were collected periodically, after pumping out approximately 1-2 L of water each sampling time to ensure sample collection from the same portion of the aquifer formation surrounding the piezometer and beyond standing water volume in the piezometer (Istok, 2013). The samples were collected from time 0 (right after completion of the solution injection) up to seven hours (Tables 6.4 & 6.5). The test volume used was constrained by what can be pumped out in a single day during the groundwater collection, and can be injected in and extracted out (in minimum of four hours) on the test day.

During the extraction phase, two sets of groundwater samples were collected at each sampling time. First, triplicates of approximately 50 mL samples were field-filtered with a 0.45 µm syringe filter and collected in PE bottles for the measurement of nitrate-nitrogen (NO_3^--N) and bromide (Br^-). A second set of duplicate samples of 120 to 180 mL (120 mL for the PNR push-pull tests only) were collected by syringe through a *t*-type luer-lock mini valve (Mühlherr and Hiscock, 1998) and transferred into evacuated vacuum pouches (100x285mm, gauge 70 µm Cas-Pak) for the extraction and analysis of dissolved nitrous oxide (N_2O) gas. The collected groundwater samples were transported in a chilly bin with ice (Daughney et al., 2006). The collected samples for hydrochemical analysis were frozen until analysis (Barns, 2010; Stenger et al., 2008). The collected samples for N_2O were kept chilled at 4 °C until the gas extraction, which was done within 24 hours of the sample collection. To extract dissolved N_2O gas from the collected groundwater samples, the phase equilibrium headspace extraction method (Addy et al., 2002; Lemon and Lemon, 1981) was adapted in which 50 - 60 mL of N_2 (50 mL of N_2 for the PNR push-pull tests only) were added to create a headspace in each of the collected sample pouches, which were then placed on a shaker for 1.5 hours at 200 rpm under 20 °C. After shaking, 25 mL of gas samples were removed from the pouches and placed into 12 mL glass vials (Labco Exetainer) for analysis in a gas chromatograph.

The groundwater parameters such as *pH*, dissolved oxygen (DO), oxidation-reduction potential (ORP), specific conductance (SPC) or electrical conductivity, and temperature were measured in-field by a multiparameter probe (YSI Professional Plus). The ORP values were converted to *Eh* by adding 200 mV (Rice et al., 2012). The DOC concentrations in groundwater samples were determined by measuring absorbance with a spectrophotometer (wavelength = 660 nm), except for PNR site samples in which the DOC was determined

much earlier generally following the potassium dichromate wet oxidation and titration (method 5220B) (Rice et al., 2012). The NO_3^--N and Br^- in groundwater samples were analysed by ion chromatography (Lachat Instruments IC5000 Ion Chromatograph). The collected dissolved N_2O gas samples from groundwater samples were analysed in a Shimadzu Gas Chromatograph (GC) 17 A (Japan) which has a ^{63}Ni-electron capture detector and detection limit of 0.05 ppm N_2O.

Among the several methods to estimate the denitrification rate from push-pull test data (Rivas et al., 2018b), this study used the best-fit slope regression line of dilution corrected concentrations of NO_3^- against time following a zero-order kinetic reaction model (Baker and Vervier, 2004; Istok, 2013; Korom et al., 2012; Tesoriero et al., 2000) for comparison purposes. First-order kinetic model may not be appropriate considering that the test solution contains approximately 10 mg L^{-1} of NO_3^--N and the lowest average NO_3^--N concentrations measured at the end of the tests at the WDV and DAN sites (where denitrification rate could be estimated) were at least 1.63 mg L^{-1}, greater than the high threshold of 1.0 to 1.5 mg of NO_3 L^{-1} by Bowman and Focht (1974). Moreover, while the lowest average NO_3^--N concentrations at the PNR site were smaller at 0.28 and 1.30 mg L^{-1} during the two push-pull tests, these are still greater than the low thresholds of 0.10 to 0.20 mg of NO_3 L^{-1} reported by Tchobanoglous et al. (2014). Thus, NO_3^--N was most likely not the limiting factor compared to electron donor availability such as DOC, which was measured to be lower at the study sites (Tables 6.4 & 6.5). The dilution-corrected concentration of NO_3^--N was obtained by multiplying the measured NO_3^--N concentration with a dilution factor, which is the ratio of the average initial concentration of the conservative tracer Br^- in the test solution and the concentration of Br^- at the respective samplings. To confirm the occurrence of the denitrification process, the measured N_2O concentrations in groundwater samples were also

determined and assessed in similar way as with the denitrification assays minus the incubation portion. Details of the procedure can be found in Chapter 5 (Rivas et al., 2018b).

6.3 Results and Discussion

6.3.1 Spatial and temporal variability of nitrate in the vadose zone

Amounts of nitrate-N (NO_3^--N) in the soil were generally found higher in the surface soils compared to the subsoil layers, with significantly higher amounts observed in the winter/spring sampling, except at the PAH site. Low NO_3^--N amount (7.01 µg g^{-1}) found at the soil surface layer at the PNR site during winter/spring was comparable to the NO_3^--N amounts measured at the DAN and WDV sites during the summer/autumn sampling (6.53-9.34 µg g^{-1}) (Table 6.3). This may be attributed to the time of sampling which was close to the end of spring (mid-November), and could indicate that most of the NO_3^- may have been leached to the deeper layers or attenuated by plant uptake and/or denitrification.

6.3.2 Spatial and temporal variability of denitrification potential in the vadose zone

6.3.2.1 Spatial variability – vadose zone

Table 6.6 summarises the results of the DEA assays conducted for the soil cores collected from different soil layers during the dry (summer/autumn) and wet (winter/spring) seasons at the study sites. The DEA in surface soils (<30 cm bgl) varied from 18,425 µg N_2O-N kg^{-1} soil d^{-1} at the PNR site to 37,182 µg N_2O-N kg^{-1} soil d^{-1} at the WDV site (Table 6.6). These DEA values were comparable to those obtained by Luo et al. (1996) for surface soil samples (0-10 cm bgl) collected in spring and summer for the Tokomaru silt loam and Manawatū fine sandy loam soils (approx. 16,000-20,000 µg N_2O-N kg^{-1} soil d^{-1}) at the Massey No. 1 Dairy Farm in

Palmerston North, in the Manawatū River catchment. Deslippe et al. (2014) also obtained comparable values from 18,960 to 23,760 µg N_2O-N kg^{-1} dry soil d^{-1} for surface soil samples (0-10 cm bgl) of Manawatū fine loam soils at the Massey No. 1 Dairy Farm. The DEA values obtained in the subsurface layers, however, were quantified as very low ranging from 2 µg N_2O-N kg^{-1} soil d^{-1} in 0.60 to 1.20 m bgl at the PNR site, to 686 µg N_2O-N kg^{-1} soil d^{-1} in 0.29 to 0.40 m bgl at the DAN site (Table 6.6). These subsurface DEA values are roughly comparable to the range of the DEA values (5-100 µg N_2O-N kg^{-1} dry soil d^{-1}) measured in the subsoil (1.20-2.35 m bgl) in the Canterbury region (Peterson et al., 2013), less than the values (700-2000 µg N_2O-N kg^{-1} soil d^{-1}) obtained in the Manawatū region subsoil (0.2-0.4 m bgl) (Luo et al., 1998), but higher than the values (1.4- 140 µg N_2O-N kg^{-1} soil d^{-1}) observed in an agricultural area in Utsunomiya, Japan (Kamewada, 2007).

The DEA values were observed to decrease significantly with the soil depth at all four sites during both sampling seasons (Table 6.6). The DEA values in the surface soils (<30 cm bgl) during the summer/autumn season were at least 28 times greater than the corresponding DEA values in the subsurface soils, whereas the DEA values in the surface soils during the winter/spring season were at least 112 times greater than the corresponding DEA values in the subsurface soils. This decreasing trend in DEA with soil depth had also been observed in other studies (Cannavo et al., 2004; Jarvis and Hatch, 1994; Limmer and Steele, 1982; Luo et al., 1998; Parkin and Meisinger, 1989). The sharp difference between the surface and underlying subsoil as well as the minor (though in most cases significant) difference among the subsurface layers have also been observed by Luo et al. (1998) in 0-0.40 m bgl in the Manawatū region, New Zealand and Sotomayor and Rice (1996) in 0-2.0 m bgl in the Flint Hills uplands in eastern Kansas.

Table 6.6 Denitrifying enzyme activity (DEA) (mean ± stdev) measured for different soil depths at the four study sites in the Manawatū River catchment, New Zealand.

Study site/depth	Summer/Autumn		Winter/Spring		Sig.
	μg N_2O-N kg^{-1} dry soil d^{-1}	kg N ha^{-1} day^{-1}	μg N_2O-N kg^{-1} dry soil d^{-1}	kg N ha^{-1} day^{-1}	
1) Pahiatua (PAH) site					
0-0.29 m	$20{,}422.4^{a}$ ± 894.5	62.01	$36{,}925.9^{a}$ ± 4305.7	111.12	0.001
0.29-0.75 m	46.3^{b} ± 37.4	0.26	40.4^{b} ± 16.9	0.17	0.969
2) Woodville (WDV) site					
0-0.18 m	$19{,}776.2^{a}$ ± 988.0	44.35	$37{,}181.5^{a}$ ± 523.5	83.00	< 0.001
0.18-0.40 m	81.7^{b} ± 2.2	0.22	50.3^{b} ± 12.9	0.17	0.007
0.40-1.00 m	8.5^{c} ± 0.5	0.08	6.0^{c} ± 1.8	0.05	0.046
1.00-1.60 m	3.4^{d} ± 1.0	0.03	5.0^{c} ± 2.4	0.05	0.317
1.60-1.85 m	4.9^{d} ± 1.0	0.02	3.7^{c} ± 2.0	0.02	0.270
1.85-1.95 m	2.1^{e} ± 0.6	<0.01	2.3^{c} ± 1.7	n/a	0.766
3) Dannevirke (DAN) site					
0-0.29 m	$23{,}291.3^{a}$ ± 849.6	76.57	$27{,}061.2^{a}$ ± 942.8	90.77	0.001
0.29-0.40 m	686.0^{b} ± 30.6	n/a	164.1^{b} ± 38.8	n/a	< 0.001
0.40-0.70 m	182.0^{c} ± 16.8	0.89	42.5^{c} ± 15.9	0.22	0.003
0.70-1.00 m	17.9^{d} ± 10.6	0.09	2.4^{d} ±1.7	0.01	0.003
4) Palmerston North (PNR) site					
0-0.30 m			$18{,}425.2^{a}$ ± 987.6	71.86	
0.30-0.60 m			61.1^{b} ± 10.3	0.25	
0.60-1.20 m			1.7^{c} ± 1.9	n/a	
1.20-2.10 m			3.2^{c} ± 0.7	n/a	

Note: Significant difference between two sampling times for each soil layer by Independent Samples *t* Test. Different letters denote significant difference (at the $p<0.05$ level) among layers at a specific site according to LSD and Tukey tests as determined by ANOVA. Entries with n/a for the equivalent reduced nitrate-N in kg N ha^{-1} day^{-1} were due to the lack of the soil bulk density data.

The results infer relatively low denitrification potential (<1 kg N ha^{-1} day^{-1}) in deeper vadose zones (>30 cm bgl) at the study sites (Table 6.6). The capacity of the vadose zone to denitrify could vary at different depths due to differences in the biological, chemical and physical factors. The biological and chemical factors include denitrifier population, amount of readily available carbon, *pH*, and presence of NO_3^-, whereas the physical factors include porosity or permeability, temperature, and water table that affect the dynamics of transport and transformation of NO_3^- (Firestone, 1982; Paramasivam et al., 1999; Tindall et al., 1995).

However, considering that DEA is a measure of the potential of the soil to denitrify with non-limiting amount of substrates and subject to the same incubation environment, the observed large differences in the DEA values between the surface (< 30 cm bgl) and subsurface (> 30 cm bgl) soils (Table 6.6) could be due to lack of the abundance and composition of denitrifying microorganisms in subsurface layers. A number of studies have found relatively higher abundance and activity of denitrifying microorganisms in the surface soils compared to the subsoils (Cannavo et al., 2004; Firestone, 1982; Fischer et al., 2013; Jahangir et al., 2012c; Paramasivam et al., 1999; Vilain et al., 2012; Yeomans et al., 1992). Moreover, consistently and significantly higher NO_3^--N in the surface soils (Table 6.3) could indicate that the denitrifying microorganisms in the surface soils are more acclimatised to facilitating the denitrification process given the constant substantial supply of electron acceptor, as opposed to the natural conditions of low NO_3^--N in the deeper vadose zone, in which denitrifiers are less likely to show substrate-induced synthesis of denitrifying enzymes (Cannavo et al., 2004).

Across the study sites, the DEA values measured in the surface soil during the summer/autumn investigation were higher (p<0.05) at the DAN site compared to the PAH and WDV sites; the latter two having no significant differences (p=0.057) (Table 6.6). However, this was not the case for the winter/spring measurements, when the DEA values measured in the surface soils were higher (p<0.05) at the PAH and WDV sites as compared to the DAN site. In the subsoil layer (0.29-0.40 m bgl) at the DAN site the DEA values were, however, measured consistently higher as compared to the other sites during both sampling times (Table 6.6). This relatively higher DEA measured in the subsurface at the DAN site could be attributed to higher DOC measured at the DAN site (Table 6.3), reflecting a greater availability of substrate for microorganisms to facilitate the denitrification process. In the

deeper vadose zones beneath the first subsoil, the DEA values at the DAN site remained significantly higher compared to the WDV and PNR sites, except the fourth layer in the winter/spring season. This could be attributed to the same factors, higher DOC and *pH* at the DAN site as compared to the WDV and PNR sites (Table 6.3). The mean DEA in the surface layer at the PNR site (18,425 µg N_2O-N kg^{-1} dry soil d^{-1}) measured during winter/spring sampling was found to be the lowest among the sites for both seasons. However, this value was not significantly different from the mean DEA values measured in the summer/autumn sampling at the WDV and PAH sites (19,776 and 20,422 µg N_2O-N kg^{-1} dry soil d^{-1}, respectively). As stated earlier, this could be due to the fact that the samples were collected at the PNR site closer to the end of spring/start of summer (mid-November; spring runs till end of November) and thus environmental conditions may have been more similar to summer/autumn. Another possible reason could be due to lower abundance of denitrifying microorganisms, which was not measured in this study.

6.3.2.2 Temporal variability – vadose zone

Table 6.6 also shows that, across the three sites in the eastern part of the catchment, the DEA measured in the surface layer increased from 19,776 to 23,291 µg N_2O-N kg^{-1} soil d^{-1} in the summer/autumn sampling to 27,061 to 37,182 µg N_2O-N kg^{-1} soil d^{-1} in the winter/spring sampling. This increase in DEA ranged from 16% (at the DAN site) to 88% (at the WDV site). The DEA analysis for the summer/autumn soil samples were conducted a bit later from the time of sampling compared to the Winter/Spring samples (difference of 7-10 days between the two seasons). This could potentially result in a slightly lower DEA in the samples collected for the summer/autumn samples. For example, Luo et al. (1996) measured from 26 to 33% decrease in the DEA values when incubations were conducted between 2 weeks to roughly a month after the sampling compared to when incubations are done within

five days from sampling. However, in this study, significant increases measured in the surface soil DEA values during the winter/spring samples especially at the WDV and PAH sites were much higher than the changes observed by Luo et al. (1996) due to the time gap between the soil sampling and incubations. The need to collect samples at a specified date due to several constraints (e.g., availability of equipment and personnel, other field work) led to some time gap between sampling and DEA incubations for the samples collected in summer/autumn. As such, possibly except for the DAN site, it is evident that the DEA in the surface soils increased from the summer/autumn samples to the winter/spring samples (Table 6.6). In an earlier study, Ruz-Jerez et al. (1994) also measured higher denitrification rates (actual, not DEA or denitrification potential) during winter from soil core samples (25 mm diameter and 75 mm deep) taken from pasture lands within the Manawatū River catchment. The increase in soil DOC (indicating higher availability of electron donor) (Table 6.3; limited data for summer/autumn not shown) and water content (leading to lower concentrations of oxygen) (data not shown) from summer/autumn to winter/spring sampling suggest favourable conditions for denitrification in the winter/spring sampling (Hashimoto and Niimi, 2001). Similarly, Ruz-Jerez et al. (1994) also attributed the high denitrification during winter to the high soil moisture above the field capacity for extended periods, as well as on the availability of NO_3^- and NO_2^-. In this study, NO_3^- was also found to increase in the winter/spring sampling, except at the PAH site wherein NO_3^- concentration was already high in the summer/autumn sampling (133 μg N g^{-1} soil; Table 6.3). The relatively smaller increase in DEA in the surface layer at the DAN site during the winter/spring sampling could reflect the decrease in *pH* from 6.13 to 5.80, which is far lower than the suggested optimum range of 7.0-8.0 (Hiscock et al., 1991; Knowles, 1982), thereby possibly affecting the activity of the microorganisms.

There was no apparent trend in the DEA values measured in the subsurface layers (> 30 cm, bgl) at the study sites (Table 6.6). In fact, DEA in the subsurface layers at the DAN site decreased. Similar with the surface layers, the concentrations of soil DOC, NO_3^-, and water content (data not shown) generally increased in the subsurface layers in the winter/spring sampling (Table 6.3). But this did not translate to a significant increase in DEA values. Although there is no data to confirm this, it is probable that, due to the usually lower number of denitrifiers in the subsurface layers (Cannavo et al., 2004; Fischer et al., 2013), changes in the environmental conditions may not have an immediate effect on the DEA in the subsurface layers. In addition, the apparent low NO_3^- concentration most of the year may entail that microorganisms were less likely to synthesise denitrifying enzymes (Cannavo et al., 2004). Thus, changes in denitrification with season seem to be more apparent in the surface soil layer than in the subsurface (Sotomayor and Rice, 1996). Other studies investigating temporal changes in denitrification in subsurface soils did not also find marked differences at different sampling seasons (Jarvis and Hatch, 1994; Luo et al., 1998; Martin et al., 1999).

6.3.3 Spatial and temporal variability of denitrification characteristics in shallow groundwaters

6.3.3.1 Groundwater depths and age

The groundwater was found to be young (0.2 yr MRT) at the PAH site and in the shallow piezometer at the DAN site (DAN-A). The groundwater was found to be relatively older at the PNR site (7 yr MRT), WDV site (20 yr MRT), and in the deepest piezometer at the DAN site (39 yr MRT) (DAN-C) (Table 6.2). It is apparent that groundwater age was younger at sites with coarse-textured soil and shallow depth to water, whereas groundwater age was older where overlain by fine-textured soils and/or vadose zone is thick (Tables 6.2 and 6.3). The significant discrepancy in the groundwater age between the two depths at the DAN site

indicates the presence of an impervious layer between DAN-A and DAN-C, most likely a layered clay (Bear, 1972) based on the measured hydraulic conductivity of 4.11 x 10^{-6} cm/s (0.0036 m day^{-1}) at the screened depth of DAN-B (Table 6.2). The presence of a hard clay layer was also experienced during the installation of the deepest piezometer DAN-C at the DAN site.

A series of push-pull tests were conducted to measure denitrification rate in the shallow groundwaters at the study sites. Tables 6.4 and 6.5 summarise the background conditions of shallow groundwaters and the parameters for push-pull tests conducted at each piezometer on the study sites. Depths to the water table were generally shallower at the PAH and DAN sites (1.43-3.38 m bgl) compared to the WDV and PNR sites (3.02-4.65 m bgl), being generally shallower during the winter/spring tests. High dissolved oxygen (DO) concentrations were found at the PAH and DAN-A sites (>2.78 mg L^{-1}) indicating oxic groundwater conditions, whereas reduced conditions were found at the WDV, PNR, and DAN-C sites (DO < 0.5 mg L^{-1}). Figures 6.2 to 6.5 show the trend in reactive (NO_3^--N) and conservative (Br^-) tracer concentrations (excluding background concentrations) for quantifying the denitrification rate at the four study sites for the two seasons. The observed trends in concentrations of the denitrification product, N_2O-N, are shown in Figure 6.6 for the push-pull tests conducted.

6.3.3.2 Spatial variability – shallow groundwater

It is apparent in Figure 6.2 that there were no clear indications of denitrification occurring in any of the three piezometers at the PAH site. At this site, the dilution-corrected NO_3^--N concentrations and the NO_3^--N/ Br^- ratio did not decrease – and even increased in the most cases – during the push-pull tests in both seasons (Figure 6.2). This is a similar trend to what was observed at the shallowest piezometer at the DAN site (DAN-A; Figure 6.4). The

observed higher dilution-corrected NO_3^--N concentrations than the test solution NO_3^--N concentrations (Figures 6.2 & 6.4) could be attributed to mixing effects of the test solution with the resident shallow groundwater. Equal dilution rate was assumed for both NO_3^--N and Br^- in the analysis but the substantial background NO_3^--N concentrations (> 5 mg L^{-1}) but low Br^- concentrations (0.03-0.13 mg L^{-1}) (Tables 6.4 & 6.5) during the push-pull tests at the PAH and DAN sites may have resulted in the faster dilution of Br^- than that of NO_3^--N, and subsequently higher dilution factor. There was no apparent other reason for such an increase in NO_3^--N during the push-pull tests. This, however, does not affect the interpretation of the results, given that the denitrification rate is assessed based on a decrease in the denitrification reactant, dilution-corrected NO_3^--N concentrations, and an increase in the denitrification product (N_2O concentrations) during the push-pull tests.

As such, no denitrification rate could be estimated from the push-pull tests conducted in the piezometers (PAH-A, PAH-B, PAH-C and DAN-A) at the PAH and DAN sites (Tables 6.4 & 6.5). This was also supported by no clear increasing trend in the observed N_2O concentrations during the push-pull tests at these piezometers (PAH-A, PAH-B, PAH-C and DAN-A) (Figures 6.6 a1, a2, c1 and c2), thus supporting that there was no or negligible denitrification occurring during the push-pull tests. This was not surprising considering that the corresponding DO concentrations in groundwaters at these sites (Tables 6.4 and 6.5) were measured much higher (ranging from 2.78 to 9.69 mg L^{-1}) than 2 mg L^{-1}, the reported limit for denitrification to occur in groundwaters (Rissmann, 2011; Rivett et al., 2008). In fact, this observation confirmed the earlier survey findings that the groundwater in the vicinity of the PAH site has low potential for denitrification (Rivas et al., 2017). The coarse-textured soils and shallow depths to the groundwater most likely contributed to the rapid recharge to groundwater as indicated by the young age of groundwater at the sites (0.2 yr) resulting in

high DO concentrations at the sites (Tables 6.3, 6.4 & 6.5). Oxidised groundwater had also been found beneath well-drained soils (Clague et al., 2015c).

The dilution-corrected NO_3^--N concentrations and NO_3^--N/Br^- ratio showed a consistent decreasing trend during the push-pull tests conducted in the WDV-A, WDV-B, WDV-C and PNR piezometers (Figures 6.3 & 6.5). This suggests the occurrence of denitrification in shallow groundwater at the WDV and PNR sites. This corresponds well with the low DO concentrations (< 0.45 mg L^{-1}) observed in shallow groundwaters at the WDV and PNR sites (Tables 6.4 and 6.5). At these sites, older groundwaters were found (>7 yrs) because of slow groundwater recharge from the surface due to the fine-textured soil and/or thick vadose zone (e.g. Clague et al., 2015c), and/or the significant proportion of groundwater found at these sites originate from areas further upstream resulting in reasonably long travel times.

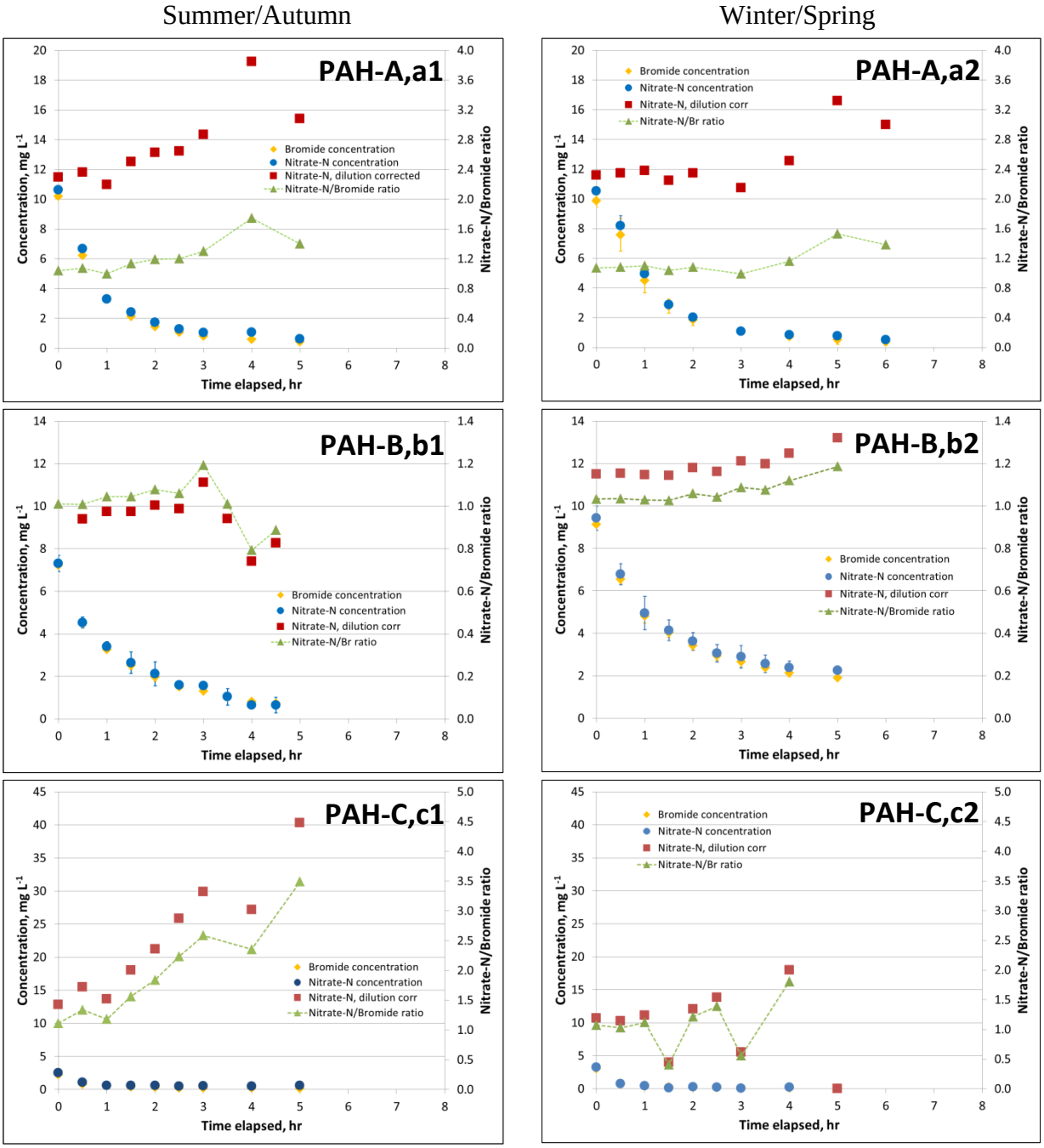


Figure 6.2 Trend in average NO_3-N and Br concentrations (excluding background concentrations) measured during the push-pull tests conducted at the Pahiatua (PAH) site in the Manawatū River catchment in (1) the Summer/Autumn 2015 and (2) the Winter/Spring 2015. The time elapsed (hr) was measured from the end of test solution injection. Error bars show the standard deviation for three replicates.

Summer/Autumn

Winter/Spring

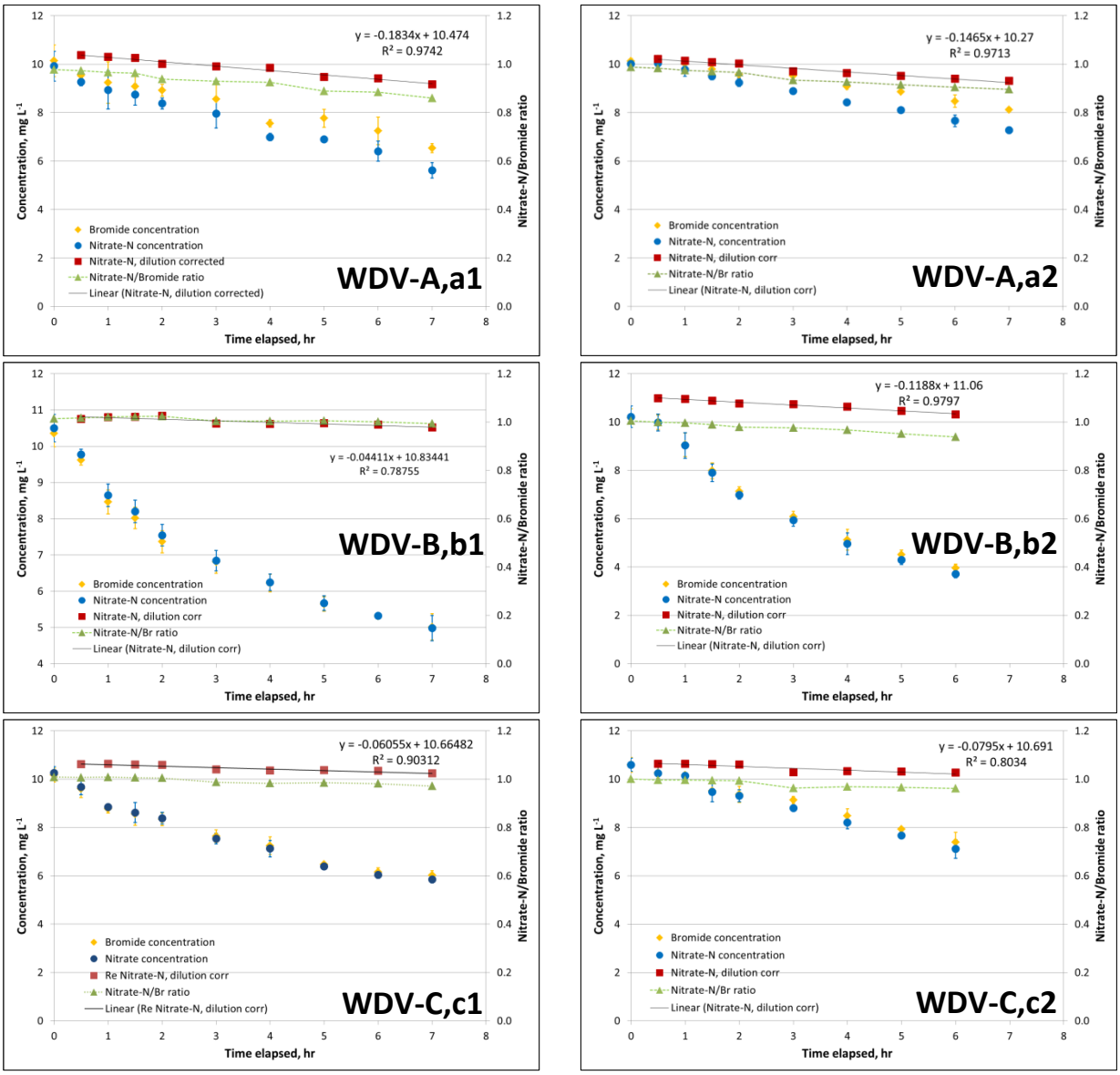


Figure 6.3 Trend in average NO_3-N and Br concentrations (excluding background concentrations) measured during the push-pull tests conducted at the Woodville (WDV) site in the Manawatū River catchment in (1) the Summer/Autumn 2015 and (2) the Winter/Spring 2015. The time elapsed (hr) was measured from the end of test solution injection. Error bars show the standard deviation for three replicates.

Summer/Autumn

Winter/Spring

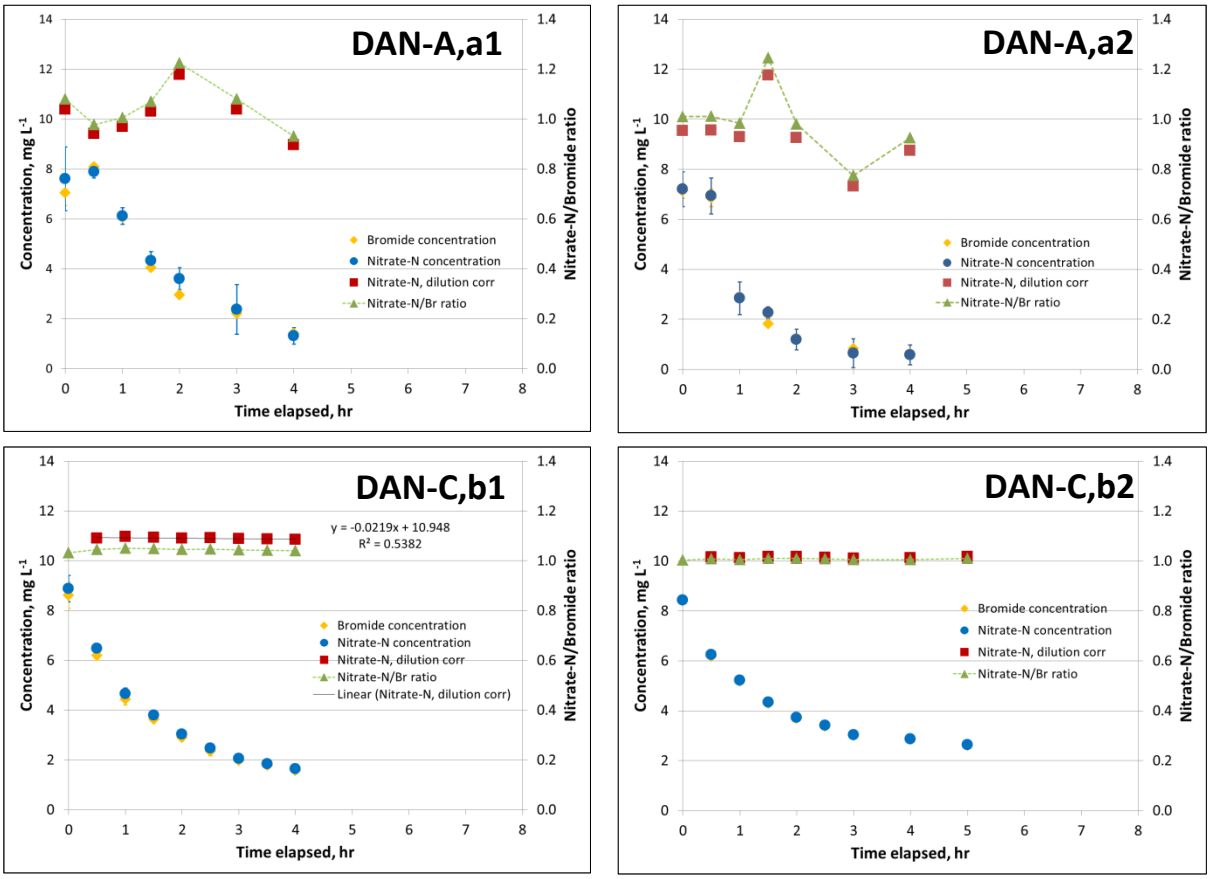


Figure 6.4 Trend in average NO_3-N and Br concentrations (excluding background concentrations) measured during the push-pull tests conducted at the Dannevirke (DAN) site in the Manawatū River catchment in (1) the Summer/Autumn 2015 and (2) the Winter/Spring 2015. The time elapsed (hr) was measured from the end of test solution injection. Error bars show the standard deviation for three replicates.

Summer/Autumn

Winter/Spring

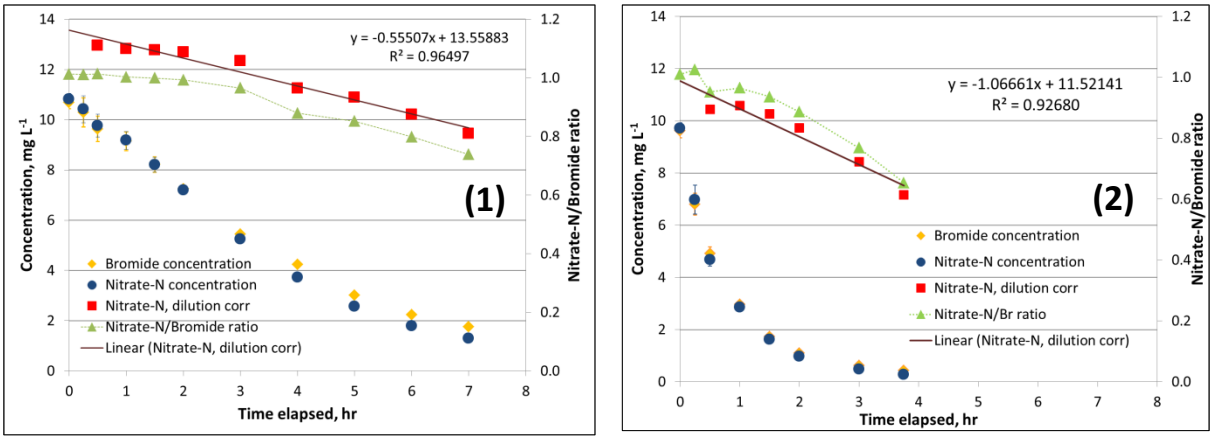


Figure 6.5 Trend in average NO_3-N and Br concentrations (excluding background concentrations) measured during the push-pull tests conducted at the Palmerston North (PNR) site in the Manawatū River catchment in (1) Summer/Autumn 2014 and (2) the Winter/Spring 2013. The time elapsed (hr) was measured from the end of test solution injection. Error bars show the standard deviation for three replicates.

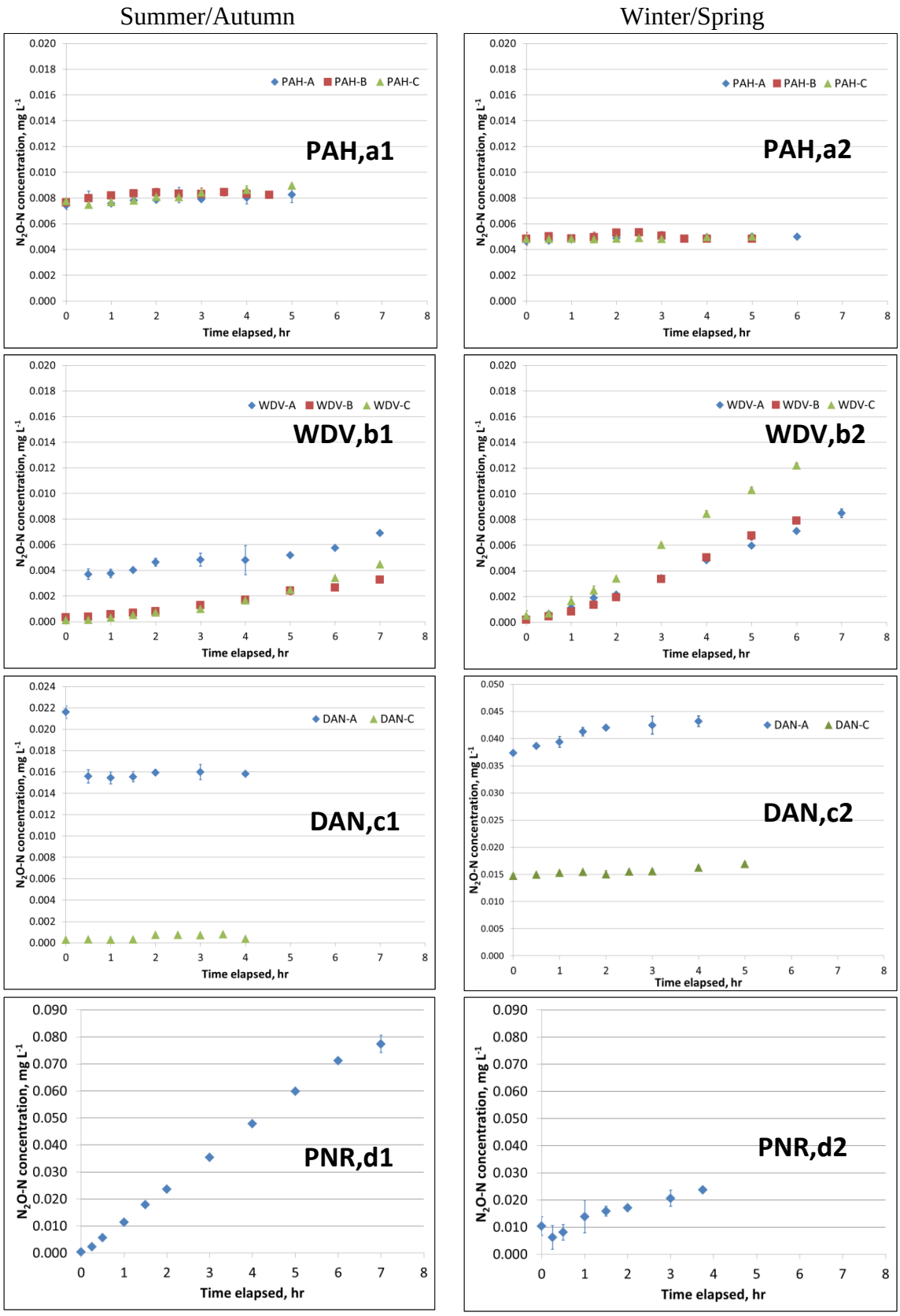


Figure 6.6 Trend in average nitrous oxide (N_2O-N) concentrations measured during the push-pull tests conducted at the four study sites in the Manawatū River catchment in (1) the Summer/Autumn and (2) Winter/Spring seasons. The time elapsed (hr) was measured from the end of test solution injection. Error bars show the standard deviation for three replicates.

Zero-order denitrification rates, estimated based on the best-fit slope regression line of the dilution-corrected NO_3^--N concentrations against time (Istok, 2013; Korom et al., 2012), were quantified ranging from 0.04 to 1.07 mg N L^{-1} h^{-1} at the WDV and PNR sites, respectively (Tables 6.4 & 6.5). These zero-order rates are comparable to the reported zero-order rates in the literature using the push-pull test technique (0.01-1.12 mg N L^{-1} h^{-1}) (Addy et al., 2002; Istok et al., 1997; Starr and Gillham, 1993; Toda et al., 2002; Trudell et al., 1986; Well et al., 2003). The denitrification rate was highest at the PNR site, particularly during the Winter/Spring test (Tables 6.4 & 6.5). Similar to the case of the WDV site with NO_3^--N $\leq$ 0.02 mg L^{-1} (Tables 6.4 & 6.5), Tesoriero et al. (2000) also found low denitrification rates at sites with low natural NO_3^- concentrations (NO_3^--poor) despite strong reducing conditions in groundwater. This observed low denitrification rates in NO_3^--poor sites such as the WDV site could be attributed to the slow response or lack of acclimatisation of microorganisms to NO_3^- additions (Tesoriero et al., 2000). The higher rates at the PNR site may be attributed to the higher DOC concentrations in shallow groundwater compared to the WDV site (Tables 6.4 & 6.5).

The observed N_2O-N concentrations also showed a consistent increasing trend during the push-pull tests conducted in the WDV-A, WDV-B, WDV-C and PNR piezometers (Figure 6.6 b1, b2, d1 and d2). At the WDV site, the observed N_2O-N concentrations increased from <0.001 to approximately 0.007 mg N_2O-N L^{-1} during the push-pull test in the Summer/Autumn (Figure 6.6 b1), and from <0.001 to 0.012 mg N_2O-N L^{-1} during the push-pull test in the Winter/Spring (Figure 6.6 b2). At the PNR site, the observed N_2O concentrations increased from <0.001 to approximately 0.077 mg N_2O-N L^{-1} during the push-pull test in the Summer/Autumn (Figure 6.6 d1), and from <0.001 to 0.024 mg N_2O-N L^{-1}

during the push-pull test in the Winter/Spring (Figure 6.6 d2). Consistent with the reduced conditions (DO < 0.5 mg L^{-1}), *pH* values range (5.89-6.86) within the preferred range by denitrifiers (5.5-8.0; Rust et al., 2000), and the presence of an electron donor (DOC values range from 0.49-3.81 mg L^{-1}) appears to provide conditions favourable for denitrification in shallow groundwater at the WDV and PNR sites (Tables 6.4 & 6.5).

At the DAN site, there were no indications of denitrification observed in the shallowest piezometer (DAN-A) (Figure 6.4). However, denitrification in the deep piezometer (DAN-C) was apparent with an estimated zero-order denitrification rate of 0.04 mg N L^{-1} h^{-1} during the Summer/Autumn test (Table 6.4). This is also supported by a slight increase in the N_2O-N concentrations observed during the push-pull test (Figure 6.6 c1). In contrast to the DAN-A, environmental conditions at the DAN-C were conducive for denitrification given the anoxic conditions (DO < 0.5 mg L^{-1}) and presence of an electron donor (DOC = 1.38 mg L^{-1}) (Table 6.4). The denitrification rate could not be estimated at the DAN-C site during the Winter/Spring test, due to the sensitivity of NO_3^--N and Br^- concentrations to measurement errors considering the very low rate of transformation as observed in the Summer/Autumn test (Figure 6.4 b1). However, the environmental conditions at the DAN-C during the Winter/Spring remained conducive for denitrification given the anoxic conditions (DO < 0.5 mg L^{-1}) and presence of electron donor (DOC = 1.14 mg L^{-1}) (Table 6.5).

This contrast in environmental conditions observed at the DAN-A and DAN-C piezometers, spaced at only about 6m, could be explained by the presence of a relatively low hydraulic connectivity layer in the subsurface environment. This is supported by a discrepancy in the estimates of groundwater age (MRT) of 0.2 yr for the DAN-A piezometer (4.0 – 4.5 m bgl), as compared to an MRT of 39 yr for the DAN-C piezometer (7.0-7.5 m bgl) (Table 6.2). A

relatively very low hydraulic conductivity (0.0036 m day^{-1}) estimated for the mid depth piezometer DAN-B (screened at 5.5-6.0 m bgl) (Table 6.2) also suggests the existence of an aquitard layer, essentially separating the subsurface environments hydrologically at the DAN site.

6.3.3.3 Temporal variability – shallow groundwaters

Comparing the Summer/Autumn and Winter/Spring measurements (Tables 6.4 & 6.5), there were no apparent significant changes in the shallow groundwater conditions observed in different piezometers at the study sites and at specific depths. The oxic groundwater conditions (DO > 2 mg L^{-1}) remained oxic in all piezometers at the PAH site and the shallowest piezometer DAN-A at the DAN site, whereas the reduced groundwater conditions (DO < 0.5 mg L^{-1}) remained reduced in all piezometers at the WDV and PNR sites and the deepest piezometers DAN-C at the DAN site (Tables 6.4 & 6.5). Accordingly, no denitrification rates were estimated at the PAH and DAN-A sites. It appears that the DO concentrations at the PAH and DAN-A sites slightly increased during the Winter/Spring experiments indicating the influence of groundwater recharge (Tables 6.4 & 6.5). A relatively fast recharge of groundwater at the PAH and DAN-A sites is also suggested by the young age of groundwater (0.2 yr MRT; Table 6.2). However, the lower rainfall and reduced groundwater recharge during the summer months does not seem to have a significant effect on the redox conditions of groundwater at the PAH and DAN-A sites, as DO concentrations remained high (> 2 mg L^{-1}) during the Summer/Autumn experiments (Table 6.4).

At the WDV and DAN-C sites, the low values and the absence of apparent trend in denitrification rates between the Summer/Autumn and Winter/Spring experiments indicate no significant changes in the denitrification characteristics at the sites between the seasons

(Tables 6.4 & 6.5). This is also supported by the insignificant changes and no clear trend in the DO and DOC values observed the Summer/Autumn and Winter/Spring experiments (Tables 6.4 & 6.5) at the WDV and DAN-C sites. This again suggests the lack of a significant influence of different seasonal climatic conditions on the denitrifications characteristics at the WDV and DAN-C sites. This is also consistent with the estimates of relatively high groundwater age (MRT) of 20 yr at the WDV sites and of 39 yr at the DAN-C site (Table 6.2).

At the PNR site, the denitrification rate was measured twice as high during the Winter/Spring experiment compared to the Summer/Autumn experiment (Tables 6.4 & 6.5). This could be due to slightly more reduced groundwater conditions considering the drop in DO and ORP values at the PNR site during Winter/Spring experiment (Table 6.5). Moreover, the presence of a small amount of NO_3^- (0.05 mg L^{-1}) at the PNR site during the Winter/Autumn test compared to being undetected (<0.01 mg L^{-1}) in the Summer/Autumn test may also have acclimatised the denitrifiers such that they were already active when the Winter/Autumn experiment was conducted (Eschenbach et al., 2015).

6.3.4 Indications from background concentrations of nitrous oxide in groundwater at the study sites

A notable observation with the piezometers at the PAH and DAN sites was the apparent high background concentrations of the N_2O-N (Tables 6.4 & 6.5). The observed background N_2O concentrations at the PAH site ranged from 5.16 to 8.73 µg N L^{-1} and at the DAN site from 0.08 to 46.93 µg N L^{-1} (Tables 6.4 & 6.5), generally much higher than the dissolved N_2O concentration of 0.22 µg N L^{-1} at equilibrium with the atmosphere at 20 °C (Jurado et al.,

2017). In contrast, low background N_2O concentrations were found at the WDV site (0.04 – 0.33 µg N L^{-1}; Tables 6.4 & 6.5). Observations of high background concentrations of N_2O in groundwater, however, are not new (Jurado et al., 2017; Mühlherr and Hiscock, 1998; Ueda et al., 1993). In a review, Jurado et al. (2017) found a broad range of values from 0 to 4004 µg N_2O-N L^{-1} in groundwater based on studies conducted across the globe. The N_2O measured in groundwater could be produced in the groundwater itself or in the overlying unsaturated zone and leached to groundwater. In terms of N_2O production in groundwater, denitrification has been identified as the main process producing N_2O in groundwater followed by the nitrification process (Jurado et al., 2017).

In this study, the higher N_2O concentrations were particularly observed in oxic groundwaters (DO > 2 mg L^{-1}) at the PAH and DAN-A sites (Tables 6.4 & 6.5). The low amounts of NO_2^-, NH_4^+ and high DO concentrations (2.78-9.69 mg L^{-1}) observed at the PAH and DAN-A sites (Tables 6.4 & 6.5), however, suggest that denitrification was not largely responsible for the high N_2O concentrations in groundwater at the PAH and DAN-A sites (e.g., Ueda et al., 1993).

Nitrification, the oxidation of ammonia or ammonium to NO_3^-, has been identified as a source of N_2O in oxic groundwaters (Ueda et al., 1993). In nitrification, both NO_3^- and N_2O are expected to increase under aerobic conditions as products of the process (Minamikawa et al., 2015). While N_2O in reduced groundwater may be reduced to N_2 in denitrification, nitrification can only result in N_2O production (Mühlherr and Hiscock, 1998) as both the high NO_3^- concentrations and aerobic conditions inhibit the reduction of N_2O to N_2 (Baggs and Philippot, 2010; Clough et al., 2005; Minamikawa et al., 2015). Mcmahon et al. (2000) also argued that the positive correlation between N_2O and NO_3^- concentrations, high DO

concentrations but low DOC concentrations, as also found in this study, indicate nitrification as the primary source of N_2O in groundwaters. However, they suggested that nitrification may have occurred in the overlying unsaturated zone considering the low ammonium ($NH4^+$) and high NO_3^- concentrations in groundwater. Mühlherr and Hiscock (1998) also used low NH_4^+ concentrations (< 0.01 mg L^{-1}) in groundwater samples to indicate that nitrification occurred in the unsaturated zone. These conditions of low NH_4^+ but high NO_3^- concentrations in groundwater are similar to what was observed at the PAH and DAN-A sites (Tables 6.4 and 6.5). Mühlherr and Hiscock (1998) also suggested that N_2O production in an aerobic aquifer is indicated by increasing N_2O with depth below the water table. However, this was not observed in this study (Tables 6.4 & 6.5) further supporting the interpretation that the high N_2O observed at the PAH and DAN-A sites were not primarily produced by nitrification process in the shallow groundwater.

As for other N_2O-producing processes in groundwater, the absence or very low concentrations of NO_2^- and NH_4^+ in groundwater (Tables 6.4 & 6.5) indicates that chemodenitrification is unlikely (Baggs and Philippot, 2010; Bremner, 1997). Moreover, the aerobic conditions (DO > 2.78 mg L^{-1}) and relatively low DOC (0.37-3.15 mg L^{-1}) but high NO_3^- concentrations (> 5.16 mg L^{-1}) observed in shallow groundwaters at the PAH and DAN-A sites do not support the occurrence of DNRA given the requirement of C/N ratio higher than 12 (Rütting et al., 2011; Storey et al., 2004; Tiedje, 1988).

It, therefore, appears that the observed N_2O-N at the PAH and DAN-A sites may have been mainly produced by nitrification and/or incomplete denitrification in the overlying vadose zones and leached as dissolved N_2O to the groundwater (Baggs and Philippot, 2010; Clough et al., 2005; Hashimoto and Niimi, 2001; Mühlherr and Hiscock, 1998; Vilain et al., 2012).

This is also supported by the observations of a decrease in NO_3^--N and NH_4^+ -N contents in soils with an increase in soil depth in the vadose zone at the study sites (Table 6.3). Although further investigation would be required to distinguish with certainty whether the observed enriched concentrations of N_2O-N in shallow groundwaters at the PAH and DAN-A sites are due to nitrification and/or incomplete denitrification in the unsaturated zone, it appears that such high N_2O-N concentrations in shallow groundwater at the PAH and DAN-A sites are mainly produced in the unsaturated zone and leached as dissolved N_2O-N to the groundwater. This is also supported by strong hydrological connectivity between the unsaturated zone and the shallow groundwater, as indicated by the young groundwater age of 0.2 yr at the PAH and DAN-A sites (Table 6.2). It has to be noted that the soils and vadose zone at the PAH and DAN-A sites have a coarse texture with significant amount of gravel and sand in the profile (Table 6.3), which support faster infiltration and percolation to groundwater.

6.3.5 Implications of denitrification characteristics on transformation and transport of nitrate in the subsurface environment

It is very clear from the investigations at the four study sites that the surface soil (< 30 cm, bgl) had the highest denitrification potential, at least 28 times and up to 11,000 times the estimated potential in the subsurface layer (> 30 cm bgl) (Table 6.6). Considering the soil bulk density and corresponding soil layer depths, the DEA values measured for the surface soil translated to NO_3^--N removal in a range of 44 to 111 kg N ha^{-1} day^{-1} at the study sites (Table 6.6). The DEA values measured for the subsurface soil layers (> 30 cm bgl), however, translated to NO_3^--N removal in a range of <0.01 to 0.89 kg N ha^{-1} day^{-1} (Table 6.6). Obviously, this study was not able to estimate DEA from all the layers in the vadose zone above the water table due to several constraints, such as difficulty to collect samples. The gap in the investigation of the entire vadose zone profile and the absence of measurements of

actual denitrification makes it difficult to translate the measured DEA values to the actual denitrification capacity of the entire vadose zone profile at the study sites. However, even with the lack of information on nutrient inputs and denitrification capacity of the whole profile, it is apparent that denitrification in the vadose zone was not enough to reduce completely the NO_3^- in the soil profile. This seems evident in the NO_3^--N concentrations in the soil-water found at the deepest investigated soil layer, ranging from 4.76 mg L^{-1} in 0.70-1.0 m bgl at the DAN site to 18.48 mg L^{-1} in 1.85-1.95 m bgl at the WDV site (Table 6.3), and in the high NO_3^- concentrations in the shallow groundwater at the PAH and DAN sites (Tables 6.4 & 6.5). Nevertheless, the huge discrepancy in NO_3^- concentrations in the soil-water at the deepest soil layers (6.16-18.48 mg L^{-1}) and in shallow groundwater (<0.01-0.05 mg L^{-1}) at the WDV and PNR sites strongly suggests the denitrification in the shallow groundwater significantly reducing the NO_3^- concentrations (Tables 6.3, 6.4 & 6.5), although dilution may also play a role. The higher NO_3^- concentrations in shallow groundwater (10.72-14.16 mg L^{-1}) at the DAN-A site compared to the concentrations in the soil-water (4.76-12.11 mg L^{-1}) could indicate that leaching of greater NO_3^- concentrations to groundwater may have occurred at different locations on the land (such as urine spots) not sampled in the vadose investigations.

At the PAH site, the coarse texture of the soil and the thinner vadose zone (water table within 2.5-3.5m bgl; Tables 6.2 & 6.3) seemed to have facilitated relatively faster transport of NO_3^- to the shallow groundwater. This is evident in the high NO_3^- concentrations in all the three piezometers of different depths at the PAH site (Tables 6.4 & 6.5), indicating that dilution was not sufficient to lower NO_3^- concentrations to insignificant amounts. Moreover, the high DO concentrations (Tables 6.4 & 6.5) and the young age of the groundwater (0.2 yr MRT; Tables 6.2) strongly suggest the influence of percolating water on the quality of shallow

groundwater. The high DO concentrations in shallow groundwater suggests the absence of denitrification potential, as confirmed in the push-pull test measurements, indicating the risk of NO_3^- contaminated groundwater reaching the surface water. This is notable considering that, in the Mangatainoka sub-catchment where the PAH site was located, the surface water sampled during base flow conditions in the Mangatainoka river was found to have a young age of 0 – 2 years of mean residence time (Morgenstern et al., 2014). Thus, the type of the hydrogeologic settings leading to oxic groundwater conditions found at the PAH site seems to be a strong candidate as priority sites for effective nitrate management to minimise the impact of land-based activities on the quality of groundwater and receiving surface water.

At the WDV site, the fine textured soil and deeper vadose zone (depth to water table > 3.5m, bgl; Tables 6.2 & 6.3) seemed to have restricted the transport of NO_3^- to the shallow groundwater (Tables 6.4 & 6.5). The thick silty and clayey formation at the WDV site restricts fast vertical movement of groundwater allowing more time for transport and transformation of NO_3^- in the subsurface environment. It is unclear though whether it's the restriction of downward flow or the possibility of higher denitrification rate considering the high DOC found in the deeper layer at the site (19.36 µg C g^{-1} soil at 1.85-1.95 m bgl; Table 6.3), or both, has more influence on the reduction and/or transport of NO_3^- in the vadose zone. Nevertheless, the estimates of relatively older shallow groundwater (20 yr MRT) at the WDV site suggests that any percolating water from the vadose zone does not have a significant influence on the quality of groundwater. Moreover, considering the reduced condition and occurrence of denitrification in groundwater at the WDV site (Tables 6.4 & 6.5), any NO_3^- reaching the groundwater may be significantly or entirely reduced in the groundwater before reaching the surface water. This is supported by very low (<0.2 mg L^{-1})

concentrations of NO_3-N observed in shallow groundwater at the WDV site (Tables 6.4 and 6.5).

Interestingly, the vadose zone profile at the PNR site is also composed of coarse-textured soils like the PAH site with sand being prominent in the profile (Table 6.3). In such soil profile with good drainage, we may expect percolating water to reach the groundwater easily along with its contaminants. However, the thicker vadose zone (water table at 4.29 m bgl, Table 6.2) at the PNR site compared to the PAH site (water table at < 3.0 m bgl Table 6.2) and the thin layers of silty soils in the generally sandy profile may have facilitated longer residence time for the transport and transformation of NO_3^- in the vadose zone at the PNR site. This is supported by the estimates of relatively older groundwater age (MRT ~ 7 yr) at the PNR site as compared to the PAH site (MRT <0.2 yr) (Table 6.2). In addition, the reduced conditions and occurrence of denitrification in shallow groundwater at the PNR site (Tables 6.4 and 6.5) may significantly or entirely reduce percolating NO_3^- in the groundwater before reaching the surface water.

The DAN site is altogether a different site having a mix of the properties of the PAH and WDV sites. The DAN site had the thinnest vadose zone with water table found at 1.4-3.2 m bgl (Tables 6.4 & 6.5). The apparent higher denitrification potential in the surface and subsurface soil layers (Table 6.6), possibly due to the higher DOC content, was not sufficient to reduce NO_3^- in the vadose zone profile possibly due to the fast percolation considering the coarse texture of the profile (Table 6.3). This is evident in the high DO content (> 2 mg L^{-1}) and younger age of groundwater (0.2 yr MRT) at the DAN-A (4.0-4.5 m, bgl) site, resulting in high NO_3^--N concentrations measured in the shallow groundwater (>10 mg N L^{-1}) (Tables 6.4 & 6.5). However, groundwater at the deeper DAN-C (7.0-7.5 m, bgl) was significantly

different with anoxic conditions (DO < 0.5 mg L^{-1}) and low NO_3^--N concentrations (< 0.01 mg N L^{-1}). It is apparent that the influence of percolating water was limited to shallow groundwater at the DAN-A site, possibly a perched water table considering the presence of very low hydraulic conductivity between the DAN-A and DAN-C piezometers (Table 6.2). This is also supported by the estimates of relatively older groundwater age (MRT ~ 39 yr) at the DAN-C site as compared to the DAN-A site (MRT <0.2 yr) (Table 6.2). It suggests a possibility of mainly lateral (horizontal) rather than downward (vertical) flow of shallow groundwater at the DAN site. While NO_3^- concentrations at the DAN-A were higher than those found at the PAH site, the lower DO concentrations, higher DOC concentrations, and lower hydraulic conductivity at DAN-A compared to the PAH site (Tables 6.2, 6.4 & 6.5) indicate the strong possibility of further NO_3^- reduction in groundwater before being discharged to the surface water. Further investigation along the groundwater flow path at the DAN site would be required to assess the risk of high NO_3^- concentrations measured in shallow (perched) groundwater reaching the surface water.

6.4 Conclusions

The targeted and effective management of the impacts of farm nitrate on receiving freshwater quality requires a sound understanding of their sources, pathways and attenuation mechanisms in their flow from farms to receiving freshwater bodies. This study investigated NO_3^- flow and its potential attenuation in the vadose and saturated zones at four pasture land sites in the Manawatū catchment in New Zealand. Results of the study revealed diverse and even contrasting denitrification characteristics across and within the study sites, affecting the transport and transformation of NO_3^- in the subsurface environment. The denitrification potential measured as DEA values was found at least 28 times higher in the surface soils (<30

cm bgl) as compared to the subsurface layers (<30 cm bgl) across the study sites. A seasonal variation in the DEA values measured for the surface soil layers was observed, with higher denitrification potential observed in the Winter/Spring than in the Summer/Autumn season. While it was unclear how the denitrification potential in the vadose zone translates to its capacity to reduce NO_3^- in actual field conditions, the physical properties such as texture and thickness of the vadose zone appeared to have influence on the transport and transformation of NO_3^- in the subsurface environment. At the PAH and DAN sites, with the coarse textured and relatively thin vadose zone, denitrification processes in the vadose profile was insufficient to reduce NO_3^- before reaching the shallow groundwater. As a result, enriched NO_3^-N concentrations (> 5 mg L^{-1}) were measured in the shallow groundwater at the PAH and DAN-A sites. While the PNR site also had a coarse-textured soil, the thicker vadose zone seemed to have contributed to mitigation of the transport of NO_3^- to shallow groundwater underneath. This was even clearer at the WDV site, where the fine-textured soils and thicker vadose zone appeared to have restricted downward flow of percolating water resulting in a longer residence time and relatively older shallow groundwater.

More interestingly, different hydrogeologic settings at the study sites appeared to have resulted in spatially variable groundwater conditions and denitrification characteristics in shallow groundwater at the sites. This has contrasting influence on the transport and transformation of NO_3^- in shallow groundwater before its eventual discharge to the surface water. At the PAH site, shallow groundwater was found as relatively young and oxic (DO > 7 mg L^{-1}) with less potential for further reduction of NO_3^- in the saturated zone. Similarly, the shallowest groundwater measured in DAN-A (4.0 – 4.5 m bgl) at the DAN site was found as relatively young and oxic with less denitrification potential in the saturated zone. At the DAN site, there was however low potential for NO_3^--contaminated shallow groundwater to reach

the relatively deeper groundwater (7.0 – 7.5 m bgl) due to the restricting low hydraulic conductivity layer. In contrast, shallow groundwater at the WDV and PNR sites have reduced conditions with high potential for further reduction of NO_3^- in the saturated zone. Very limited seasonal influence was observed on the quality and denitrification characteristics of shallow groundwater at the study sites. The oxic and reduced groundwater conditions measured at the PAH/DAN-A and WDV/PNR/DAN-C sites, respectively, were essentially unchanged between the Summer/Autumn and Winter/Spring seasons measurements. This could be further investigated by conducting high spatial and temporal, such as monthly, groundwater monitoring and analysis to measure redox status of shallow groundwater at the study area.

It is very clear from this study that different hydrogeologic settings have a strong influence on transport and transformation of NO_3^- flows in the subsurface environment. NO_3^- leached from agricultural lands in some cases may be easily transported with very low attenuation to the groundwater underneath and to the receiving surface water, as in the PAH site in this study. However in other cases, NO_3^- leached from agricultural soils may be reduced or 'attenuated' in the subsurface environment like at the WDV and PNR sites, resulting in minimal impact to the quality of receiving surface water. This highlights the importance of investigating both the vadose and saturated zones to improve our understanding of denitrification characteristics in the subsurface environment and their implications on nitrate transformation and transport from agricultural lands to receiving water bodies. This study recommends further research to assess and map effects of different hydrogeologic settings on transport and attenuation of NO_3^- in the subsurface environment of sensitive agricultural catchments. Such improved understanding offers a sound basis for formulating efficient and

targeted nitrate management actions to support the better use of agricultural lands while minimising the impact on water quality across agricultural landscapes.

CHAPTER 7

SYNTHESIS AND RECOMMENDATIONS FOR FUTURE WORK

7.1 Introduction

A sound understanding of the transport, transformations and effects of nitrate is a key component of managing the influence of agricultural production on receiving water quality. But there is yet limited knowledge available for effective management of the processes and pathways that lead to N contamination of rivers and lakes in New Zealand's agricultural catchments. The literature review (Chapter 2) suggested that the amount of nitrate (NO_3^-) leached to groundwater and subsequently transported to surface waters depends strongly on the NO_3^- attenuation characteristics of its flow pathways from land to surface waters (Puckett, 2004). Denitrification – the microbial-mediated transformation of water soluble NO_3^- to gaseous N forms – has been identified as an important NO_3^- attenuation process in the subsurface environment (Anderson et al., 2014; Jahangir et al., 2013, 2012c; Rivett et al., 2008). This capacity of the subsurface environment to reduce NO_3^- by denitrification varies in space and time (Oehler et al., 2007; Pinay et al., 1993; Seitzinger et al., 2006). A sound knowledge of the characteristics of the subsurface environment and its implications for denitrification capacity is, therefore, essential to the potential to manage and mitigate NO_3^- contamination in agricultural catchments. But there is currently little known about the occurrence, spatial and temporal variability, and factors contributing to denitrification potential in the subsurface environment in New Zealand agricultural landscapes, particularly in the Manawatū River catchment where N contamination is particularly problematic.

The study focused on; the occurrence and spatial distribution of denitrification in groundwaters, the evaluation and development of reliable and practical methods for quantifying subsurface denitrification in the vadose and saturated zones, the quantification of subsurface denitrification in the vadose and saturated zones (shallow groundwaters), and the assessment of hydrogeological factors responsible for the variability in subsurface denitrification characteristics at the study sites. Given its degraded status, the Manawatū River catchment was a very appropriate setting for this investigation of subsurface denitrification.

7.2 Occurrence of and potential for denitrification in the subsurface environment

A groundwater survey was conducted to assess indications of the occurrence of, and the potential for, denitrification in groundwater in the study area, with particular focus on the eastern part (Tararua GWMZ) of the Manawatū catchment. A number of existing geographical information, field surveys, laboratory measurements and statistical (Principal Components Analysis and Analysis of Variance) methods were combined to confirm findings and obtain supporting evidence for the features that determine the denitrification characteristics of the subsurface environment, with particular focus on groundwater (Chapter 3).

Results of the study showed that denitrification potential varies in the subsurface environment (below root zone) in the Tararua GWMZ. Oxic groundwater conditions with low denitrification potential were observed in the southern part (Mangatainoka sub-catchment), whereas areas of anoxic/reduced groundwater with high potential to denitrify were found in

the middle and northern parts of the catchment. An analysis of different hydrogeological factors indicates that soil characteristics and aquifer materials that allow faster movement of percolating water or groundwater (e.g., well-drained soil, gravel type rock) correlated with enriched dissolved oxygen (DO) concentrations in groundwater, such as in the southern part of the catchment. These oxic groundwater conditions limit subsurface denitrification. Low DO and NO_3^- concentrations were found in groundwater in the middle and northern parts of the Tararua GWMZ where the combinations of soil and rock types had poor drainage characteristics (e.g. poor soil drainage, and alluvium and/or loess rock types). Limited similar studies also found high DO concentrations in shallow groundwater beneath well-drained soils in the Waikato region (Clague et al., 2015c).

The above results offer insight into the need for variable nitrate management policy and practices to account for the variability in the nitrate reduction potential of the subsurface environment as seen in the Tararua GWMZ. The logical step forward to inform appropriate policy and management options is to quantify denitrification in the subsurface environment. The groundwater survey was used to select sites with low or high denitrification potential for detailed measurements and quantification of denitrification in both the unsaturated and saturated zones in the study area.

7.3 Quantifying denitrification in the vadose and saturated zones

Reliable measurements of denitrification rates are required to obtain strong evidence and a sound understanding of the capability of the subsurface environment to reduce NO_3^-. A review of existing literature (Chapter 2) showed that approaches and methods for measuring and estimating denitrification rates both in the vadose and saturated zones differ in their

protocols and procedures. Among the several methods available for quantifying denitrification in soil-water systems, the acetylene inhibition (AI) method was adopted in this study both in laboratory denitrification assays and an *in situ* single-well, push-pull test technique. This method was selected for the following reasons: relatively low cost, ability to analyse large number of samples, and it is versatile enough for use in laboratory, field and remote site investigations (Tiedje et al., 1989). The AI method is a reasonably accurate measurement of denitrification rates in soil-water systems, demonstrated by several studies that have observed no significant difference between the denitrification rates of surface soils as measured by the AI and ^{15}N tracer methods (Mosier et al., 1986; Parkin et al., 1985; Tiedje et al., 1989). The continued use of the AI method in recent denitrification studies supports its popularity and usefulness. However, very few studies have applied and evaluated the AI method for measurements of denitrification rates in either the deeper vadose layers below the root zone or in shallow groundwater.

7.3.1 A novel technique for measuring denitrifying enzyme activity in the subsurface soils

Previous studies of subsurface soil denitrification that have employed the AI method have varied significantly in terms of the amount of substrate used as a source of NO_3^- and the electron donor, the pre-incubation of samples, acetylene concentration, etc. Moreover, challenges have been encountered with the popular Erlenmeyer flasks (125 mL) for denitrification assays with subsurface soils. Thus, this study evaluated a novel incubation assay technique using vacuum pouches for measurement and quantification of denitrifying enzyme activity (DEA) of the subsurface soils (Chapter 4). Advantages of using the vacuum pouch technique includes the ability to use larger amounts of soil in assays, the maintenance of atmospheric pressure throughout the process, the ability to process much larger number of

assays than when using flasks, simpler steps with less use of syringes and needles, and the ability to extract large gas sample volumes, which better represents the headspace composition in the analysis.

Results of this study (Chapter 4) showed that the use of either the vacuum pouch or Erlenmeyer flask is appropriate for DEA measurements in surface soils (<0.3 m bgl). However, the vacuum pouch technique is more accurate and practical for subsurface soils (0.3 to 2.0 m bgl), where relatively lower DEA values are expected. The vacuum pouch technique gave a better representation of the headspace composition resulting in less variation and more certainty in the DEA values measured for subsurface soils. In terms of the optimum amount of substrate for DEA assays, a combination of 75 µg N and 400 µg C g^{-1} dry soil provided the optimum DEA in subsurface soils, as compared to the recommended 50 µg N and 300 µg C g^{-1} dry soil as determined in earlier studies for surface soils. This study contributes to the development of a more standardised measurement of denitrification in subsurface soils which allows for a more accurate assessment of the capability of the vadose zone to attenuate or reduce the NO_3^- that leaches from agricultural soils.

7.3.2 Quantifying denitrification rate in shallow groundwater using the singe-well, push-pull test technique

The single-well, push-pull test technique is a practical method that is commonly used to measure denitrification in a groundwater system. However, the results of a push-pull test can vary depending on the denitrification tracer (whether using reactant or product) and kinetic model (zero- or first-order) used in the analysis to estimate the denitrification rate. These differences in method may lead to erroneous understanding of the denitrification characteristics of the subsurface environment. In Chapter 5, this study evaluated the use of

different tracers and kinetic models to estimate denitrification rates from single-well, push-pull test data in shallow groundwater systems (e.g., <10 m below ground level) at two study sites.

On the one hand, in general terms, the study results showed that the single-well, push-pull test was able to measure denitrification rates in shallow groundwater in the Manawatū River catchment. On the other hand, there were discrepancies in estimated denitrification rates based on different denitrification tracers (whether using reactant or product), and kinetic model (zero- or first-order) used for the analysis of push-pull test data obtained. The denitrification rates estimated using the measurements of denitrification reactant (NO_3^- reduction) were significantly higher (6 to 60 times) than the rates estimated using the measurements of denitrification product (nitrous oxide production). The zero-order rates estimate using reactant were comparable to reported zero-order rates in the literature using the push-pull test technique (0.01-1.12 mg N L^{-1} h^{-1}) (Rivas et al., 2014). Relying on the measurement of only denitrification products (in this case N_2O) without measuring all other N species (e.g., N_2, NO_2^-, etc.) during the experiment could lead to erroneous quantification of denitrification in groundwater system. In this regard, the denitrification rate based on measurements of the denitrification reactant (NO_3^- reduction) may provide a more representative value of the denitrification characteristics of shallow groundwater. This uncertainty over the magnitude of the denitrification aside, the use of acetylene in the push-pull tests and subsequent quantification of N_2O as the denitrification product provided supporting evidence for the occurrence or non-occurrence of denitrification in shallow groundwater systems.

The study results also showed that the estimates of denitrification rates differed depending on whether a zero-order or first-order kinetic model was assumed for the analysis of push-pull test data. However, in some instances as shown in this study, either a zero-order or a first-order model may appear to be valid to analyse and estimate denitrification rate from push-pull tests. These discrepancies in estimates of denitrification rates may have implications for the simulation of NO_3^- transport and transformation in groundwater systems, and requires further investigations (which was beyond the scope of this study).

7.4 Variability in denitrification characteristics in the subsurface environment and the contributing factors

A sound understanding of subsurface denitrification, its occurrence in the vadose and saturated (groundwater) zones, its spatial and temporal variability, and influencing factors, are key components in the management and mitigation of any adverse impacts of land use and management practices on receiving water quality and ecosystem health. This study investigated both the vadose and saturated zones to characterise and quantify denitrification in the subsurface environment of the Manawatū River catchment (Chapter 6). A total of four study sites were selected and established for detailed measurements of denitrification in areas of low or high groundwater denitrification potential as determined in the earlier stage of this thesis research (Chapter 3). While all sites were under pasture, three were on dairy farms (Pahiatua, Dannevirke, and Palmerston North) and one was on a sheep and beef farm (Woodville). Soil type and texture varied from silt loam and clay loam soils at the Woodville site to stony silt loam soils at the Pahiatua and Dannevirke sites, to coarse-textured fine sandy loam soils at the Palmerston North site. Alluvium rock type was identified at the Woodville and Palmerston sites, whereas the rock type at the Pahiatua and Dannevirke sites was loess

over gravel. Vadose zone investigations were carried out with soil samples collected using a percussion corer, while piezometers were installed at the sites for shallow groundwater investigations.

At these sites, the vacuum pouch denitrification assays and push-pull test methods developed in this thesis (Chapters 4 and 5) were used for the detailed investigations of denitrification in the vadose and saturated zones. These measurements were conducted at different depths i.e. for both the vadose (0-2.1 m below ground level (bg)) and saturated (4.0-7.5 m bgl) zones, and at different seasons (wet and dry) to gain an understanding of the spatial and temporal variability in subsurface denitrification characteristics at the study sites.

7.4.1 Spatial and temporal variability

Investigations of the vadose zone at the four sites revealed that, in terms of DEA, the denitrification potential in the surface soils (< 0.3 m bgl) was at least 28 times greater than in the subsurface soils (> 0.3 m bgl). It was also apparent that the denitrification potential in the surface soil at the Pahiatua, Woodville and Dannevirke study sites was greater during the wet season (winter/spring) than in the dry season (summer/autumn). No temporal comparison could be made for the Palmerston North site due to a lack of data. This temporal variability was less apparent in the subsurface soil (>0.3 m bgl) where the DEA values were low; these values translated to a rate of NO_3-N reduction in the range of <0.01 to 0.89 kg N ha^{-1} day^{-1}. It is to be noted that the absence of measurements of actual denitrification makes it difficult to translate the measured DEA values to actual denitrification capacity of the vadose zone at the study sites. However, the measured DEA values suggested denitrification rates in the vadose zone was not sufficient to completely reduce the nitrate in the soil profile as evidenced by the high nitrate concentrations in the shallow groundwater at the Pahiatua and Dannevirke sites.

The results from push-pull tests suggested the presence of oxic groundwaters with no indications of denitrification at the Pahiatua (4.0-6.5 m bgl) and Dannevirke (4.0-4.5 m bgl) sites. In comparison, reduced groundwater with strong indications of denitrification was found at the Woodville (4.5-7.5 m bgl), Palmerston North (6.0-6.5 m bgl), and Dannevirke (7.0-7.5 m bgl) sites. The presence of a layer with very low hydraulic conductivity at the Dannevirke site (5.5-6.0 m bgl) appears to have caused a degree of separation of the waters at the two depths (4.0-4.5 m bgl vs 7.0-7.5 m bgl) resulting in different denitrification characteristics at shallow and deeper groundwater depths at this site. Overall, the denitrification rates measured in shallow groundwaters at the study sites varied from 0.04 mg N L^{-1} h^{-1} to 1.07 mg N L^{-1} h^{-1}. Furthermore, no seasonal influence was observed on the denitrification characteristics of shallow groundwater at the study sites as both oxic and reduced groundwater remained unchanged between the two seasons investigated.

7.4.2 Contributing factors

For the vadose zone investigations, it was apparent that denitrification potential in the surface soil (< 0.3 m bgl) was much greater than in the subsurface soils (> 0.3 m bgl) across the study sites. This could be attributed to the greater concentration of denitrifying microorganisms in the surface soil compared to the subsurface soil. In addition, greater dissolved organic carbon (DOC) content appears to influence the denitrification potential across the soils, even the subsurface soils. Soil moisture content, which is indicative of the oxygen status in the soil-water system, was also found to influence the denitrification characteristics of the subsurface soils. These results confirm the findings of several studies that have identified the aforementioned factors as key determinants of denitrification in soil-water systems.

The most revealing factor identified in this study is the effect of different hydrogeological properties on the denitrification characteristics of the subsurface environment. The physical properties of the subsurface soil such as texture and thickness of the vadose zone appeared to have a significant influence on the transport and transformation of NO_3^- in the subsurface environment at the study sites. Where the vadose zone is thin and composed of coarse-textured soils, percolation of NO_3^- was evident in large NO_3^- concentrations in oxic and young, shallow groundwaters at the Pahiatua and Dannevirke sites. On the other hand, where soil texture is fine and/or the vadose zone is thick (depth to water table greater than 3 m), low NO_3^- concentrations were measured in the reduced groundwater such as at the Woodville and Palmerston North sites. Denitrification was found to occur in these reduced groundwaters. These results strongly suggest that different hydrogeologic settings influence the spatial variability of denitrification characteristics in the subsurface environment.

While this study did not investigate the influence of nutrient inputs (e.g., fertilisers or animal excreta) or the sources of the electron donor such as carbon, it is clear from observations made that hydrogeological properties such as soil texture and rock type have a strong influence on the fate and transport of NO_3^- from farms to receiving waters. While the quantity of nutrient input and sources of carbon may enhance denitrification conditions, it is apparent from this study that the reduced groundwater conditions that are conducive to subsurface denitrification are mainly due to the influence of hydrogeological characteristics on water movement, either rapid or slow, through the soil profile and groundwater system.

Overall, the results of this study suggest that denitrification characteristics in the subsurface environment of the study catchment vary spatially due to several factors but mainly due to

hydrogeological properties. Some areas were found to have low potential to reduce NO_3^-, whereas some areas have high potential for denitrification to occur.

7.5 Implications for land management to mitigate nitrate contamination of freshwater resources

The knowledge of subsurface denitrification characteristics gained in this study is crucial to inform measures to manage and mitigate the impacts of agricultural activities on groundwater and surface water quality not only in the Manawatū catchment, but other agricultural catchments across New Zealand and, for that matter, worldwide. The clear spatial variability found in the potential of the subsurface environment for nitrate attenuation, mainly due to the different hydrogeological characteristics, entails that specific measures appropriate for the local hydrogeological settings are made. A uniform or 'one size fits all' approach will not be adequate for effective nitrate management, even within a landscape or a farm. In order to achieve improved water quality, reliable and accurate characterisation of the hydrogeological properties of the vadose and saturated zones is essential. While New Zealand has existing soil (Fundamental Soil Layers or S-map) and geology (QMap) maps that are useful, finer resolutions of these maps with particular focus on depth-specific characteristics is critical to gain a clear understanding of the potential for denitrification in the subsurface environment. Equipped with this hydrogeological information and corresponding denitrification characteristics, land managers will be able to identify the areas where status quo or 'business as usual' may continue or areas where limited agricultural activities or specific measures are required to minimise the impact on water quality.

7.6 Recommendations for future work

In addition to the key findings described above and the implications on land management, this thesis suggests the following future research work to further advance our limited knowledge of subsurface denitrification and its potential to mitigate the impacts of agricultural activities on receiving water quality across the Manawatū catchment and other catchments across New Zealand's agricultural landscapes:

- Establishing the vacuum pouch technique as the standard to measure DEA and actual denitrification rates of subsoils

In this study, the vacuum pouch technique was assessed and compared with the commonly used flask technique to measure DEA with only two types of soil profiles namely, Manawatū fine sandy loam and Rangitikei silt loam both of which are found in the Manawatū catchment. It was further applied to measure DEA of different vadose zone layers at the selected four study sites in the catchment and consistent results were obtained. Further application of the vacuum pouch technique in comparison with flask technique using other soil types to measure DEA of surface and subsurface soils would increase the data set to provide further validity of its use and help establish this technique as a standard for DEA measurements. Moreover, it would be interesting to see how the vacuum pouch technique performs in measurements of actual denitrification rates in subsurface soils with no supplemental sources of N and C in incubation assays. This will help develop a more standardised measurement of denitrification in low denitrification activity soils.

- Assess the effects of different estimates of denitrification rates on the simulation of nitrate fate and transport in subsurface environment

Assessing the impacts of different denitrification rates as estimated by zero-order or first-order kinetics on the simulation of NO_3^- transport and transformation is an important area of future research. More accurate estimates of denitrification kinetics could inform simulations of NO_3^- transport and transformation processes in shallow groundwater systems.

- More detailed spatial and temporal assessment of denitrification characteristics in shallow groundwater

This study investigated both the vadose and saturated zones in an integrated manner to characterise and quantify subsurface denitrification at four selected sites in the Manawatū River catchment. This suggests that different hydrogeological properties such as texture and thickness of the vadose zone appear to have a significant influence on groundwater redox conditions (reduced or oxic) and transport and transformation of NO_3^- in the subsurface environment at the study sites. Further application of such measurements and assessments for different combinations of catchment characteristics and hydrogeological settings is recommended in the Manawatū catchment and elsewhere. This new knowledge and information will help build our capability to assess and map subsurface denitrification potential in the Manawatū catchment and other catchments across New Zealand agricultural landscapes and worldwide.

While no clear seasonal variation in the denitrification characteristics was found in shallow groundwater at the study sites, detailed temporal monitoring (e.g., monthly) of groundwater hydrochemistry to study temporal variability of the denitrification characteristics of shallow groundwater is recommended. A comprehensive understanding of any seasonal variations in subsurface denitrification characteristics is an essential aid to managing and mitigating the impacts of agricultural activities on receiving water.

- Impacts or contributions of subsurface denitrification characteristics in terms of NO_3^- transport in different flow pathways

As revealed in the investigations at the study sites, the hydrogeological settings affect the water flow pathways. For instance, a low permeability layer in the vadose zone may restrict percolation and induce lateral movement of water during rainfall events resulting in shorter pathways to receiving surface waters. This highlights the importance of a better characterisation of different water flow pathways and their potential to affect transport and transformation of NO_3^- in subsurface environment.

REFERENCES

Addiscott, T.M., Addiscott, T.M., Benjamin, N., 2004. Nitrate and human health. Soil Use Manag. 20, 98–104. doi:10.1079/SUM2004256

Addy, K., Kellogg, D.Q., Gold, A.J., Groffman, P.M., Ferendo, G., Sawyer, C., 2002. In situ push-pull method to determine ground water denitrification in riparian zones. J. Environ. Qual. 31, 1017–1024. doi:10.2134/jeq2002.1017

Alcalá, F.J., Custodio, E., 2008. Using the Cl/Br ratio as a tracer to identify the origin of salinity in aquifers in Spain and Portugal. J. Hydrol. 359, 189–207. doi:10.1016/j.jhydrol.2008.06.028

Anderson, T.R., Groffman, P.M., Kaushal, S.S., Walter, M.T., 2014. Shallow groundwater denitrification in riparian zones of a headwater agricultural landscape. J. Environ. Qual. 43, 732–744. doi:10.2134/jeq2013.07.0303

Appelo, C., Postma, D., 2005. Geochemistry, Groundwater and Pollution, 2nd ed. A.A. Balkema Publishers, Amsterdam, The Netherlands.

Arp, D.J., 2009. Nitrification. Encycl. Life Sci. doi:10.1002/9780470015902.a0021154

Ascott, M.J., Wang, L., Stuart, M.E., Ward, R.S., Hart, A., 2016. Quantification of nitrate storage in the vadose (unsaturated) zone: a missing component of terrestrial N budgets. Hydrol. Process. 30, 1903–1915. doi:10.1002/hyp.10748

Baggs, E., Philippot, L., 2010. Microbial terrestrial pathways to nitrous oxide, in: Smith, K. (Ed.), Nitrous Oxide and Climate Change. Earthscan, London, pp. 5–35.

Baker, M.A., Vervier, P., 2004. Hydrological variability , organic matter supply and denitrification in the Garonne River ecosystem. Freshw. Biol. 49, 181–190.

Ballantine, D.J., Davies-Colley, R.J., 2014. Water quality trends in New Zealand rivers: 1989-2009. Environ. Monit. Assess. 186, 1939–1950. doi:10.1007/s10661-013-3508-5

Barkle, G., Clough, T., Roland Stenger, 2007. Denitrification capacity in the vadose zone at three sites in the Lake Taupo catchment, New Zealand. Aust. J. Soil Res. 45, 91–99.

Barns, G., 2010. On Single Well Forced Gradient Tracer Tests: Implications of Aquifer Physical Heterogeneity and Tracer Behavior. The University of Sheffield.

Bear, J., 1972. Dynamics of Fluids in Porous Media. Dover Publications, Inc, New York.

Beauchamp, E., Bergstrom, D., 1993. Denitrification, in: Carter, M. (Ed.), Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, Florida, pp. 351–357.

Bekesi, G., 2001. Manawatu-Wanganui, in: Rosen, M., White, P. (Eds.), Groundwaters of New Zealand. New Zealand Hydrological Society, Wellington, New Zealand, pp. 387–396.

Bekesi, G., Mcconchie, J., 2002. The use of aquifer-media characteristics to model vulnerability to contamination, Manawatu region , New Zealand. Hydrogeol. J. 10, 322–331. doi:10.1007/s10040-002-0189-5

Bekins, B., Warren, E., Michael Godsy, E., 1998. A Comparison of Zero-Order, First-Order and Monod Biotransformation Models. Ground Water 36, 261–268.

Bellini, M.I., Gutiérrez, L., Tarlera, S., Scavino, A.F., 2013. Isolation and functional analysis of denitrifiers in an aquifer with high potential for denitrification. Syst. Appl. Microbiol. 36, 505–516. doi:10.1016/j.syapm.2013.07.001

Bishop, P., Kusumo, B., Calvelo-Pereira, R., Burkitt, L., Hedley, C., Hedley, M., Saggar, S., Camps, M., 2014. Assessment of denitrification potentials of grazed pasture soils, in: Currie, L.D., Christensen, C.L. (Eds.), Nutrient Management for the Farm, Catchment and Community. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand, p. 7.

Blakemore, L., Searle, P., Daly, B., 1987. Methods for Chemical Analysis of Soils. NZ Soil Bureau, Lower Hutt, New Zealand.

Böhlke, J., 2003. Sources, transport, and reaction of nitrate, in: Lindsey, B., Phillips, S., Donnelly, C., Speiran, G., Plummer, L.N., Böhlke, J., Focazio, M., Burton, W., Busenberg, E. (Eds.), Residence Times and Nitrate Transport in Ground Water Discharging to Streams in the Chesapeake Bay Watershed. US Geological Survey, Denver, pp. 25–38.

Bohlke, J., Wanty, R., Tuttle, M., Delin, G., Landon, M., 2002. Denitrification in the recharge area and discharge area of a transient agricultural nitrate plume in a glacial outwash sand aquifer, Minnesota. Water Resour. Res. 38, 10-1-10–26.

Boone, R., Grigal, D., Sollins, P., Ahrens, R., Armstrong, D., 1999. Soil sampling, preparation, archiving, and quality control, in: Philip Robertson, G., Coleman, D., Bledsoe, C., Sollins, P. (Eds.), Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York, pp. 3–28.

Bouman, B.A.M., Castañeda, A.R., Bhuiyan, S.I., 2002. Nitrate and pesticide contamination of groundwater under rice-based cropping systems: Past and current evidence from the Philippines. Agric. Ecosyst. Environ. 92, 185–199. doi:10.1016/S0167-8809(01)00297-3

Bouwer, H., 1989. The Bouwer and Rice slug test - an update. Ground Water 27, 304–309.

Bowman, R.A., Focht, D.D., 1974. The influence of glucose and nitrate concentrations upon denitrification rates in sandy soils. Soil Biol. Biochem. 6, 297–301.

Brady, N., Weil, R., 2002. The nature and properties of soils, 13th ed. Prentice Hall, New Jersey.

Bragan, R., Starr, J., Parkin, T., 1997. Shallow groundwater denitrification rate measurement by acetylene block. J. Environ. Qual. 26, 1531–1538.

Bremner, J.M., 1997. Sources of nitrous oxide in soils. Nutr. Cycl. Agroecosystems 49, 7–16. doi:10.1023/A:1009798022569

Burbery, L., 2004. Reactive Single-Well Tests for Determination of Natural Attenuation Rates. Lancaster University.

Burbery, L., Cassiani, G., Andreotti, G., Ricchiuto, T., Semple, K.T., 2004. Single-well reactive tracer test and stable isotope analysis for determination of microbial activity in a fast hydrocarbon-contaminated aquifer. Environ. Pollut. 129, 321–330. doi:10.1016/j.envpol.2003.10.017

Burbery, L.F., Flintoft, M.J., Close, M.E., 2013. Application of the re-circulating tracer well test method to determine nitrate reaction rates in shallow unconfined aquifers. J. Contam. Hydrol. 145, 1–9. doi:10.1016/j.jconhyd.2012.11.006

Burbery, L.F., Wang, F., 2010. A re-circulating tracer well test method for measuring reaction rates in fast-flowing aquifers: Conceptual and mathematical model. J. Hydrol. 382, 163–173. doi:10.1016/j.jhydrol.2009.12.027

Buss, S., Rivett, M., Morgan, P., Bemment, C., 2005. Attenuation of nitrate in the sub-surface environment. Bristol.

Cakin, A., Gokcen, G., Eroğlu, A.E., Baba, A., 2012. Hydrogeochemistry and Environmental Properties of Geothermal Fields. Case Study: Balçova, Izmir-Turkey. Energy Sources, Part A Recover. Util. Environ. Eff. 34, 732–745. doi:10.1080/15567031003681903

Cannavo, P., Richaume, A., Lafolie, F., 2004. Fate of nitrogen and carbon in the vadose zone: in situ and laboratory measurements of seasonal variations in aerobic respiratory and denitrifying activities. Soil Biol. Biochem. 36, 463–478. doi:10.1016/j.soilbio.2003.10.023

Castaldelli, G., Colombani, N., Vincenzi, F., Mastrocicco, M., 2013. Linking dissolved organic carbon, acetate and denitrification in agricultural soils. Environ. Earth Sci. 68, 939–945. doi:10.1007/s12665-012-1796-7

Castle, K., Arah, J.R.M., Vinten, A.J.A., 1998. Denitrification in intact subsoil cores. Biol. Fertil. Soils 28, 12–18. doi:10.1007/s003740050457

Cavari, B.Z., Phelps, G., 1977. Denitrification in Lake Kinneret in the presence of oxygen. Freshw. Biol. 7, 385–391. doi:10.1111/j.1365-2427.1977.tb01686.x

Cheng, D., He, G., Liu, M., Chen, H., He, J., Lin, J., 2010. Estimation method of denitrification rate constant in aquifer. 2010 4th Int. Conf. Bioinforma. Biomed. Eng. iCBBE 2010 3–5. doi:10.1109/ICBBE.2010.5517951

Cheong, J.-Y., Hamm, S.-Y., Lee, J.-H., Lee, K.-S., Woo, N.-C., 2012. Groundwater nitrate contamination and risk assessment in an agricultural area, South Korea. Environ. Earth Sci. 66, 1127–1136. doi:10.1007/s12665-011-1320-5

Clagnan, E., Thornton, S.F., Rolfe, S.A., Tuohy, P., Peyton, D., Wells, N.S., Fenton, O., 2018. Influence of artificial drainage system design on the nitrogen attenuation potential of gley soils: Evidence from hydrochemical and isotope studies under field-scale conditions. J. Environ. Manage. 206, 1028–1038. doi:10.1016/j.jenvman.2017.11.069

Clague, J., Stenger, R., Clough, T.J., 2015a. Evaluation of the stable isotope signatures of nitrate to detect denitrification in a shallow groundwater system in New Zealand. Agric. Ecosyst. Environ. 202, 188–197. doi:10.1016/j.agee.2015.01.011

Clague, J., Stenger, R., Clough, T.J., 2015b. Denitrification in the shallow groundwater system of a lowland catchment: A laboratory study. Catena 131, 109–118. doi:10.1016/j.catena.2015.03.012

Clague, J., Stenger, R., Clough, T.J., 2013. The Impact of Relict Organic Materials on the Denitrification Capacity in the Unsaturated–Saturated Zone Continuum of Three Volcanic Profiles. J. Environ. Qual. 42, 145–154. doi:10.2134/jeq2012.0239

Clague, J., Stenger, R., Moorhead, B., 2015c. Invesitigating how soil drainage class affects the redox status of shallow groundwater, in: New Zealand Hydrological Society Annual Conference: From Data to Knowledge. NZ Hydrological Society, Hamilton, New Zealand, pp. 40–41.

Clark, M., Roygard, J., 2008. Land Use and Land Use Capability in the Manawatu-Wanganui Region: Internal Technical Report to Support Policy Development. Palmerston North, New Zealand.

Close, M.E., Abraham, P., Humphries, B., Lilburne, L., Cuthill, T., Wilson, S., 2016. Predicting groundwater redox status on a regional scale using linear discriminant analysis. J. Contam. Hydrol. 191, 19–32. doi:10.1016/j.jconhyd.2016.04.006

Clothier, B., Mackay, A., Carran, A., Gray, R., Parfitt, R., Francis, G., Manning, M., Duerer, M., Green, S., 2007. Farm Strategies for Contaminant Management. Palmerston North, New Zealand.

Clough, T.J., Sherlock, R.R., Cameron, K.C., Ledgard, S.F., 1996. Fate of urine nitrogen on mineral and peat soils in New Zealand. Plant Soil 178, 141–152. doi:10.1007/BF00011172

Clough, T.J., Sherlock, R.R., Rolston, D.E., 2005. A review of the movement and fate of N2O in the subsoil. Nutr. Cycl. Agroecosystems 72, 3–11. doi:10.1007/s10705-004-7349-z

Collins, S., Singh, R., Rivas, A., Palmer, A., Horne, D., Manderson, A., Roygard, J., Matthews, A., 2017. Transport and potential attenuation of nitrogen in shallow groundwaters in the lower Rangitikei catchment , New Zealand. J. Contam. Hydrol. 206, 55–66. doi:10.1016/j.jconhyd.2017.10.002

Corre, M.D., Schnabel, R.R., Shaffer, J.A., 1999. Evaluation of soil organic carbon under forests, cool-season and warm-season grasses in the northeastern US. Soil Biol. Biochem. 31, 1531–1539. doi:10.1016/S0038-0717(99)00074-7

Counotte, G.H., Prins, R.A., 1979. Calculation of k and v from substrate concentration versus time plot. Appl. Environ. Microbiol. 38, 758–60.

Crepin, J., Johnson, R., 1993. Soil sampling for environmental assessment, in: Carter, M. (Ed.), Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, Florida, pp. 5–18.

Culley, J., 1993. Density and compressibility, in: Carter, M. (Ed.), Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, Florida, pp. 529–541.

Daughney, C., Baker, T., Jones, A., Hanson, C., Davidson, P., Thompson, M., Reeves, R.R., Zemansky, G.M., 2007. Comparison of groundwater sampling methods for State of the Environment monitoring in New Zealand. J. Hydrol. New Zeal. 46, 19–31.

Daughney, C., Jones, A., Baker, T., Hanson, C., Davidson, P., Zemansky, G., Reeve, R., Thompson, M., 2006. A National Protocol for State of the Environment Groundwater Sampling in New Zealand. Ministry for the Environment, Wellington, New Zealand.

Daughney, C., Meilhac, C., Zarour, H., 2009. Spatial and temporal variations and trends in groundwater quality in the Manawatu-Wanganui Region. Taupo, New Zealand.

Daughney, C.J., Reeves, R.R., 2005. Definition of hydrochemical facies in the New Zealand National Groundwater Monitoring Programme. J. Hydrol. 44, 105–130.

Deslippe, J.R., Jamali, H., Jha, N., Saggar, S., 2014. Denitrifier community size, structure and activity along a gradient of pasture to riparian soils. Soil Biol. Biochem. 71, 48–60. doi:10.1016/j.soilbio.2014.01.007

Di, H.J., Cameron, K.C., 2002. Nitrate leaching in temperate agroecosystems: sources ,

factors and mitigating strategies. Nutr. Cycl. Agroecosystems 46, 237–256.

Di, H.J., Cameron, K.C., Moore, S., Smith, N.P., 1998. Nitrate leaching from dairy shed effluent and ammonium fertiliser applied to a free-draining pasture soil under spray or flood irrigation. New Zeal. J. Agric. Res. 41, 263–270. doi:10.1080/00288233.1998.9513310

Domagalski, J.L., Phillips, S.P., Bayless, E.R., Zamora, C., Kendall, C., Wildman, R.A., Hering, J.G., 2008. Influences of the unsaturated, saturated, and riparian zones on the transport of nitrate near the Merced River, California, USA. Hydrogeol. J. 16, 675–690. doi:10.1007/s10040-007-0266-x

Drury, C., Myrold, D., Beauchamp, E., Reynolds, W., 2008. Denitrification techniques for soils, in: Carter, M., Gregorich, E. (Eds.), Soil Sampling and Methods of Analysis. CRC Press, Boca Raton, Florida, pp. 471–493.

Duncan, R., 2014. Regulating agricultural land use to manage water quality: The challenges for science and policy in enforcing limits on non-point source pollution in New Zealand. Land use policy 41, 378–387. doi:10.1016/j.landusepol.2014.06.003

E.Union, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of European Communities.

E.Union, 1991. Council Directive of 12 December 1991 concerning the protection of water against pollution caused by nitrates from agricultural sources. Official Journal of European Communities.

Ellis, S., Howe, M.T., Goulding, K.W.T., Mugglestone, M.A., Dendooven, L., 1998. Carbon and nitrogen dynamics in a grassland soil with varying pH: effect of pH on the denitrification potential and dynamics of the reduction enzymes. Soil Biol. Biochem. 30, 359–367.

Elmi, A.A., Astatkie, T., Madramootoo, C., Gordon, R., Burton, D., 2005. Assessment of Denitrification Gaseous End-Products in the Soil Profile under Two Water Table Management Practices Using Repeated Measures Analysis. J. Environ. Qual. 34, 446–454.

Elmi, A.A., Madramootoo, C., Hamel, C., Liu, A., 2003. Denitrification and nitrous oxide to nitrous oxide plus dinitrogen ratios in the soil profile under three tillage systems. Biol. Fertil. Soils 38, 340–348. doi:10.1007/s00374-003-0663-9

Elwan, A., Singh, R., Horne, D., Roygard, J., Clothier, B., 2015. Nitrogen attenuation factor: can it tell a story about nutrients in different subsurface environments?, in: Currie, L.,

Christensen, C. (Eds.), Moving Farm Systems to Improved Nutrient Attenuation. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, p. 12.

Eppinger, R., Walraevens, K., 1998. Mobility and removal of nitrate in heterogeneous Eocene aquifers. Groundw. Qual. Remediat. Prot. 250, 11–18.

Eschenbach, W., Well, R., Walther, W., 2015. Predicting the denitrification capacity of sandy aquifers from in situ measurements using push – pull 15 N tracer tests. Biogeosciences 12, 2327–2346. doi:10.5194/bg-12-2327-2015

Espanto, P., 2015. Assessment of the transport and transformation of nitrogen in the unsaturated and saturated zones under two dairy farms in the Manawatu River catchment. Massey University.

Felber, R., Conen, F., Flechard, C.R., Neftel, A., 2012. Theoretical and practical limitations of the acetylene inhibition technique to determine total denitrification losses. Biogeosciences 9, 4125–4138. doi:10.5194/bg-9-4125-2012

Fenton, O., Richards, K.G., Kirwan, L., Khalil, M.I., Healy, M.G., 2009. Factors affecting nitrate distribution in shallow groundwater under a beef farm in South Eastern Ireland. J. Environ. Manage. 90, 3135–3146. doi:10.1016/j.jenvman.2009.05.024

Finkelstein, M., Verma, D., 2001. Exposure estimation in the Presence of non detectable values: Another Look. Am. Ind. Hyg. Assoc. J. 62, 195–198. doi:10.1080/15298660108984622

Firestone, M., 1982. Biological denitrification, in: Stevenson, F., Bremner, J., Hauck, R., Keeney, D. (Eds.), Nitrogen in Agricultural Soils. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, Wisconsin, pp. 289–326.

Fischer, D., Uksa, M., Tischler, W., Kautz, T., Köpke, U., Schloter, M., 2013. Abundance of ammonia oxidizing microbes and denitrifiers in different soil horizons of an agricultural soil in relation to the cultivated crops. Biol. Fertil. Soils 49, 1243–1246. doi:10.1007/s00374-013-0812-8

Florinsky, I. V, Mcmahon, S., Burton, D.L., 2004. Topographic control of soil microbial activity: a case study of denitrifiers. Geoderma 119, 33–53. doi:10.1016/S0016-7061(03)00224-6

Flury, M., Papritz, A., 1993. Bromide in the Natural Environment: Occurrence and Toxicity. J. Environ. Qual. 22, 747–758.

Food and Agriculture Organization of the United Nations (FAO), 2015. World fertilizer

trends and outlook to 2018, Food and Agriculture Organization of United Nations. Food and Agriculture Organization of the United Nations (FAO), Rome.

Freeze, R., Cherry, J., 1979. Groundwater. Prentice Hall, Inc., New Jersey.

Ghani, A., Dexter, M., Perrott, K.W., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol. Biochem. 35, 1231–1243. doi:10.1016/S0038-0717(03)00186-X

Ghasemi, A., Zahediasl, S., 2012. Normality tests for statistical analysis: A guide for non-statisticians. Int. J. Endocrinol. Metab. 10, 486–489. doi:10.5812/ijem.3505

Gillham, R.W., Starr, R.C., Miller, D.J., 1990. Device for in situ determination of geochemical parameters. Ground Water 28, 858–862.

Green, C., Puckett, L., Bohlke, J., Bekins, B., Phillips, S., Kauffman, L., Denver, J., Johnson, H., 2008. Limited Occurrence of Denitrification in Four Shallow Aquifers in Agricultural Areas of the United States. J. Environ. Qual. 37, 994–1009.

Grimaldi, C., Fossey, M., Thomas, Z., Fauvel, Y., Merot, P., 2011. Nitrate attenuation in soil and shallow groundwater under a bottomland hedgerow in a European farming landscape. Hydrol. Process. doi:10.1002/hyp

Groffman, P.M., Altabet, M.A., Bohlke, J.K., Butterbach-Bahl, K., David, M.B., Firestone, M.K., Giblin, A.E., Kana, T.M., Nielsen, L.P., Voyteck, M.A., 2006. Methods for Measuring Denitrification: Diverse Approaches to a Difficult Problem. Ecol. Appl. 16, 2091–2122. doi:10.1890/1051-0761(2006)016[2091:MFMDDA]2.0.CO;2

Groffman, P.M., Holland, E., Myrold, D.D., Robertson, P.G., Zou, X., 1999. Denitrification, in: Robertson, P., Coleman, D., Bledsoe, C., Sollins, P. (Eds.), Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York, pp. 272–288.

Gu, B., Ge, Y., Chang, S.X., Luo, W., Chang, J., 2013. Nitrate in groundwater of China: Sources and driving forces. Glob. Environ. Chang. 23, 1112–1121. doi:10.1016/j.gloenvcha.2013.05.004

Gueler, C., Thyne, G.D., McCray, J.E., Turner, A.K., 2002. Evaluation of graphical and multivariate statistical methods for classification of water chemistry data. Hydrogeol. J. 10, 455–474. doi:10.1007/s10040-002-0196-6

Guggenmos, M.R., Daughney, C.J., Jackson, B.M., Morgenstern, U., 2011. Regional-scale identification of groundwater-surface water interaction using hydrochemistry and multivariate statistical methods, Wairarapa Valley, New Zealand. Hydrol. Earth Syst. Sci. 15, 3383–3398. doi:10.5194/hess-15-3383-2011

Gusman, A., Marino, M., 1999. Analytical Modeling of Nitrogen Dynamics in Soils and

Ground Water. J. Irrig. Drain. Eng. 125, 330–337.

Haag, D., Kaupenjohann, M., 2001. Landscape fate of nitrate fluxes and emissions in Central Europe A critical review of concepts, data, and models for transport and retention. Agric. Ecosyst. Environ. 86, 1–21.

Haggerty, R., Schroth, M., Istok, J., 1998. Simplified method of "push-pull" test data analysis for determining in situ reaction rate coefficients. Ground Water 36, 314–324. doi:10.1111/j.1745-6584.1998.tb01097.x

Hashimoto, T., Niimi, H., 2001. Seasonal and vertical changes in denitrification activity and denitrifying bacterial populations in surface and subsurface upland soils with slurry application. Soil Sci. Plant Nutr. 47, 503–510. doi:10.1080/00380768.2001.10408414

Hayakawa, A., Nakata, M., Jiang, R., Kuramochi, K., Hatano, R., 2012. Spatial variation of denitrification potential of grassland, windbreak forest, and riparian forest soils in an agricultural catchment in eastern Hokkaido, Japan. Ecol. Eng. 47, 92–100. doi:10.1016/j.ecoleng.2012.06.034

Heinen, M., 2006. Application of a widely used denitrification model to Dutch data sets. Geoderma 133, 464–473. doi:10.1016/j.geoderma.2005.08.011

Helsel, D., 2010. Much Ado About Next to Nothing: Incorporating Nondetects in Science. Ann. Occup. Hyg. 54, 257–262. doi:10.1093/annhyg/mep092

Helsel, D., Hirsch, R., 2002. Statistical Methods in Water Resources, in: Techniques of Water-Resources Investigations of the United States Geological Survey, Book 4: Hydrologic Analysis and Interpretation. U.S. Geological Survey, Denver, Colorado, p. 510.

Hénault, C., Devis, X., Lucas, J.L., Germon, J.C., 1998. Influence of different agricultural practices (type of crop, form of N-fertilizer) on soil nitrous oxide emissions. Biol. Fertil. Soils 27, 299–306. doi:10.1007/s003740050437

Her, J.-J., Huang, J.-S., 1995. Influences of carbon source and C/N ratio on nitrate/nitrite denitrification and carbon breakthrough. Bioresour. Technol. 54, 45–51.

Hill, A., Devito, K., Campagnolo, S., Sanmugadas, K., 2000. Subsurface denitrification in a forest riparian zone: Interactions between hydrology and supplies of nitrate and organic carbon. Biogeochemistry 51, 193–223. doi:10.1023/a:1006476514038

Hiscock, K., 2005. Hydrogeology: Principles and Practice. Blackwell Publishing, Malden, MA.

Hiscock, K.M., Lloyd, J.W., Lerner, D.N., 1991. Review of natural and artificial denitrification of groundwater. Water Res. 25, 1099–1111. doi:10.1016/0043-

1354(91)90203-3

Holden, P.A., Fierer, N., 2005. Microbial Processes in the Vadose Zone. Vadose Zo. J. 4, 1–21. doi:10.2136/vzj2005.0001

Hoogendoorn, C.J., Betteridge, K., Ledgard, S.F., Costall, D.A., Park, Z.A., Theobald, P.W., 2011. Nitrogen leaching from sheep-, cattle- and deer-grazed pastures in the Lake Taupo catchment in New Zealand. Anim. Prod. Sci. 51, 416–425. doi:10.1071/AN10179

Hoyle, F., Murphy, D., Sheppard, J., n.d. Labile carbon [WWW Document]. URL http://soilquality.org.au/factsheets/labile-carbon (accessed 3.5.13).

Hughes, B., Wilson, K., Rissmann, C., Rodway, E., 2016. Physiographics of Southland: Development and application of a classification system for managing land use effects on water quality in Southland. Invercargill, New Zealand.

Hvorslev, M., 1951. Time Lag and Soil Permeability in Ground-Water Observations. Vicksburg, Mississippi.

Istok, J.D., 2013. Push-Pull Tests for Site Characterization. Springer Berlin Heidelberg. doi:10.1007/978-3-642-13920-8

Istok, J.D., Humphrey, M., Schroth, M., Hyman, M., O'Reilly, K., 1997. Single-well, "push-pull" test for in situ determination of microbial activities. Ground Water 35, 619–631.

Jahangir, M.M., Minet, E., Johnston, P., Coxon, C., 2014. In situ denitrification rates in shallow groundwater beneath a spring barley - mustard cover crop system, in: Geophysical Research Abstracts. pp. EGU2014-3952–1.

Jahangir, M.M.R., Johnston, P., Addy, K., Khalil, M., Groffman, P., Richards, K., 2013. Quantification of In Situ Denitrification Rates in Groundwater Below an Arable and a Grassland System. Water. Air. Soil Pollut. 224, 1693. doi:10.1007/s11270-013-1693-z

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Hennessy, D., Humphreys, J., Fenton, O., Richards, K.G., 2012a. Groundwater: A pathway for terrestrial C and N losses and indirect greenhouse gas emissions. Agric. Ecosyst. Environ. 159, 40–48. doi:10.1016/j.agee.2012.06.015

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Richards, K.G., 2012b. Linking hydrogeochemistry to nitrate abundance in groundwater in agricultural settings in Ireland. J. Hydrol. 448–449, 212–222. doi:10.1016/j.jhydrol.2012.04.054

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Richards, K.G., 2010. Assessing groundwater denitrification under two contrasting land uses in South-East Ireland, in: Advances in Animal Biosciences. p. 87.

Jahangir, M.M.R., Khalil, M.I., Johnston, P., Cardenas, L.M., Hatch, D.J., Butler, M., Barrett,

M., O'flaherty, V., Richards, K.G., 2012c. Denitrification potential in subsoils: A mechanism to reduce nitrate leaching to groundwater. Agric. Ecosyst. Environ. 147, 13–23. doi:10.1016/j.agee.2011.04.015

Jarvis, S., Hatch, D., 1994. Potential for denitrification at depth below long-term grass swards. Soil Biol. Biochem. 26, 1629–1636.

Jarvis, S.C., Hatch, D.J., Lovell, R.D., 2001. An improved soil core incubation method for the field measurement of denitrification and net mineralization using acetylene inhibition. Nutr. Cycl. Agroecosystems 59, 219–225. doi:10.1023/A:1014401932276

Jha, N., Saggar, S., Giltrap, D., Tillman, R., Deslippe, J., 2017. Soil properties impacting denitrifier community size, structure, and activity in New Zealand dairy-grazed pasture. Biogeosciences 14, 4243–4253. doi:10.5194/bg-2016-390

Jha, N., Saggar, S., Tillman, R., Giltrap, D., 2011. Preliminary studies to measure denitrification enzyme activity and denitrification rate in New Zealand pasture soils, in: Currie, L.D., Christensen, C.L. (Eds.), Adding to the Knowledge Base for the Nutrient Manager. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, p. 8.

Jha, N., Saggar, S., Tillman, R.W., Giltrap, D., 2012. Changes in Denitrification Rate and N 2 O / N 2 Ratio With Varying Soil Moisture Conditions, in: L.D. Currie, Christensen, C.L. (Eds.), Advanced Nutrient Management: Gains from the Past - Goals for the Future. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand, p. 15.

Johnson, R.A., Wichern, D.W., 2007. Applied Multivariate Statistical Analysis, 6th ed, The design and statistical analysis of animal experiments. Pearson Education, Inc. doi:10.1111/b.9781444331899.2011.00023.x

Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38, 991–999. doi:10.1016/j.soilbio.2005.08.012

Jurado, A., Borges, A. V., Brouyère, S., 2017. Dynamics and emissions of N2O in groundwater: A review. Sci. Total Environ. 584–585, 207–218. doi:10.1016/j.scitotenv.2017.01.127

Jurgens, B.C., McMahon, P.B., Chapelle, F.H., Eberts, S.M., 2009. An Excel® workbook for identifying redox processes in ground water, U.S. Geological Survey Open-File Report. Reston, Virginia.

Kamewada, K., 2007. Vertical distribution of denitrification activity in an Andisol upland

field and its relationship with dissolved organic carbon: Effect of long-term organic matter application. Soil Sci. Plant Nutr. 53, 401–412. doi:10.1111/j.1747-0765.2007.00148.x

Kelley, C.J., Keller, C.K., Evans, R.D., Orr, C.H., Smith, J.L., Harlow, B.A., 2013. Nitrate-nitrogen and oxygen isotope ratios for identification of nitrate sources and dominant nitrogen cycle processes in a tile-drained dryland agricultural field. Soil Biol. Biochem. 57, 731–738. doi:10.1016/j.soilbio.2012.10.017

Khalil, M.I., Richards, K.G., 2011. Denitrification enzyme activity and potential of subsoils under grazed grasslands assayed by membrane inlet mass spectrometer. Soil Biol. Biochem. 43, 1787–1797. doi:10.1016/j.soilbio.2010.08.024

Khanitchaidecha, W., 2010. Influence of Carbon Source on Biological Nitrogen Removal by Immobilised Bacteria. J. Water Resour. Prot. 02, 527–531. doi:10.4236/jwarp.2010.26059

Khdyer, I., Cho, C., 1983. Nitrification and denitrification of nitrogen fertilizers in a soil column. Soil Sci. Soc. Am. J. 47, 1134–1139.

Killick, M., 2013. An analysis of the relationship of apparent electrical conductivity to soil moisture in alluvial Resent Soils, lower North Island, New Zealand. Massey University.

Kim, K.H., Yun, S.T., Choi, B.Y., Chae, G.T., Joo, Y., Kim, K., Kim, H.S., 2009. Hydrochemical and multivariate statistical interpretations of spatial controls of nitrate concentrations in a shallow alluvial aquifer around oxbow lakes (Osong area, central Korea). J. Contam. Hydrol. 107, 114–127. doi:10.1016/j.jconhyd.2009.04.007

Klemedtsson, L., Svensson, B.O.H., Rosswall, T., 1987. Dinitrogen and nitrous oxide produced by denitrification and nitrification in soil with and without barley plants. Plant Soil 319, 303–319. doi:10.1007/BF02370877

Knowles, R., 1982. Denitrification. Microbiol. Rev. 46, 43–70.

Korom, S., 1992. Natural Denitrification in the Saturated Zone: A Review. Water Resour. Res. 28, 1657–1668.

Korom, S.F., Schlag, A.J., Schuh, W.M., Schlag, A.K., 2005. In Situ Mesocosms: Denitrification in the Elk Valley Aquifer. Ground Water Monit. Remediat. 25, 79–89.

Korom, S.F., Schuh, W.M., Tesfay, T., Spencer, E.J., 2012. Aquifer denitrification and in situ mesocosms: Modeling electron donor contributions and measuring rates. J. Hydrol. 432–433, 112–126. doi:10.1016/j.jhydrol.2012.02.023

Krantz, D., Powars, D., 2000. Hydrogeologic Setting and Potential for Denitrification in Ground Water, Coastal Plain of Southern Maryland. Baltimore, Maryland.

Krieger, F.W., 1992. Sedimentology and Paleoenvironmental Analysis of Castlecliffian Strata in the Dannevirke Basin. Massey University.

Kulabako, N.R., Nalubega, M., Thunvik, R., 2007. Study of the impact of land use and hydrogeological settings on the shallow groundwater quality in a peri-urban area of Kampala, Uganda. Sci. Total Environ. 381, 180–199. doi:10.1016/j.scitotenv.2007.03.035

Lambrakis, N., Antonakos, A., Panagopoulos, G., 2004. The use of multicomponent statistical analysis in hydrogeological environmental research. Water Res. 38, 1862–1872. doi:10.1016/j.watres.2004.01.009

Landon, M.K., Green, C.T., Belitz, K., Singleton, M.J., Esser, B.K., 2011. Relations of hydrogeologic factors, groundwater reduction-oxidation conditions, and temporal and spatial distributions of nitrate, Central-Eastside San Joaquin Valley, California, USA. Hydrogeol. J. 19, 1203–1224. doi:10.1007/s10040-011-0750-1

Lapham, W., Wilde, F., Koterba, M., 1997. Guidelines and Standard Procedures for Studies of Ground-water Quality: Selection and Installation of Wells, and Supporting Documentation, USGS Water-Resources Investigations Report 96-4233. Reston, Virginia. doi:10.1007/s13398-014-0173-7.2

Ledein, E., Ausseil, O., Roygard, J., 2007. Identifying Point Source and Non-Point Source Contributions to Nutrient Loadings in Water Ways in Three Catchments in the Manawatu-Wanganui Region: Technical Report to Support Policy Development. Palmerston North, New Zealand.

Ledgard, S., Schils, R., Eriksen, J., Luo, J., 2009. Environmental impacts of grazed clover/grass pastures. Irish J. Agric. Food Res. 48, 209–226.

Ledgard, S.F., Steel, K., Journeaux, P.R., 2000. Use of OVERSEER to compare farm systems and countries for nutrient balances, losses and efficiency, in: Currie, L., Loganathan, P. (Eds.), Soil Research - a Knowledge Industry for Land-Based Experts. Occasional Report No. 13. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 83–91.

Lee, J.M., Begg, J.G., 2002. Geology of the Wairarapa Area. Institute of Geological & Nuclear Sciences Limited, Lower Hutt, New Zealand.

Lemon, E., Lemon, D., 1981. Nitrous oxide in freshwaters of the Great Lakes Basin. Limnol. Oceanogr. 26, 867–879.

Lewandowski, A., Montgomery, B., Rosen, C., Moncrief, J., 2008. Groundwater nitrate contamination costs: A survey of private well owners. J. Soil Water Conserv. 63, 153–

161.

Liao, L., Green, C.T., Bekins, B.A., Böhlke, J.K., 2012. Factors controlling nitrate fluxes in groundwater in agricultural areas. Water Resour. Res. 48, 1–18. doi:10.1029/2011WR011008

LIC, DairyNZ, 2016. New Zealand Dairy Statistics 2015-16. Hamilton, New Zealand.

Limmer, A., Steele, K., 1982. Denitrification potentials: measurement of seasonal variation using a short-term anaerobic incubation technique. Soil Biol. Biochem. 14, 179–184.

Liu, C.W., Lin, K.H., Kuo, Y.M., 2003. Application of factor analysis in the assessment of groundwater quality in a blackfoot disease area in Taiwan. Sci. Total Environ. 313, 77–89. doi:10.1016/S0048-9697(02)00683-6

Luo, J., Tillman, R.W., Ball, P.R., 2000. Nitrogen loss through denitrication in a soil under pasture in New Zealand. Soil Biol. Biochem. 32, 497–509.

Luo, J., Tillman, R.W., Ball, P.R., 1999. Factors regulating denitrification in a soil under pasture. Soil Biol. Biochem. 31, 913–927. doi:10.1016/S0038-0717(99)00013-9

Luo, J., Tillman, R.W., White, R.E., Ball, P.R., 1998. Variation in denitrification activity with soil depth under pasture. Soil Biol. Biochem. 30, 897–903.

Luo, J., White, R.E., Ball, P.R., Tillman, R.W., 1996. Measuring denitrification activity in soils under pasture: optimizing conditions for the short-term denitrification enzyme assay and effects of soil storage on denitrification activity. Soil Biol. Biochem. 28, 409–417.

Mantovi, P., Fumagalli, L., Beretta, G. Pietro, Guermandi, M., 2006. Nitrate leaching through the unsaturated zone following pig slurry applications. J. Hydrol. 316, 195–212. doi:10.1016/j.jhydrol.2005.04.026

Martin, T.L., Kaushik, N.K., Trevors, J.T., Whiteley, H.R., 1999. Review: denitrification in temperate climate riparian zones. Water Resour. Res. 111, 171–186.

Marton, J., Herbert, E., Craft, C., 2012. Effects of salinity on denitrification and greenhouse gas production from laboratory-incubated tidal forest soils. Wetlands 32, 347–357. doi:https://doi.org/10.1007/s13157-012-0270-3

Mastrocicco, M., Colombani, N., Salemi, E., Castaldelli, G., 2011. Reactive Modeling of Denitrification in Soils with Natural and Depleted Organic Matter. Water, Air, Soil Pollut. 222, 205–215. doi:10.1007/s11270-011-0817-6

Matiatos, I., Alexopoulos, A., Godelitsas, A., 2014. Multivariate statistical analysis of the hydrogeochemical and isotopic composition of the groundwater resources in northeastern Peloponnesus (Greece). Sci. Total Environ. 476–477, 577–590.

doi:10.1016/j.scitotenv.2014.01.042

Maynard, D.G., Kalra, Y.P., 1993. Nitrate and Exchangeable Ammonium Nitrogen, in: Carter, M. (Ed.), Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, Florida, pp. 25–38.

Mcaleer, E.B., Coxon, C.E., Richards, K.G., Jahangir, M.M.R., Grant, J., Mellander, P.E., 2017. Science of the Total Environment Groundwater nitrate reduction versus dissolved gas production : A tale of two catchments. Sci. Total Environ. 586, 372–389. doi:10.1016/j.scitotenv.2016.11.083

McArthur, K., Clark, M., 2007. Nitrogen and Phosphorus Loads to Rivers in the Manawatu-Wanganui Region : An Analysis of Low Flow State : Technical Report to Support Policy Development. Palmerston North, New Zealand.

McCarty, G.W., Mookherji, S., Angier, J.T., 2007. Characterization of denitrification activity in zones of groundwater exfiltration within a riparian wetland ecosystem. Biol. Fertil. Soils 43, 691–698. doi:10.1007/s00374-006-0151-0

McLarin, W., Bekesi, G., Brown, L., McConchie, J., 1999. Nitrate contamination of the unconfined aquifer, Manakau, Horowhenua, New Zealand. J. Hydrol. (New Zealand) 38, 211–235.

McLay, C.D.A., Dragten, R., Sparling, G., Selvarajah, N., 2001. Predicting groundwater nitrate concentrations in a region of mixed agricultural land use: A comparison of three approaches. Environ. Pollut. 115, 191–204. doi:10.1016/S0269-7491(01)00111-7

Mcmahon, P.B., Bruch, B.W., Becker, M.F., Pope, L.M., Dennehy, K.F., 2000. Occurrence of nitrous oxide in the central High Plains aquifer, 1999. Environ. Sci. Technol. 34, 4873–4877. doi:10.1021/es001233t

McMahon, P.B., Chapelle, F.H., 2008. Redox processes and water quality of selected principal aquifer systems. Ground Water 46, 259–271. doi:10.1111/j.1745-6584.2007.00385.x

Mcphillips, L.E., Groffman, P.M., Goodale, C.L., Walter, M.T., 2015. Hydrologic and Biogeochemical Drivers of Riparian Denitrification in an Agricultural Watershed. Water. Air. Soil Pollut. 226, 169. doi:10.1007/s11270-015-2434-2

Mees, J., Gomes, S.D., Hasan, S., Gomes, B.M., Boas, M., 2014. Nitrogen removal in a SBR operated with and without pre-denitrification: effect of the carbon:nitrogen ratio and the cycle time. Environ. Technol. 35, 115–123. doi:10.1080/09593330.2013.816373

Mehnert, E., Dey, W.S., Hwang, H., Keefer, D.A., Holm, T.R., Sanford, R.A., Mcdonald, J.M., Shiffer, S.M., 2006. Mass Balance of Nitrogen and Phosphorus in an Agricultural

Watershed: The Shallow Groundwater Component. Champaign, Illinois.

Meixner, T., Brooks, P., Hogan, J., Soto, C., Simpson, S., 2012. Carbon and Nitrogen Export from Semiarid Uplands to Perennial Rivers: Connections and Missing Links, San Pedro River, Arizona, USA. Geogr. Compass 6, 546–559. doi:10.1111/j.1749-8198.2012.00510.x

Mengis, M., Schiff, S.L., Harris, M., English, M.C., Aravena, R., Elgood, R.J., MacLean, A., 1999. Mutiple Geochemical and Isotopic Approaches for Assessing Groundwater NO3-Elimination in a Riparian zone. Ground Water. doi:10.1111/j.1745-6584.1999.tb01124.x

MfE, 2014. National Policy Statement for Freshwater Management 2014. Ministry for the Environment, New Zealand.

Minamikawa, K., Wagai, R., Nishimura, S., Yagi, K., 2015. Heterotrophic denitrification constrains the upper limit of dissolved N2O-nitrate concentration ratio in agricultural groundwater. Nutr. Cycl. Agroecosystems 101, 181–191. doi:10.1007/s10705-014-9668-z

Minet, E., Coxon, C.E., Goodhue, R., Richards, K.G., Kalin, R.M., Meier-augenstein, W., 2012. Evaluating the utility of 15N and 18O isotope abundance analyses to identify nitrate sources: A soil zone study. Water Res. 46, 3723–3736. doi:10.1016/j.watres.2012.03.004

Mohamed, M.A.A., Terao, H., Suzuki, R., Babiker, I.S., Ohta, K., Kaori, K., Kato, K., 2003. Natural denitrification in the Kakamigahara groundwater basin, Gifu prefecture, central Japan. Sci. Total Environ. 307, 191–201. doi:10.1016/S0048-9697(02)00536-3

Monaghan, R.M., Hedley, M.J., Di, H.J., McDowell, R.W., Cameron, K.C., Ledgard, S.F., 2007. Nutrient management in New Zealand pastures— recent developments and future issues. New Zeal. J. Agric. Res. 50, 181–201. doi:10.1080/00288230709510290

Morgenstern, U., Brown, L.J., Begg, J.G., Daughney, C.J., Davidson, P., 2007. Flaxbourne catchment groundwater residence time, flow pattern, and hydrochemistry trends, GNS Science Report 2007/14. Lower Hutt, New Zealand.

Morgenstern, U., Daughney, C.J., 2012. Groundwater age for identification of baseline groundwater quality and impacts of land-use intensification – The National Groundwater Monitoring Programme of New Zealand. J. Hydrol. 456–457, 79–93. doi:10.1016/j.jhydrol.2012.06.010

Morgenstern, U., Martindale, H., Stewart, M., Trompetter, V., van der Raaij, R., Toews, M., Townsend, D., 2017. Groundwater Dynamics Source & Hydrochemical Processes as Inferred from Horizons Regional Age Tracer Data. Palmerston North, New Zealand.

Mosier, A., Klemedtsson, L., 1994. Measuring denitrification in the field, in: Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties. Soil Science Society of America, Inc., Madison, Wisconsin, pp. 1047–1065.

Mosier, A.R., Guenzi, W.D., Schweizer, E.E., 1986. Field Denitrification Estimation by Nitrogen-15 and Acetylene Inhibition Techniques. Soil Sci. Soc. Am. J. 50, 831–833.

Mühlherr, I., Hiscock, K., 1998. Nitrous oxide production and consumption in British limestone aquifers. J. Hydrol. 211, 126–139.

Murray, P.J., Hatch, D.J., Dixon, E.R., Stevens, R.J., Laughlin, R.J., Jarvis, S.C., 2004. Denitrification potential in a grassland subsoil: effect of carbon substrates. Soil Biol. Biochem. 36, 545–547. doi:10.1016/j.soilbio.2003.10.020

Necpalova, M., Fenton, O., Casey, I., Humphreys, J., 2012. N leaching to groundwater from dairy production involving grazing over the winter on a clay-loam soil. Sci. Total Environ. 432, 159–172. doi:10.1016/j.scitotenv.2012.05.091

Nolan, B., Ruddy, B., Hitt, K., Helsel, D., 1997. Risk of nitrate in ground waters of the United States - A national perspective. Environ. Sci. Technol. 31, 2229–2236. doi:10.1021/es960818d

Nolan, B.T., 1999. Nitrate Behavior in Ground Waters of the Southeastern USA. J. Environ. Qual. 28, 1518–1527. doi:10.2134/jeq1999.00472425002800050017x

Oehler, F., Bordenave, P., Durand, P., 2007. Variations of denitrification in a farming catchment area. Agric. Ecosyst. Environ. 120, 313–324. doi:10.1016/j.agee.2006.10.007

Orr, A., 2014. Hydrogeological influences on the fate and transport of nitrate in groundwater. Queen's University Belfast.

Ottley, C.J., Davison, W., Edmunds, W.M., 1997. Chemical catalysis of nitrate reduction by iron (II). Geochim. Cosmochim. Acta 61, 1819–1828. doi:10.1016/S0016-7037(97)00058-6

Paramasivam, S., Alva, A.K., Fares, A., Sajwan, K.S., 2002. Vadose Zone Processes and Chemical Transport Fate of Nitrate and Bromide in an Unsaturated Zone of a Sandy Soil under Citrus Production. J. Environ. Qual. 31, 671–681.

Paramasivam, S., Alva, A.K., Prakash, O., Cui, S.L., 1999. Denitrification in the vadose zone and in surficial groundwater of a sandy entisol with citrus production. Plant Soil 208, 307–319.

Parkin, T., Meisinger, J., 1989. Denitrification below the crop rooting zone as influenced by surface tillage. J. Environ. Qual. 18, 12–16.

Parkin, T.B., 1987. Soil Microsites as a Source of Denitrification Variability. Soil Sci. Soc.

Am. J. 51, 1194–1199. doi:10.2136/sssaj1987.03615995005100050019x

Parkin, T.B., Sexstone, A.J., Tiedje, J.M., 1985. Comparison of Field Denitrification Rates Determined by Acetylene-Based Soil Core and Nitrogen-15 Methods. Soil Sci. Soc. Am. J. 49, 94–99.

Pattle Delamore Partners Ltd., 2013a. Report on Horizons Groundwater Level Monitoring Network and Groundwater Quantity Management Issues. Palmerston North, New Zealand.

Pattle Delamore Partners Ltd., 2013b. Report on Horizons Groundwater Quality Monitoring Network. Palmerston North, New Zealand.

Pauwels, H., Kloppmann, W., Foucher, J., Martelat, A., Fritsche, V., 1998. Field tracer test for denitrification in a pyrite-bearing schist aquifer. Appl. Geochemistry 13, 767–778. doi:10.1016/S0883-2927(98)00003-1

Payne, J., 1981. Denitrification. Wiley, New York.

Pell, M., Stenberg, B., Stenstrom, J., Torstensson, L., 1996. Potential denitrification activity assay in soil - with or without chloramphenicol? Soil Biol. Biochem. 28, 393–398.

Petersen, R., Calvin, L., 1986. Sampling, in: Klute, A. (Ed.), Methods of Soil Analysis: Part 1 Physical and Mineralogical Methods. Soil Science Society of America, Inc., Madison, Wisconsin, pp. 33–51.

Peterson, M.E., Curtin, D., Thomas, S., Clough, T.J., Meenken, E.D., 2013. Denitrification in vadose zone material amended with dissolved organic matter from topsoil and subsoil. Soil Biol. Biochem. 61, 96–104. doi:10.1016/j.soilbio.2013.02.010

Pfeiffer, S.M., Bahr, J.M., Beilfuss, R.D., 2006. Identification of groundwater flowpaths and denitrification zones in a dynamic floodpain aquifier. J. Hydrol. 325, 262–272. doi:10.1016/j.jhydrol.2005.10.019

Pinay, G., Roques, L., Fabre, A., 1993. Spatial and temporal patterns of denitrification in a riparian forest. J. Appl. Ecol. 30, 581–591. doi:10.2307/2404238

Porubsky, W.P., Joye, S.B., Moore, W.S., Tuncay, K., Meile, C., 2011. Field measurements and modeling of groundwater flow and biogeochemistry at Moses Hammock, a backbarrier island on the Georgia coast. Biogeochemistry 104, 69–90. doi:10.1007/s10533-010-9484-8

Powlson, D., 1993. Understanding the soil nitrogen cycle. Soil Use Manag. 9, 86–94.

Puckett, L., 2004. Hydrogeologic controls on the transport and fate of nitrate in ground water beneath riparian buffer zones: results from thirteen studies across the United States. Water Sci. Technol. 49, 47–53.

Puckett, L., Cowdery, T., 2002. Transport and Fate of Nitrate in a Glacial Outwash Aquifer in Relation to Ground Water Age, Land Use Practices, and Redox Processes. J. Environ. Qual. 31, 782–796.

Puckett, L.J., Cowdery, T.K., Lorenz, D.L., Stoner, J.D., 1999. Estimation of Nitrate Contamination of an Agro-Ecosystem Outwash Aquifer Using a Nitrogen Mass-Balance Budget. J. Environ. Qual. 28, 2015–2025.

Puckett, L.J., Hughes, W.B., 2005. Transport and fate of nitrate and pesticides: hydrogeology and riparian zone processes. J. Environ. Qual. 34, 2278–2292. doi:10.2134/jeq2005.0109

Puckett, L.J., Zamora, C., Essaid, H., Wilson, J.T., Johnson, H.M., Brayton, M.J., Vogel, J.R., 2008. Transport and fate of nitrate at the ground-water/surface-water interface. J. Environ. Qual. 37, 1034–1050. doi:10.2134/jeq2006.0550

Qin, S., Hu, C., Oenema, O., 2012. Quantifying the underestimation of soil denitrification potential as determined by the acetylene inhibition method. Soil Biol. Biochem. 47, 14–17. doi:10.1016/j.soilbio.2011.12.019

Quinn, J.M., Wilcock, R.J., Monaghan, R.M., McDowell, R.W., Journeaux, P., 2009. Grassland farming and water quality in New Zealand. Tearmann Irish J. Agric. Res. 7, 69–88.

Rasiah, V., Armour, J.D., Yamamoto, T., Mahendrarajah, S., Heiner, D.H., 2003. Nitrate dynamics in shallow groundwater and the potential for transport to off-site water bodies. Water. Air. Soil Pollut. 147, 183–202. doi:10.1023/A:1024529017142

Rawlinson, Z., Begg, J., 2014. Hydrogeology of the Upper Manawatu and Mangatainoka catchments, Tararua. Lower Hutt, New Zealand.

Raymond, N., Bonin, P., Bertrand, J., 1992. Comparison of methods for measuring denitrifying activity in marine sediments from the Western Mediterranean coast. Oceanol. Acta 15, 137–143.

Reddy, K.R., Patrick, W.H., Phillips, R.E., 1978. The Role of Nitrate Diffusion in Determining the Order and Rate of Denitrification in Flooded Soil: I. Experimental Results. Soil Sci. Soc. Am. J. 42, 268–272.

Rice, E., Baird, R., Eaton, D., Clesceri, L. (Eds.), 2012. Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association, American Water Works Association, Water Environment Federation.

Rissmann, C., 2011. Regional Mapping of Groundwater Denitrification Potential and Aquifer Sensitivity. Invercargill, New Zealand.

Rivas, A., Singh, R., Bishop, P., Horne, D., Roygard, J., Hedley, M., Centre, F. and L.R., 2014. Measuring denitrification in the subsurface environment of manawatu river catchment, in: Currie, L., Christensen, C. (Eds.), Nutrient Management for the Farm, Catchment and Community. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, p. 13p.

Rivas, A., Singh, R., Bishop, P., Horne, D., Roygard, J., Hedley, M.J., 2018a. A novel acetylene inhibition incubation technique for accurate quantification of denitrifying enzyme activity in subsurface soils. Soil Biol. Biochem. (under revision).

Rivas, A., Singh, R., Horne, D., Bishop, P., Hedley, M., Roygard, J., 2013. Evaluation of Acetylene Inhibition Method to Measure Denitrification in Unsaturated Zone, in: Water & Weather: Solutions for Health, Wealth & Environment. NZ Hydrological Society, Palmerston North, New Zealand, pp. 183–184.

Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., Hedley, M.J., 2018b. Quantification of denitrification rate in shallow groundwater using the single-well push-pull test technique. J. Contam. Hydrol. (under revision).

Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., Hedley, M.J., 2017. Denitrification potential in the subsurface environment in the Manawatu River catchment, New Zealand: Indications from oxidation-reduction conditions, hydrogeological factors, and implications for nutrient management. J. Environ. Manage. 197, 476–489. doi:10.1016/j.jenvman.2017.04.015

Rivett, M.O., Buss, S.R., Morgan, P., Smith, J.W.N., Bemment, C.D., 2008. Nitrate attenuation in groundwater : A review of biogeochemical controlling processes. Water Res. 42, 4215–4232. doi:10.1016/j.watres.2008.07.020

Roygard, J., Clark, M., 2012. Supplementary Statement by Jon Roygard and Maree Clark on Nutrient Load Scenarios and Methodology.

Roygard, J., McArthur, K., 2008. A Framework for Managing Non-Point Source and Point Source Nutrient Contributions to Water Quality. Palmerston North, New Zealand.

Rust, C.M., Aelion, C.M., Flora, J.R. V, 2000. Control of pH during denitrification in subsurface sediment microcosms using encapsulated phosphate buffer. Water Res. 34, 1447–1454.

Rütting, T., Boeckx, P., Müller, C., Klemedtsson, L., 2011. Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. Biogeosciences 8, 1779–1791. doi:10.5194/bg-8-1779-2011

Ruz-Jerez, B.E., White, R.E., ball, P.R., 1994. Long-term measurement of denitrification in

three contrasting pastures grazed by sheep. Soil Biol. Biochem. 26, 29–39. doi:10.1016/0038-0717(94)90192-9

Ryden, J., 1983. Denitrification loss from a grassland soil in the field receiving different rates of nitrogen as ammonium nitrate. J. Soil Sci. 34, 355–365. doi:10.1111/j.1365-2389.1983.tb01041.x

Ryden, J.C., Skinner, J.H., Nixon, D.J., 1987. Soil core incubation system for the field measurement of denitrification using acetylene-inhibition. Soil Biol. Biochem. 19, 753–757.

Saggar, S., Jha, N., Deslippe, J., Bolan, N.S., Luo, J., Giltrap, D.L., Kim, D., Zaman, M., Tillman, R.W., 2013. Denitrification and N2O:N2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. Sci. Total Environ. 465, 173–195. doi:10.1016/j.scitotenv.2012.11.050

Sanchez-Perez, J., Bouey, C., Sauvage, S., Teissier, S., Antiguedad, I., Vervier, P., 2003. A standardised method for measuring in situ denitrification in shallow aquifers: numerical validation and measurements in riparian wetlands. Hydrol. Earth Syst. Sci. 7, 87–96.

Schofield, J.C., 1960. Boron in some New Zealand ground waters. New Zeal. J. Geol. Geophys. 3, 98–104. doi:10.1080/00288306.1960.10423148

Schroth, M.H., Istok, J.D., 2006. Models to Determine First-Order Rate Coefficients from Single-Well Push-Pull Tests. Ground Water 44, 275–283. doi:10.1111/j.1745-6584.2005.00107.x

Schroth, M.H., Istok, J.D., 2005. Approximate Solution for Solute Transport during Spherical-Flow Push-Pull Tests. Ground Water 43, 280–284.

Schroth, M.H., Istok, J.D., Conner, G., Hyman, M., Haggerty, R., O'Reilly, K., 1998. Spatial variability in in situ aerobic respiration and denitrification rates in a petroleum-contaminated aquifer. Ground Water 36, 924–937.

Schroth, M.H., Kleikemper, J., Bolliger, C., Bernasconi, S.M., Zeyer, J., 2001. In situ assessment of microbial sulfate reduction in a petroleum-contaminated aquifer using push – pull tests and stable sulfur isotope analyses. J. Contam. Hydrol. 51, 179–195.

Schullehner, J., Hansen, B., Thygesen, M., Pedersen, C., Sigsgaard, T., 2018. Nitrate in drinking water and colorectal cancer risk: a nationwide population-based cohort study. Int. J. Cancer. doi:10.1002/ijc.31306

Schulte, R.P.O., Creamer, R.E., Donnellan, T., Farrelly, N., Fealy, R., Donoghue, C.O., 2014. Functional land management: A framework for managing soil-based ecosystem services for the sustainable intensification of agriculture. Environ. Sci. Policy 38, 45–58.

doi:10.1016/j.envsci.2013.10.002

Schumacher, B., 2002. Methods for the determination of total organic carbon (TOC) in soils. Las Vegas, Nevada.

Schürmann, A., Schroth, M., Saurer, M., Bernasconi, S.M., Zeyer, J., 2003. Nitrate-consuming processes in a petroleum-contaminated aquifer quantified using push-pull tests combined with 15N isotope and acetylene-inhibition methods. J. Contam. Hydrol. 66, 59–77. doi:10.1016/S0169-7722(03)00007-X

Seitzinger, S., Harrison, J., Bohlke, J., Bouwman, A., Lowrance, R., Peterson, B., Tobias, C., Van Drecht, G., 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecol. Appl. 16, 2064–2090.

Seitzinger, S.P., 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. Limnol. Oceanogr. 33, 702–724. doi:10.4319/lo.1988.33.4part2.0702

Selbie, D., Buckthought, L., Shepherd, M., 2015. The Challenge of the Urine Patch for Managing Nitrogen in Grazed Pasture Systems. Adv. Agron. 129, 229–292. doi:https://doi.org/10.1016/bs.agron.2014.09.004

Sheldrick, B., Wang, C., 1993. Particle Size Analysis, in: Carter, M. (Ed.), Soil Sampling and Methods of Analysis. Boca Raton, Florida, pp. 499–517.

Siemens, J., Haas, M., Kaupenjohann, M., 2003. Dissolved organic matter induced denitrification in subsoils and aquifers? Geoderma 113, 253–271. doi:10.1016/S0016-7061(02)00364-6

Šimek, M., Jíšová, L., Hopkins, D.W., 2002. What is the so-called optimum pH for denitrification in soil? Soil Biol. Biochem. 34, 1227–1234. doi:10.1016/S0038-0717(02)00059-7

Singh, B., Ryden, J., Whitehead, D., 1989. Denitrification potential and actual rates of denitrification in soils under long-term grassland and arable cropping. Soil Biol. Biochem. 21, 897–901.

Singh, R., Elwan, A., Horne, D., Manderson, A., Patterson, M., 2017. Predicting Land-Based Nitrogen Loads and Attenuation in the Rangitikei River Catchment - the Model Development, in: Currie, L., Hedley, M. (Eds.), Science and Policy: Nutrient Management Challenges for the Next Generation. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 1–13.

Singh, R., Rivas, A., Espanto, P., Elwan, A., Horne, D.J., Matthews, A., Clothier, B., 2014. Assessment of transport and transformation of nitrogen in the subsurface environment of

Manawatu River catchment – work in progress, in: Currie, L., Christensen, C. (Eds.), Nutrient Management for the Farm, Catchment and Community. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, p. 11.

Singleton, M., Esser, B., Moran, J.E., Hudson, G.B., McNab, W.W., Harter, T., 2007. Saturated Zone Denitrification: Potential for Natural Attenuation of Nitrate Contamination in Shallow Groundwater Under Dairy Operations. Environ. Sci. Technol. 41, 759–765.

Smid, E., Beauchamp, E., 1976. Effects of temperature and organic matter on denitrification in soil. Can. J. Soil Sci. 56, 385–391.

Smith, M.S., Tiedje, J.M., 1979. Phases of Denitrification Following Oxygen Depletion in Soil. Soil Biol. Biochem. 11, 261–268. doi:10.1016/0038-0717(79)90071-3

Smith, R.L., Duff, J.H., 1988. Denitrification in a sand and gravel aquifer. Appl. Environ. Microbiol. 54, 1071–1078.

Smith, R.L., Garabedian, S.P., Brooks, M.H., 1996. Comparison of denitrification activity measurements in groundwater using cores and natural-gradient tracer tests. Environ. Sci. Technol. 30, 3448–3456. doi:10.1021/es960042g

Snodgrass, M., Kitanidis, P., 1998. A method to infer in situ reaction rates from push-pull experiments. Ground Water 36, 645–650.

Sotomayor, D., Rice, C.W., 1996. Denitrification in Soil Profiles beneath Grassland and Cultivated Soils. Soil Sci. Soc. Am. J. 60, 1822–1828.

Spalding, R.F., Parrott, J.D., 1994. Shallow groundwater denitrification. Sci. Total Environ. 141, 17–25. doi:10.1016/0048-9697(94)90014-0

Srinandan, C.S., Glen, D., Srivastava, N., Nayak, B., Nerurkar, A.S., 2012. Carbon sources influence the nitrate removal activity, community structure and biofilm architecture. Bioresour. Technol. 117, 292–299. doi:10.1016/j.biortech.2012.04.079

Starr, R., Gillham, R., 1993. Denitrification and Organic Carbon Availability in Two Aquifers. Ground Water 31, 934–947. doi:10.1111/j.1745-6584.1993.tb00867.x

Stenger, R., Barkle, G., Burgess, C., Wall, A., Clague, J., 2008. Low nitrate contamination of shallow groundwater in spite of intensive dairying: the effect of reducing conditions in the vadose zone – aquifer continuum. J. Hydrol. (New Zealand) 47, 1–24.

Stenger, R., Bidwell, V., Wall, A., Clague, J., Moorhead, B., 2009. Nitrogen contamination of surface waters: What are the important flowpaths in the Toenepi dairying catchment? Hamilton, New Zealand.

Stevenson, B.A., Schipper, L.A., Mcgill, A., Clark, D., 2011. Denitrification and Availability

of Carbon and Nitrogen in a Well-drained Pasture Soil Amended with Particulate Organic Carbon. J. Environ. Qual. 40, 923–930. doi:10.2134/jeq2010.0463

Storey, R.G., Williams, D.D., Fulthorpe, R.R., 2004. Nitrogen processing in the hyporheic zone of a pastoral stream. Biogeochemistry 69, 285–313. doi:10.1023/B:BIOG.0000031049.95805.ec

Strebel, O., Duynisveld, W.H.M., Böttcher, J., 1989. Nitrate pollution of groundwater in western Europe. Agric. Ecosyst. Environ. 26, 189–214. doi:10.1016/0167-8809(89)90013-3

Sullivan, L.O., Creamer, R.E., Fealy, R., Lanigan, G., Simo, I., Fenton, O., Carfrae, J., Schulte, R.P.O., 2015. Functional Land Management for managing soil functions: A case-study of the trade-off between primary productivity and carbon storage in response to the intervention of drainage systems in Ireland. Land use policy 47, 42–54. doi:10.1016/j.landusepol.2015.03.007

Suthar, S., Bishnoi, P., Singh, S., Mutiyar, P.K., Nema, A.K., Patil, N.S., 2009. Nitrate contamination in groundwater of some rural areas of Rajasthan, India. J. Hazard. Mater. 171, 189–199. doi:10.1016/j.jhazmat.2009.05.111

Taylor, C.B., Trompetter, V.J., Brown, L.J., Bekesi, G., 2001. The Manawatu aquifers, North Island, New Zealand: Clarification of hydrogeology using a multidisciplinary environmental tracer approach. Hydrol. Process. 15, 3269–3286. doi:10.1002/hyp.272

Taylor, J.P., Wilson, B., Mills, M.S., Burns, R.G., 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biol. Biochem. 34, 387–401.

Tchobanoglous, G., David Stensel, H., Tsuchihashi, R., Burton, F., Abu-Orf, M., Bowden, G., Pfrang, W., 2014. Wastewater Engineering: Treatment and Resource Recovery, 5th ed. McGraw Hill Education, New York.

Tesoriero, A.J., Liebscher, H., Cox, S.E., 2000. Mechanism and rate of denitrification in an agricultural watershed: Electron and mass balance along groundwater flow paths. Water Resour. Res. 36, 1545–1559.

Tesoriero, A.J., Puckett, L.J., 2011. O 2 reduction and denitrification rates in shallow aquifers. Water Resour. Res. 47, 1–17. doi:10.1029/2011WR010471

Thayalakumaran, T., Bristow, K.L., Charlesworth, P.B., Fass, T., 2008. Geochemical conditions in groundwater systems: Implications for the attenuation of agricultural nitrate. Agric. Water Manag. 95, 103–115. doi:10.1016/j.agwat.2007.09.003

Thomas, S., Dann, R., Close, M., 2012. Nitrous Oxide Dynamics in a Deep Soil-Alluvial

Gravel Vadose Zone Following Nitrate Leaching. Soil Sci. Soc. Am. J. 76, 1333–1346. doi:10.2136/sssaj2011.0349

Tidswell, S., Conwell, C., Milne, J., 2012. Groundwater quality in the Wellington region State and trends Groundwater quality in the Wellington region, Publication No. GW/EMI-T-12/140. Wellington, New Zealand.

Tiedje, J., 1994. Denitrifiers, in: Weaver, R., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A. (Eds.), Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties. Soil Science Society of America, Inc., Madison, Wisconsin, pp. 245–267.

Tiedje, J., 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium, in: Zehnder, A. (Ed.), Biology of Anaerobic Microorganisms. Wiley-Interscience Publication, New York, pp. 179–244.

Tiedje, J., 1982. Denitrification, in: Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties. ASA-SSSA, pp. 1011–1026.

Tiedje, J., Simkins, S., Groffman, P., 1989. Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. Plant Soil 115, 261–284. doi:10.1007/BF02202594

Tindall, J.A., Petrusak, R.L., Mcmahon, P.B., 1995. Nitrate Transport and Transformation Processes in Unsaturated Porous-Media. J. Hydrol. 169, 51–94. doi:10.1016/0022-1694(94)02666-Y

Toda, H., Mochizuki, Y., Kawanishi, T., Kawashima, H., 2002. Denitrification in shallow groundwater in a coastal agricultural area in Japan. Nutr. Cycl. Agroecosystems 63, 167–173.

Torrentó, C., Cama, J., Urmeneta, J., Otero, N., Soler, A., 2010. Denitrification of groundwater with pyrite and Thiobacillus denitrificans. Chem. Geol. 278, 80–91. doi:10.1016/j.chemgeo.2010.09.003

Trudell, M., Gillham, R., Cherry, J., 1986. An in-situ study of the occurrence and rate of denitrification in a shallow unconfined sand aquifer. J. Hydrol. 83, 251–268.

Twining, B.S., Mylon, S.E., Benoit, G., 2007. Potential role of copper availability in nitrous oxide accumulation in a temperate lake. Limnol. Oceanogr. 52, 1354–1366.

Uddameri, V., Honnungar, V., Hernandez, E.A., 2014. Assessment of groundwater water quality in central and southern Gulf Coast aquifer, TX using principal component analysis. Environ. Earth Sci. 71, 2653–2671. doi:10.1007/s12665-013-2896-8

Ueda, S., Ogura, N., Yoshinari, T., 1993. Accumulation of nitrous oxide in aerobic

groundwaters. Water Res. 27, 1787–1792.

van Cleemput, O., 1998. Subsoils: chemo-and biological denitrification, N2O and N2 emissions. Nutr. Cycl. Agroecosystems 52, 187–194. doi:10.1023/A:1009728125678

van Meter, K.J., Basu, N.B., Veenstra, J.J., Burras, C.L., 2016. The nitrogen legacy: emerging evidence of nitrogen accumulation in anthropogenic landscapes. Environ. Res. Lett. 11, 1–12. doi:10.1088/1748-9326/11/3/035014

Vidon, P., Hill, A.R., 2004. Denitrification and patterns of electron donors and acceptors in eight riparian zones with contrasting hydrogeology. Biogeochemistry 71, 259–283. doi:10.1007/s10533-004-9684-1

Vilain, G., Garnier, J., Roose-Amsaleg, C., Laville, P., 2012. Potential of denitrification and nitrous oxide production from agricultural soil profiles (Seine Basin, France). Nutr. Cycl. Agroecosystems 92, 35–50. doi:DOI 10.1007/s10705-011-9470-0

Vilain, G., Garnier, J., Tallec, G., Cellier, P., 2010. Effect of slope position and land use on nitrous oxide (N2O) emissions (Seine Basin, France). Agric. For. Meteorol. 150, 1192–1202. doi:10.1016/j.agrformet.2010.05.004

Well, R., Augustin, J., Davis, J., Griffith, S.M., Meyer, K., Myrold, D.D., 2001. Production and transport of denitrification gases in shallow ground water. Nutr. Cycl. Agroecosystems 60, 65–75.

Well, R., Augustin, J., Meyer, K., Myrold, D.D., 2003. Comparison of field and laboratory measurement of denitrification and N2O production in the saturated zone of hydromorphic soils. Soil Biol. Biochem. 35, 783–799. doi:10.1016/S0038-0717(03)00106-8

Well, R., Myrold, D.D., 2002. A Proposed Method for Measuring Subsoil Denitrification In Situ. Soil Sci. Soc. Am. J. 66, 507–518.

Woodward, K.B., Fellows, C.S., Conway, C.L., Hunter, H.M., 2009. Nitrate removal, denitrification and nitrous oxide production in the riparian zone of an ephemeral stream. Soil Biol. Biochem. 41, 671–680. doi:10.1016/j.soilbio.2009.01.002

Woodward, S.J.R., Stenger, R., Bidwell, V.J., 2013. Dynamic analysis of stream flow and water chemistry to infer subsurface water and nitrate fluxes in a lowland dairying catchment. J. Hydrol. 505, 299–311. doi:10.1016/j.jhydrol.2013.07.044

Wu, W., Ma, B., 2015. Integrated nutrient management (INM) for sustaining crop productivity and reducing environmental impact: A review. Sci. Total Environ. 512–513, 415–427. doi:10.1016/j.scitotenv.2014.12.101

Yang, X., Huang, S., Wu, Q., Zhang, R., 2012. Nitrate reduction coupled with microbial

oxidation of sulfide in river sediment. J. Soils Sediments. doi:10.1007/s11368-012-0542-9

Yeomans, J., Beauchamp, E., 1982. Acetylene as a possible substrate in the denitrification process. Can. J. Soil Sci. 62, 139–144.

Yeomans, J.C., Bremner, J., McCarty, G.W., 1992. Denitrification capacity and denitrification potential of subsurface soils. Commun. Soil Sci. Plant Anal. 23, 919–927.

Yoshinari, T., Hynes, R., Knowles, R., 1977. Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. Soil Biol. Biochem. 9, 177–183.

Yu, H., Song, Y., Xi, B., Du, E., He, X., Tu, X., 2012. Denitrification potential and its correlation to physico-chemical and biological characteristics of saline wetland soils in semi-arid regions. Chemosphere 89, 1339–1346. doi:10.1016/j.chemosphere.2012.05.088

Yu, K., Struwe, S., Kjøller, A., Chen, G., 2008. Denitrification rate determined by nitrate disappearance is higher than determined by nitrous oxide production with acetylene blockage. Ecol. Eng. 32, 90–96. doi:10.1016/j.ecoleng.2007.09.006

Yuan, L., Pang, Z., Huang, T., 2012. Integrated assessment on groundwater nitrate by unsaturated zone probing and aquifer sampling with environmental tracers. Environ. Pollut. 171, 226–233. doi:10.1016/j.envpol.2012.07.027

Zaman, M., Nguyen, M.L., Gold, A.J., Groffman, P.M., Kellogg, D.Q., Wilcock, R.J., 2008. Nitrous oxide generation , denitri fi cation , and nitrate removal in a seepage wetland intercepting surface and subsurface fl ows from a grazed dairy catchment. Aust. J. Soil Res. 46, 565–577. doi:10.1071/SR07217

Zarour, H., 2008. Groundwater Resources in the Manawatu-Wanganui Region: Technical Report to Support Policy Development. Palmerston North, New Zealand.

Zhang, X., Xu, Z., Sun, X., Dong, W., Ballantine, D., 2013. Nitrate in shallow groundwater in typical agricultural and forest ecosystems in China, 2004-2010. J. Environ. Sci. (China) 25, 1007–1014. doi:10.1016/S1001-0742(12)60139-9

Zhang, Y., Slomp, C.P., Peter, H., Bostick, B., Passier, H.F., Böttcher, M.E., Omoregie, E.O., Lloyd, J.R., Polya, D.A., Cappellen, P. Van, 2012. Isotopic and microbiological signatures of pyrite-driven denitri fi cation in a sandy aquifer. Chem. Geol. 300–301, 123–132. doi:10.1016/j.chemgeo.2012.01.024

Zhang, Y., Slomp, C.P., Peter, H., Passier, H.F., Cappellen, P. Van, 2009. Denitrification coupled to pyrite oxidation and changes in groundwater quality in a shallow sandy

aquifer. Geochim. Cosmochim. Acta 73, 6716–6726. doi:10.1016/j.gca.2009.08.026

Zhu, J.G., Liu, G., Han, Y., Zhang, Y.L., Xing, G.X., 2003. Nitrate distribution and denitrification in the saturated zone of paddy field under rice/wheat rotation. Chemosphere 50, 725–732. doi:10.1016/S0045-6535(02)00212-6

Zou, X.M., Ruan, H.H., Fu, Y., Yang, X.D., Sha, L.Q., 2005. Estimating soil labile organic carbon and potential turnover rates using a sequential fumigation – incubation procedure. Soil Biol. Biochem. 37, 1923–1928. doi:10.1016/j.soilbio.2005.02.028

APPENDICES

Appendix A. Summary of Approaches for Measuring Denitrification

Approach	Description	Applications/Advantages	Limitations/Shortcomings
Mass balance approach	• Denitrification is deduced from measurements of different fluxes of N and changes in storage • Simplified method may only account for the amount of nitrate lost	+ applicable for field, stream reach, watershed, or regional scales + may utilise secondary data from literature + provide information on potential importance of denitrification	- difficulty in measuring or estimating all types of N fluxes; dilution and the type of nitrate removal process are usually not accounted - limited in obtaining quantitative rates of denitrification - more appropriate for longer time scale of measurements due to limitation in measuring small fluxes - may not be able to specify where denitrification occurred or the temporal dynamics of denitrification
In situ gradient of environmental tracers (atmospheric or terrestrial)	• Environmental tracers are used to indicate elapse time in order to calculate denitrification rate • Ideally, naturally existing tracers are used instead of introduced	+ largely applicable for groundwater systems + spatial scale is large enough to account for heterogeneity + may provide information on nitrate mass gradients or processes independent of denitrification + denitrification rate could be computed	- system may be too complex to interpret - choice of tracer depends on the abundance of ambient concentration (introduced tracer); hence requiring preliminary assessment - tracer data may be ambiguous - limited application in vadose zone due to the transport component of the method
Acetylene inhibition method	• Acetylene is used to inhibit the reduction of N_2O to N_2 to simplify measurement of the end-product of denitrification given the abundance of N_2 in ambient conditions • Denitrification rate is determined from the production of N_2O with time	+ applicable for terrestrial and aquatic systems from small sample sizes to field scale of limited coverage + relatively simple to carry out and allows running a large number of measurements to account for variability + lower cost and availability of instrument to detect and measure N_2O + may be used to identify denitrification hotspots + can be used to determine any of the measures of denitrification	- applicability in environments with low nitrate may be constrained with the possible inhibition of nitrification - methodological constraints: incomplete and slow diffusion of acetylene in active microsites; decomposition and consumption of acetylene by microbes; acetylene scavenging of NO; incomplete inhibition of N_2O reduction

^{15}N tracer method	• ^{15}N-labelled nitrate (or ammonium) is added into the system and denitrification rate is determined from proportions of nitrate in reactants and/or products ($^{15}N_2O$ and/or $^{15}N_2$)	+ provides reliable estimates of denitrification; considered one of the best for soil studies + can be used in situ where soil sampling is difficult	- high cost of material (^{15}N-labelled nitrate) and analysis (mass spectrometers) - actual denitrification rate could not be measured due to nitrate added; increased N availability may result to overestimation of denitrification in low N systems - sample preparation and collection techniques are time consuming - instantaneous and homogeneous mixing of added ^{15}N-labelled nitrate may never be achieved
Direct N_2 quantification	• Denitrification end-product, N_2, is measured in a gas-tight incubation system • For aquatic systems such as groundwater, N_2 measurements is coupled with Ar measurements to determine excess N_2 which could be attributed to denitrification (N_2:Ar method)	+ applicable for both terrestrial and aquatic systems; may be conducted in situ and in the laboratory + non-destructive and no additional substrates or inhibitors are required + may be used in combination with ^{15}N tracer and acetylene inhibition method + can be applied in both low and high denitrification rates + rather simple as long as gas-tight incubation system is available	- for incubation experiments, requires a complex gas flow incubation system (very gas-tight system that connects incubation samples to analytical instruments given the high N_2 background concentration) - limited amount of samples in each run - application for *in situ* investigations in groundwater systems seems limited to investigations along a flow path - calculation of denitrification rates for in situ investigations requires measurements of groundwater age tracers
Stoichiometric approaches	• Denitrification is determined from the difference between observed DIN and expected DIN, the latter is deduced from mass balance of reaction in the decomposition of organic matter • Redfield ratio is used as basis of elemental composition of organic matter	+ applicable for large scale measurements + applicable for measurements with sediments + denitrification may be determined from secondary/published data	- largely restricted to marine environments where elemental concentration of primary producers is more constrained - applicability for investigations with sediments is constrained by the uncertainty of the C:N ratio of the substrate - measurements may yield not simply denitrification but the net difference between fixation and denitrification
Stable isotope-based methods	• Samples are collected for analysis of stable N and O isotopes of nitrate • Occurrence of denitrification is determined from the enrichment of $\delta^{15}N$ and $\delta^{18}O$ due to isotopic fractionation as the lighter isotope	+ applicable for in situ or laboratory investigations, but more applicable for groundwater and open water systems	- costly isotope analysis limiting the number of samples to run - measurements are primarily indicative; calculation for denitrification rate is much less straightforward

	(e.g., ^{14}N) tends to be denitrified before the heavier isotope (^{15}N); $\delta^{15}N$:$\delta^{18}O$ ratio indicating denitrification is in the range of 1:3 to 2:1		
Molecular approaches	• Focuses on investigating the abundance, composition and role of different enzymes in denitrification	+ molecular characterisation, in combination with denitrification rates and quantitative measures of environmental controls, helps in evaluating the abiotic and biotic controls that drive N transformation	- more sophisticated due to the small amount of sample to be handled - requires specialised techniques and instrumentation

Source: mainly based from Groffman et al., 2006; Addy et al., 2002; Drury et al., 2008; Felber et al., 2012; Fukada et al., 2003; Groffman et al., 1999; Mengis et al., 1999; Mohamed et al., 2003; Well & Myrold, 2002

Appendix B. Summary of In Situ Groundwater Denitrification Rate Measurement Techniques

B.1 Investigation following flow pathways

Method [Reference]	Application [Type of DR]	Description and Parameters	Rate Computation	Pros (+) and Cons (-)
Nitrate gradient method [Tesoriero et al., 2000]	Piezometers along flow path [**actual** denitrification rate]	Monitoring nitrate concentration from a set of piezometers along groundwater flow path; nitrate and a host of other parameters	Average DR computed from **nitrate concentrations upgradient and downgradient well pair and groundwater travel time**	+ no injection of test solution required + simple - no other sources or sinks along the flow path - requires several number of bores - requires **modelling** to determine travel time
Excess N_2 gradient method [Tesoriero et al., 2000]	Piezometers along flow path [**actual** denitrification rate]	Monitoring concentration of excess N2 from a set of piezometers along groundwater flow path; N_2, Ar, nitrate and other parameters	Zero-order DR calculated from the **excess N_2 divided by groundwater travel time**	See pros and cons above + more accurate DR estimation than nitrate gradient method - more expensive and laborious that nitrate gradient method
Piezometer nest with excess N_2 [Green et al., 2008]	Piezometer nests along flow path [**actual** denitrification rate]	Monitoring concentration of excess N_2 from a set of piezometer nests along groundwater flow path; N_2, Ar, CFCs (11, 12, 113)	Zero-order DR calculated from the **excess N_2 divided by the water age**	Same pros and cons for excess N2 gradient method - costly borehole installation
Injection method with well pair [Pauwels et al., 1998]	A pair of injection and sampling well separated by 15m along flow path [denitrification **capacity** rate]	Injection of test solution with bromide and nitrate; bromide, nitrate, ammonium, etc. monitored; 2D model used to simulate BTCs	First-order DR obtained from **slope of a line fit in a plot of natural logarithm of relative nitrate concentration vs time**	+ large aquifer material investigated - need to ascertain flow path for capturing injected solution
Injection method with pair of multilevel wells [Smith et al., 1996; see also Killingstad et al., 2002 and Smith et al., 2001 on file]	A pair of injection and 15 sampling wells separated by 10m along flow path [**actual** denitrification rate]	Injection of test solution with bromide and acetylene; used a transport model to simulate tracer BTC; NO_3, N_2O, Br, etc.	DR calculated from nitrous oxide production rate adjusted to match simulated N_2O BTCs	+ better capture of injected solution - more borehole required with cost and time implications
Two-well re-circulating Well Test [Burbery et al., 2013; Burbery & Wang, 2010]	A pair of injection and extraction well [denitrification **capacity** rate]	Similar to injection-extraction pair, except that water is re-circulated; Monitoring	First-order nitrate reaction rate determined through parameter estimation using non-linear	+ applicable in high hydraulic conductivity or lateral flow conditions

Method [Reference]	Application [Type of DR]	Description and Parameters	Rate Computation	Pros (+) and Cons (-)
		concentrations of conservative tracer and nitrate	least-squared error optimisation	+ larger aquifer material investigated - difficult to ascertain direction of groundwater flow and compete capture of recirculated water - complex heterogeneity of aquifer material may affect complete capture of injected solution

B.2 Localised or discrete investigations

Method [Reference]	Application [Type of DR]	Description and Parameters	Rate Computation	Pros (+) and Cons (-)
Simple push-pull method [Tesoriero et al., 2000]	Single well or piezometer [denitrification **capacity** rate]	Test solution with nitrate and bromide injected and extracted from the same well; DO , nitrate, nitrite, ammonium, and bromide monitored over 15h	DR determined as the **slope of the line fitted on the plot of corrected nitrate concentration over time**	+ relatively simple - all nitrate lost considered as denitrified; nitrate lost due to other processes is neglected - may not be applicable in formations with high K ? N and O isotope of residual nitrate could provide information on whether denitrification indeed occurred
Push-pull method with tracer BTC [Istok et al., 1997]	Single well or piezometer [denitrification **capacity** rate]	Test solution with nitrate and bromide injected and extracted from the same well; monitored bromide, nitrate, nitrite and N2O during extraction	Zero-order DR for reactants (and products) computed by **dividing the quantity of reactant consumed** or product formed **by the test solution injection volume and by the mean residence time** for the test solution in the aquifer	Same as above (simple push-pull method)
Acetylene-based push-pull method [Sanchez-Perez et al., 2003]	Single well or piezometer [**actual** denitrification rate – given that there is enough nitrate for the process]	Test solution with bromide and acetylene injected and extracted through a packer system; DO, nitrate, bromide, nitrous oxide	DR computed as the **maximum slope of the line tangent to the curve of corrected N_2O concentrations plotted against**	+ relatively simple and fast + DR value is more reliable than nitrate-based values - applicable for aquifers with K

Method [Reference]	Application [Type of DR]	Description and Parameters	Rate Computation	Pros (+) and Cons (-)
		and DOC monitored over a short period (4h)	**time**	from 10-2 to 10-4 m/s - relatively more laborious and expensive than nitrate-based methods **- other possible sources of N2O could not be accounted** - all other acetylene-related limitations
Isotope-based push-pull method [Addy et al., 2002; Well et al., 2003]	Mini-piezometer (but may be applicable for regular well or piezometer) [denitrification **capacity** rate]	Test solution with bromide and 15N-labelled nitrate; DO, nitrate, bromide, DOC, N_2O, N_2 over an appropriate incubation period	DR computed as: **total mass of ^{15}N gas per volume of water pulled/(dry mass of soil per volume of water pulled x incubation period)**; DR is the sum of $^{15}N_2O$-N and $^{15}N_2$-N rates	+ applicable for low denitrification rates + more accurate DR values - more laborious and expensive than all other push-pull methods - concerns with N2 sampling and measurements ? may adapt the method of Sanchez-Perez et al. (2003) to account for dilution
Isotope and acetylene-based push-pull method [Well et al., 2003]	PVC probe or steel drive probe [denitrification **capacity** rate]	Test solution with bromide, acetylene and ^{15}N-labelled nitrate; nitrate, bromide, N_2, N_2O	DR is **production rate of gases corrected for dilution**; see paper for formulas	See pros and cons of isotope-based and acetylene-based methods
Simple drive point injection method [Trudell et al., 1986]	Stainless steel drive point [**enhanced** denitrification rate]	Test solution with nitrate and bromide; nitrate, bromide and bicarbonate monitored over 450h	DR determined from **the slope of the line tangent to the curve of corrected nitrate concentration against time**	See simple push-pull method - suitable in sites with high denitrification activity and low hydraulic gradient
Acetylene-based drive point injection method [Toda et al., 2002]	Same drive point as above [denitrification **capacity** rate]	Test solution with nitrate, bromide and acetylene; nitrate, nitrite, bromide, and nitrous oxide monitored over 5 days	DR determined from **initial and final nitrate concentrations** (corrected for dispersion) – checked with stoichiometry for nitrite and nitrous oxide	Pros and cons of nitrate-based and acetylene-based methods apply
Simple cluster method [Bragan et al., 1997; Woodward et al., 2009 with 9 capture wells per injection well]	Piezometer nest with 1 injection well and 3 sampling wells of the same depths [denitrification **capacity** rate]	Test solution with nitrate and bromide; nitrate, bromide, nitrous oxide monitored over 6 days	DR based on **nitrate-N loss computed with correction for dilution in a specific time window**	+ hydrology is preserved + 2 methods can be used simultaneously - higher cost for borehole - duration depends on formation
Acetylene-based cluster method	Piezometer nest with 1 injection	Test solution with nitrate,	DR determined from **peak**	+ hydrology is preserved

Method [Reference]	Application [Type of DR]	Description and Parameters	Rate Computation	Pros (+) and Cons (-)
[Bragan et al., 1997]	well and 3 sampling wells of the same depths [denitrification **capacity** rate]	bromide, and acetylene; nitrate, bromide, nitrous oxide, acetylene monitored over 6 days	**nitrous oxide concentration multiplied by bromide travel rate and divided by the travel distance**	+ 2 methods can be used simultaneously - higher cost for borehole - duration depends on formation - other acetylene-related limitations
Acetylene-based in situ microcosm [Gillham et al., 1990; Starr & Gillham, 1993	In situ microcosm [denitrification **capacity** rate]	Test solution with nitrate, bromide, and acetylene; nitrate, N_2O, ammonium, bromide, DO, DOC, acetylene monitored over 10 days	DR is determined in two ways: **nitrate reduction and N_2O production over the period of experiment** (may be corrected for dilution depending on the recovery of bromide)	+ easy interpretation of results due to the isolated system + reduces difficulties in controlling environmental variables - small aquifer volume - limited to reasonably permeable geologic materials - hydrology is disrupted
Isotope-based in situ microcosm [Mengis et al., 1999]	In situ microcosm [denitrification **capacity** rate]	Test solution with ^{15}N-labelled nitrate and bromide; nitrate, ammonium, bromide, and ^{15}N-N_2 monitored over 7 days	DR computed based on **^{15}N-N_2 and ^{14}N-N_2 produced**; not clear how computation was made (may need to adapt calculation of other isotope-based studies)	See pros and cons of using in situ microcosm and isotope-based methods
Simple in situ mesocosm [Korom et al., 2005; 2012]	In situ mesocosm [denitrification **capacity** rate]	Test solution with nitrate and bromide; nitrate and bromide monitored for 270 days	DR computed in 3 ways: (zero-order) based on **corrected nitrate lost over incubation duration**, or **average rate from the slope of the line fitted to the plot of corrected nitrate concentration and time**; (first-order) slope of the line fitted on the plot of relative nitrate concentration	+ large enough to incorporate microsites + minimised hydrodynamic mixing associated with flow path + applicable for slow denitrification rates + large samples can be obtained - laborious and costly installation - may require longer time to monitor depending on rate in the aquifer
Multilevel device [Korom et al., 2012]	Multilevel wells (based on Pickens et al., 1978) [**actual**	No test solution; nitrate, and isotopic composition of nitrate	DR determined by **plotting the depth-averaged concentration**	+ simple mass balance approach - need to be conducted where

Method [Reference]	Application [Type of DR]	Description and Parameters	Rate Computation	Pros (+) and Cons (-)
	denitrification rate]		**of nitrate over time and fitting the data with a first-order rate model**; N and O isotopes of nitrate for inferring if denitrification is indeed occurring	there are no further inputs of nitrate - applicable for formations that are isotropic in horizontal direction - negligible dilution required
Single well push-pull method with confining lysimeter [Zaman et al., 2008]	Single well (may be used to measure actual although Zaman used ^{15}N –labelled nitrate)	Dosing solution with conservative tracer and nitrate source; dissolve gases also measured in water samples collected (^{15}N [N2O+N_2], N_2O)	Denitrification rate (zero-order) based on the concentrations of nitrate and conservative tracer	+ dosing or injection solution conserve within the system - additional field setup (confining lysimeter) which may be challenging and costly when investigating deeper profiles

Appendix C. Summary of denitrification studies in New Zealand

C.1 Root zone (pasture/crop lands; 0.60m root zone depth)

Location	Depth, m	Soil series /texture	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
DairyNZ Scott Farm, central Waikato	0-0.10	Well-drained Horotiu sandy loam	Dairy pasture (rye grass, white clover)	Acetylene inhibition method;	10g soil added with 20mL solution containing 0.2g glucose, 0.1g KNO3 L-1, 0.125 chloramphenicol; incubated for 75 min at 25deg in a rotary shaker	DEA (Tiedje et al., 1989)	1.09 kg N_2O-N ha^{-1} h^{-1} (undisturbed control); 1.04 (disturbed control), 1.36 (sawdust), 2.03 (woody mulch)	Stevenson et al., 2011
DairyNZ Scott Farm, central Waikato	0-0.15	Well-drained Horotiu sandy loam	Dairy pasture (rye grass, white clover)	Acetylene inhibition method; intact cores in 3.2cm x 15cm perforated PVC liners	Acetylene added; incubated for 6 h at soil temperature during collection	Actual denitrification rate	2.47 x 10^{-3} kg N_2O-N ha^{-1} h^{-1} (undisturbed control); 2.48 (disturbed control), 2.60 (sawdust), 2.38 (woody mulch)	Stevenson et al., 2011
Massey University Dairy Farm 4, Palmerston North	0-0.075	Poorly-drained Tokomaru silt loam	Rye grass, white clover pasture	Acetylene inhibition method; intact cores in 2.5cm x 7.5cm PVC	Acetylene added; incubated for 24 h on site at a shaded portion close to the paddock	Actual denitrification rate	21 µg N_2O-N kg^{-1} d^{-1} (control site); 42 (grazed site)	Luo et al., 2000
Palmerston North, Manawatū (Massey University site)	0-0.40	Tokomaru silt loam	Pasture (samples collected in autumn and spring)	Acetylene inhibition method (10% v/v); composite (disturbed) samples in 125 mL Erlenmeyer flask	Unamended; amended with 50 µg nitrate-N g^{-1} (20deg in the dark; not shaken?)	DEA (no chloramphenicol; 5-hr incubation)	0.03-5.50 µg N_2O-N g^{-1} soil d^{-1} (0-0.10); 0.04-1.0 (0.10-0.20); 0.03-0.07 (0.20-0.40)	Luo et al., 1998
Palmerston	0-0.40	Tokomaru silt	Pasture	Acetylene	Amended with	DEA (no	3.5-10.8 µg N_2O-	Luo et al., 1998

Location	Depth, m	Soil series /texture	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
North, Manawatū (Massey University site)		loam	(samples collected in autumn and spring)	inhibition method (10% v/v); composite (disturbed) samples in 125 mL Erlenmeyer flask	300 µg glucose-C g^{-1}; Amended with 50 µg nitrate-N g^{-1} and 300 µg glucose-C g^{-1} (20deg in the dark; not shaken?)	chloramphenicol; 5-hr incubation)	N g^{-1} soil d^{-1} (0-0.10); 1.0-5.0 (0.10-0.20); 0.70-2.0 (0.20-0.40)	
Palmerston North, Manawatū (AgResearch Institute site)	0-0.40	Manawatū sandy loam	Pasture (samples collected in autumn and spring)	Acetylene inhibition method (10% v/v); composite (disturbed) samples in 125 mL Erlenmeyer flask	Unamended; amended with 50 µg nitrate-N g^{-1} (20deg in the dark; not shaken?)	DEA (no chloramphenicol; 5-hr incubation)	0.30-7.50 µg N_2O-N g^{-1} soil d^{-1} (0-0.10); 0.03-0.25 (0.10-0.20); 0.02-0.05 (0.20-0.40)	Luo et al., 1998
Palmerston North, Manawatū (AgResearch Institute site)	0-0.40	Manawatū sandy loam	Pasture (samples collected in autumn and spring)	Acetylene inhibition method (10% v/v); composite (disturbed) samples in 125 mL Erlenmeyer flask	Amended with 300 µg glucose-C g^{-1}; Amended with 50 µg nitrate-N g^{-1} and 300 µg glucose-C g^{-1} (20deg in the dark; not shaken?)	DEA (no chloramphenicol; 5-hr incubation)	4.0-11.0 µg N_2O-N g^{-1} soil d^{-1} (0-0.10); 1.0-3.10 (0.10-0.20); 0.30-1.05 (0.20-0.40)	Luo et al., 1998
Palmerston North, Manawatū (Massey University site)	0.20-0.40	Tokomaru silt loam	Pasture (samples collected March in 1993)	Acetylene inhibition method (10% v/v); intact core samples (2 cm diameter)	No amendment (incubation at field moisture and temperature)	Actual denitrification rate (24-hr incubation)	0.0005-0.0008 µg N_2O-N g^{-1} soil d^{-1} (before rainfall); 0.0011-0.0015 µg N_2O-N g^{-1} soil d^{-1} (after rainfall)	Luo et al., 1998
Lincoln, Canterbury	0.40-0.85	Pahau silt loam (mottled sandy clay)	Italian ryegrass (ungrazed)	Acetylene inhibition method (10% v/v); composite (disturbed) samples in 250 mL Schott bottle	50 µg nitrate-N g^{-1}; 300 µg glucose-C g^{-1}; incubated at 20degC; 100rpm	Denitrification potential (8-hr incubation; no chloramphenicol)	2.4 µg N_2O-N kg^{-1} h^{-1} (with anaerobic pre-incubation: 10.3 [24hr]; 12.5 [48hr]; 13.1 [96hr]; 15.2 [168hr])	Peterson et al., 2013
Lake Taupo,	0-0.5m	Oruanui loamy	Sheep grazing	^{15}N-tracer method;	^{15}N-KNO_3	Denitrification	0-0.45 nmol N g^{-1}	Clague et al.,

Location	Depth, m	Soil series /texture	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
Waikato (Waihora field site)		sand (Podzolic Orthic Pumice Soil subgroup)	(2009) to low intensity calf and heifer grazing	composite samples in 0.5L mason jars (not shaken?)	solution (10 µ N g^{-1} dry soil); anaerobic incubation (argon); pre-incubation (24 hr at 25 deg before substrate addition)	capacity	soil h^{-1} ($^{15}N_2O$ flux)	2013
Rangiatea site, Lake Taupo, Waikato	0-0.9	Oruanui hill soils (Yellow-brown pumice)	Large sheep and beef farms	^{15}N-tracer method; composite samples in 0.5L mason jars (not shaken?); 48-hr incubation	^{15}N-KNO_3 solution (5-13 µg NO_3-N g^{-1} dry soil); anaerobic incubation (argon); pre-incubation (24 hr at 28 deg before substrate addition)	Denitrification capacity	0.7-1.0e-5 µmol N_2O-N g^{-1} soil h^{-1} (0-0.4m); 45.2e-5-6.8e-5 (0.4-0.9m)	Barkle et al., 2007
Waihora site, Lake Taupo, Waikato	0.0-0.9	Oruanui loamy sand (Yellow-brown pumice)	Large sheep and beef farms	^{15}N-tracer method; composite samples in 0.5L mason jars (not shaken?); 48-hr incubation	^{15}N-KNO_3 solution (5-13 µg NO_3-N g^{-1} dry soil); anaerobic incubation (argon); pre-incubation (24 hr at 28 deg before substrate addition)	Denitrification capacity	0 µmol N_2O-N g^{-1} soil h^{-1} (0-0.4); 5.2e-5-1.32e-4 (0.4-0.9m)	Barkle et al., 2007
Kinloch site, Lake Taupo, Waikato	0.0-0.6	Waipahihi sand (Yellow-brown pumice)	Sheep and horse grazing	^{15}N-tracer method; composite samples in 0.5L mason jars (not shaken?); 48-hr incubation	^{15}N-KNO_3 solution (5-13 µg NO_3-N g^{-1} dry soil); anaerobic incubation (argon); pre-incubation (24 hr at 28 deg before substrate addition)	Denitrification capacity	0.7-1.0e-5 µmol N_2O-N g^{-1} soil h^{-1} (0-0.6m);	Barkle et al., 2007
Lincoln,	0-0.6	Pahau silt loam	Oats, potatoes	*In situ* collection of	Treatments are the	Actual N_2O	0-0.0032 g N_2O m^{-}	Thomas et al.,

Location	Depth, m	Soil series /texture	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
Canterbury		(Mottled Argillic Pallic); sand and sandy gravels from 1.10m		N_2O	actual fertiliser applications on site	production rate (not necessarily denitrification rate as acetylene use was absent and N_2 rate was not measured)	[2] h^{-1} (0.6m);	2012
Palmerston North	0-0.075	Mixed mesic Dystric Eutrochrept (freely drained sandy loam)	Sheep-grazed grass-clover, herbal ley, grass +N400 (ryegrass)	Acetylene inhibition method; intact cores	In situ soil temperature and moisture content, incubated for 24 hours; (grass+N400 land was supplied with 400 kgN ha-1 yr-1 as urea)	Actual denitrification	20-40 g N_2O-N ha^{-1} d^{-1} (grass-clover; herbal ley); 40-300 (grass+N400) (May-Aug)	Ruz-Jerez et al., 1994
Ruakura Research Center, Waikato	0-0.30	Bruntwood silt loam	Pasture (ryegrass, white clover)	Gas chambers on surface of lysimeters (18cm dia x 30cm long)	In situ application of synthetic urine at 500 kg N ha-1	Actual denitrification (surface layer)	1.3 kg N ha^{-1} d^{-1} (peak flux at 18 days; no water table); 0.8 kg N ha^{-1} d^{-1} (peak flux at 18 days; water table at 0.10m below surface)	Clough et al., 1996
Massey No. 1 Dairy Farm, Palmerston North	0-0.10	Manawatū fine loam/Manawatū sandy loam	Pasture (ryegrass and white clover); riparian areas	Acetylene inhibition method; composite sample (10g) Acetylene inhibition method; (200g dry)	DEA: 50 µg NO_3-N g^{-1} fresh soil; 250 µg glucose-C g^{-1} fresh soil; chloramphenicol	DEA; and Actual (?) denitrification rate	DEA: 0.016-0.99 mg N_2O-N kg^{-1} DW soil h^{-1} DR: 0.21-12.2 µg N_2O-N kg^{-1} DW soil h^{-1}	Deslippe et al., 2014

C.2 Vadose zone (intermediate) [pasture/crop lands; starting at 0.60m bgl down to groundwater table]

Location	Depth, m	Soil series /texture	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
Kinloch site, Lake Taupo, Waikato	0.6-11.7	Waipahihi sand (Yellow-brown pumice)	Sheep and horse grazing	^{15}N-tracer method; composite samples in 0.5L mason jars (not shaken?); 48-hr incubation	^{15}N-KNO_3 solution (5-13 µg NO_3-N g^{-1} dry soil); anaerobic incubation (argon); pre-incubation (24 hr at 28 deg before substrate addition)	Denitrification capacity	1.3e-5-1.6e-5 µmol N_2O-N g^{-1} soil h^{-1} (0.6-1.2m); 0.8e-5-1.0e-5(1.2-1.9m); 1.3e-5-4.5e-5 (approx. 4m) Overall range: 0.7e-5-4.8e-5	Barkle et al., 2007
Lincoln, Canterbury	0.6-2.20	Pahau silt loam (Mottled Argillic Pallic); sand and sandy gravels from 1.10m	Oats, potatoes	*In situ* collection of N_2O	Treatments are the actual fertiliser applications on site	Actual N_2O production rate (not necessarily denitrification rate as acetylene use was absent and N_2 rate was not measured)	0-0.0002 g N_2O m^{-2} h^{-1} (1.5m)	Thomas et al., 2012
Waihora site, Lake Taupo, Waikato	0.9-3.8	Oruanui loamy sand (Yellow-brown pumice)	Large sheep and beef farms	^{15}N-tracer method; composite samples in 0.5L mason jars (not shaken?); 48-hr incubation	^{15}N-KNO_3 solution (5-13 µg NO_3-N g^{-1} dry soil); anaerobic incubation (argon); pre-incubation (24 hr at 28 deg before substrate addition)	Denitrification capacity	2.5e-5-3.6e-5 µmol N_2O-N g^{-1} soil h^{-1} (0.9-1.3m); 5.5e-5-7.0e-5 (1.3-1.8m) Overall range: 0-7.0e-5	Barkle et al., 2007
Lincoln, Canterbury	0.40-0.85; 2.05-2.30; 4.60-4.80	Pahau silt loam	Italian ryegrass (ungrazed)	Acetylene inhibition method (10% v/v); composite (disturbed) samples in 250 mL Schott bottle	250 µg nitrate-N g^{-1}; 20 mL of soil extract (16-25 mg kg^{-1} CWE or HWE); incubated at 20degC; 100rpm	Denitrification potential (7 days incubation; no chloramphenicol)	440-500 µg N_2O-N kg^{-1} h^{-1} (at the period of rapid rate; 36-96hr; with extract from pasture); 55-130 µg N_2O-N kg^{-1} h^{-1} (with extract from	Peterson et al., 2013

Location	Depth, m	Soil series /texture	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
							fallow lands)	
Rangiatea site, Lake Taupo, Waikato	0.9-5.4	Oruanui hill soils (Yellow-brown pumice)	Large sheep and beef farms	^{15}N-tracer method; composite samples in 0.5L mason jars (not shaken?); 48-hr incubation	^{15}N-KNO_3 solution (5-13 µg NO_3-N g^{-1} dry soil); anaerobic incubation (argon); pre-incubation (24 hr at 28 deg before substrate addition)	Denitrification capacity	0.7e-5-1.2e-5 µmol N_2O-N g^{-1} soil h^{-1} (0.9-1.3m); 0.1e-5-0.4e-5 (1.3-1.8m) Overall range: 0.1e-5-1.5e-5	Barkle et al., 2007
Lincoln, Canterbury	1.20-1.40	Pahau silt loam (small gravels in sand)	Italian ryegrass (ungrazed)	Acetylene inhibition method (10% v/v); composite (disturbed) samples in 250 mL Schott bottle	50 µg nitrate-N g^{-1}; 300 µg glucose-C g^{-1}; incubated at 20degC; 100rpm	Denitrification potential (8-hr incubation; no chloramphenicol)	0.2 µg N_2O-N kg^{-1} h^{-1} (with anaerobic pre-incubation: 0.7 [24hr]; 1.1 [48hr]; 0.9 [96hr]; 1.3 [168hr])	Peterson et al., 2013
Lincoln, Canterbury	2.05-2.35	Pahau silt loam (sandy gravel with very small clay; iron rich open framework gravel)	Italian ryegrass (ungrazed)	Acetylene inhibition method (10% v/v); composite (disturbed) samples in 250 mL Schott bottle	50 µg nitrate-N g^{-1}; 300 µg glucose-C g^{-1}; incubated at 20degC; 100rpm	Denitrification potential (8-hr incubation; no chloramphenicol)	4.2 µg N_2O-N kg^{-1} h^{-1} (with anaerobic pre-incubation: 2.8 [24hr]; 3.5 [48hr]; 2.7 [96hr]; 3.0 [168hr])	Peterson et al., 2013
Lincoln, Canterbury	4.27-4.60	Pahau silt loam (sandy gravel; very sandy)	Italian ryegrass (ungrazed)	Acetylene inhibition method (10% v/v); composite (disturbed) samples in 250 mL Schott bottle	50 µg nitrate-N g^{-1}; 300 µg glucose-C g^{-1}; incubated at 20degC; 100rpm	Denitrification potential (8-hr incubation; no chloramphenicol)	1.0 µg N_2O-N kg^{-1} h^{-1} (with anaerobic pre-incubation: 1.3 [24hr]; 1.3 [48hr]; 1.3 [96hr]; 1.1 [168hr])	Peterson et al., 2013
Lincoln, Canterbury	4.60-4.80	Pahau silt loam (open framework gravel with clayey base)	Italian ryegrass (ungrazed)	Acetylene inhibition method (10% v/v); composite (disturbed) samples	50 µg nitrate-N g^{-1}; 300 µg glucose-C g^{-1}; incubated at 20degC; 100rpm	Denitrification potential (8-hr incubation; no chloramphenicol)	2.6 µg N_2O-N kg^{-1} h^{-1} (with anaerobic pre-incubation: 3.7 [24hr]; 3.1 [48hr]; 2.9 [96hr]; 2.3	Peterson et al., 2013

Location	Depth, m	Soil series /texture	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
				in 250 mL Schott bottle			[168hr])	

C.3 Saturated zone (below water table)

Location	Depth, m bgl	Aquifer matrix/ soil type	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
Mapara, Waikato	6.9-9.8	Unwelded ignimbrite with silty peat bed		Re-circulating well test (two-well) method	0.74 kg Bromide; 0.75 kg N-Nitrate; injection rate 0.14 L min^{-1}	Denitrification capacity	First-order rate: 0.089 d^{-1}	Burbery et al., 2013
Rangiatea, Waikato	12.0-16.0	Unwelded ibnimbrite		Re-circulating well test (two-well) method	0.81 kg Bromide; 0.80 kg N-Nitrate; injection rate 0.04 L min^{-1}	Denitrification capacity	First-order rate: 0.015 d^{-1}	Burbery et al., 2013
Kuratau, Waikato	2.0-5.5	Pumiceous gravelly sand		Re-circulating well test (two-well) method	0.57 kg Bromide; 0.58 kg N-Nitrate; injection rate 0.06 L min^{-1}	Denitrification capacity	First-order rate: 0.025 d^{-1}	Burbery et al., 2013
Edendale, Southland	14.0-17.0	Alluvial sandy gravel		Re-circulating well test (two-well) method	53.76 kg Bromide; no N-Nitrate; injection rate 0.77 L min^{-1}	Actual denitrification	First-order rate: 0.0004 d^{-1}	Burbery et al., 2013
Rangiatea, Waikato		Unwelded ibnimbrite		Single well push-pull method			First-order rate: 0.118 d^{-1}	Burbery et al., 2013; Hadfield & Gibbs, 2007
Mapara, Waikato		Unwelded ignimbrite with silty peat bed		Single well push-pull method			First-order rate: 0.367 d^{-1}	Burbery et al., 2013; Hadfield & Gibbs, 2007
Lake Taupo, Waikato (Waihora field site)	2.4	Oruanui loamy sand (Podzolic Orthic Pumice Soil subgroup)	Sheep grazing (2009) to low intensity calf and heifer grazing	^{15}N-tracer method; composite samples in 0.5L mason jars (not shaken?); incubation of	^{15}N-KNO_3 solution (10 µ N g^{-1} dry soil); anaerobic incubation	Denitrification capacity	0-0.10 nmol N g^{-1} soil h^{-1} ($^{15}N_2O$ flux)	Clague et al., 2013

Location	Depth, m bgl	Aquifer matrix/ soil type	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
				samples from saturated zone	(argon); pre-incubation (24 hr at 25 deg before substrate addition)			
Kiwitahi, Waikato	0.20-0.35 below the water level (?) – wetland was saturated to the surface	Topehaehae silt loam, Kiwitahi silt loam	Seepage wetland	Single well push-pull method with ^{15}N-tracer; piezometers within 0.5m diameter PVC lysimeters of 1.2m long	10mL dosing solution with 30mg/l Br- (as LiBr), 12 mg/L 15N-labelled NO3- as KNO3 (99 atom% 15N)	Enhanced denitrification rate (or denitrification capacity)	1110 (±584) µg N_2O and N_2 L^{-1} d^{-1}	Zaman et al., 2008
Kiwitahi, Waikato	0-0.7	Topehaehae silt loam, Kiwitahi silt loam	Seepage wetland	DEA (Tiedje et al., 1989); acetylene inhibition method		DEA	8.5 mg N_2O-N/kg soil h^{-1}(0-0.1); 5.6 (0-0.2); 2.2 (0.2-0.4); 0.54 (0.4-0.7)	Zaman et al., 2008

C.4 Wetlands/Riparian zones

Location	Depth, m bgl	Soil series/type	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
Whatawhata Research Station, Hamilton, Waikato	0-0.10 and 0.10-0.15; bulked by depth	Waingaro steepland soil (clay loam topsoil)	Riparian wetland (permanently wet swale); vegetated with floating sweetgrass, rush, sedge, and lotus			DEA (Tiedje et al., 1981)	4.1 (±0.3 SD) mg N kg^{-1} soil h^{-1}; 5.7 (±1.8 SD) mg N kg^{-1} soil h^{-1} (Burns & Nguyen, 2002)	Rutherford & Nguyen, 2004

Location	Depth, m bgl	Soil series/type	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
Lake Taupo Cathcment Control Scheme (Mangakowiriwiri stream)	0-0.05 (surface soil)	Taupo sandy loam (Typic udivitrand)	Riparian zone; native (shrubs and ferns), grazed (exotic pasture), set-aside area (native tussock, some remnant pasture)	Acetylene inhibition method	Nitrate added, anaerobic incubation	DEA (same as Cooper, 1990)	0.38 (0.16-1.1) mg N kg^{-1} h^{-1} (native); 0.58 (0.30-2.4) (grazed); 0.41 (0.19-1.1) (set-aside)	Cooper et al., 1995
Whangamata, Tairua Forest, Coromandel Peninsula	0-0.30 (mixed; water saturated)	Wharekawa soils (Whangamata volcanic ash overlying older Waihi ash weathered to allophane clay)	Riparian zone (mosaic of organic and mineral soil patches)	Acetylene inhibition method; anaerobic incubation with Ar		Actual denitrification (on site incubation for 1 hr)	520 (±500 SD) ng N g^{-1} h^{-1} (1.12 g N m^{-2} d^{-1})	Schipper et al., 1993
Whangamata, Tairua Forest, Coromandel Peninsula	0-0.30 (mixed; water saturated)	Wharekawa soils (Whangamata volcanic ash overlying older Waihi ash weathered to allophane clay)	Riparian zone (mosaic of organic and mineral soil patches)	Same as measurement of on-site denitrification without acetylene		N_2O production rate (natural)	73 (±100 SD) ng N g^{-1} h^{-1} (0.16 g N m^{-2} d^{-1})	Schipper et al., 1993
Whangamata, Tairua Forest, Coromandel Peninsula	0-0.30 (mixed; water saturated)	Wharekawa soils (Whangamata volcanic ash overlying older Waihi ash weathered to allophane clay)	Riparian zone (mosaic of organic and mineral soil patches)	Acetylene inhibition method (approx. 10% v/v)	0.5mg KNO_3 in 5mL distilled water for 20g wet soil (no carbon source??)	DEA, anaerobic incubation for 75min at 25deg at 200 rev/min	810 (±440 SD) ng N g^{-1} h^{-1} (1.12 g N m^{-2} d^{-1})	Schipper et al., 1993
Kiwitahi, Waikato	0.20-0.35 below the water	Topehaehae silt loam, Kiwitahi silt loam	Riparian seepage wetland	Single well push-pull method with ^{15}N-tracer;	10mL dosing solution with 30mg/l Br- (as	Enhanced denitrification rate (or denitrification	1110 (±584) µg N_2O and N_2 L^{-1} d^{-1}	Zaman et al., 2008

Location	Depth, m bgl	Soil series/type	Land use	Method	Treatments	Measure of Denitrification	Denitrification Rate	Reference
	level (?) – wetland was saturated to the surface			piezometers within 0.5m diameter PVC lysimeters of 1.2m long	LiBr), 12 mg/L ^{15}N-labelled NO3- as KNO3 (99 atom% ^{15}N)	capacity)		
Kiwitahi, Waikato	0-0.7	Topehaehae silt loam, Kiwitahi silt loam	Riparian seepage wetland	DEA (Tiedje et al., 1989); acetylene based		DEA	8.5 mg N_2O-N/kg soil h^{-1}(0-0.1); 5.6 (0-0.2); 2.2 (0.2-0.4); 0.54 (0.4-0.7)	Zaman et al., 2008
Scotsman Valley, Waikato (near Hamilton)	A horizon and sub-soil horizon	Tauwhare soils, Aeric haplaquepts	Riparian zone	Acetylene inhibition method	Nitrate added (Tiedje, 1982), shaken (no carbon source??)	DEA, anaerobic incubation at 25deg for 2 h	0.044 (±0.031 SD) µg N g^{-1} h^{-1} (mineral soil, A horizon); 0.013 (±0.013 SD) µg N g^{-1} h^{-1} (mineral soil, subsoil horizon); 2.61 (±1.66 SD) µg N g^{-1} h^{-1} (organic soil)	Cooper, 1990
Scotsman Valley, Waikato (near Hamilton)	Full depth of organic soil (0.3-0.8m)	Tauwhare soils, Aeric haplaquepts	Riparian zone	Acetylene inhibition method (10% v/v); 10g wet soil (not intact)	Anaerobic incubation, shaken for 2 min before and after incubation	Actual denitrification rate (in situ), anaerobic incubation (He) at 18deg C for 1 h	0-1.35 (±0.42 SD) µg N g^{-1} h^{-1} (organic soil)	Cooper, 1990

Appendix D. Supplementary Results

D.1 Well and groundwater quality data for the groundwater survey conducted in 2014 in the Tararua Groundwater Management Zone of Manawatū River catchment, New Zealand.

Well ID	Well depth	Depth to water	Temp	Bar. Pressure	DO	Sp. Cond.	pH	ORP (Eh)	NH_4^+-N	HCO_3^-	Bromide	Chloride	Boron	Calcium
Unit	m	m	°C	mbar	mg L^{-1}	µS cm^{-1}		mV	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
1			11.9	978.1	1.96	316.7	6.54	305.4	0.142	86.5	0.294	53.39	0.065	10.32
2	23.0		14.2	981.7	2.85	108.4	5.03	425.7	0.007	12.2	0.108	16.66	0.010	3.37
3	3.4	2.94	15.4	981.4	2.14	154.0	5.32	505.8	0.007	17.1	0.094	13.05	0.027	13.55
4	2.6	1.25	16.1	981.9	0.21	241.6	6.56	277.7	0.279	103.6	0.166	11.27	0.013	31.99
5	6.3	3.24	14.4	982.4	4.24	130.7	5.10	532.1	0.013	8.5	0.113	15.43	0.010	7.88
6	6.3	4.63	16.0	984.9	4.71	71.2	6.02	425.9	0.007	14.6	0.046	8.80	0.009	4.00
7	6.3	3.75	15.7	992.7	5.07	73.5	5.76	366.2	0.007	17.1	0.050	8.73	0.011	4.45
8	4.7	2.90	15.6	986.4	3.55	77.2	5.80	371.1	0.021	19.5	0.052	8.98	0.012	4.67
9	6.6	4.52	14.5	985.4	7.69	124.5	5.25	400.6	0.007	9.8	0.081	11.21	0.014	8.22
10	7.1	6.12	14.9	998.3	7.20	92.2	5.08	448.7	0.010	6.1	0.093	12.33	0.013	1.91
11	5.8	5.45	14.8	1000.8	5.70	147.6	5.96	256.7	0.007	28.1	0.096	13.04	0.015	10.30
12	14.4		16.0	1000.0	8.54	135.6	5.34	469.1	0.007	11.0	0.103	13.82	0.014	6.71
13	4.6	3.65	15.2	987.1	8.35	147.9	5.35	515.6	0.007	9.8	0.102	14.15	0.013	9.64
14	11.6	5.65	13.9	989.2	5.28	95.1	5.46	361.7	0.007	17.1	0.059	9.90	0.013	4.82
15	5.0	2.50	14.9	988.7	8.50	146.9	5.31	554.3	0.007	9.8	0.099	14.14	0.013	9.62
16	5.0	1.43	16.2	1005.3	0.62	93.0	6.03	236.7	0.093	14.6	0.054	7.11	0.006	3.02
17	36.0	23.28	13.5	991.3	5.43	122.4	5.38	369.1	0.007	25.6	0.085	16.05	0.012	2.90
18	6.0	3.28	14.8	991.3	0.15	269.3	5.70	368.0	0.134	36.6	0.117	46.42	0.033	13.91
19	10.3	5.96	13.9	1008.8	4.26	224.3	6.01	344.5	0.007	87.8	0.098	14.60	0.011	32.08
20	4.5	3.05	17.6	998.2	4.59	99.9	5.81	360.7	0.007	30.5	0.057	10.16	0.013	7.34
21	8.8	1.65	16.2	994.0	5.38	207.4	6.02	356.7	0.007	59.8	0.077	14.42	0.004	18.40

Well ID	Well depth	Depth to water	Temp	Bar. Pressure	DO	Sp. Cond.	pH	ORP (Eh)	NH_4^+-N	HCO_3^-	Bromide	Chloride	Boron	Calcium
Unit	m	m	°C	mbar	mg L^{-1}	µS cm^{-1}		mV	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
22	8.1	5.54	14.1	1011.7	0.25	114.6	6.20	198.9	0.209	123.2	0.360	77.99	0.048	36.40
23	7.1	3.12	17.4	1013.6	2.51	171.4	5.53	342.7	0.007	32.9	0.109	16.80	0.019	10.69
24	6.4	4.25	16.0	1014.9	5.21	114.6	5.97	350.8	0.007	34.2	0.068	10.96	0.013	9.65
25	4.9	2.21	15.8	1011.8	0.21	369.2	6.20	156.1	0.168	86.6	0.142	47.05	0.050	24.10
26	10.6	7.53	14.1	1005.9	0.57	326.6	5.99	297.9	0.027	86.6	0.143	37.05	0.037	18.10
27	4.4	2.04	15.6	1010.6	2.17	175.3	5.81	352.8	0.012	42.7	0.090	18.01	0.013	10.37
28	135.0		12.9	1006.8	5.74	141.1	6.17	348.9	0.007	48.8	0.068	12.84	0.009	7.21
29	10.5	6.05	14.5	1011.2	0.41	303.3	5.90	353.3	0.024	70.7	0.252	49.58	0.036	21.00
30	5.4	3.70	14.3	1001.3	0.40	292.2	6.53	157.9	0.036	120.7	0.090	20.99	0.077	21.89
31	9.9	5.15	19.1	1003.0	0.57	202.1	5.92	262.7	0.030	81.7	0.080	12.41	0.028	16.72
32	5.4	4.33	14.5	1002.7	0.66	226.6	5.94	175.7	0.007	67.1	0.100	22.54	0.162	16.47
33	27.0		15.6	1004.2	0.37	523.6	6.75	27.4	0.115	201.1	0.207	62.78	0.482	45.99
34	4.7	3.43	17.9	999.4	7.41	362.6	5.90	465.6	0.007	34.2	0.217	57.86	0.059	19.60
35	12.0		15.5	1002.8	0.21	329.6	6.64	155.0	0.929	146.3	0.110	24.27	0.061	16.13
36	12.0	6.42	13.9	1002.1	3.24	322.1	5.83	333.5	0.007	54.9	0.114	28.60	0.031	23.30
37	28.8		14.3	1001.8	0.18	352.8	6.61	137.7	0.256	136.5	0.138	25.43	0.057	23.70
38	8.0		14.2	1001.9	0.20	313.7	6.26	210.3	0.076	150.0	0.140	18.418	0.035	22.30
39	17.0		15.4	1003.3	4.96	186.7	5.92	377.9	0.007	45.1	0.092	20.19	0.025	12.10
40	121.1		13.9	999.1	0.18	929.0	8.35	97.8	2.300	462.6	0.308	74.95	1.450	7.05
41	5.9	4.42	16.3	1007.1	1.30	435.6	6.06	391.2	0.007	93.9	0.114	21.56	0.017	17.40
42	9.0	3.91	14.9	1004.3	0.33	244.8	6.16	114.8	0.158	76.8	0.144	25.52	0.032	11.10
43	45.0		12.7	992.9	0.07	295.8	7.04	125.0	1.440	160.7	0.050	10.34	0.018	9.46
44	91.5		21.2	987.9	0.12	642.0	6.94	204.8	0.627	306.9	0.144	46.65	0.065	0.26
45	34.0		13.8	1002.3	1.49	115.9	7.36	246.0	0.007	235.6	0.103	24.80	0.018	85.06
46	15.0		11.8	969.1	9.05	70.0	6.18	440.6	0.007	20.7	0.026	7.07	0.004	4.99
47	6.0		13.8	993.1	0.21	114.1	7.92	28.0	3.968	255.7	0.073	17.18	0.122	3.01
48	14.0		10.9	982.8	8.82	73.5	6.31	418.4	0.007	25.6	0.030	7.11	0.004	5.54

Well ID	Well depth	Depth to water	Temp	Bar. Pressure	DO	Sp. Cond.	pH	ORP (Eh)	NH_4^+-N	HCO_3^-	Bromide	Chloride	Boron	Calcium
Unit	m	m	°C	mbar	mg L^{-1}	µS cm^{-1}		mV	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
49	3.3	1.63	14.4	987.0	8.65	68.4	6.65	333.0	0.007	24.4	0.019	5.69	0.016	5.99
50	6.4	1.59	15.3	987.5	6.49	63.9	6.05	351.6	0.015	19.5	0.022	5.69	0.017	4.99
51	54.0		19.0	974.0	0.43	1308.0	7.45	292.2	7.374	589.7	0.128	35.66	0.475	76.24
52	10.0		14.6	986.5	0.13	208.9	6.59	113.7	0.288	95.1	0.104	9.20	0.011	15.10
53	21.0		12.7	985.7	0.57	155.7	6.31	185.1	0.044	46.3	0.056	10.48	0.009	8.81
54	27.0		14.5	973.7	0.22	333.7	6.44	115.3	0.046	154.9	0.097	18.98	0.024	22.74
55	35.0		13.6	964.2	0.24	274.2	6.95	38.5	0.253	124.2	0.069	15.61	0.019	21.44
56	33.0	10.47	12.7	968.6	0.23	189.5	6.57	90.9	0.076	76.8	0.100	16.71	0.013	11.61

Note: Values below the detection limit have been adjusted to DL*(1/√2).

D.1 Well and groundwater quality data for the groundwater survey conducted in 2014 in the Tararua Groundwater Management Zone of Manawatū River catchment, New Zealand (cont'n.).

Well ID	DIC	Iron	Magnesium	Manganese	Potassium	Silica	Sodium	DOC	NO_3-N	NO_2-N	Sulphate	Total_Alk
Unit	mg L-^{1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
1	19.80	0.251	3.01	0.224	1.38	24.08	48.55	0.30	0.348	0.009	2.96	71
2	11.04	0.030	2.67	0.014	0.95	16.84	10.57	0.30	1.006	0.001	8.92	10
3	14.87	0.004	1.98	0.043	3.89	8.22	8.74	1.00	5.032	0.004	15.68	14
4	24.88	0.247	2.91	0.289	6.07	9.07	7.24	2.01	0.271	0.014	13.28	85
5	2.06	0.005	2.06	0.024	2.14	9.56	8.20	0.36	4.129	0.001	9.42	7
6	2.87	0.004	1.16	0.004	1.55	6.46	5.55	0.65	0.455	0.011	4.38	12
7	4.844	0.004	1.20	0.004	1.00	7.84	5.84	0.30	0.595	0.001	4.82	14
8	3.53	0.004	1.29	0.004	1.19	7.08	5.50	0.62	0.332	0.001	4.81	16
9	6.77	0.004	2.56	0.004	2.11	10.27	7.64	0.30	3.860	0.001	9.06	8
10	7.21	0.004	1.75	0.007	1.21	15.70	9.37	0.30	3.351	0.001	3.18	5
11	9.12	0.009	2.06	0.008	3.04	11.29	8.12	0.40	3.797	0.007	9.98	23
12	7.03	0.037	2.59	0.011	2.93	10.84	8.81	0.30	5.037	0.003	10.29	9

Well ID	DIC	Iron	Magnesium	Manganese	Potassium	Silica	Sodium	DOC	NO_3-N	NO_2-N	Sulphate	Total_Alk
Unit	mg L-$^{-1}$	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
13	7.09	0.004	3.29	0.004	1.37	11.24	8.59	0.30	5.426	0.001	10.15	8
14	3.09	0.004	1.95	0.004	1.65	10.17	6.83	0.35	1.605	0.001	8.67	14
15	5.25	0.004	3.05	0.004	1.88	11.80	8.87	0.30	5.318	0.001	10.16	8
16	11.21	6.190	0.84	0.284	0.46	11.40	6.45	0.30	0.072	0.006	5.08	12
17	11.65	0.016	1.60	0.016	5.41	81.28	14.58	0.80	0.599	0.001	7.52	21
18	6.31	0.289	5.00	1.007	3.93	12.31	21.39	8.75	0.424	0.002	19.77	30
19	24.32	0.004	2.95	0.004	1.87	13.20	14.20	0.11	2.158	0.001	10.92	72
20	6.64	0.004	1.70	0.004	1.45	7.94	7.55	0.90	0.529	0.001	5.88	25
21	10.79	0.004	4.35	0.004	2.02	13.39	11.16	0.67	2.898	0.001	17.51	49
22	30.18	2.056	10.10	0.843	3.10	39.50	37.50	1.19	0.007	0.001	18.62	101
23	16.32	0.004	3.28	0.004	3.59	16.90	13.10	0.80	3.138	0.003	14.08	27
24	9.60	0.004	2.04	0.004	1.65	9.09	8.72	0.59	0.489	0.001	6.74	28
25	25.60	0.800	8.45	0.417	3.16	31.70	30.90	0.64	0.003	0.001	30.81	71
26	24.14	0.132	7.64	0.095	2.60	26.60	31.77	0.80	0.027	0.001	27.27	71
27	15.03	0.004	3.94	0.004	4.33	26.13	14.28	0.30	2.962	0.003	9.95	35
28	13.64	0.013	1.82	0.004	0.85	37.39	17.87	0.30	0.868	0.003	6.32	40
29	19.70	0.036	5.32	0.004	3.19	22.76	20.33	1.07	0.130	0.001	11.15	58
30	28.74	0.358	5.78	0.940	2.89	32.50	23.70	1.80	1.080	0.016	6.92	99
31	26.67	1.059	3.95	0.243	2.49	27.66	13.40	1.00	0.052	0.001	11.07	67
32	23.18	1.856	4.34	0.108	4.21	25.87	17.00	1.60	0.055	0.002	16.34	55
33	41.37	0.030	6.75	0.066	1.80	29.81	49.90	1.10	0.001	0.001	2.28	165
34	10.02	0.010	6.85	0.004	3.30	21.25	32.10	1.80	4.652	0.001	31.04	28
35	29.96	0.194	5.77	0.362	1.71	36.80	35.40	1.30	0.002	0.001	0.00	120
36	16.89	0.008	10.10	0.024	2.28	19.60	19.20	1.00	11.620	0.003	20.69	45
37	29.73	0.216	9.21	0.480	2.47	32.70	28.50	1.30	0.003	0.001	20.05	112
38	35.67	0.076	11.50	1.346	1.82	31.20	23.20	1.50	0.007	0.001	9.90	123
39	13.31	0.004	5.02	0.004	3.02	16.30	13.70	1.50	1.013	0.001	15.41	37
40	91.55	0.037	4.63	0.004	3.55	11.80	205.36	3.42	0.001	0.001	0.03	396

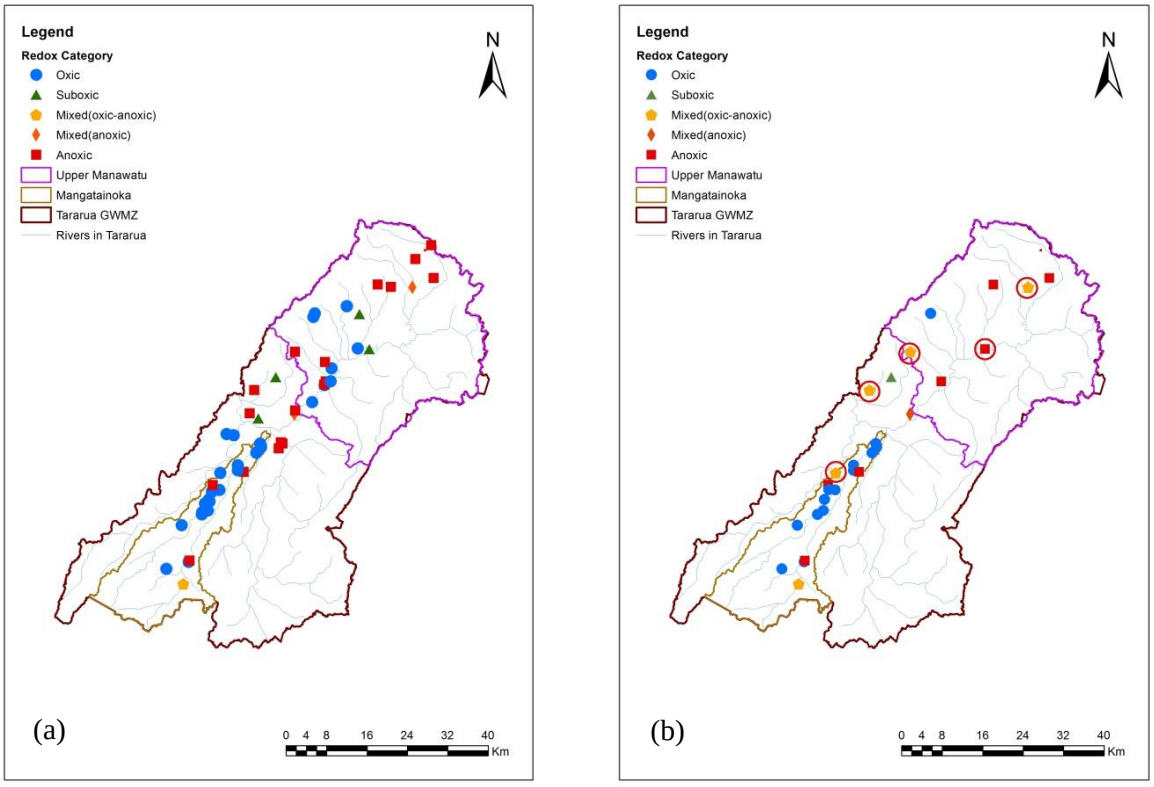
Well ID	DIC	Iron	Magnesium	Manganese	Potassium	Silica	Sodium	DOC	NO_3-N	NO_2-N	Sulphate	Total_Alk
Unit	mg L-$^{-1}$	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
41	26.59	0.004	7.55	0.004	13.8	19.20	47.10	1.32	15.827	0.002	39.04	77
42	23.37	5.530	4.94	0.561	1.71	35.30	23.80	1.07	0.003	0.001	6.59	63
43	31.18	0.039	5.72	0.362	3.95	57.10	39.50	1.53	0.001	0.005	0.00	132
44	63.94	0.034	0.03	0.004	5.58	65.09	146.21	0.97	0.006	0.007	6.95	252
45	46.00	0.005	6.16	0.022	2.13	21.79	18.50	1.14	5.100	0.025	32.65	194
46	5.168	0.005	1.26	0.004	0.83	10.09	5.15	0.22	0.156	0.006	4.34	17
47	48.67	0.077	1.38	0.046	5.35	62.30	94.37	2.21	0.001	0.006	4.32	213
48	5.35	0.004	1.27	0.004	0.70	9.09	5.77	0.31	0.193	0.001	4.68	21
49	5.03	0.004	1.31	0.004	0.67	10.08	5.02	0.30	0.035	0.006	4.69	20
50	5.34	0.004	1.23	0.004	0.79	10.55	4.85	0.30	0.100	0.009	4.28	16
51	120.3	0.009	35.27	0.083	21.64	28.68	135.12	2.20	1.026	0.284	160.88	486
52	21.75	1.140	6.54	0.693	2.88	66.10	10.96	0.53	0.001	0.005	5.48	78
53	11.53	2.180	4.67	0.257	2.06	57.40	10.80	0.40	0.001	0.004	15.21	38
54	35.18	0.157	14.95	0.982	1.89	35.92	20.81	0.55	0.007	0.006	15.28	127
55	25.44	0.052	11.59	0.662	1.97	45.71	14.67	0.30	0.002	0.002	14.50	102
56	17.40	0.064	7.63	0.593	1.08	44.36	10.92	0.14	0.001	0.005	2.24	63

Note: Values below the detection limit have been adjusted to DL*(1/√2).

D.2 Pearson correlation coefficients (*r*) between selected groundwater quality parameters measured in the Tararua GWMZ during February-March, 2014.

	SPC	pH	DIC	Well Depth	WL	DO	NH_4^+-N	HCO_3^-	Br^-	Cl^-	B^-	Ca^{2+}	Fe^{2+}	Mg^+	Mn^{2+}	K^+	SiO_2	Na^+	DOC	NO_3^--N	NO_2^--N	SO_4^{2-}	Alk (T)
pH	.557**																						
DIC	.886**	.752**																					
Well Depth	.425**	.407**	.504**																				
WL	.098	-.222	.155	.799**																			
DO	-.472**	-.567**	-.583**	-.223	-.027																		
NH_4^+-N	.628**	.746**	.734**	.289*	-.177	-.766**																	
HCO_3^-	.685**	.846**	.823**	.408**	.105	-.766**	.728**																
Br^-	.493**	.136	.434**	.025	.298	-.482**	.355**	.440**															
Cl^-	.610**	.299*	.533**	.150	.299	-.498**	.412**	.567**	.874**														
B^-	.754**	.562**	.761**	.218	.179	-.535**	.609**	.658**	.591**	.736**													
Ca^{2+}	.286*	.205	.268*	-.076	-.057	-.318*	.112	.431**	.384**	.356**	.285*												
Fe^{2+}	.126	.267*	.204	-.020	-.014	-.716**	.527**	.392**	.353**	.312*	.287*	.175											
Mg^+	.326*	.163	.265*	.009	.238	-.357**	.208	.367**	.380**	.358**	.310*	.860**	.249										
Mn^{2+}	.174	.303*	.253	-.021	.047	-.771**	.586**	.464**	.308*	.268*	.271*	.407**	.818**	.499**									
K^+	.595**	.245	.569**	.136	.223	-.414**	.381**	.500**	.489**	.482**	.491**	.298*	.075	.290*	.140								
SiO_2	.291*	.422**	.415**	**.397****	**.527****	**-.625****	.533**	.620**	.321*	.393**	.327*	.111	.542**	.265*	.562**	.348**							
Na^+	.790**	.646**	.825**	.382**	.316	-.622**	.709**	.807**	.644**	.797**	.809**	.177	.302*	.241	.284*	.605**	.590**						
DOC	.478**	.388**	.488**	.121	-.041	-.539**	.472**	.539**	.427**	.540**	.621**	.289*	.290*	.279*	.320*	.619**	.216	.588**					
NO_3^--N	-.159	-.555**	-.296*	-.258	.011	.618**	-.644**	-.497**	-.005	-.119	-.304*	.057	-.626**	-.032	-.557**	.150	-.490**	-.282*	-.161				
NO_2^--N	.371**	.465**	.481**	.094	-.317	-.189	.335*	.343**	-.127	-.073	.164	.150	.029	.044	.170	.283*	.111	.178	.112	.002			
SO_4^{2-}	.233	-.173	.158	-.084	-.030	.007	-.172	.063	.186	.211	.021	.425**	-.030	.333*	.027	.439**	-.002	.051	.137	.379**	.261		
Alk(T)	.686**	.847**	.825**	.409**	.104	-.765**	.729**	1.000**	.440**	.567**	.660**	.430**	.391**	.367**	.462**	.500**	.619**	.808**	.540**	-.497**	.343**	.061	
Temp	.348**	-.067	.269*	-.114	-.437**	-.087	.089	.093	.160	.151	.221	-.099	-.057	-.221	-.165	.387**	-.082	.189	.288*	.151	.132	.313*	.092

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed); Data used have been transformed to conform with normality requirement. SPC – specific conductance or electrical conductivity; Depth – depth of well below ground level; WL – depth to water table below ground level; DIC – dissolved inorganic carbon; DOC – dissolved organic carbon



D.3 Comparison of redox categories in 28 samples collected in the Tararua GWMZ at two sampling times, (a) Summer 2013/2014, and (b) Summer 2014/2015. Encircled wells indicate samples with different redox category in the second sampling.

D.4 Redox sensitive species in groundwater with respect to different land use types in the Tararua GWMZ during February-March, 2014.

Parameter	Unit	Dairy (n=28)				Sheep and/or Beef (n=28)				Sig.
		Min	Max	Mean	SE Mean	Min	Max	Mean	SE Mean	
DO	mg L^{-1}	0.07	8.35	2.78	0.51	0.12	9.05	3.30	0.64	0.388
ORP (Eh)	mV	28.00	532.10	287.30	25.71	27.4	554.30	303.57	25.96	0.912
NH_4-N	mg L^{-1}	0.007	3.968	0.320	0.165	0.007	7.374	0.371	0.262	0.827
HCO_3^-	mg L^{-1}	6.10	462.65	88.02	18.52	9.76	589.67	89.06	22.70	0.769
Fe^{2+}	mg L^{-1}	0.0035	5.5300	0.3316	0.2013	0.0035	6.1900	0.5012	0.2416	0.617
Mn^{2+}	mg L^{-1}	0.0035	1.3460	0.1774	0.22	0.0035	1.0070	0.2225	0.0636	0.611
DOC	mg L^{-1}	0.11	3.42	0.85	0.14	0.22	8.75	1.14	0.30	0.645
NO_3^--N	**mg L^{-1}**	**0.001**	**15.827**	**2.245**	**0.705**	**0.001**	**5.318**	**1.177**	**0.336**	**0.075**
NO_2^--N	mg L^{-1}	0.0014	0.0252	0.0036	0.0009	0.0014	0.2838	0.0142	0.0100	0.200
SO_4^{2-}	mg L^{-1}	0.004	39.039	11.387	1.904	0.004	160.885	16.194	5.511	0.993

Note: t-test was performed using the transformed values of the parameters. Sig. – significance level.

D.5 Coefficient of variation (CV) of denitrification enzyme activity (DEA) values for the Manawatu sandy loam soil (sampled in November 2014) at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand.

Soil depth	Incubation technique	Substrate (N and C concentrations) treatments								
		50 µg N g^{-1}			75 µg N g^{-1}			100 µg N g^{-1}		
		200 µg C g^{-1}	300 µg C g^{-1}	400 µg C g^{-1}	200 µg C g^{-1}	300 µg C g^{-1}	400 µg C g^{-1}	200 µg C g^{-1}	300 µg C g^{-1}	400 µg C g^{-1}
		1	2	3	4	5	6	7	8	9
0-0.3 m	Vacuum Pouch	**2.9**	**2.1**	4.5	**1.7**	**1.9**	**5.4**	7.6	**5.2**	**3.8**
	Flask	**10.7**	**10.5**	1.9	**5.7**	**7.2**	**5.7**	7.4	**5.9**	**4.6**
0.3-0.6 m	Vacuum Pouch	**8.2**	**86.1**	**10.3**	**25.2**	**49.3**	**16.9**	**30.5**	**4.5**	**48.7**
	Flask	**46.2**	**104.9**	**127.2**	**127.4**	**80.7**	**116.1**	**43.7**	**16.2**	**135.8**
0.6-1.2 m	Vacuum Pouch	-	30.1	50.2	-	53.9	114.1	**36.1**	34.3	**0**
	Flask	13.8	21.2	33.9	21.3	8.2	54.7	**64.5**	20.0	**43.1**
1.2-2.1 m	Vacuum Pouch	35.4	60.9	34.6	**20.8**	**29.2**	**20.8**	**46.3**	74.6	35.9
	Flask	24.4	18.5	15.9	**54.5**	**60.2**	**32.9**	**57.0**	24.0	10.5

Note: Bold numbers indicate the pairs in which the CV of DEA with flask incubation is higher than the CV with pouch incubation. CV of DEA with flasks incubations were greater than the CV with vacuum pouch incubations in 22 pairs, compared to 12 pairs where CV with the pouch incubations were greater.

D.6 Coefficient of variation (CV) of denitrification enzyme activity (DEA) values for the Rangitikei silt loam soil (sampled in June 2013) at the Massey No. 1 Dairy Farm, Palmerston North, New Zealand.

Depth	Incubation technique	Treatments		
		50 µg N, 300 µg C	75 µg N, 300 µg C	100 µg N, 300 µg C
		2	5	8
0-0.3 m	Vacuum Pouch	**12.4**	**10.9**	**2.2**
	Flask	**26.7**	**40.1**	**30.8**
1.0-2.0 m	Vacuum Pouch	31.1	28.9	49.7
	Flask	21.1	19.9	10.5

Note: Bold numbers indicate the pairs in which the CV of DEA with flask incubation is higher than the CV with pouch incubation.

D.7 Effect of the use of formaldehyde (0.5%) on N_2O-N concentrations in groundwater samples collected during the push-pull test conducted in (a) October 2013 and (b) May 2014 at Massey No. 1 Dairy Farm, Palmerston North.

(a)

Time elapsed, min	**N_2O-N concentration (mg L^{-1})**	
	Without formaldehyde	With formaldehyde
0	0.00803	0.01282
15	0.00312	0.00927
30	0.01011	0.00610
60	0.01813	0.00966
60	0.01458	0.01709
120	0.01791	0.01631
180	0.01854	0.02273
225	0.02388	0.02363

(b)

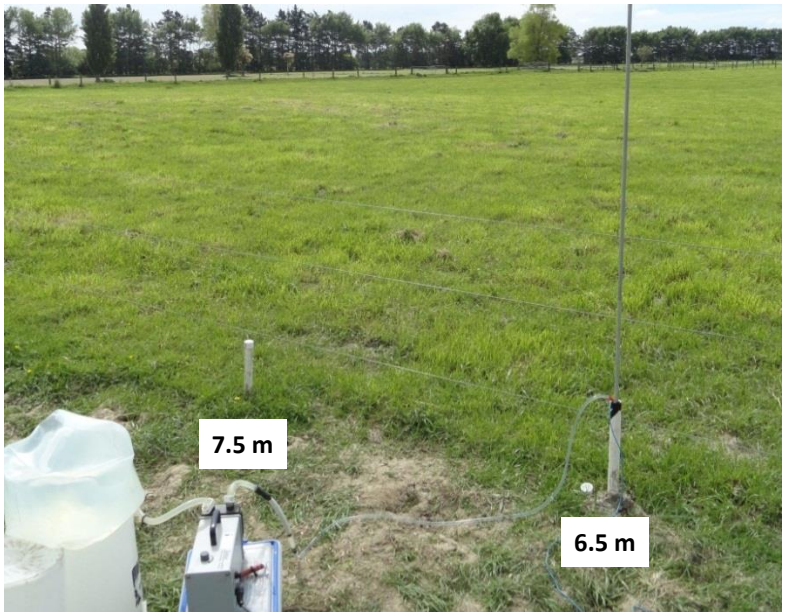
Time elapsed, min	**N_2O-N concentration (mg L^{-1})**	
	Without formaldehyde	With formaldehyde
0	0.00040	0.00033
15	0.00240	0.00228
30	0.00601	0.00535
60	0.01187	0.01094
60	0.01816	0.01762
120	0.02354	0.02370
180	0.03624	0.03466
240	0.04847	0.04726
300	0.06020	0.05949
360	0.07138	0.07109
420	0.07513	0.07968

It is clear that there is no significant difference in N_2O concentrations whether formaldehyde is used or not in groundwater samples to limit further microbial activity in the collected samples during storage. Results of paired *t*-test for the two tests conducted in October 2013 and May 2014 show *p* values of 0.821 and 0.801, respectively. This indicates that as long as gas samples are extracted within 24 hours of the completion of the push-pull test from the groundwater samples, there is no significant difference in the nitrous oxide concentration in groundwater.

Appendix E. Characteristics of the Study Sites

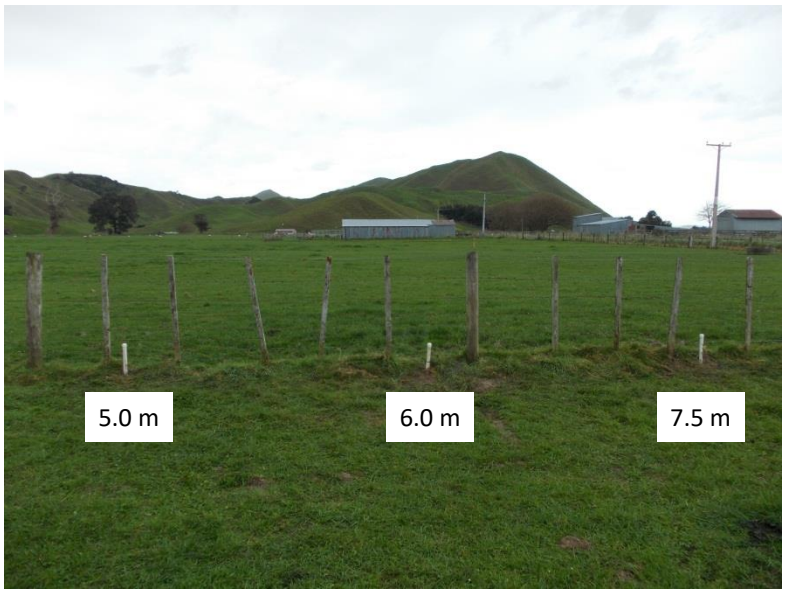
E.1 Piezometers installed at the four study sites in the Manawatū River Catchment

Palmerston North Site

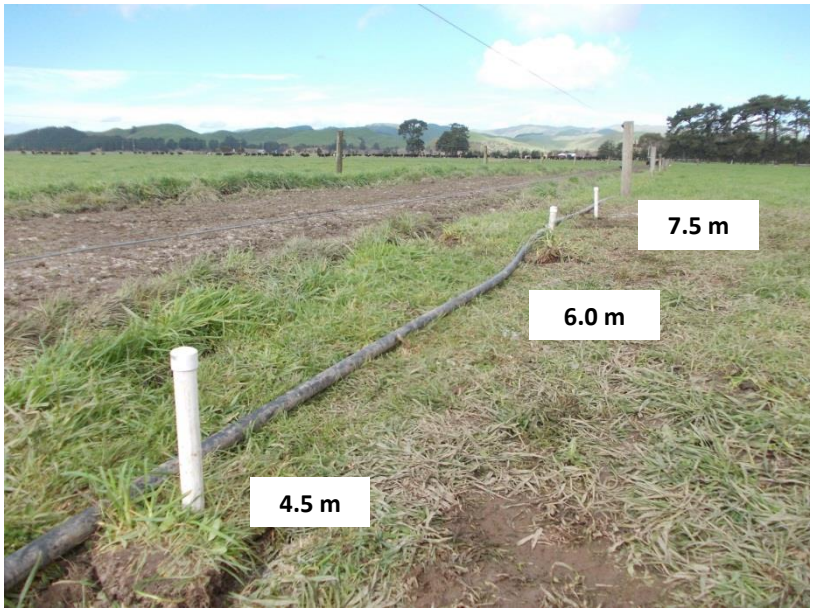


Pahiatua Site

Woodville Site



Dannevirke Site

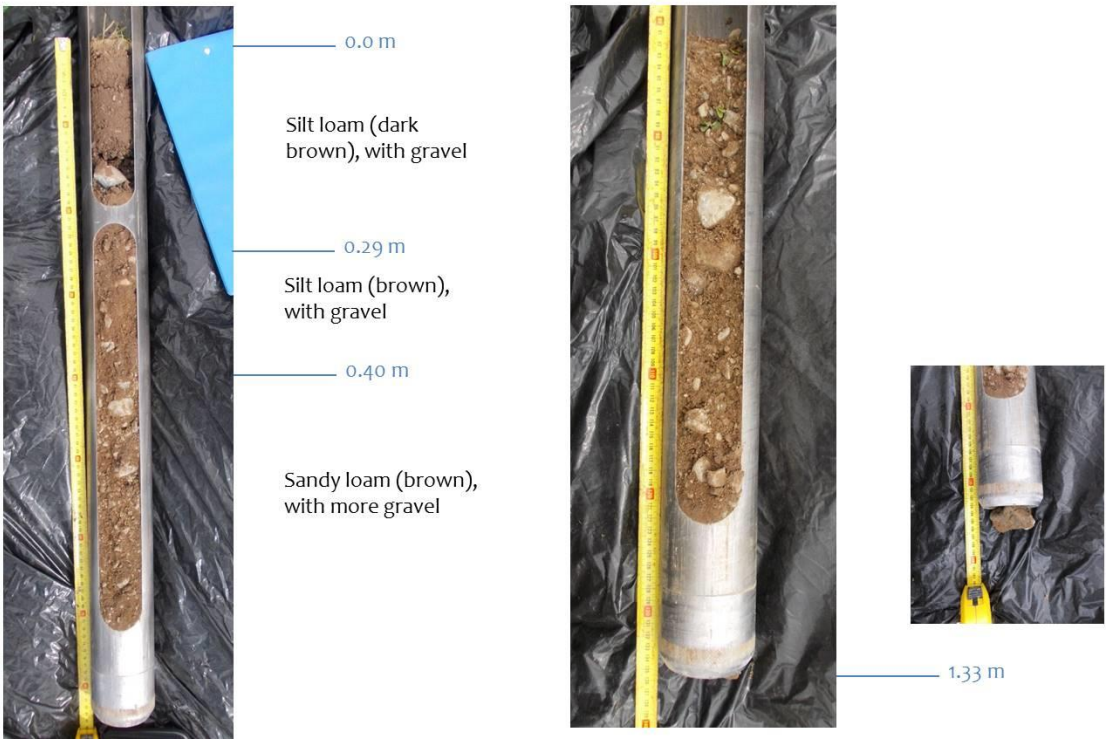


E.2 Characteristics of the soil profile at the four study sites in the Manawatū River Catchment

1. Pahiatua site

2. Woodville site

3. Dannevirke site



4. Palmerston North site

Soil texture class based on investigation by hand following Milne et al. (1995). *Soil Description Handbook (Revised Ed.)*. Lincoln, Canterbury: Manaaki Whenua Press.

Appendix F. Statement of Contribution to Doctoral Thesis Containing Publications

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: ALDRIN A. RIVAS

Name/Title of Principal Supervisor: DR. RANVIR SINGH

Name of Published Research Output and full reference:

Rivas, A., Singh, R., Horne, D., Roygard, J., Matthews, A., Hedley, M.J., 2017. Denitrification potential in the subsurface environment in the Manawatu River catchment, New Zealand: Indications from oxidation-reduction conditions, hydrogeological factors, and implications for nutrient management. Journal of Environmental Management 197, 476–489. doi:10.1016/j.jenvman.2017.04.015

In which Chapter is the Published Work: CHAPTER 3

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: 80%

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  The Candidate contributed in the design of the study; planned all the activities; led the survey to assess suitability of wells for sampling; led the collection of groundwater samples; checked and analysed the data; interpreted the results with inputs from co-authors; wrote the manuscript; revised and finalised the manuscript based on inputs/comments from co-authors and journal referees

Aldrin A. Rivas — Candidate's Signature

15 Feb 2018 — Date

Ranvir Singh — Principal Supervisor's signature

16 Feb 2018 — Date