Investigating Nitrate Attenuation Capacity and Processes in Pastoral Hill Country Landscapes

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This thesis is dedicated to my husband Ebele, and my son Gabriel. Thanks for your love and encouragement!

ABSTRACT

The presence of agricultural nutrients, particularly nitrate, in ground and surface waters is an issue of increasing concern for the degradation of water quality in New Zealand. Thus, several studies have focused on the management of pastoral agricultural systems to limit the leaching and availability of nitrate in receiving waters. Such studies, however, are rare for pastoral hill country landscapes which occupy more than 60% of New Zealand agricultural area and hence have the potential to impact on water quality. Therefore, this thesis aims to assist in filling this knowledge gap by investigating the influence of hill country landscape features on nitrate attenuation in pastoral hill country.

Denitrification has, for decades, been identified as an important nitrate attenuation process in soil-water systems. Its effectiveness below the topsoil is, however, limited by the supply of dissolved organic carbon (DOC). Pastoral hill country landscape features such as soil type, topography, wet areas, land use, and climate have the capacity to impact on the availability of DOC for denitrification in both the topsoil and subsoil. This study examined the contribution of these landscape features on the dynamics of DOC, and the effect on denitrification in the soil profile (100 cm).

The Massey University's Agricultural Experiment Station (Tuapaka farm) was the case study farm used in this thesis. In order to investigate the effect of soil type and slope on DOC concentration and denitrification capacity, soil samples were collected (from three depths down to 100 cm) from the lowest to the highest elevation (50-360 m) in the farm. The sampled locations comprised of three slope classes (low, medium and high) and eight soil types (Tokomaru, Ohakea, Shannon, Tuapaka, Halcombe, Korokoro, Ramiha and Makara), grouped into three drainage classes (poorly-, imperfectly-, and well-drained). The results of the study indicated that compared to slope, soil type had a greater effect on denitrification capacity within the farm. This effect of soil type was mainly associated with soil parent material, as the Ramiha soil which had a higher carbon (C) storage capacity (due to its high content of short-range order constituents), also had the highest amount of DOC (105 mg kg^{-1}, within the 30-60 cm soil depth) and thus the highest denitrification capacity (10 µg kg^{-1} h^{-1}). The findings of this experiment

imply that farms or catchments with soil types similar to the Ramiha soil may have a greater capacity to attenuate nitrate losses to receiving waters.

The contribution of hill country wet areas (seepage wetland and hillside seeps) to nitrate attenuation was assessed by first comparing the DOC concentration of the wet areas to that of an adjacent dry area. This showed that mean DOC concentration of the surface 30 cm soil depth was in the following order: seepage wetland (498 mg kg^{-1}) > hillside seep (172 mg kg^{-1}) > dry area (109 mg kg^{-1}). A subsequent more detailed examination of the seepage wetland and dry area showed that the denitrification capacity of the seepage wetland within the 0-30 and 30-60 cm soil depths was 7 and 69 times higher, respectively, than that of the dry area. The higher DOC concentration and the presence of readily-decomposable DOC in the seepage wetland contributed to its higher denitrification capacity. This contrasting nitrate attenuation capacity of the seepage wetland versus that of the dry area highlights the potential contribution of seepage wetlands to nitrate attenuation for improved water quality in pastoral hill country landscapes.

Land use change from pasture to forage cropping, which is increasingly being adopted in New Zealand hill country, has the potential to influence the dynamics and leaching of DOC for subsurface denitrification. However, there is limited research understanding on the effect of land use change (forage crop establishment) on DOC dynamics and leaching in pastoral hill country. Therefore, a study was designed to investigate soil DOC dynamics and denitrification capacity as influenced by the establishment of a forage crop (swede, *Brassica napobrassica* Mill.) via the surface sowing technique (no cultivation). This experiment was carried out in two stages. The first stage monitored the short-term changes in DOC concentration and chemistry immediately after spraying out pasture with selected agrochemicals (active ingredients: glyphosate, dicamba, diazinon and organomodified polydimethyl siloxane). The results showed that the agrochemicals increased DOC concentration only within the surface 5 cm soil depth (by ~ 20 mg kg^{-1}) on days 1 and 6 after the agrochemicals were applied. This increase in topsoil DOC concentration was most likely due to a direct contribution of C from the agrochemical, an indirect C contribution through the displacement of adsorbed organic molecules, and the decomposition of root necromass. DOC chemistry was, however, not

altered by the applied agrochemicals. These findings were further confirmed by a follow-up experiment which used $\delta^{13}C$ isotope technique to measure leached DOC from an organic material (plant residue) added to the topsoil. This showed that one week after the application of organic material, only a negligible amount ($\leq$ 5%) of C derived from the organic material was detected in the subsoil (20-60 cm depth) DOC, compared to > 20% detected in the surface 20 cm soil depth, suggesting the limited leaching of exogenous DOC (under the experimental condition studied) due to its rapid turnover in the topsoil.

The second stage of the forage crop establishment experiment monitored temporal changes in DOC concentration and denitrification capacity within a year of forage crop establishment. The results indicated that DOC concentration and denitrification capacity of both topsoil and subsoil layers were generally not affected by the establishment of the forage crop. However, an increase in rainfall and soil moisture, after periods of soil water deficit, increased the DOC concentration of the soil. Forage crop establishment resulted in an initial increase (by > 55%) in the nitrate concentration of the surface 20 cm soil depth, most likely due to poor nitrogen (N) utilisation by the growing brassica forage crop. However, the higher nitrate concentrations were only detected in the topsoil and thus the risk of increased nitrate leaching was assumed to be negligible.

This thesis has highlighted the variations that exist in the DOC concentration and denitrification capacity of the different soils within hill country landscapes and thus suggests that these soils require contrasting management practices for effective water quality outcomes. In addition, the potential contribution of hill country seepage wetlands to nitrate attenuation shown in this thesis suggests that management strategies that preserve and enhance these pastoral hill country landscape features should be promoted to attenuate the losses of nitrate to receiving waters. Furthermore, this thesis has demonstrated that the common land use change from pasture to a forage crop, to supplement animal feed production in New Zealand hill country, is unlikely to have any significant impact on the DOC concentration and denitrification capacity of the soil profile (100 cm), within a one-year period. The observed results suggest that this practice is also not likely to negatively impact on water quality via nitrate leaching. However, larger-scale forage crop trials would be required to validate these findings.

The findings of this thesis suggest that some hill country landscape features have the potential to attenuate nitrate losses to receiving waters. This information is critical for improving hill country N management for better water quality outcomes, which could potentially credit farmers under possible N loss regulations.

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RELEVANT PUBLICATIONS AND PRESENTATIONS

Publications to date

Peer-reviewed journal articles

- **Chibuike, G.**, Burkitt, L., Camps-Arbestain, M., Bishop, P., Bretherton, M. & Singh, R. (2019). Effect of forage crop establishment on dissolved organic carbon dynamics and leaching in a hill country soil. *Soil Use and Management*. https://doi.org/10.1111/sum.12497

- **Chibuike, G.**, Burkitt, L., Bretherton, M., Camps-Arbestain, M., Singh, R., Bishop, P., Hedley, C. & Roudier, P. (2019). Dissolved organic carbon concentration and denitrification capacity of a hill country sub-catchment as affected by soil type and slope. *New Zealand Journal of Agricultural Research* 62(3): 354-368.

Conference/ workshop proceedings

- **Chibuike, G.**, Burkitt, L., Camps-Arbestain, M., Singh, R., Bretherton, M., Bishop, P. & Shen, Q. (2019). Nitrate attenuation capacity of a hill country seepage wetland and adjacent areas as influenced by the concentration and chemistry of dissolved organic carbon. In: *Nutrient Loss Mitigations for Compliance in Agriculture*, Occasional Report No. 32, 7p. (Eds L. D. Currie and C. L. Christensen). Palmerston North, New Zealand: Fertilizer and Lime Research Centre, Massey University.

- **Chibuike, G.**, Burkitt, L., Bretherton, M., Camps-Arbestain, M., Singh, R., Bishop, P., Hedley, C. & Roudier, P. (2018). The effect of soil type and slope on the dissolved organic carbon concentration and denitrification capacity of a hill country sub-catchment. In: *Farm Environmental Planning – Science, Policy and Practice*, Occasional Report No. 31, 13p. (Eds L. D. Currie and C. L. Christensen). Palmerston North, New Zealand: Fertilizer and Lime Research Centre, Massey University.

Presentations

- **Chibuike, G.**, Burkitt, L., Camps-Arbestain, M., Singh, R., Bretherton, M. & Bishop, P. (2019). "Nitrate attenuation capacity of a hill country seepage

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- **Chibuike, G.**, Burkitt, L., Camps-Arbestain, M., Singh, R., Bretherton, M. & Bishop, P. (2018). "Assessment of the nitrate attenuation capacity of a seepage wetland in a hill country landscape." Presented at the New Zealand Society of Soil Science Conference, 3-6 December 2018, Napier, New Zealand.

- **Chibuike, G.**, Burkitt, L., Singh, R., Camps-Arbestain, M., Bretherton, M. & Bishop, P. (2018). "Monitoring the leaching of dissolved organic carbon in a hill country soil." Presented at the School of Agriculture & Environment Symposium, 19 November 2018, Palmerston North, New Zealand.

- **Chibuike, G.**, Burkitt, L., Bretherton, M., Camps-Arbestain, M., Singh, R. & Bishop, P. (2018). "The effect of soil type and slope on the dissolved organic carbon content and denitrification capacity of a hill country sub-catchment." Presented at the 31st Annual Fertiliser and Lime Research Centre (FLRC) Workshop, 7-9 February 2018, Palmerston North, New Zealand.

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- **Chibuike, G.**, Burkitt, L., Singh, R., Camps-Arbestain, M., Bretherton, M. & Bishop, P. (2017). "Seasonal variations in the denitrification capacity of a hill country soil as influenced by land use change." Presented at the Institute of Agriculture & Environment Symposium, 10-12 April 2017, Palmerston North, New Zealand.

- **Chibuike, G.**, Burkitt, L., Camps-Arbestain, M., Bishop, P., Bretherton, M. & Singh, R. (2016). "Short-term effect of land use change on dissolved organic carbon dynamics in pastoral hill country." Presented at the Joint Conference for

the New Zealand Society of Soil Science & Soil Science Australia, 12-16 December 2016, Queenstown, New Zealand.

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$AgSO_4$	Silver sulphate
Al	Aluminium
Al_o	Acid ammonium oxalate-extractable aluminium
Al_p	Sodium pyrophosphate-extractable aluminium
ANOVA	Analysis of variance
C	Carbon
CO_2	Carbon dioxide
DEA	Denitrification enzyme activity
DNA	Deoxyribonucleic acid
DNRA	Dissimilatory nitrate reduction to ammonium
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
E2	Absorbance measured at 250 nm
E3	Absorbance measured at 365 nm
E4	Absorbance measured at 465 nm
E6	Absorbance measured at 665 nm
EC	Electrical conductivity
Fe	Iron
Fe_o	Acid ammonium oxalate-extractable iron
Fe_p	Sodium pyrophosphate-extractable iron
GC	Gas chromatograph
GDP	Gross domestic product
GLM	General linear model
H_2SO_4	Sulphuric acid
$HgSO_4$	Mercuric sulphate
HWEC	Hot water-extractable carbon
IRMS	Isotope ratio mass spectrometer
$K_2Cr_2O_7$	Potassium dichromate
K_2SO_4	Potassium sulphate
KCl	Potassium chloride
$KCr(SO_4)_2.12H_2O$	Chromium potassium sulphate dodecahydrate
KHP	Potassium hydrogen phthalate
Ksat	Saturated hydraulic conductivity
MBC	Microbial biomass carbon
Mn	Manganese
MP-AES	Microwave plasma-atomic emission spectrometer
N	Nitrogen
n	Number of observations
N_2	Dinitrogen
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
NO	Nitric oxide
NO_2^-	Nitrite
NO_3	Nitrate
O_2	Oxygen
P	Phosphorus

p, *p*-value	Probability value
r	Pearson correlation coefficient
R^2	Coefficient of determination
RCBD	Randomised Complete Block Design
rpm	Revolutions per minute
S	Sulphur
Si	Silicon
Si_o	Acid ammonium oxalate-extractable silicon
SO_4^{2-}	Sulphate
SOM	Soil organic matter
sUVa	Ratio of absorbance measured at 254 nm and dissolved organic carbon concentration
TDN	Total dissolved nitrogen
TOC	Total organic carbon
USDA	United States Department of Agriculture
UV	Ultraviolet

CHAPTER 1: Introduction

1.1 Background and rationale of the study

Hill country farms, which support sheep and beef cattle production, occupy 66% of New Zealand's agriculture and forestry land (MPI, 2012). Agricultural production on these farms contributes substantially to the country's gross domestic product (GDP) – generating about $7.5 billion annually in export earnings (Morris, 2013). As the Government's Business Growth Agenda aims to significantly increase the value of New Zealand's Primary Industry exports by 2025 (MPI, 2018), increasing agricultural intensification is expected on pastoral hill country landscapes. The lower growth rate of pasture during winter compared to late spring/early summer also creates pressure to increase animal feed production in hill country farms. Therefore, practices such as the replacement of perennial pasture with forage crops, for increased animal feed production, is rapidly being adopted in hill country (Houlbrooke *et al.*, 2009; Fraser *et al.*, 2016; Burkitt *et al.*, 2017). This increase in agricultural production as well as the unique features and complexity of these landscapes (steep slopes, fragile soils that are prone to erosion, and presence of grazing animals) is likely to result in increased losses of nutrients to water bodies via runoff, erosion and leaching, thus significantly affecting water quality (Ledgard and Hughes, 2012; Saggar *et al.*, 2015) and leading to serious environmental consequences such as eutrophication (Vitousek *et al.*, 1997). The presence of farm nutrients (particularly nitrate) in ground and surface waters is an issue of increasing concern for the degradation of water quality in New Zealand (Selvarajah *et al.*, 1994; McLarin *et al.*, 1999; Davies-Colley, 2013). It is, therefore, important to understand the processes that control nitrate attenuation in hill country in order to recommend adequate management options that reduce nitrate leaching and its impact on ground and surface water quality.

Despite the potential for nitrate leaching loss in hill country, there are avenues for nitrate attenuation within hill country which can mitigate nitrate loss to receiving waters. For instance, seepage wetlands, which are common in hill country landscapes, have the potential to increase denitrification and thus reduce the loss of nitrate to ground and surface waters (Zaman *et al.*, 2008). Although denitrification has been identified as an important nitrate attenuation process, its efficiency, especially below the root zone, is limited by the supply of dissolved organic carbon (DOC) which is the main energy

source for denitrifying bacteria (Starr and Gillham, 1993; Barkle *et al.*, 2007; Peterson *et al.*, 2013). The dynamics of DOC within the root zone is influenced by several factors ranging from soil and environmental factors, to land management factors (Kalbitz *et al.*, 2000). These factors indirectly control the leaching and availability of DOC for denitrification below the root zone. For instance, practices such as the change from perennial pasture to forage crops for improved feed production in hill country could significantly impact on the leaching and availability of DOC in subsoils (Ghani *et al.*, 2007).

Currently, integrative studies comparing the influence of several landscape features, particularly topography, soil type and wet areas on the distribution of DOC in hill country are limited in literature. There is also very limited research understanding of how the increasing prevalence of land use changes in pastoral hill country influence DOC availability and the denitrification capacity of hill country soils. Improving research understanding in these areas is critical as it would enable catchment scale assessment and enhancement of the landscape capacity to attenuate nitrate. This would result in the targeted management of nitrate losses to ground and surface waters, and allow farmers to gain environmental credits in a future nitrogen (N) loss regulated system.

1.2 Research hypotheses and objectives

1.2.1 Research hypotheses

1. Different soil types interact with slope classes to influence the DOC concentration and denitrification capacity of hill country at the catchment scale.
2. Naturally occurring wet areas (hillside seeps and seepage wetlands) in hill country landscapes have higher DOC concentration and denitrification capacity than the surrounding drier areas.
3. Land use change from pasture to forage cropping affects DOC availability and the occurrence of denitrification, both within and below the root zone.

1.2.2 Research objectives

This research aims to investigate the influence of hill country landscape features on DOC concentration and the potential for nitrate loss via denitrification. In particular, the study will investigate:

1. The influence of soil type and slope on the DOC concentration and denitrification capacity of pastoral hill country.
2. The denitrification capacity of hill country wet areas and the role of DOC in nitrate attenuation within these landscape features.
3. The effect of the annual establishment of forage crops (land use change) on DOC dynamics in the root zone, its transport below the root zone, and the impact on soil denitrification capacity.

1.3 Thesis outline

This thesis consists of 8 chapters which are briefly described as follows:

Chapter 1 is the introductory chapter which discusses the background and rationale of the research. It states the study hypotheses and objectives, and concludes with an outline of the entire thesis.

Chapter 2 reviews the literature on N balance in pastoral hill country and nitrate attenuation processes. It generally discusses the catchment features that influence denitrification and more specifically, describes what influences denitrification below the root zone. It highlights the research gaps in nitrate attenuation in pastoral hill country and describes the various methods used for measuring DOC and denitrification – the two main parameters investigated in this thesis. Finally, it describes the research farm where all the experimental work was conducted.

Chapters 3-6 address the specific objectives of the research. These chapters were written as stand-alone chapters for publication in peer-reviewed journals. **Chapter 3** examines the effects of soil type and slope on DOC concentration and denitrification capacity of a pastoral hill country farm. **Chapter 4** investigates the DOC and denitrification capacity of hill country wet areas and compares these to that of adjacent drier areas. **Chapter 5** reports on the first phase of a forage crop establishment trial in

pastoral hill country. More specifically, it examines the effect of agrochemicals (used for clearing out pasture before forage crop establishment) on DOC dynamics in a hill country soil. **Chapter 6** presents results of the second (final) phase of the forage crop establishment trial. It reports on the temporal variations in DOC concentration and denitrification capacity of a hill country soil after the replacement of perennial pasture with a forage crop.

Chapter 7 describes a follow-up experiment which was designed based on results obtained in Chapters 5 and 6. It was also written as a stand-alone chapter which investigates the leaching of DOC (after the application of an organic amendment to the topsoil) in order to better understand the origin of DOC found in the subsoil.

Chapter 8 discusses the major findings of Chapters 3-7, and recommends areas for further research on nitrate attenuation in pastoral hill country.

CHAPTER 2: Review of literature

2.1 Nitrogen balance in pastoral hill country

Pastoral hill country is described as an area of land with > 12° slope, located below the tree line, and is grazed by sheep and/or beef cattle (Hoogendoorn *et al.*, 2011a). The contrasting micro-topographic units within hill country (rolling to steep slopes and areas with relatively flat land) lead to spatial variability in the distribution of nitrogen (N) within hill country. However, N accumulation generally occurs at lower slope regions due to surface runoff and animal grazing and resting habits (Bowatte, 2003).

Biological N fixation from pasture legumes has historically been one of the primary sources of N input in New Zealand hill country (Bowatte, 2003; Lambert *et al.*, 2012). However, the use of fertiliser N has increased in recent years. For instance Parliamentary Commissioner for the Environment (2004) reported that the average annual fertiliser N input to hill country farms increased from 0.7 kg N ha^{-1} in 1996 to in 5.7 kg N ha^{-1} in 2002. Inputs from animal urine and dung also contribute substantially to the N balance of hill country soils. For instance, Hoogendoorn *et al.* (2011b) noted that > 80% of pasture N ingested by grazing animals in hill country is returned to pasture in the form of urine and dung. Non-symbiotic N fixation is another form of N input in pastoral hill country. Grant and Lambert (1979) reported that about 21 kg N ha^{-1} yr^{-1} was fixed by non-symbiotic N fixation and atmospheric deposition on a poorly developed hill country pasture dominated by browntop (*Agrostis tenuis* Sibth.) and other low-fertility-tolerant grasses, with legume contents of 1-2%.

Hoogendoorn *et al.* (2011b) summarised the major N translocations within hill country to include: (i) plant uptake; (ii) N returned in litter, and (iii) N ingestion by grazing animals, retention in animal product and excretion in urine and dung. The N transformation processes included mineralisation, immobilisation and nitrification, while the major N output pathways were ammonia (NH_3) volatilisation, denitrification and leaching. Bowatte (2003) noted that about 21-34% of N can be lost from NH_3 volatilisation in hill country, especially from urine patches. Leaching losses of 38-274 kg N ha^{-1} yr^{-1} were measured on a New Zealand high-fertility hill country experimental site receiving N fertiliser at the rate of 300 kg N ha^{-1} yr^{-1} (Parfitt *et al.*, 2009). In the same vein, Saggar *et al.* (2015) estimated nitrous oxide (N_2O) emissions from animal excreta inputs on New Zealand hill country soils and recorded approximately 5 Gg N_2O

yr^{-1} for the year 2012. Very limited research has been undertaken to understand how denitrification influences N losses (especially below the root zone) in pastoral hill country and how this contributes to the improvement of water quality in this landscape.

After consideration of the above N input, translocation, transformation and output processes in a 1 ha hill country paddock comprised of 12% flat land, 46% easy (25°) slope and 42% steep (45°) slope, Bowatte (2003) reported that N outputs were likely to exceed N inputs in pastoral hill country, with pasture utilisation and excretal deposition being the major determinants of the overall N balance in the paddock. Understanding what influences denitrification (a major N output process) in hill country will form a major component of the present study.

2.2 Nitrate attenuation via denitrification

Denitrification is the microbial transformation of nitrate (NO_3^-) to dinitrogen (N_2) via a multi-stage process shown in the following equation (Saggar *et al.*, 2013):

	NO_3^- reductase		NO_2^- reductase		NO reductase		N_2O reductase	
NO_3^-	→	**NO_2^-**	→	**NO**	→	**N_2O**	→	**N_2**
Nitrate		Nitrite		Nitric oxide		Nitrous oxide		Dinitrogen

In order for denitrification to occur in any environment, four basic requirements need to be fulfilled; they include an anoxic environment, the presence of NO_3^- (electron acceptor), the presence of denitrifying bacteria, and suitable electron donors (Stenger *et al.*, 2013). Depending on the electron donor available, denitrification can either be autotrophic, i.e. in the presence of inorganic substrates such as reduced iron (Fe), manganese (Mn) and sulphur (S), or heterotrophic, i.e. in the presence of organic substrates such as DOC. Heterotrophic denitrification has been identified as the most common form of denitrification (Sgouridis *et al.*, 2011).

Denitrification can also take place in the absence of microbes (chemodenitrification). This process involves the abiotic conversion of ammonium (NH_4^+) to NO_2^- or the reaction of NO_2^- with amines and metals to yield gaseous N products (Reddy and

DeLaune, 2008). However, this is a less common form of denitrification which is favoured under acidic conditions (Kumar, 2008; Reddy and DeLaune, 2008). Since microbes are ubiquitous in soils, references made to denitrification in most agricultural studies refer to the biological form of denitrification.

Denitrification may stop at the production of the intermediate gases, i.e. NO or N_2O (incomplete denitrification) rather than the final product, N_2 (complete denitrification) owing to changes in the availability of any of the four basic requirements. Incomplete denitrification is more likely to occur in the topsoil due to the presence of high oxygen (O_2) and NO_3^- concentrations (Brady and Weil, 2002; Rivett *et al.*, 2008). These intermediate by-products of denitrification (especially N_2O) have been linked with stratospheric ozone depletion (Portmann *et al.*, 2012; Wang *et al.*, 2014). In addition, N_2O is a powerful greenhouse gas with a global lifetime of about 114 years and a global warming potential equivalent to 298 times that of carbon dioxide (CO_2), when considering a 100-year timeframe (IPCC, 2007). Thus several studies have focused on understanding the factors that influence denitrification at the root zone, i.e. topsoil (Ruz-Jerez *et al.*, 1994; Luo *et al.*, 1996; 1998; 1999b; 1999a; 2000; Jha *et al.*, 2011; 2012) with the intention of controlling the emission of these gases, especially N_2O.

Conversely, denitrification below the root zone does not readily result in the emission of N_2O because of the more stable reduced conditions in this environment, and also because the limited gas exchange between the subsurface soil layers and the atmosphere restricts the release of any produced N_2O into the atmosphere (Stenger *et al.*, 2013). Indeed, subsurface denitrification (below the root zone) has been reported to be the most important NO_3^- attenuation process that results in significant NO_3^- reduction, which in turn leads to an improvement of water quality (Rivett *et al.*, 2008). Therefore, research focusing on subsurface denitrification has been receiving attention in recent years (Barkle *et al.*, 2007; Stenger *et al.*, 2008; Burbery *et al.*, 2013; Clague *et al.*, 2013; Peterson *et al.*, 2013; Collins *et al.*, 2017; Rivas *et al.*, 2017). However, such studies for pastoral hill country are very limited.

2.3 Nitrate attenuation processes other than denitrification

2.3.1 Plant uptake

Uptake of NO_3^- by plant roots may play a key role in NO_3^- attenuation at the root zone, since this nutrient is important for plant growth and development. In the subsurface environment however, its impact may not be obvious, except for deep rooted plants that go further down the soil profile (Rivett *et al.*, 2008). Conversely, the influence of plant uptake in grassland ecosystems may be minimal because of the presence of shallower root zone; however, Zaman *et al.* (2008) reported that plant uptake played a significant role in decreasing the amount of NO_3^- that leached into groundwater on a seepage wetland located on a dairy pasture farm in Kiwitahi, New Zealand. The impact of plant uptake in nutrient attenuation is more obvious in riparian zones, where this mechanism/pathway may surpass denitrification in the removal of NO_3^- from the environment (Sabater *et al.*, 2003). Despite the important role played by plant uptake in NO_3^- attenuation (in a system with plants), it is however not a permanent approach to NO_3^- removal, since organic matter from plants is returned into the soil, at harvest or via the grazing cycle, resulting in the release of NH_4^+ into the system. However, the organic matter from plants could also be a source of DOC, thereby enhancing denitrification and resulting in the net attenuation of NO_3^- from the system.

2.3.2 Dissimilatory nitrate reduction to ammonium (DNRA)

Both DNRA and denitrification are dissimilatory NO_3^- reduction processes – dissimilatory in the sense that N, in the form of NO_3^-, is not assimilated into biomass but is rather used as a terminal electron acceptor for electron transport. However, in the case of DNRA, NH_4^+ is the end product, while N_2 (and sometimes NO or N_2O) is the end product of denitrification. Another difference between these pathways is that DNRA is carried out by obligate anaerobes, while denitrification is carried out by facultative anaerobes (Hill, 1996); thus DNRA does not readily occur in unsaturated environments (Rivett *et al.*, 2008). In addition, DNRA is the favoured pathway when NO_3^- is the limiting factor, while denitrification is favoured when C is the limiting factor (Kelso *et al.*, 1997). The NH_4^+ produced from DNRA can easily be returned to the system via direct plant uptake or it can be oxidised to NO_3^- under aerobic conditions (Rivett *et al.*, 2008); thus making DNRA a temporary pathway for NO_3^- attenuation.

2.3.3 Assimilation of nitrate into microbial biomass

Assimilation of NO_3^- into microbial biomass is another attenuation process, though significant NO_3^- reduction through this process is difficult to achieve, especially in the presence of NH_4^+, because most heterotrophs prefer NH_4^+ to NO_3^- for their growth (Hill, 1996). However, in the presence of readily biodegradable organics, assimilation of NO_3^- into microbial biomass could play a significant role in NO_3^- attenuation (Kelso *et al.*, 1999; Hu *et al.*, 2000). Similar to DNRA and plant uptake, assimilation into microbial biomass is a temporary means of NO_3^- attenuation because N (in the form of NH_4^+) is released back into the system when the microbes die (Rivett *et al.*, 2008).

2.3.4 Alternative processes

The concentration of NO_3^- in the environment could also be reduced via dilution, however this depends on certain catchment features such as climate and topography as well as hydrological connectivity within the catchment (Ocampo *et al.*, 2006). Similarly, movement of NO_3^- to water bodies may be retarded by its adsorption to variable surface charge of allophane and other poorly crystalline materials in Andisols (Katou *et al.*, 1996). However, NO_3^- attenuation via this process may not be significant compared to other attenuation processes. Studies have also showed that NO_3^- reduction could occur via the oxidation of iron and manganese (Fe^{2+} and Mn^{2+} oxidation), especially under anoxic and near neutral conditions (Luther *et al.*, 1997; Melton *et al.*, 2014).

2.4 Catchment features that affect denitrification

2.4.1 Soil properties

i. Soil physical properties

One of the major physical properties influencing denitrification is soil moisture content. The importance of soil moisture in denitrification has been studied for decades (Bremner and Shaw, 1958; Myrold, 1988; De Klein and Van Logtestijn, 1996; Jha *et al.*, 2012). The work carried out by Miller *et al.* (2008) showed that under high soil moisture content, complete denitrification is accomplished because of an increase in the concentration of N_2O reductase which facilitates the reduction of N_2O to N_2, thus

reducing the N_2O/N_2 ratio. Soil moisture promotes denitrification mainly by decreasing soil O_2 availability via the occupation of pore spaces (Saggar *et al.*, 2013). Water-filled pore space (WFPS) is commonly used in the assessment of soil denitrification capacity, with higher WFPS values reported to increase soil denitrification capacity (van Groenigen *et al.*, 2005; Mekala and Nambi, 2017; Owens *et al.*, 2017). Substrate mobility and hence availability for denitrification can also be improved by higher soil moisture content (Luo *et al.*, 1999a). Soil texture is a key factor that influences soil water holding capacity. Studies have shown that fine textured soils have higher rates of denitrification (Groffman and Tiedje, 1991; De Klein and Van Logtestijn, 1996). This is because fine textured soils have smaller pores which retain water at higher tension than coarse textured soils, thus providing an anoxic environment suitable for denitrification. Furthermore, the solubility and hence availability of NO_3^- and carbon (C), have been shown to increase in fine textured soils since these soils are able to hold water for longer periods, compared to coarse textured soils (De Klein and Van Logtestijn, 1996).

Other soil physical properties that influence denitrification are bulk density (Buchkina *et al.*, 2013), porosity (Jha *et al.*, 2012), structure and aggregate stability (Laudone *et al.*, 2011). In general, when soil physical conditions are not suitable, incomplete denitrification occurs i.e. denitrification stops at the production of N_2O (Jha *et al.*, 2012).

ii. Soil chemical properties

Soil pH is an important soil chemical property that influences denitrification (Thomsen *et al.*, 1994; Yamulki *et al.*, 1997; Čuhel *et al.*, 2010). Acidic pH stops denitrification at the N_2O stage, while neutral to basic soil pH encourages the completion of the process and thus the production of N_2 (Rivett *et al.*, 2008). In a study carried out by Čuhel *et al.* (2010) on a pastoral grassland in Czech Republic, the $N_2O/(N_2O + N_2)$ ratio increased with increasing acidity. This corresponds to the work of Koskinen and Keeney (1982) who reported that at pH 4.6 and 5.4, N_2O was the major denitrification product on a silt loam, whereas at higher pH (6.9), N_2 was the main denitrification product. The increase in N_2O concentration under acidic pH is mainly because this pH range interferes with the denitrifiers' ability to assemble N_2O reductase enzyme, which is needed for the conversion of N_2O to N_2 (Liu *et al.*, 2014). Although the ideal soil pH for agricultural

pasture production has been reported to range from 5.8 to 6.0 (Roberts *et al.*, 1996), Rust *et al.* (2000) noted that the ideal pH for heterotrophic denitrification ranges from 5.5 to 8.0. However, denitrification can occur outside this pH range because microorganisms may adapt to certain soil pH conditions as evidenced by the work of Yamulki *et al.* (1997). Here, the authors observed that N_2O emission occurred at pH 3.9 on a silty clay loam. The authors attributed this denitrification to adaptation of the microbial community to low pH as well as to chemodenitrification, which is common in soils with low pH (Beauchamp *et al.*, 1989). After carrying out an extensive review on the influence of soil pH on denitrification, ŠImek and Cooper (2002) concluded that the concept of an ideal soil pH for denitrification does not exist without considerations of other soil factors.

Availability of a suitable energy source (organic C) is necessary for heterotrophic denitrification to occur. The rate of denitrification is related to the amount of DOC rather than the total amount of organic C present in a system (Rivett *et al.*, 2008). The availability of DOC is closely related to the amount of soil organic matter (SOM). Hence, a clayey soil which tends to retain more organic matter may show higher rates of denitrification compared to a sandy soil, though this may not always be the case due to the influence of other soil and environmental factors. Brettar *et al.* (2002) noted that the amount of SOM on a floodplain correlated positively with the rate of denitrification. In most cases, it is the land use and soil management practices rather than the soil type that influences the availability of DOC for denitrification in the subsoil (McCarty and Bremner, 1993; Bhogal and Shepherd, 1997). This will be discussed further in section 2.4.2.

The amount of NO_3^- available in a soil also influences the rate of denitrification. Blackmer and Bremner (1978) reported that low NO_3^- concentration (10 to 20 µg NO_3^--N g^{-1}) delayed the reduction of N_2O to N_2, while an excess amount of NO_3^- (50 to 2000 µg NO_3^--N g^{-1}) completely stopped the conversion of N_2O to N_2. They stated that the inhibitory effect of NO_3^- on the reduction of N_2O increases with a decrease in soil pH. Wang *et al.* (2013b) examined the effect of seven different NO_3^- concentrations (ranging from 10 to 250 mg N kg^{-1} dry soil) on denitrification on a silt clay calcic Cambisol. They observed that increasing the concentration of NO_3^- enhanced denitrification potential. Although the concentration of NO_3^- used in the above studies

varied, it was clear that "too little", or an "excess" of NO_3^-, was not suitable for denitrification. In addition to NO_3^-, denitrifying bacteria require other nutrients such as phosphorus (P) as well as micronutrients for effective metabolism (Hunter, 2003; Rivett *et al.*, 2008). This implies that a fertile soil (i.e. a soil with the essential plant nutrients present in the right proportions and at the right pH) may enhance denitrification, although this requires further research to better understand the mechanisms involved in this relationship.

iii. Soil biological properties

The influence of soil biological properties on denitrification is dependent on the soil physicochemical properties. In other words, when the soil physical and chemical properties are conducive for denitrification, the growth and activities of denitrifying bacteria will be enhanced, subsequently improving the process of denitrification. Jha *et al.* (2012) measured denitrification in different soils and reported that soils which had higher microbial biomass carbon (MBC) and denitrification enzyme activity (DEA) produced more N_2 during denitrification, when soil conditions were suitable.

iv. Parent material

The parent material from which a soil is formed also influences denitrification through its effect on nutrient content. Groffman and Hanson (1997) measured denitrification on wetland soils formed from different parent materials. They reported higher denitrification on soils formed on nutrient-rich parent materials on Rhode Island. The result of this study corresponds to that of Xu and Cai (2007) who recorded low denitrification capacity of subtropical soils derived from parent materials that were not very fertile. The presence of short-range order constituents (e.g. allophane) in soil parent material could potentially increase the soil denitrification capacity, since such a soil would have a high capacity to store organic C (Dahlgren *et al.*, 2004). Denitrification may also be affected in certain environments if microbial movement is hindered by small soil pore sizes (Whitelaw and Rees, 1980; West and Chilton, 1997). Though a number of studies have examined how parent material affects denitrification/nutrient attenuation, such studies for New Zealand soils are rare in the literature; therefore, more research is needed in this area.

2.4.2 Land use and soil management practices

When compared to other land use types such as cropping and forests, pasture grasslands have been shown to have higher rates of denitrification (Sgouridis *et al.*, 2011; Vallejo *et al.*, 2011). The higher denitrification in pasture grassland is mainly due to increases in the availability of DOC and other nutrients through the deposition of urine and dung, root exudates and microbial biomass of dense pasture roots, and anoxic condition caused by soil compaction during grazing (Bhandral *et al.*, 2007; Recous *et al.*, 2010; Sgouridis *et al.*, 2011; Vallejo *et al.*, 2011).

The type of management practice carried out on pasture grasslands affects its capacity for denitrification. For instance, Sgouridis *et al.* (2011) reported that the availability of DOC was significantly higher in grazed pastures compared to mowed pastures. As a consequence, denitrification was higher in the grazed pasture. Patra *et al.* (2006) equally reported that management practices (grazing and mowing) and plant species affected the species of microbes present in grasslands. Though denitrification was not significantly affected by the interaction between management practices and plant species in their study, it can be deduced that herbivores (which are selective in their grazing habit) could affect denitrification through their influence on the type of plant present in the field. Urine additions to the soil during grazing also influences the rate of denitrification, as urine not only represent a potential NO_3^- source, but also causes an increase in pH (during the mineralisation of urea to NH_4^+), thus affecting the solubility of DOC (Carter, 2007; De Klein and Eckard, 2008).

Soil compaction and pugging (probable under high stocking density) greatly impacts soil physical properties (Singleton *et al.*, 2000) and thus is likely to promote anoxic soil conditions, which in turn promotes denitrification, especially during winter. Bhandral *et al.* (2007) recorded higher N_2O flux on a compacted grassland soil compared to a similar soil that was not compacted. Similarly Menneer *et al.* (2005) reported that animal treading on a pasture soil resulted in a short-term increase in the rate of denitrification. The authors (Menneer *et al.*, 2005) excluded animal excretion in their experiment and thus attributed the increase in denitrification to reduced soil aeration in addition to lower N utilisation caused by reduced plant growth.

Other soil management practices such as the use of fertilisers and the shift from pastures to cropping (and vice versa) could also influence denitrification on pasture grasslands. Applying the right quantity of N fertiliser at the right time will limit the detrimental effect (excess or deficiency) of NO_3^- availability on denitrification (Velthof *et al.*, 1997; Ledgard *et al.*, 1999). In addition, the form of N fertiliser applied to the soil may also affect denitrification. Velthof *et al.* (1997) recorded higher denitrification rates in soils amended with NO_3^- fertilisers compared to those amended with NH_4^+ fertilisers.

In a study comparing land use change from grassland to annual crop in France, Recous *et al.* (2010) showed that converting from a 5-year grassland to annual cropping (corn-wheat-barley rotation) resulted in a significant decline in SOM and DEA. Conversely, when the arable land was reconverted to grassland, there was an increase in SOM, though this effect was not significant within the time scale of the experiment (36 months). This increase was attributed to the roots of perennial grasses which accumulate in the upper soil horizon, and to crop residues which were left on the soil surface to decompose. A similar study in New Zealand (Haynes and Francis, 1990) showed that short-term changes in cropping systems, i.e. 2 to 4 years of arable cropping to 2 to 4 years of pasture farming did not allow for significant changes to be observed in terms of SOM build up in pasture and its decline in arable cropping; though the authors noted that pasture significantly increased soil N (via fixation by pasture legumes) and structural stability (through formation of pores and binding agents by plant roots). However, long-term monitoring (10 to 18 years) of these land use types showed that the pasture soils had considerably higher SOM compared to the arable soils (Haynes *et al.*, 1991), and this could in turn lead to differences in the denitrification capacity of these soils.

The replacement of perennial pasture with forage crops for increased feed production is a common agricultural intensification practice in New Zealand (Houlbrooke *et al.*, 2009). Although this practice is more common on flat lands, it is increasingly being adopted in hill country (Fraser *et al.*, 2016; Burkitt *et al.*, 2017). Due to the steep and uncultivable terrain of hill country, forage crop establishment is typically accomplished via the aerial spray and surface sowing technique (i.e. using helicopter, without cultivation). This method of crop establishment has allowed large areas of hill country land to be converted from perennial pasture to forage crop within short time frames.

Land use changes have been reported to affect the SOM and DOC content of soils (Haynes and Swift, 1990; Haynes *et al.*, 1991; Ghani *et al.*, 2007; Schipper *et al.*, 2007). However, there is a dearth of information on the effect of such changes on denitrification in pastoral hill country soils, especially when the spray and surface sowing technique is used for crop establishment. This thesis (Chapters 5 and 6) will assist in filling this knowledge gap.

2.4.3 Topography

Topography affects the distribution of soil moisture, thus it influences the occurrence and rate of denitrification in any catchment. Studies examining the impact of topography on denitrification show that the highest denitrification occurs at the bottom of slopes (van Kessel *et al.*, 1993; Beaujouan *et al.*, 2002; Gu *et al.*, 2011; Luo *et al.*, 2013). This greater denitrification at the slope bottom compared to the mid and upper slope have been attributed to the higher moisture and nutrient content of the slope bottom caused by lateral movement of water (Holst *et al.*, 2007). These suitable conditions enhance the growth and activities of the denitrifying bacteria. Broughton and Gross (2000) found an active microbial community at a slope bottom characterised by higher moisture, N, C and plant biomass compared to the upper and mid slopes.

Ocampo *et al.* (2006) carried out a study to quantify the balance between biochemical and hydrological controls of NO_3^- removal in a riparian agricultural sub-catchment grazed by sheep in Western Australia. They used a numerical model of NO_3^- transport and reaction to show that different mechanisms were responsible for nutrient removal on two different topographical settings (steep and flat slopes). On the steep slope, NO_3^- removal was mostly influenced by hydrological process, i.e. dilution, because water transport through the soil was fast compared to reaction time, thus NO_3^- removal by denitrification was low. In contrast, on the flat slope, NO_3^- removal by denitrification was more of a biochemical process, i.e. denitrification; here, the rate of water transport was slow compared to reaction time, thus NO_3^- removal was high. This corresponds to the research by Messer *et al.* (2012) on a riparian buffer in North Carolina. The authors reported that reduced velocities on flat topographies increased groundwater residence time which subsequently increased denitrification in these areas.

On pastoral landscapes, topography influences the distribution of animals and this determines the intensity of nutrient attenuation on different areas on the landscape. Animals tend to graze and rest on flat/low slopes than on steep/higher slopes, thus depositing more C and other nutrients (via urine and dung) in the low slope areas (Saggar *et al.*, 1990; Saggar *et al.*, 1999). Therefore areas with flat slopes experience higher nutrient attenuation than areas with steep slopes (Kelliher *et al.*, 2014; Saggar *et al.*, 2015). Hoogendoorn *et al.* (2011a) in their study on hill land units in New Zealand reported that N_2O emissions from low slopes was 5 to 20 times higher than that obtained from both moderate and high slopes combined.

Using digital terrain modelling, Florinsky *et al.* (2004) studied the effect of topography on the activities of denitrifiers under different soil moisture conditions in grasslands of North America. They observed that under wetter soil conditions, denitrification rate and other microbial activities increased due to gravity and slope-controlled supply of nutrients to microbes. On the other hand, under drier soil conditions, denitrification rate and microbial activity was reduced and did not depend on moisture distribution and land surface morphology.

2.4.4 Wetlands

Both natural and artificial wetlands have been used for decades to attenuate N entering water bodies (Gersberg *et al.*, 1983; Romero *et al.*, 1999; Tanner *et al.*, 2005; Zaman *et al.*, 2008). Due to the nature of wetlands (i.e. anoxic environment, presence of nutrients and aquatic plants) NO_3^- removal could be through denitrification, DNRA, plant uptake and assimilation to microbial biomass (Bowman *et al.*, 1989; Matheson *et al.*, 2003). Denitrification however, is known to be the dominant removal process (Zaman *et al.*, 2008).

Several factors affect the ability of wetlands to efficiently remove nutrients. International Water Association (2000) reported that treatment performance of wetlands varies depending on the forms and concentration of nutrient inputs, soil types, vegetation, climatic conditions and flow characteristics such as inter-event times and hydraulic residence. For artificial wetlands, treatment performance may change during construction and maturation. Tanner *et al.* (2005) reported that a constructed wetland

established to attenuate nutrients from a dairy pasture was able to significantly remove NO_3^- from the farm, though it became a source of NH_4^+ during establishment.

Zaman *et al.* (2008) reported that a seepage wetland in a grazed dairy catchment in New Zealand was a source of N_2O when NO_3^- levels were non-limiting but also became a sink of N_2O (i.e. it readily removed N_2O through reduction to N_2) when NO_3^- levels were low. Similarly, Xue *et al.* (1999) noted that when the NO_3^- loading rate to a constructed wetland was low due to reduced precipitation, virtually all of the NO_3^- could be denitrified. On the other hand, during a period of high NO_3^- input resulting from increased precipitation, most of the added NO_3^- could not be denitrified. Both studies equally stressed the importance of water residence time in determining the denitrification capacity of wetlands. During periods of high inflow events, water residence time is reduced which decreases the contact time between the high NO_3^- concentrations in water and the denitrifying microbes, hence, resulting in a decrease in NO_3^- removal.

The availability of DOC greatly influences the NO_3^- attenuation capacity of wetlands. Ingersoll and Baker (1998) reported increases in NO_3^- removal efficiencies with increasing C additions (dried plant residue) in a laboratory wetland microcosm experiment. Using plant residue to increase the availability of DOC for enhanced denitrification was also reported by McCarty and Bremner (1993). Increases in denitrification due to the planting of crops in wetlands vary depending on the crop species. This is mainly due to their ability to supply organic material through the root exudates. For instance, Bastviken *et al.* (2005) reported that soils planted with pondweed (*Elodea Canadensis*) showed greater denitrification potential than those planted with broadleaf cattail (*Typha latifolia*) and common reed (*Phragmites australis*). The contribution of DOC to the NO_3^- attenuation capacity of hill country wetlands is yet to be investigated, even though seepage wetlands are major features of hill country landscapes.

2.4.5 Climate

The ideal temperature for denitrification to occur has been reported to be between 25 to 35°C (Brady and Weil, 2002). However denitrification processes could occur across a

wide range of temperatures due to adaptation of bacteria to specific environments (Rivett *et al.*, 2008). For instance, Carter (2014) monitored the effect of temperature on NO_3^- removal from denitrification beds in Karata, New Zealand, and observed a NO_3^- removal rate of 11.9 g N m^{-3} day^{-1} and 1.6 g N m^{-3} day^{-1} at temperatures of 22°C and 12°C, respectively. Pfenning and McMahon (1997) equally reported that lowering the incubation temperature from 22 to 4°C led to an approximately 77% reduction in the rate of N_2O produced in riverbed sediments in Colorado. Similarly Xie *et al.* (2003) reported that at temperatures below 10°C, denitrification was adversely affected. Lind (1983) noted that denitrification in the subsoil at an *in situ* temperature of 10°C was significantly lower than that carried out at a laboratory temperature of 25°C. These observations are in line with the general rule of thumb that for every 10°C rise in temperature, reaction rate is doubled. However, this change in the rate of denitrification with temperature is dependent on other factors such as soil type, topography, and availability of substrates which is mostly dictated by land use (Lind, 1983; Pfenning and McMahon, 1997).

When considering the loss of NO_3^- to water bodies, it is important to take into account the effect of seasonal distribution of rainfall and the impact on soil moisture. For instance, Velthof *et al.* (1996) reported that in managed grasslands in the Netherlands, large N_2O losses were recorded during spring, summer and autumn, while relatively small losses were observed in winter. The authors attributed these variations to changes in weather conditions (rainfall pattern and groundwater levels) in addition to other factors such as land use and management practices. Similarly, Venterink *et al.* (2002) in their study on an abandoned wet meadow in Sweden observed that soil drying resulted in denitrification rates that were three times lower than in continuously wet soil. They equally reported that rewetting the dried soil significantly increased denitrification to about 160 mg N m^{-2} d^{-1}. Conversely, Fromin *et al.* (2010) observed that in the Rhone River delta, South-eastern France, potential denitrification increased in the first 5 weeks of drought before it subsequently decreased. They argued that denitrifying bacteria were able to maintain their enzymatic ability to denitrify even after extended periods of aerobic and dry conditions. It is also worth mentioning the effect of the interaction between rainfall and soil type on denitrification. A fine textured soil is likely to retain more water during rainfall and will also retain more water during drought, compared to

a coarse textured soil; this will subsequently result in greater denitrification in the fine textured soil.

2.5 What influences denitrification below the root zone?

2.5.1 Organic carbon supply

Organic C is an essential requirement for heterotrophic denitrification. It has been identified as the most important factor limiting denitrification below the root zone (Starr and Gillham, 1993; Rivett *et al.*, 2008; Peterson *et al.*, 2013). The availability of C for denitrification is more important than the total C content; thus, in most cases, the rate of denitrification is closely related to the supply of DOC rather than the total organic C (Rivett *et al.*, 2008). DOC generally decreases with increasing depth (Luo *et al.*, 1998); this is likely to mean that less C is available for denitrification below the root zone – with a number of studies showing that C availability limits denitrification in the subsurface environment (Jarvis and Hatch, 1994; Barkle *et al.*, 2007; Clague *et al.*, 2013).

Peterson *et al.* (2013) reported that the addition of a DOC substrate (water-soluble organic matter extracted from soil sample) to the vadose zone of a site in Canterbury, New Zealand significantly increased the rate of denitrification. However, they noted that the efficiency of this process was affected by the thickness of the overlying subsoil which regulated the supply of DOC to the vadose zone. In this study, the authors used bioavailable soil C sources (extracted with cold water at 20°C for 30 minutes) which are representative of the DOC that would normally be transported to the subsurface environment as opposed to studies that have utilised other readily available C sources (e.g. glucose) that were not obtained from the soil (Rivas *et al.*, 2014a; Clague *et al.*, 2015a). Conversely, in a study in northwest Germany, Siemens *et al.* (2003) noted that leached DOC from agricultural soils is unlikely to affect denitrification potential in groundwater due to the low bioavailability of the leached DOC. The authors observed that a substantial amount of the DOC within the vadose zone was either retained through sorption or mineralised.

Studies on some New Zealand pasture soils show that about 100 to 1600 kg C ha^{-1} yr^{-1} in the form of DOC could be lost via leaching (Ghani *et al.*, 2007; 2010). When DOC

pools from other land use systems (cropping and forestry) were compared to pasture, the pasture soils were shown to have higher DOC fluxes (Ghani *et al.*, 2007). This could have implications on the amount of DOC that would be bioavailable for subsurface denitrification, i.e. after consideration of the amount that could be retained via sorption to mineral surfaces. In a study in the Waikato region of New Zealand, Quinn and Stroud (2002) reported higher DOC concentrations in a stream draining a grazed pasture land compared to streams draining native forest and mixed (grazed pasture plus forest) land use types. The authors observed similar groundwater DOC concentrations on all the land use types and thus stated that the absence of riparian trees on the pasture land was likely to have contributed to the higher DOC concentration on the stream draining the pasture land.

In a similar study, Jahangir *et al.* (2013) compared groundwater denitrification on two contrasting land use types (grazed grassland and arable farming) in southeast Ireland, where they recorded higher denitrification rates in the groundwater underlying the grazed grassland. This was likely due to the higher DOC content of the grassland, although other hydrogeochemical properties such as lower saturated hydraulic conductivity and dissolved oxygen (DO) content may have also increased denitrification in this groundwater. In addition, the plant species and density in the topsoil could have also affected the bioavailability of DOC for subsurface denitrification. For instance, Premrov *et al.* (2009), who similarly carried out their research in southeast Ireland, reported that the use of cover crops (as winter cover) significantly increased groundwater DOC concentrations during winter recharge. The authors attributed this increase to several factors, including plant litter and root exudates originating from the cover crop, and a possible stimulation of microbial activity or productivity under the cover crop. These studies show the importance of the overlying land use practice on subsurface denitrification. It would be interesting to study the impact of different land use change and practices on DOC availability in pastoral landscapes and the effect of these practices on subsurface denitrification, since such studies are scarce in the literature.

The presence of Paleosols (buried soils) has been shown to increase denitrification in the subsurface environment. Barkle *et al.* (2007) recorded higher denitrification capacity in a Paleosol layer in the Lake Taupo catchment in Waikato, New Zealand. Similarly,

Clague *et al.* (2013) monitored denitrification in the same catchment and noted a significant increase in the rate of shallow groundwater denitrification due to the presence of relict organic matter located 2.46 to 2.66 m below the soil surface. The authors noted that spatial variability associated with the buried relict organic matter (common in the area due to periodic volcanic eruptions) made it difficult to assess denitrification on the catchment scale. Lignite (brown coal) has also been reported to play a significant role in groundwater denitrification (Postma *et al.*, 1991); however, denitrification with the aid of this C source is not so common, especially in New Zealand. In addition, lignite is a fossil C source and its use as a soil amendment is controversial because of its connection with greenhouse gas emission (Palumbo *et al.*, 2004).

2.5.2 Nitrate concentration

Excess NO_3^- (2000 µg NO_3^--N g^{-1} dry soil) has been reported to inhibit denitrification by terminating the reaction at the N_2O stage. This inhibitory effect was shown to increase with decreasing soil pH (Blackmer and Bremner, 1978). However, Korom *et al.* (2005) reported that when NO_3^- concentrations are more than 1 mg NO_3^--N L^{-1}, the kinetics of denitrification in aquifers becomes independent of concentration (zero order); possibly because other factors such as enzyme activity become limiting.

Nitrate requirement for denitrification varies with depth. For instance, Luo *et al.* (1996), who sampled surface (10 cm depth) pasture soils in New Zealand reported that optimum DEA occurred at a NO_3^- concentration of 50 µg NO_3^--N g^{-1} dry soil. Conversely, Rivas *et al.* (2014a), who also sampled pasture soils in New Zealand, reported that optimum DEA occurred at a NO_3^- concentration of 75 µg NO_3^--N g^{-1} dry soil, when samples were collected from a depth of 100 to 200 cm. The findings of these studies contrast with that of Clague (2013) who reported that a NO_3^- concentration of 40 µg NO_3^--N g^{-1} dry soil inhibited denitrification from depths of 0 to 5 m below the soil surface. The author reported that this NO_3^- concentration increased the N_2O/N_2 ratio during the course of the experiment. These studies show that the effect of NO_3^- concentration on denitrification in any system is case specific and is also dependent on other properties of the system such as pH and DOC concentration (Blackmer and Bremner, 1978; Clague, 2013). Therefore, it is important to investigate how denitrification is influenced by the

fragile soils of pastoral hill country landscapes, as there is very limited research understanding in this area.

2.5.3 Presence of denitrifying microbes

Denitrifiers are ubiquitous in the natural environment and are able to reduce NO_3^-, provided environmental conditions are suitable for their growth and activity (Rivett *et al.*, 2008). Cannavo *et al.* (2004) reported that denitrification in the vadose zone of a site in France (after maize harvest and incorporation of maize residue) was dependent on the population of the denitrifying organisms and the specific denitrifying activity of these organisms. They noted that the presence and activity of denitrifiers were influenced by the concentration of NO_3^- and DOC as well as on the seasonal distribution of rainfall and temperature. In this study, it was observed that while the supply of DOC limited the activities of denitrifiers in the upper part of the profile, NO_3^- concentration controlled denitrification in the lower part of the profile (100 to 160 cm).

Rivett *et al.* (2008) noted that the activities of denitrifiers can be influenced by aquifer pore spaces. The pore spaces in intergranular aquifers are known to be regions of high biomass and metabolic activities, because they provide suitable surfaces for microbial growth (Blakey and Towler, 1988). However, microbial activities may be restricted by the small pore sizes of some aquifer matrices. For instance, Whitelaw and Rees (1980) reported that movement of bacteria (about 1 µm in diameter) was restricted in a Cretaceous Chalk aquifer matrix characterised by an average pore size diameter of 0.2 to 0.7 µm.

The influence of microbial populations on denitrification was reported by Clague *et al.* (2015a). The authors recorded an increase in denitrification potential in subsoils (collected from a depth of 2.4 to 3.7 m) after 72 hours of incubation. They argued that the microbial population of the samples was not able to accomplish any significant denitrification at 24 and 36 hours of incubation, thus indicating a low microbial population. However, with the increase in the incubation period (72 hours), sufficient growth in the microbial population was likely to have triggered the increase in denitrification potential. The findings of this study do not correspond to that of Luo *et al.* (1996) who recommended 5 hours as the optimum period of incubation for

measurement of DEA on topsoils (0 to 10 cm). These studies appear to indicate that microbial population decreases with depth with direct implications for denitrification in the subsurface environment.

It is important to note that high microbial populations do not necessarily mean high denitrifying population as evidenced by the study carried out by Clague (2013). The author noted that even though high hot water-extractable carbon (HWEC) – an indicator of microbial biomass – was extracted from a layer of soil sampled from approximately 4 to 5 m below the soil surface, the rate of denitrification from this layer was low compared to some other layers (closer to the soil surface) with smaller HWEC. This result could indicate that other factors, such as nutrients, may have been limiting at depth; however, it also shows that the influence of microbial population on denitrification would be better understood if the microbial species present in a particular environment are properly identified.

2.5.4 Dissolved oxygen concentration

Oxygen is a major factor limiting denitrification in subsurface environments (Rivett *et al.*, 2008). When concentrations of this element are very low, N_2 is usually the end product of denitrification; however, when O_2 concentrations are variable or not very low, denitrification stops at the formation of NO or N_2O (Brady and Weil, 2002). Several studies have reported increasing rates of denitrification at low DO concentrations (Trevors and Starodub, 1987; Gómez *et al.*, 2002; Stenger *et al.*, 2012). A DO concentration of < 2 mg L^{-1} is required for complete denitrification to proceed in any environment, provided that all other environmental conditions are suitable (Rivett *et al.*, 2008; Stenger *et al.*, 2013).

Böhlke and Denver (1995), who worked on a glacier outwash sand aquifer in Minnesota, reported that groundwater denitrification occurred at a DO concentration of > 2 mg L^{-1}. This may have been because denitrification occurred in microsites which had lower DO concentrations compared to the entire groundwater system. This is evidenced by the study by Jacinthe *et al.* (1998), where small patches of organic matter (located within the aquifer matrix) served as "hotspots" for denitrification. These patches of organic matter were assumed to be more anoxic than the entire aquifer

matrix, thus they enhanced the activities of most denitrifiers in the aquifer. This study corresponds to that of Clague *et al.* (2013), where layers of buried organic materials (associated with a Paleosol) exhibited higher denitrification rates compared to other layers within the soil profile. However, the authors did not measure the DO concentration of the different layers, but used the Childs Test – which indicates the presence of Fe^{2+} (Childs, 1981) – as a measure of the redox condition of the profile; thus, comparison of the DO concentrations of the layers could not be made.

2.5.5 Electron donors other than organic carbon

Reduced forms of Fe and S can serve as electron donors for denitrification (autotrophic denitrification by *Thiobacillus denitrificans*). Studies have shown that these electron donors can contribute significantly to denitrification in the subsurface environment (Pauwels *et al.*, 1998; Schwientek *et al.*, 2008; Korom *et al.*, 2012). Occasionally, a change in lithology along a flow path may result in the presence of two or more electron donors occurring within an aquifer (Böhlke *et al.*, 2002). Some of these multiple electron donor systems may contain both organic and inorganic elements. In such cases, heterotrophic denitrification may occur above a zone of autotrophic denitrification as reported by Weymann *et al.* (2010). In this study, both DOC and S were found in the matrix of a sand and gravel aquifer. Pauwels *et al.* (2000) also reported a similar pattern of denitrification in a schist aquifer. On the other hand, Korom *et al.* (2012) noted that both heterotrophic and autotrophic denitrification occurred simultaneously in an unconfined glaciofluvial aquifer containing DOC, pyrite and non-pyritic Fe^{2+} as electron donors. However, the contribution of DOC to denitrification in this aquifer surpassed that of the other electron donors. This is probably because DOC is thermodynamically the stronger electron donor, but differences in space may allow the oxidation of other electron donors that are thermodynamically less favourable.

Autotrophic denitrification may be the dominant form of denitrification in a groundwater system when C sources are minimal or present in forms that are not bioavailable. For instance, Postma *et al.* (1991) reported that sulphide (from pyrite) was the dominant electron donor in a groundwater system, even though organic matter (lignite) was more abundant (but not readily available) in the aquifer matrix. Experiments carried out in volcanic profiles in the Waikato region of New Zealand

(Clague, 2013) suggest that reduced forms of Fe and S may have contributed to groundwater denitrification in this region, because high amounts of these reduced forms were present below the root zone. However, heterotrophic denitrification dominated the NO_3^- reduction process, due to the presence of relict organic matter found within the subsurface environment.

2.5.6 Indirect effect of other factors on subsurface denitrification

Although the basic requisites for denitrification are the presence of NO_3^-, suitable electron donors, denitrifying microbes, and an anoxic environment (Stenger *et al.*, 2013), other factors such as climate and geology can also indirectly affect denitrification, and in most cases, the effect that these factors have on denitrification, are not mutually exclusive.

Studies have shown that denitrification below the root zone is affected by seasonal variations in rainfall (van Kessel *et al.*, 1993; Velthof *et al.*, 1996; Clague, 2013). High rainfall could increase the supply of NO_3^- and DOC in the subsurface environment, and this may result in higher rates of denitrification. However, the supply of these nutrients to the subsurface environment may be influenced by the soil type as well as the availability of these nutrients within the root zone (van Kessel *et al.*, 1993; Velthof *et al.*, 1996).

High rainfall events could also lead to the dilution of NO_3^- (rather than denitrification) because of an increase in the flow rate along a flow path which reduces the reaction time within an aquifer; however, this strongly depends on the topography of the landscape (Pauwels *et al.*, 2000; Ocampo *et al.*, 2006; Messer *et al.*, 2012; Stenger *et al.*, 2012). To some extent, it also depends on the age and physical characteristics of the aquifer material. Puckett and Cowdery (2002) reported that older aquifers with longer residence times indicate that significant denitrification could occur in such aquifers, hence leading to the improvement of the overall water quality, on the catchment scale. Furthermore, acid rain caused by sulphur dioxide emissions has been reported to reduce DOC concentrations in water bodies (Monteith *et al.*, 2007). The aquifer matrix could also influence denitrification by supplying the electron donors needed for denitrification (Pauwels *et al.*, 2000; Weymann *et al.*, 2010; Korom *et al.*, 2012) as well as by

determining the species of microbes that will be present for denitrification (Whitelaw and Rees, 1980).

Given that denitrification occurs across a wide range of temperatures, typically 2 to 50°C (Brady and Weil, 2002) and that temperature is relatively stable in the subsurface environment compared to the topsoil, it is not always easy to observe changes in rates of denitrification as a result of temperature variations below the root zone (Rivett *et al.*, 2008). However, Saunders and Kalff (2001) reported that a 5°C rise in temperature resulted in a 10 fold increase in the denitrification rate in the sediments of a lake in Canada.

Rivett *et al.* (2008) also noted that denitrification in the subsurface environment could be influenced by pH (because most denitrifiers prefer a neutral to basic pH) and the supply of nutrients such as P and micronutrients (needed for the growth of the denitrifying microbes). Hunter (2003) reported that a phosphate–P concentration of 0.16 mg L^{-1} was required for denitrification to occur in a groundwater system in southeast Colorado, USA. Lower phosphate–P concentrations resulted in a large accumulation of NO_2^- in the groundwater system. The experiment by Wang *et al.* (2013a) illustrates the importance of copper in denitrification. In this study, copper deficiency was shown to lower the activity of N_2O reductase; thus the reduction of N_2O to N_2 (i.e. complete denitrification) was hindered. It is important to note that the extent to which these factors (pH and nutrient supply) affect denitrification will likely depend on the species of microbes involved in the denitrification process.

2.6 Research gaps in nitrate attenuation in pastoral hill country

Based on the above literature review, current research gaps in NO_3^- attenuation in pastoral hill country landscapes are summarised below:

1. The effect of land use change and soil management practices on the availability of DOC (and NO_3^-) and the subsequent effect on denitrification have not been fully explored, especially on pastoral hill country where the shift from pasture to forage cropping is rapidly becoming a common agricultural practice.

2. There is a paucity of information on the influence of different parent materials/soil types on the amount of DOC and denitrification in New Zealand hill country soils.
3. Slope plays a critical role in the distribution of nutrients in any landscape. There is limited research on how slope interacts with other landscape features (such as soil type) to influence the distribution of DOC and the occurrence of denitrification on pastoral hill country.
4. Wet areas (seepage wetlands and hillside seeps) are significant features of hill country landscapes and are known for their high nutrient attenuation capacity. However, limited information is available on the contribution of DOC to NO_3^- attenuation in these unique landscape features.

Since the main focus of this research is to understand how DOC and denitrification are influenced by hill country landscape features, sections 2.7 and 2.8 will explore the methods for measuring these two parameters (DOC and denitrification).

2.7 Measurement of dissolved organic carbon (DOC) in soil

2.7.1 Methods for the *in situ* sampling for soil DOC

Different devices such as suction cups, suction plates, resin boxes, wick samples and lysimeters, can be used to directly collect soil water for subsequent DOC analysis (Weihermuller *et al.*, 2007). Suction cups/tubes are the most commonly used, because they are relatively easy to install, compared to other devices (Weihermuller *et al.*, 2007). These cups can be made from a variety of materials ranging from membranes to sintered and ceramic materials (Dorrance *et al.*, 1991). Materials made with oxide ceramic, adhesives and elastomeric bonds are to be avoided when sampling for DOC, in order to avoid specific sorption and contamination from plasticisers; on the other hand, materials (that do not sorb DOC) such as glass, nylon and stainless steel can be used for DOC sampling (Weihermuller *et al.*, 2007). The installation of these devices into the soil can be done in a number of ways viz. vertically, horizontally or at 45° (Mitchell *et al.*, 2001), while maintaining good hydraulic contact between the cup and the surrounding soil.

Water extraction with suction cups involves imposing a negative pressure to the soil through the application of suction to the cup, using a vacuum system (Weihermuller *et al.*, 2007). Factors that may influence the amount of water extracted from the soil through suction cups include the soil type and the water content of the soil at the time of sampling (Warrick and Amoozegar-Fard, 1977; Weihermüller *et al.*, 2005). Suction cups do not work well on soils with high potential for preferential flow i.e. soils that are not fairly homogeneous (Wang *et al.*, 2012). Severson and Grigal (1976) reported a tendency of suction cups to sample macropores at the expense of micropores. The small cross sectional area of the cups equally makes it difficult to obtain a representative sample of the entire soil pores.

Lysimeters (also known as soil columns) are vessels filled with either disturbed or undisturbed soil (Weihermuller *et al.*, 2007). They are used for monitoring changes in soil solute concentrations, as the solutes leach through the soil. Lysimeters filled with disturbed soils result in changes in soil physical properties, and this may influence the amount of nutrient leached out of the soil (Johnson *et al.*, 1995). Lysimeters could be installed in the field, greenhouse, in a special lysimeter facility or in the laboratory (Meissner *et al.*, 2000; Weihermuller *et al.*, 2007). Schoen *et al.* (1999) reported that lysimeters did not account for lateral water flow; in addition, the vertical boundaries of the device resulted in the creation of preferential flowpaths that were not representative of actual field conditions. However, several modifications to the design and installation of lysimeters have been used to minimise the occurrence of these problems (Titus and Mahendrappa, 1996).

Based on the drainage characteristics of lysimeters, they can be classified into free-draining (zero tension) and suction-controlled (tension) lysimeters (Weihermuller *et al.*, 2007). Suction-controlled lysimeters continuously drain larger quantity of water compared to the free-draining lysimeter (Vereecken and Dust, 1998); however, they are more expensive and difficult to install. In addition, solutes may interact with the material used in exerting pressure at the base of the lysimeter (Weihermuller *et al.*, 2007).

On the other hand, free-draining lysimeters are easier and cheaper to install. However, because the lower boundary is exposed to atmospheric pressure, water tends to accumulate in this region before drainage occurs (Abdou and Flury, 2004). This may lead to the creation of temporary anoxic conditions at the lower boundary, resulting to changes in soil nutrient concentrations that may differ from natural field conditions for some soils (Giesler *et al.*, 1996; Lewis and Sjöstrom, 2010). Sanderman *et al.* (2008) recorded lower soil DOC concentrations from suction-controlled lysimeters compared to free-draining lysimeters. The authors attributed this difference to the fact that the suction-controlled lysimeters sampled relatively smaller capillaries with higher water retention capacities compared to the free-draining lysimeters, where macropores were equally sampled. This study showed that the suction-controlled lysimeter may not always sample larger quantity of water/nutrients from the soil; thus, highlighting the importance of the lysimeter design in soil water sampling.

An alternative method of sampling for soil nutrients (DOC) involves installing subsurface drainage at a plot or paddock scale to collect all drainage/leachate which moves below the root zone. In addition, soil core samplers can be used to collect soil samples from the field for subsequent extraction in the laboratory. The use of soil core samplers does not require any installation process; thus, it is an easy and inexpensive method of sampling for soil DOC. In addition, soil pores are well represented and DOC concentration at different depth within the soil profile can be easily monitored. However, because subsequent sample extraction involves agitation of the soil, this method may overestimate the amount of DOC that would be used for microbial activity. For instance, Ghani *et al.* (2013) observed significantly higher DOC concentration in samples extracted from soil cores compared to those leached from free-draining lysimeters. The authors argued that soil aggregates from the cores were broken prior to extraction, resulting in greater surface area for DOC extraction, compared to the undisturbed soils in the lysimeters.

2.7.2 Laboratory extraction procedures

After soil sample collection using soil cores, DOC in the soil sample can be extracted by shaking the soil with a suitable solvent at a high soil to solution ratio for a short

period of time. Subsequent centrifugation and filtration of the sample is used to separate the solution phase (Jones and Willett, 2006; Peterson *et al.*, 2013; Clague *et al.*, 2015a). DOC may be lost or degraded during extraction, leading to an underestimation of the nutrient concentration (Jones and Willett, 2006). Similarly, DOC content may be overestimated when microbial cells are released during extraction. For instance, studies have shown that soil extraction using hot water (80°C for 16 hours) give higher DOC concentration compared to extraction done with cold water at room temperature for ≤ 1 hour (Ghani *et al.*, 2013; Peterson *et al.*, 2013; Clague *et al.*, 2015a). This hot water extraction method is an estimate of the soil microbial biomass and thus overestimates the DOC content of the soil.

Error during filtration may also cause an overestimation of DOC concentration. DOC is usually taken to be the organic C smaller than 0.45 µm (Thurman, 1985). This size limitation is the lower limit for bacteria and the upper limit for viruses (Thurman, 1985; Kleber *et al.*, 2015). Therefore, in order to exclude the interference of the microbial components during analysis, filtration is usually carried out with a filter of 0.45 µm pore size (Ghani *et al.*, 2013; Clague *et al.*, 2015a). However, because some microorganisms such as archaea can be smaller than 0.45 µm (Krieg, 2005), Kleber *et al.* (2015) recommended a filter pore size of 0.2 µm, in order to exclude all microorganisms and thus avoid an overestimation of DOC.

Jones and Willett (2006) examined the effect of sample preparation and extraction on the recovery of DOC from some UK soils, with the aim of establishing a standardised method of extraction. The experiment was carried out on different soil types (Cambisol, Podzol and Gleysol). In brief, the authors observed the following: compared to not sieving, sieving (< 2 mm or < 1 mm) significantly ($p < 0.05$) increased the amount of DOC recovered from the soil; compared to field-moist condition, air-drying resulted in a 3 to 10-fold increase in the amount of DOC; repeated freezing and thawing resulted in DOC aggregation; the extraction temperature (2 and 20°C) had little effect on the concentration of DOC; the amount of DOC recovered from the soil remained relatively constant above a soil:extract ratio of 1:4; a gradual increase in the amount of DOC extracted from the soil, over a 24-hour shaking period.

At the end of the study, the authors concluded that differences in soil type responses to the different treatments (methods of sample preparation and extraction) made it difficult to propose a standardised experimental procedure; thus, soil extracts can only give estimates of the actual DOC concentration in the soil. However, despite this difficulty, the authors recommended that extraction should be carried out as soon as possible after sample collection, on unsieved, field-moist soils. They equally suggested that distilled water, 0.5 M potassium sulphate (K_2SO_4) or 2 M potassium chloride (KCl) could be used for extraction, at a soil:extract ratio of 1:5, with constant shaking for 1 hour at 20°C.

2.7.3 Analytical methods

After soil sample extraction, the extracts are subjected to further analysis using any of the following methods: wet oxidation, dry combustion, and the spectrophotometric method. The wet oxidation method involves oxidising organic C to CO_2 using a chemical oxidant, with subsequent measurement of the released CO_2 or consumed oxidant (Ciavatta *et al.*, 1991). Chemical oxidants which can be used for this analysis include peroxide, dichromate and persulphate (Wangersky, 1994); however, the most common method of wet oxidation involves the use of potassium dichromate, with further quantification of the amount of dichromate consumed either by titration with a reducing agent, or by the calorimetric method (Bolan *et al.*, 1996).

Wet oxidation using the dichromate method is usually criticised for underestimating the concentration of organic C because of the incomplete combustion of organic C (Bolan *et al.*, 1996). However, this can be corrected by the application of external heat and the use of a correction factor (Fernandes *et al.*, 2015). Another source of error in the determination of DOC using the dichromate method is that the presence of reduced inorganic substances (especially chloride) can constitute a positive interference, since they are also oxidised; however, this can be corrected by the addition of mercuric sulphate which masks the effect of the chloride (Goerlitz and Brown, 1984). Addition of a silver compound (silver sulphate) can equally mask the effect of chloride in the dichromate method (Tzeng and Chen, 1993); this compound also serves as a catalyst for the oxidation process (Boyles, 1997).

Dry combustion (also known as high temperature oxidation) works on a similar principle with that of the wet oxidation method, i.e. oxidation of organic C to CO_2; however, oxidation is carried out by the application of heat at very high temperature (>1400^{o}C), in the presence of oxygen (Bolan *et al.*, 1996). The CO_2 released is measured by titration after absorbing in an alkali, or by infrared detector, or by weight gain after absorbing in ascarite (Bremner and Tabatabai, 1971; Bolan *et al.*, 1996). Dry combustion results in the complete oxidation of some recalcitrant organic compounds that may not be accessed with the wet oxidation method (Wangersky, 1994). Therefore, the dry combustion method of analysis gives accurate results for DOC concentration, but it requires the use of expensive apparatus.

The spectrophotometric method involves the use of a spectrophotometer to measure the absorption of light by DOC (Stewart and Wetzel, 1981). It works on the assumption that DOC concentration is proportional to ultraviolet (UV) absorbance (Deflandre and Gagné, 2001). However, this is not always true, due to the presence of interfering substances such as NO_3^- and Fe, in addition to spatial variability in the molecular composition of DOC (Ogura and Hanya, 1966; Deflandre and Gagné, 2001; Maloney *et al.*, 2005). Bolan *et al.* (1996) reported that light absorption per unit DOC increased with increases in the relative molecular weight of organic substances contained in DOC extracts.

Moore (1987) used the spectrophotometric method (UV absorbance = 330 nm) to monitor the DOC content of some streams and forest soils in the Maimai and Larry River catchments in Westland, New Zealand. They reported that this method was best suited for stream samples compared to soil water samples, probably because the soil contained a wide range of organic compounds with variable absorbances. However, Deflandre and Gagné (2001) measured DOC concentration of sediment samples in Quebec, Canada, and reported a significant ($p < 0.0001$), strong correlation ($R^2 = 0.911$) between DOC concentration measured with the spectrophotometric method (UV absorbance = 254 nm) and that measured with the dry combustion method. This implies that estimates of DOC concentration can be made (using a representative subset from a larger set of samples) from the mathematical relationship between spectrophotometric readings and measurements obtained using the dry combustion (or wet oxidation)

method. This is particularly important in experiments with large sample sizes, as it reduces the drudgery, health risk and cost involved in analysing individual samples, especially when using the dichromate wet oxidation method.

In addition to being used for the determination of DOC concentration, the spectrophotometric method can also be used as a measure of DOC quality/chemistry, i.e. it provides information on the complexity of DOC (Peacock *et al.*, 2014) and its bioavailability (Austnes *et al.*, 2010). Three indices commonly used as measures of DOC quality are (i) sUVa index: absorbance measured at 254 nm divided by DOC concentration (mg L^{-1}), (ii) E2/E3 index: ratio of absorbance measured at 250 nm and 365 nm, and (iii) E4/E6 index: ratio of absorbance measured at 465 nm and 665 nm. The sUVa index has been reported to be positively correlated with molecular weight and conjugated unsaturated C systems, such as those in aromatic molecules, while E2/E3 and E4/E6 indices have been reported to be negatively correlated with molecular weight and aromaticity (Peuravuori and Pihlaja, 1997; Weishaar *et al.*, 2003; Wallage *et al.*, 2006).

2.8 Methods for measuring denitrification

Two main terminologies (approaches) associated with measuring denitrification in the field and laboratory are; denitrification capacity and denitrification potential. Denitrification capacity experiments involve measurement of a system's inherent ability to reduce NO_3^-, without the addition of an electron donor (in most cases, organic C). On the other hand, denitrification potential experiments measure denitrification when electron donors are not limiting, i.e. an additional C source is introduced into the environment. Denitrification potential experiments are used to determine if a system is C and/or microbe limited (Yeomans *et al.*, 1992; Luo *et al.*, 1996; Clague *et al.*, 2015a).

Different techniques for measuring denitrification are described in this section, with emphasis on the advantages and disadvantages of each method. Different ways of manipulating each method in order to improve its accuracy for any given environment are highlighted.

2.8.1 Acetylene inhibition technique

This is the most common method of measuring denitrification and it has been widely adopted in New Zealand (Table 2.1). It involves the addition of an inhibitor (acetylene) into a system to prevent the reduction of N_2O to N_2; the N_2O, which is less abundant than N_2 in the atmosphere, can then be easily measured (Groffman *et al.*, 2006). Usually, acetylene is injected into an enclosed soil or water system, and the accumulation of N_2O over an incubation period (usually 1 to 24 h) is measured (Tiedje *et al.*, 1989; Luo *et al.*, 1999b).

Acetylene inhibition technique is a simple method that can be adopted in the field and laboratory. It can be used to measure both the capacity and potential of a system to undergo denitrification and is particularly useful in denitrification potential experiments (Luo *et al.*, 1996; 1999b; Groffman *et al.*, 2006; Jha *et al.*, 2011). It allows large number of samples to be measured over a wide range of ecosystems, including soil and water systems; thus, it enables both spatial and temporal variations associated with denitrification to be investigated (Groffman *et al.*, 2006).

Acetylene also inhibits the production of NO_3^- during nitrification, thus limiting the amount of NO_3^- available for denitrification. This constitutes a major problem in the use of this technique, as it can lead to an underestimation of denitrification, especially in systems with small and/or variable NO_3^- pools (Seitzinger *et al.*, 1993; Groffman *et al.*, 2006). Thus, this method is suitable for use in pastoral soils characterised by large N pools (Ghani *et al.*, 2007). The use of acetylene could also result in the scavenging of NO and this could lead to an underestimation of denitrification (Bollmann and Conrad, 1997). Prolonged use of acetylene within a system can lead to the adaptation of the denitrifying microbes to the added acetylene which they use as a C source; this subsequently overestimates the rate of denitrification (Tiedje *et al.*, 1989). Furthermore, contamination of acetylene with acetone and gases such as methane (Hyman and Arp, 1987; Gross and Bremner, 1992), as well as the slow diffusion of acetylene into saturated and/or fine-textured soils (Jury *et al.*, 1982) are other problems associated with the use of this technique (Groffman *et al.*, 2006). Several modifications to the acetylene inhibition technique have been proposed in recent years, in order to overcome some of these limitations and thus improve the efficiency of this technique. These

modifications range from the use of an appropriate amount of NO_3^- and C source in denitrification potential experiments, to the reduction of the incubation period, and also the use of vacuum pouches rather than conical flasks in laboratory denitrification experiments (Luo *et al.*, 1996; Rivas *et al.*, 2014a). The use of vacuum pouches in laboratory denitrification experiments allows for larger mass of soil to be incubated and larger gas sample volumes to be collected after incubation, relative to when using conical flasks. Thus, this results in N_2O concentrations that do not fall below the detection limit of the gas chromatograph (GC), and this is particularly useful for systems with low NO_3^- concentrations e.g. subsoils (Rivas *et al.*, 2014a).

2.8.2 Isotope tracer technique

Using stable isotopes (especially ^{15}N) in measuring denitrification involves the addition of ^{15}N-labelled nitrate ($^{15}NO_3^-$) in a system, with subsequent measurement of the produced ^{15}N-labelled dinitrogen, $^{15}N_2$ (Hauck and Melsted, 1956). Further modification of this technique involves the simultaneous addition of both $^{15}NO_3^-$ and ^{15}N-labelled ammonium ($^{15}NH_4^+$) into a system, in order to prevent the underestimation of denitrification (Hauck *et al.*, 1958; Nishio *et al.*, 1983). However, although this method may improve the measurement of denitrification in N-rich environments, such as grasslands, the addition of both $^{15}NO_3^-$ and $^{15}NH_4^+$ may lead to an overestimation of denitrification in N-limited systems, since this increases the availability of N in these systems (Groffman *et al.*, 2006).

Other stable isotopes of elements such as O_2, C, and S provide information about the major electron donors in a system; thus, they give indirect evidence of the occurrence of denitrification (Böhlke and Denver, 1995; Aravena and Robertson, 1998; Böhlke *et al.*, 2002; Groffman *et al.*, 2006). It is possible to use stable isotopes of two different elements to measure denitrification in a particular system. This dual isotope approach, expressed as a ratio and denoted by the delta symbol (δ), estimates denitrification from the correlation between the isotopes e.g. $\delta^{15}N/\delta^{18}O$.

Using dual isotope technique not only detects denitrification, but it also identifies the source of NO_3^- in a system. This is basically because of isotopic fractionation effect,

meaning that different NO_3^- sources have identifiable isotopic signatures (Kendall, 1998). Isotope tracer technique has been identified as one of the best approach for soil studies (Groffman *et al.*, 2006). However, application of this technique is limited due to the expensive equipment required for its operation as well as its laborious procedures (Groffman *et al.*, 2006). The interpretation of this technique may also be limited when groundwater flowpaths are not well understood (Stenger *et al.*, 2013; Clague *et al.*, 2015b).

2.8.3 Direct dinitrogen (N_2) quantification

Quantifying denitrification via this approach is somewhat difficult because of the high amount of N_2 (79%) that naturally exists in the atmosphere (Groffman *et al.*, 2006). Hence, in order to effectively measure denitrification using this method, core samples of soils are enclosed/incubated under reduced atmospheric N_2, usually in gas-tight vessels and the vessel is flushed with an inert gas (usually helium or argon) to remove background concentration of N_2 before subsequent measurement of N_2 and N_2O produced during denitrification (Butterbach-Bahl *et al.*, 2002).

Direct N_2 quantification is a non-destructive method that does not require the use of inhibitors and isotopic substrates; hence, it does not interfere with the activities of soil microbes, enabling denitrification to be monitored under natural or semi-natural conditions (Groffman *et al.*, 2006). However, the complex processes involved in providing a gas-tight incubation vessel is a major limitation in the use of this technique.

Furthermore, Scholefield *et al.* (1997) noted that direct N_2 quantification is only suitable for measuring denitrification in systems with large N pool, since small changes in denitrification products cannot be detected with this method. However, with the use of non-radioactive pulsed-discharged helium ionisation detector, N_2 fluxes in areas with low rates of denitrification can be monitored (Cárdenas *et al.*, 2003). Thus, with the appropriate gas-tight vessel, direct N_2 quantification can be a simple means of measuring denitrification in both soil and water systems (Groffman *et al.*, 2006).

2.8.4 Mass balance approach

In this method, denitrification is estimated from the difference between N inputs and outputs in a system. It is assumed that the system is in a steady state, i.e. it is a closed system, so that only input and outputs can be quantified (Groffman *et al.*, 2006). In terrestrial ecosystems, major sources of input include fertiliser application, biological and non-biological N fixation, atmospheric deposition, and decomposition of animal manure; while outputs (besides denitrification) include harvest and export of crop and biomass, animal consumption, leaching, and runoff to water bodies (David *et al.*, 2001).

Changes as a result of storage within a system are usually ignored when using the mass balance approach, because they are assumed to be small and thus difficult to estimate, especially over a short time scale (Groffman *et al.*, 2006). However, in agricultural soils, N storage pools (especially in the upper 1 m of soil) are extremely large; thus, estimates of denitrification could be affected by relatively small changes resulting from storage (David *et al.*, 2001; Groffman *et al.*, 2006).

Mass balance approach does not detect minute (but important) changes in denitrification fluxes, especially in pristine terrestrial ecosystems, where N input and output are small (Groffman *et al.*, 2006). In addition, spatial and temporal variations in denitrification are difficult to monitor with this approach (McIsaac and Hu, 2004). However, in constrained systems such as reservoirs, where input, output and storage can be controlled to a reasonable extent, denitrification can be effectively estimated using the mass balance approach (Jossette *et al.*, 1999). In general, estimates of denitrification using the mass balance approach can be improved by increasing the time scale of the experiment, i.e. using the mean data from multiple years, rather than estimating balances on a yearly basis (Groffman *et al.*, 2006).

2.8.5 Conservative tracer technique

Conservative tracers such as bromide and chloride, which are chemically and biologically inert, give ideas on the mechanisms involved in the reduction in NO_3^- concentration of (shallow) groundwater systems (Schipper *et al.*, 1993; Rutherford and Nguyen, 2004; Zaman *et al.*, 2008; Burbery *et al.*, 2013). For instance, when chloride or

bromide concentration remains stable and the concentration of NO_3^- significantly decreases within a system, the reduction may be attributed to denitrification. On the other hand, when the concentration of the conservative tracer and NO_3^- reduces at the same rate, yielding a constant ratio (e.g. nitrate:bromide) with time, other process such as dilution, could be responsible for the reduction in NO_3^- concentration.

These conservative tracers can be monitored at their natural concentration (Schipper *et al.*, 1993) or they can be artificially added to the soil (Rutherford and Nguyen, 2004). In areas where the groundwater flow is fast and microbial reactions occur at a slow pace, tracers are added through an injection well, and subsequent sampling could be done through a series of monitoring wells at various locations along a flowpath (Clague, 2013). Conversely, when groundwater flow is slow and the rate of microbial reaction is high, tracers can be added to the groundwater system using the push-pull technique. This basically involves injecting water with known concentrations of NO_3^- and a conservative tracer into a groundwater system via a piezometer, waiting for a period of time (incubation period) and subsequently withdrawing the injected water (from the same spot) and analysing for changes in the concentration of NO_3^- and the conservative tracer (Istok *et al.*, 1997).

Push-pull tests may not be representative of the entire groundwater system, due to changes in concentration of electron donor and hydraulic conductivity along a flowpath (Clague, 2013). However, modifications to this technique have been adopted in recent studies; this may involve the addition of a C source to measure the groundwater denitrification potential, addition of $^{15}NO_3^-$ to the tracer solution in order to assess the input and removal of NO_3^- in the groundwater system, or even increasing the number of piezometers in a site location, though this may have cost implications (Addy *et al.*, 2002; Zaman *et al.*, 2008; Clague, 2013).

2.8.6 Microbe-mediated approach

Microbes play a key role in the denitrification process; thus, understanding the structure, abundance and diversity of these organisms is important in assessing denitrification in any given environment. Microbial communities involved in

denitrification can be estimated by measuring the most probable number of denitrifying bacteria (Lensi *et al.*, 1995), or more commonly, by measuring the DEA. Under a C-rich and anoxic environment, denitrifying microbes produce enzymes at a rate proportional to the amount of NO_3^- in the system (Downey, 1966). DEA is a biochemical assay which quantifies the activities of these enzymes; thus, it is an indication of the amount of NO_3^- being denitrified (Schipper *et al.*, 1993).

Most times, DEA is used interchangeably with denitrification potential assays because of the presence of non-limiting C sources; however, a typical DEA assay involves the addition of an enzyme inhibitor (e.g. chloramphenicol) to ensure that new enzymes are not synthesised due to addition of a C source (Smith and Tiedje, 1979). DEA is mostly used in conjunction with the acetylene inhibition technique for the determination of the denitrification potential of both surface and subsurface environments (Table 2.1).

The molecular approach to measuring denitrification is a more in-depth method of assessing the denitrifying community. The main aim of this approach is to create a better understanding of how the composition and physiology of denitrifiers affect the denitrification process and vice versa (Groffman *et al.*, 2006). Hence, relationships can be established between the distribution/abundance of denitrifying microbes and other factors (such as soil properties and land use practices) that affect denitrification (Jha *et al.*, 2013a; 2013b; Morales *et al.*, 2015a; 2015b).

Molecular measurements basically involve extracting the deoxyribonucleic acid (DNA) of the denitrifying microbes, followed by a series of polymerase chain reaction-dependent or -independent techniques to determine the denitrifier community structure and gene abundance (Jha, 2015). DNA sequencing could also be employed to study the composition of soil denitrifying communities without isolating the individual microbes (Jones *et al.*, 2013). However, when dealing with a large number of samples, DNA sequencing can be laborious in terms of laboratory and computational work (Jha, 2015). In general, molecular studies on denitrification have shown that denitrifying microbes are abundant in the environment (especially terrestrial environment). The composition of these microbes is complex and is subject to both spatial and temporal variations (Groffman *et al.*, 2006).

2.8.7 Other approaches

Other approaches to measuring denitrification, especially in (shallow) groundwater systems include the interpretation of hydrochemical data and the use of mathematical models (Table 2.1). Concentration of redox sensitive parameters such as DO, Fe, sulphate (SO_4^{2-}), and Mn can be used to assess the denitrification capacity of soil-water systems (Stenger *et al.*, 2013; Rivas *et al.*, 2014b).

DO concentration below 2 mg L^{-1} indicates that a system is reduced and thus would support the activities of denitrifying microbes (Clague, 2013). When a low NO_3^- concentration is recorded in such a system, it could be assumed that denitrification contributed to it. However, a low NO_3^- concentration does not necessarily mean that denitrification is occurring, especially in older groundwater, as this may mean that the water was recharged when little NO_3^- was present in the system (Stenger *et al.*, 2013). The concentration of DOC in a system can also be used to predict the denitrification potential of a system. For instance, low concentrations of NO_3^-, in the presence of high DOC concentration may indicate that denitrifying bacteria have enough electron donor to carry out denitrification, thus resulting in a rapid reduction in NO_3^- concentration (Thayalakumaran *et al.*, 2008).

On a catchment scale, quantification of denitrification is done through modelling which integrates both hydrological and biogeochemical data obtained from laboratory and field measurements. Denitrification models are able to show that even though an area has a high denitrification capacity, such an area would not significantly impact the groundwater quality, if the water passing through it is low in NO_3^- or the groundwater flux in such an area is small (Stenger *et al.*, 2013).

2.9 Description of research farm

All the experimental work reported in this thesis was conducted at Massey University's Agricultural Experiment Station, Tuapaka, a sheep and beef cattle hill country farm located approximately 15 km north-east of Palmerston North, lower North Island, New Zealand (40°21'20.1"S, 175°44'19.6"E) (Figure 2.1). The New Zealand State Highway 57 (SH 57) forms the north-west border of the farm, followed by the Manawatu River.

The hilly landscape units on the farm form part of the intermediate slopes of the Tararua Ranges at the southern end of the Manawatu Gorge. The farm is about 470 ha in size, comprising of relatively flat areas (31 paddocks) at lower elevations (50-100 m), to hilly and steep slopes (54 paddocks) at higher elevations (360 m) (Hedley *et al.*, 2014).

The farm has a humid temperate climate with an annual average rainfall of 1100 mm, and predominantly dry summers (Massey University, 2016). Pollok and McLaughlin (1986) noted that the research farm consists of nine different soil series, namely Ramiha, Korokoro, Shannon, Tuapaka, Tokomaru, Ohakea, Kairanga, Makara steepland, and Halcombe hill and steepland soils. Variants of the Korokoro, Shannon, and Ohakea soils are also present in the farm. The soils of the farm belong to the Brown and Pallic soil orders in the New Zealand soil classification system. These soils are formed on loess, greywacke, volcanic ashes, and/or variable parent materials.

The vegetation in the flat area is primarily comprised of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). In the hilly and steep areas, the vegetation is mainly browntop (*Agrostis capillaries* L.) and crested dogstail (*Cynosurus cristatus* L.), with perennial ryegrass and white clover (Massey University, 2016). Rushes (*Juncus edgariae* L.) also occur in the wet areas (seepage wetlands) of the farm.

Fertiliser application to the hill areas consists of an annual application of approximately 25 kg N ha^{-1} and 20 kg P ha^{-1} applied by aeroplane. However, the soils in the flat area receive higher rates of lime and fertiliser, compared to the hill areas of the farm. These flat area soils are also mole- and tile-drained, and are usually rotationally cropped.

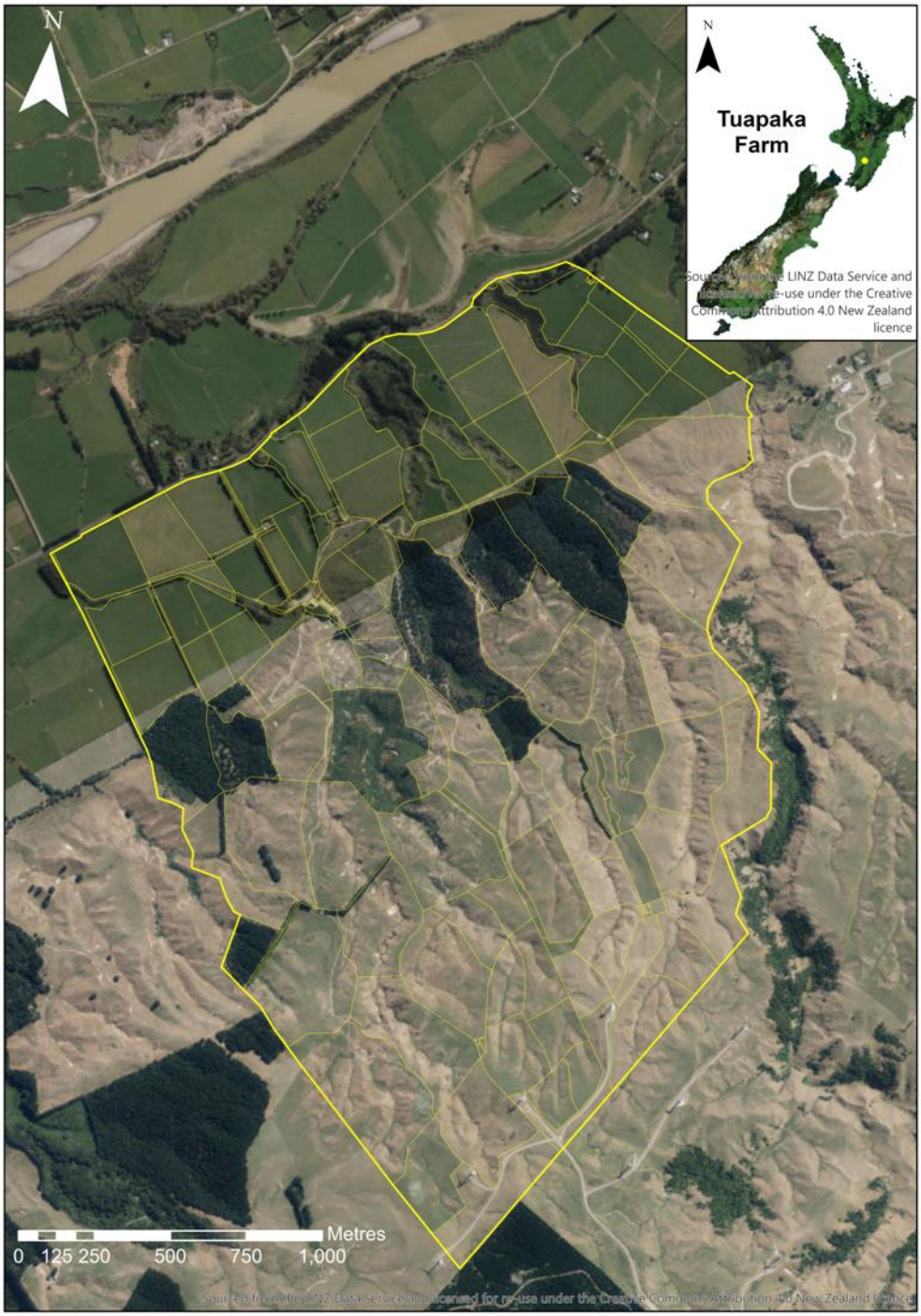

Figure 2.1: Satellite map of research farm (yellow dot on insert indicates the farm location within New Zealand)

2.10 Conclusion

Denitrification is an important NO_3^- attenuation process that results in the removal of NO_3^- from soil-water systems. Its efficiency, especially below the root zone, is limited by the supply of DOC. Several pastoral hill country landscape features such as soils,

topography, seepage wetlands and land use have the potential to influence the DOC concentration and denitrification capacity of hill country soils. However, very limited research has been undertaken to ascertain the contribution of these hill country landscape features to nitrate attenuation.

The subsequent chapters will focus on examining the effect of hill country landscape features on the DOC concentration and denitrification capacity of the topsoil and subsoil layers of different soils in a pastoral hill country landscape. For the purpose of accuracy, DOC concentration will be determined with either the dry combustion method or modified versions of the wet oxidation method, in this thesis. The acetylene inhibition technique will be used in DEA assays to measure denitrification, since this is an appropriate method for the laboratory determination of the denitrification capacity of pastoral soils.

Table 2.1: Summary of methods used in measuring denitrification in some New Zealand studies

Region	Zone of activity	Soil type or parent material	Land use	Method used for measuring denitrification	Reference
Bay of Plenty					
Rotorua	Lake sediment	-	Dairy; forest	Acetylene inhibition technique (DEA)	Bruesewitz *et al.* (2011)
Canterbury					
Lincoln	Vadose zone	Alluvial gravel	Ungrazed ryegrass	Acetylene inhibition technique (DEA)	Peterson *et al.* (2013)
Mayfield; Lismore; Paparua	Soil	Silt loam and stony silt loam	Dairy	Molecular technique; Acetylene inhibition technique (DEA)	Jha *et al.* (2012; 2013a); Morales *et al.* (2015a)
Manawatu-Wanganui					
Palmerston North	Soil	Fine sandy loam	Sheep	Acetylene inhibition technique	Ruz-Jerez *et al.* (1994)
Palmerston North	Soil	Manawatu fine loam; Manawatu sandy loam	Dairy; riparian zone	Molecular technique; acetylene inhibition technique (DEA)	Deslippe *et al.* (2014)
Palmerston North	Soil	Manawatu silt loam	Sheep	Direct N_2 quantification	Phillips *et al.* (2014)
Palmerston North	Soil	Tokomaru silt loam; Manawatu fine sandy loam	Dairy	Acetylene inhibition technique (DEA)	Luo *et al.* (1996; 1998; 1999a; 1999b; 2000); Jha *et al.* (2011; 2012)
Palmerston North; Longburn	Soil	Tokomaru silt loam; Manawatu fine sandy loam	Dairy	Molecular approach; acetylene inhibition technique (DEA)	Jha *et al.* (2013a; 2013b); Morales *et al.* (2015a; 2015b)
Palmerston North; Tararua	Soil and vadose zone; shallow ground water	Rangitikei silt loam and sand; Brown and Gley soils	Dairy	Acetylene inhibition technique (DEA); interpretation of hydrochemical data	Rivas *et al.* (2014a; 2014b; 2017)
Rangitikei	Shallow groundwater	Gravel, sand, and tertiary rocks (sandstone and mudstone)	Sheep and beef cattle; dairy	Conservative tracer technique; acetylene inhibition technique; interpretation of hydrochemical data	Collins *et al.* (2017)
Southland					
Edendale	Shallow groundwater	Fluvio-glacial gravels	Not stated	Conservative tracer technique	Burbery *et al.* (2013)

DEA: denitrification enzyme activity

Table 2.1 (cont'd): Summary of methods used in measuring denitrification in some New Zealand studies

Region	Zone of activity	Soil type or parent material	Land use	Method used for measuring denitrification	Reference
Waikato					
Cambridge	Shallow groundwater (denitrification wall)	Sandy loam to silty clay	Farm irrigated with dairy effluent	Acetylene inhibition technique (DEA)	Schipper *et al.* (2004); Long (2011)
Hamilton	Soil	Horotiu silt loam	Dairy	Acetylene inhibition technique	Watkins *et al.* (2013)
Hamilton	Soil	Silt loam	Dairy	Mass balance; acetylene inhibition technique	Ledgard *et al.* (1999); Menneer *et al.* (2005)
Kiwitahi	Soil (constructed wetland)	Rotokauri silt loam	Dairy	Acetylene inhibition technique (DEA)	Tanner *et al.* (2005)
Kiwitahi	Soil (seepage wetland)	Topehaehae silt loam; Kiwitahi silt loam	Dairy	Isotope tracer technique; conservative tracer technique; acetylene inhibition technique (DEA)	Zaman *et al.* (2008); Zaman and Nguyen (2010)
Lake Taupo Catchment: Waihora; Rangiatea; Kinloch	Vadose zone	Sand; loamy sand	Sheep and beef cattle; sheep and horse	Isotope tracer technique	Barkle *et al.* (2007)
Lake Taupo catchment: Waihora; Toenepi	Shallow groundwater	Volcanic deposit	Sheep and beef cattle; Dairy	Isotope tracer technique	Clague *et al.* (2013; 2015a; 2015b)
Mapara; Rangiatea; Kuratau	Shallow groundwater	Volcanic lithology	Not stated	Conservative tracer technique	Burbery *et al.* (2013)
Otorohonga	Soil	Silt loam	Dairy	Acetylene inhibition technique (DEA)	Jha *et al.* (2012)
Otorohonga; Horotiu; Te Kowhai	Soil	Silt loam	Dairy	Molecular technique; acetylene inhibition technique (DEA)	Jha *et al.* (2013a; 2013b); Morales *et al.* (2015a; 2015b)
Toenepi; Waihora	Shallow groundwater	Volcanic deposit	Dairy; sheep and beef cattle	Interpretation of hydrochemical data; isotope tracer technique; direct N_2 quantification, modelling	Stenger *et al.* (2013)
Whangamata	Soil (riparian zone)	Dystrochrepts (volcanic ash overlying allophane clay)	Pine forest	Conservative tracer technique	Schipper *et al.* (1993)
Whatawhata	Soil (riparian wetland)	Clay loam; clay	Sheep and beef cattle	Conservative tracer technique; acetylene inhibition technique (DEA)	Rutherford and Nguyen (2004)

DEA: denitrification enzyme activity

CHAPTER 3:
The effect of soil type and slope on the dissolved organic carbon concentration and denitrification capacity of a pastoral hill country farm

Research highlights

- Regardless of slope and soil drainage class, denitrification capacity was highest in the soil with the highest dissolved organic carbon (DOC) concentration (the Ramiha soil).
- The Ramiha soil has a high capacity to store carbon (C) due to its high content of short range order constituents such as allophane.
- Compared to slope, soil type had a greater impact on denitrification in this study because the effect of slope was masked by the pattern of soil distribution within the farm.

Peer-reviewed publication from this chapter:
Chibuike, G., Burkitt, L., Bretherton, M., Camps-Arbestain, M., Singh, R., Bishop, P., Hedley, C. & Roudier, P. (2019). Dissolved organic carbon concentration and denitrification capacity of a hill country sub-catchment as affected by soil type and slope. *New Zealand Journal of Agricultural Research* 62(3): 354-368.

3.1 Introduction

The contrasting micro-topographical units within hill country landscapes lead to spatial variability in the distribution of nitrate within hill country. Although nitrate accumulation generally occurs at low slope regions, due to animal grazing and resting habits (Bowatte, 2003; Crofoot *et al.*, 2010; Hickson *et al.*, 2016), the denitrification capacity of the specific soils within this region, among other factors, determines the amount of nitrate leached into groundwater.

In most cases, well-drained soils have a greater tendency to leach more nitrate compared to poorly-drained soils, while poorly-drained soils tend to have a higher denitrification capacity, mainly due to the abundance of oxygen-limiting conditions (high soil moisture). When soils with varying drainage capability occur on a particular micro-topographical unit/region, there tends to be spatial variability in the denitrification

capacity within that region. Understanding how the interaction between slope and drainage affect denitrification and therefore the leaching and availability of nitrate, will assist regulators to accurately assess hill country landscapes in terms of their capacity to attenuate nitrogen (N) and limit the contamination of water bodies.

Previous studies on denitrification have shown that the availability of dissolved organic carbon (DOC) is an important factor that limits denitrification below the topsoil (Yeomans *et al.*, 1992; McCarty and Bremner, 1993; Jahangir *et al.*, 2012). Furthermore, it has been reported that soil type (as influenced by the parent material) – and to some extent, slope – is an important factor that affects the storage and distribution of organic carbon–C (and by extension DOC) in the soil profile (Dahlgren *et al.*, 2004; Gray *et al.*, 2016). Knowledge of the DOC concentration of the various soil and slope combinations in hill country landscapes will help in the prediction of their denitrification capacity which in turn will help in effective nutrient management for improved water quality.

Although a number of New Zealand studies have examined the effect of slope and soil type (in particular soil drainage class) on denitrification in hill country landscapes, these studies have focused on either nitrous oxide (N_2O) emissions from the topsoil, i.e. ≤ 30 cm depth, or the study of hill country denitrification from a regional and national perspective (Hoogendoorn *et al.*, 2011a; Luo *et al.*, 2013; Saggar *et al.*, 2015). Information on subsurface denitrification in hill country as affected by soil type and slope is therefore absent.

Based on the aforementioned research gap, this study tested the hypothesis that hill country soil types interact with slope classes to influence soil DOC concentration and denitrification capacity. The objective of the study was to investigate the effect of soil type (drainage class) and slope on topsoil and subsoil DOC concentration and denitrification capacity in a pastoral hill country farm. A farm-specific approach was adopted to account for the spatial variability that exists within a farm, for effective nitrate and water quality management at the farm level.

3.2 Materials and methods

3.2.1 Site description

The study took place at Massey University's Agricultural Experiment Station, Tuapaka, which is a sheep and beef cattle farm located approximately 15 km north-east of Palmerston North, lower North Island, New Zealand (40°21'20.1"S, 175°44'19.6"E). The farm has a humid temperate climate with an annual average rainfall of 1100 mm, and predominantly dry summers (Massey University, 2016). It is about 470 ha in size and comprises of relatively flat areas at lower elevations (50-100 m), to hilly and steep slopes at higher elevations (360 m) (Hedley *et al.*, 2014). Nine different soil types (soil series) and some variants of these series are present on the farm; these soils have been described by Pollok and McLaughlin (1986).

3.2.2 Experimental design and sample collection

A previous experiment on Tuapaka farm (Hedley *et al.*, 2014) investigated the total C content of 50 locations, from the lowest to highest elevation in the farm. In the present study, which took place during spring (November 2016), these 50 locations were resampled at three soil depths (0-30, 30-60, 60-100 cm), using a soil core of ~ 4 cm in diameter. Visible urine patches and dung from grazing animals were avoided during soil sample collection. The soil samples were kept cool, transported to the laboratory and stored below 4°C for subsequent analyses (DOC and denitrification analyses were carried out within one week of soil sample collection).

Eight different soil types belonging to two soil orders (Pallic and Brown) were sampled in this experiment. These soils were distributed across three slope classes as described by Hoogendoorn *et al.* (2011a), i.e. low (1-12°), medium (12-25°) and high (> 25°) slopes (Figure 3.1). The soil types were grouped into three soil drainage classes, i.e. poorly-drained, imperfectly-drained and well-drained. A combination of the soil drainage and slope classes gave rise to five treatments, namely Poor/Low, Imperfect/Medium, Imperfect/High, Well/Medium and Well/High (Table 3.1). The soil parent materials are briefly described in Table 3.2.

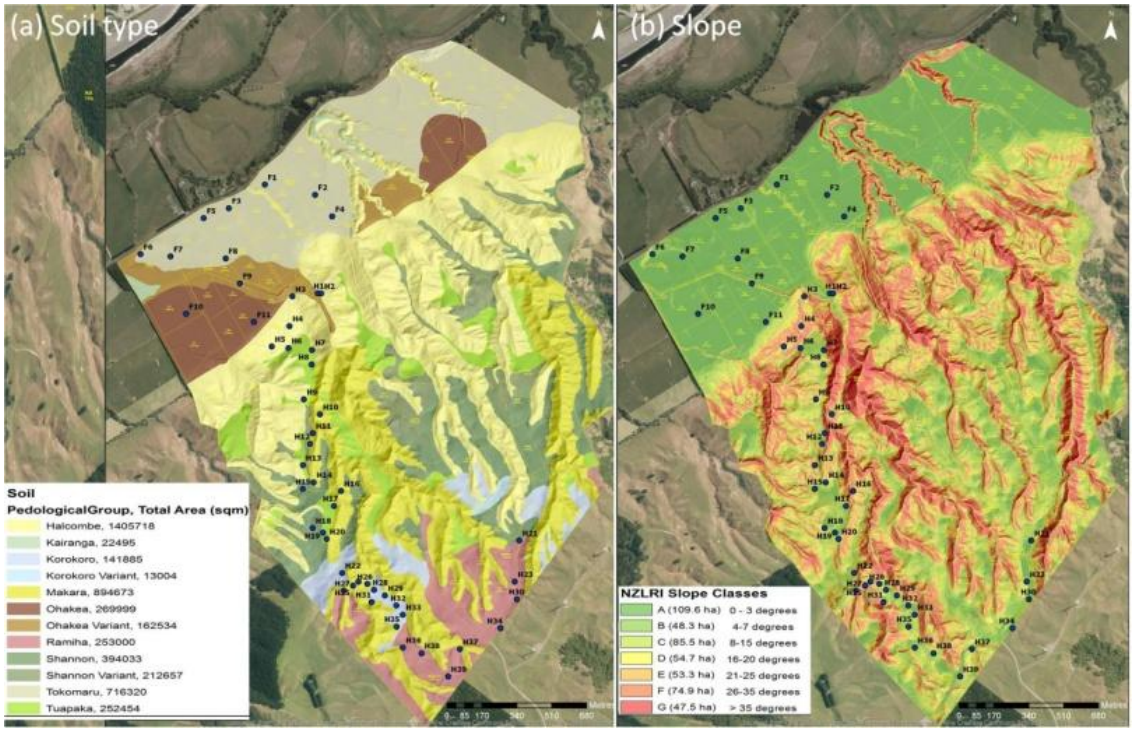

Figure 3.1: Maps showing the different soil types and slopes in the study area. *Blue dots represent the sampled locations*.

Table 3.1: Soil drainage and slope classes of the sampled locations

Soil Name	Number of observations (n)	Soil drainage class	Slope class	Treatment
Tokomaru	8	Poorly-drained	Low	Poor/Low
Ohakea	3	Poorly-drained	Low	Poor/Low
Shannon	2	Imperfectly-drained	Medium	Imperfect/Medium
Tuapaka	8	Imperfectly-drained	Medium	Imperfect/Medium
Halcombe	5	Imperfectly-drained	High	Imperfect/High
Korokoro	5	Well-drained	Medium	Well/Medium
Ramiha	7	Well-drained	Medium	Well/Medium
Makara	12	Well-drained	High	Well/High

Slope class = Low: 1-12°; Medium: 12-25°; High: > 25°

Table 3.2: Brief description of soil parent material

Soil name	New Zealand soil order	Soil description (Pollok and McLaughlin, 1986)
*Tokomaru	Pallic soil	The soil is formed from loess, sometimes with the addition of loessial and sandy materials that contain the Aokautere Ash, at depth. It is characterised by medium textured topsoil. It possesses a fragipan, or compacted horizon, at depth. A thick, highly mottled, fine-textured subsoil lies above the fragipan.
*Ohakea	Pallic soil	This soil is formed from loess interbedded with lenses of redeposited loessial material, sand and gravel that lacks the Aokautere Ash.
Shannon	Pallic soil	It is formed from loess overlying marine sand. The profile lacks a morphologically distinct eluvial horizon and possesses mottled subsoil.
Tuapaka	Pallic soil	It is formed from loess overlying marine sands and gravels. It is characterised by a morphologically distinct eluvial horizon, with strongly mottled subsoil, commonly with clay skins.
Halcombe	Pallic soil	The soil is formed from a variety of parent materials. The primary parent material is loess, with deposits of underlying sand, conglomerate and tephra. It is characterised by generally mottled subsoil.
Korokoro	Brown soil	This soil is formed from loess (< 1 m thick) overlying greywacke bedrock. Its subsoil gives a weak to moderate positive response to the field test for allophane, indicating the presence of volcanic ash in the loess that overlies the greywacke base.
Ramiha	Brown soil	It is formed from a mixture of loess and volcanic ash. It contains allophane as the principal clay mineral.
Makara	Brown soil	This soil is formed essentially out of greywacke.

*These soils are located on flat topography and receive higher rates of lime and fertiliser compared to the other soils within the farm.

3.2.3 Laboratory analyses

Soil samples were thoroughly mixed to achieve sample homogeneity. Ten grams of the homogenised fresh soil sample was extracted with 25 mL of deionised water (1:2.5

wt/v) at room temperature by shaking in a 50 mL extraction tube, on a rotatory shaker for 1 h. The agitated sample was subsequently centrifuged at 5000 rpm for 10 min and filtered with Whatman No. 41 filter paper. Thereafter, the filtered sample was centrifuged at 5000 rpm for 2 h (this second centrifugation separated more particulate organic matter than a 0.22 and 0.45 µm filter, on a sub-set of 10 samples). The centrifuged sample was decanted and analysed for DOC concentration.

The concentration of DOC in the extract was determined by the semi-automated dichromate method described by O'Dell (1993), with some modification as follows: 10 mL of the extract was pipetted into a 100 mL digestion tube to which two grains of anti-bumping granules, 1.5 mL of digestion solution (5.1 g $K_2Cr_2O_7$ + 84 mL conc. H_2SO_4 + 16.7 g $HgSO_4$ + 500 mL deionised water) and 10 mL of catalyst solution (5 g $AgSO_4$ + 500 mL conc. H_2SO_4) were added consecutively. The solution was mixed with a vortex mixer, covered with a glass funnel and placed on a thermostatically controlled (150°C) digestion block for 2 h. After digestion, the solution was allowed to cool at room temperature, made up to 25 mL with deionised water and mixed with a vortex mixer. The absorbance of the sample solution was read at a wavelength of 420 nm with a Philips PU 8625 UV/VIS spectrophotometer (Biolab Scientific Ltd.). The DOC concentration was then determined by plotting a calibration curve, which was obtained by making serial dilutions from a stock solution (100 mg DOC L^{-1}) of a primary standard – potassium hydrogen phthalate (KHP).

Denitrification enzyme activity (DEA) was used to determine the denitrification capacity of the soil. This was carried out via the acetylene inhibition method, while using the vacuum pouch incubation technique described by Rivas *et al.* (2014a). In brief, this analysis was performed as follows: 20 g of fresh soil (dry weight equivalent) was weighed into a polyethylene pouch (10 × 28.5 cm) fitted with a luer-lock valve. The pouch was heat sealed and subsequently vacuumed with a syringe via the luer-lock valve. The vacuumed pouch was then flushed with 50 mL of dinitrogen (N_2). Thereafter, 20 mL of acetylene, 20 mL of DEA solution (containing 50 µg NO_3^--N g^{-1} dry soil and 10 mg L^{-1} chloramphenicol) and 180 mL of N_2 were consecutively inserted into the pouch via the luer-lock valve. Acetylene was added to the mixture to arrest the denitrification reaction at the N_2O stage since it is easier to sample and measure N_2O

concentrations compared to N_2, because of low levels of N_2O in the atmosphere. Chloramphenicol served as an enzyme inhibitor to prevent *de novo* synthesis of enzymes during incubation (Dendooven *et al.*, 1994). The pouch/sample was incubated in the dark at 20°C, on a rotary shaker (160 rpm). Gas samples (25 mL) were collected from the pouch at 2 h intervals, i.e. 0 (initial gas sample before incubation), 2, 4 and 6 h of incubation. The gas sample was compressed into a 12 mL vac-vial for subsequent N_2O analysis via a Shimadzu Gas Chromatograph (GC) 17 A (Japan) equipped with a ^{63}Ni electron capture detector, and operating at a column and detector temperature of 55 and 330°C, respectively. The concentration of N_2O in the gas sample was obtained by plotting a calibration curve of N_2O standard gases, which were obtained from serial dilutions of a standard N_2O gas (100 mg L^{-1}). Thereafter, the mass of N_2O in the pouch headspace was calculated as follows:

Mass of N_2O (µg) = concentration of N_2O (µg L^{-1}) from GC

× volume of gas in pouch (L)

× 0.544 Bunsen coefficient

Denitrification capacity was subsequently calculated from the slope of N_2O mass and incubation time, divided by the mass of soil.

Nitrate concentration in the extracts was determined by continuous flow analysis (Technicon® AutoAnalyser II). Ammonium concentrations were considered negligible (< 0.1 mg kg^{-1}) and hence were not reported. The pH of the extract was read with a table-top standard pH meter (Meter Lab®) and the concentrations of water-extractable (total) aluminium (Al), iron (Fe) and manganese (Mn) were read with a 4200 Microwave Plasma-Atomic Emission Spectrometer – MP-AES (Agilent Technologies).

Soil samples were analysed for their total C and N concentrations using the elementar® (vario MICRO cube). In order to have a better understanding of the properties of the soil types, sodium pyrophosphate and acid ammonium oxalate extractions of Fe and Al (and also silicon–Si in ammonium oxalate) were carried out as described by Blakemore *et al.* (1987). The sodium pyrophosphate extraction involved extracting soil at a 1:100 wt/v (soil/extractant) ratio. This entailed shaking (overnight) the mixture of soil and extractant on a rotatory shaker, with subsequent centrifugation (15000 rpm for 30 min)

and filtration. Acid ammonium oxalate extraction involved using the same extraction ratio (1:100 wt/v), shaking the soil and extractant mixture in the dark for 4 h, with subsequent filtration. Thereafter, Fe, Al, and Si concentrations in the sample extracts (filtrates) were determined using the above-mentioned MP-AES. The concentration of organic C in the sodium pyrophosphate extracts was determined by the semi-automated dichromate method as described above. The gravimetric soil moisture content was also determined and hence nutrient concentrations were corrected to oven-dry soil basis.

All laboratory analyses were carried out using appropriate quality control protocols, including the use of sample duplicates, blank, reference and spiked samples.

3.2.4 Statistical analyses

Analysis of Variance (ANOVA) with Tukey comparison procedure ($p = 0.05$) was used to detect differences between treatment means. The relationship between denitrification capacity and other measured soil/water-extract parameters was determined with the Pearson correlation technique. Multiple regression analysis with the best subsets option was used to identify the best predictors of the denitrification capacity of the soil. All analyses were carried out with Minitab statistical software (17.2.1 Minitab, Inc.).

3.3 Results

3.3.1 Description of soil chemical properties

Compared to the other soils, Tokomaru and Ohakea (Poor/Low, i.e. poorly-drained soils at low slopes) had the lowest total C concentration (17 g kg^{-1} at the surface 30 cm) (Table 3.3). These soils also had the highest pH (6.1-7.1 at the topsoil). Conversely, Ramiha soil (Well/Medium) had the highest total C concentration (55 g kg^{-1} at the surface 30 cm), consistent with its [δ]proto-andic properties (12 g kg^{-1} < Al_o + ½Fe_o < 20 g kg^{-1}) (IUSS Working Group WRB, 2015). As expected, total C, N and sodium pyrophosphate-extractable C (C_p) decreased with soil depth, with C_p being 1/3 of total C in the surface 30 cm depth of the soils.

[δ]Soils with proto-andic properties contain allophane in insufficient amounts (12 g kg^{-1} < Al_o + ½Fe_o < 20 g kg^{-1}) to be fully described as allophanic soil (with andic properties). An example of a soil with proto-andic properties is the Ramiha soil, which is an allophanic brown soil.

Table 3.3: Selected soil chemical properties

Soil name	Soil depth (cm)	pH	Total C (g kg^{-1})	Total N (g kg^{-1})	C_p (g kg^{-1})	Al_p (g kg^{-1})	Fe_p (g kg^{-1})	Al_o (g kg^{-1})	Fe_o (g kg^{-1})	Si_o (g kg^{-1})	Total C/Total N	C_p/Total C	Al_p/Al_o	Al_o+½Fe_o (g kg^{-1})
Tokomaru	0-30	7.10	17.3	1.82	5.56	0.95	2.75	1.62	2.39	1.25	9.51	0.32	0.59	2.82
	30-60	6.86	3.3	0.65	1.72	0.66	1.72	2.00	3.19	0.66	5.08	0.52	0.33	3.60
	60-100	6.73	1.1	0.47	0.95	0.57	1.30	2.32	2.54	3.68	2.34	0.86	0.25	3.59
Ohakea	0-30	6.07	16.7	1.53	5.58	0.78	3.13	1.80	5.23	2.17	10.92	0.33	0.43	4.42
	30-60	6.08	5.2	0.69	2.34	0.53	3.80	1.78	4.78	1.32	7.54	0.45	0.30	4.17
	60-100	5.99	1.0	0.24	0.84	0.55	1.94	1.26	3.85	3.58	4.17	0.84	0.44	3.19
Shannon	0-30	4.63	29.3	2.50	8.45	1.49	5.35	2.33	3.66	0.09	11.72	0.29	0.64	4.16
	30-60	4.49	8.5	1.20	3.11	1.11	3.97	3.24	3.79	0.67	7.08	0.37	0.34	5.14
	60-100	4.35	3.4	0.99	1.38	3.31	1.75	3.04	1.77	0.89	3.43	0.41	1.09	3.93
Tuapaka	0-30	5.29	27.2	2.88	7.87	1.38	4.80	2.34	4.00	1.03	9.44	0.29	0.59	4.34
	30-60	5.26	6.9	1.38	1.91	0.70	2.96	2.12	3.23	2.56	5.00	0.28	0.33	3.74
	60-100	5.01	3.7	1.04	1.18	0.78	1.37	2.81	2.52	2.96	3.56	0.32	0.28	4.07
Halcombe	0-30	5.84	19.9	1.96	5.81	0.60	2.14	1.58	5.41	1.58	10.15	0.29	0.38	4.29
	30-60	5.95	6.7	1.23	2.01	0.68	3.34	2.32	3.54	0.77	5.45	0.30	0.29	4.09
	60-100	5.85	3.5	0.82	1.40	1.04	2.50	2.40	2.30	1.77	4.27	0.40	0.43	3.55
Korokoro	0-30	5.09	25.3	2.59	11.79	1.60	8.83	2.41	3.64	1.56	9.77	0.47	0.66	4.23
	30-60	5.02	11.0	1.43	5.90	1.71	8.97	4.97	3.66	3.03	7.69	0.54	0.34	6.80
	60-100	5.00	4.5	1.01	2.82	2.18	6.24	5.14	2.87	3.91	4.46	0.63	0.42	6.58
Ramiha	0-30	5.07	54.6	4.83	14.22	6.67	7.74	8.17	7.63	1.87	11.30	0.26	0.82	11.99
	30-60	4.89	24.3	2.47	12.12	5.36	8.17	8.54	8.86	0.60	9.84	0.50	0.29	12.97
	60-100	5.18	6.3	1.20	3.36	2.00	4.08	5.61	8.56	6.39	5.25	0.53	0.36	9.89
Makara	0-30	5.71	24.4	2.28	8.64	2.05	6.47	2.92	3.48	1.58	10.70	0.35	0.70	4.66
	30-60	5.53	8.5	1.29	3.49	1.72	5.55	3.83	4.33	1.53	6.59	0.41	0.45	6.00
	60-100	5.42	3.3	0.97	2.04	1.33	4.21	2.44	2.07	2.23	3.40	0.62	0.55	3.48

Subscript p: sodium pyrophosphate extracts; subscript o: acid ammonium oxalate extracts; values for Al_p/Al_o are molar ratios.

3.3.2 Variations in DOC concentration

The DOC concentrations of the treatments generally decreased with increasing soil depth (Figure 3.2). These concentrations were similar across treatments in the surface 30 cm soil depth. Further down the profile (30-60 cm), however, the Well/Medium treatment had significantly ($p \leq 0.05$) higher DOC concentration than all other treatments. A similar trend was observed within the 60-100 cm soil depth, where the Poor/Low treatment had significantly ($p \leq 0.05$) lower DOC concentration compared to the other treatments (except the Imperfect/Medium treatment).

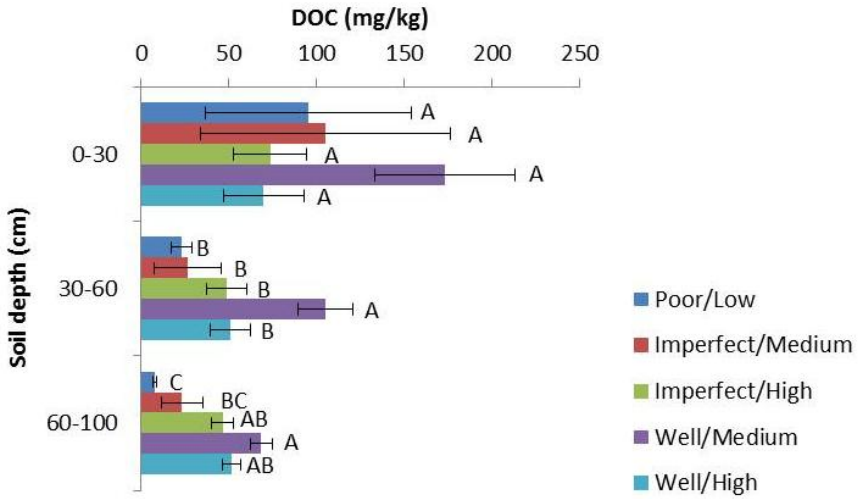

Figure 3.2: Variations in DOC concentration as influenced by soil drainage and slope classes. *Different letters denote significant difference between treatments for a particular soil depth ($p \leq 0.05$). Error bars are standard error of the mean ($n = 5$).*

3.3.3 Variations in denitrification capacity

There was more than 50% greater denitrification in the surface 30 cm of the soil compared to other soil depths (Figure 3.3a). However, significant ($p \leq 0.05$) differences in denitrification capacity were observed only within the 30-60 cm soil depth, with higher denitrification occurring in the Well/Medium treatment compared to the Imperfect/Medium and Well/High treatments.

When soil type and slope were considered separately (Figure 3.3b and c), the Ramiha soil had significantly ($p \leq 0.05$) higher denitrification capacity compared to the other soil types, in the surface 60 cm soil depth. No significant differences within slope classes were observed at this soil depth.

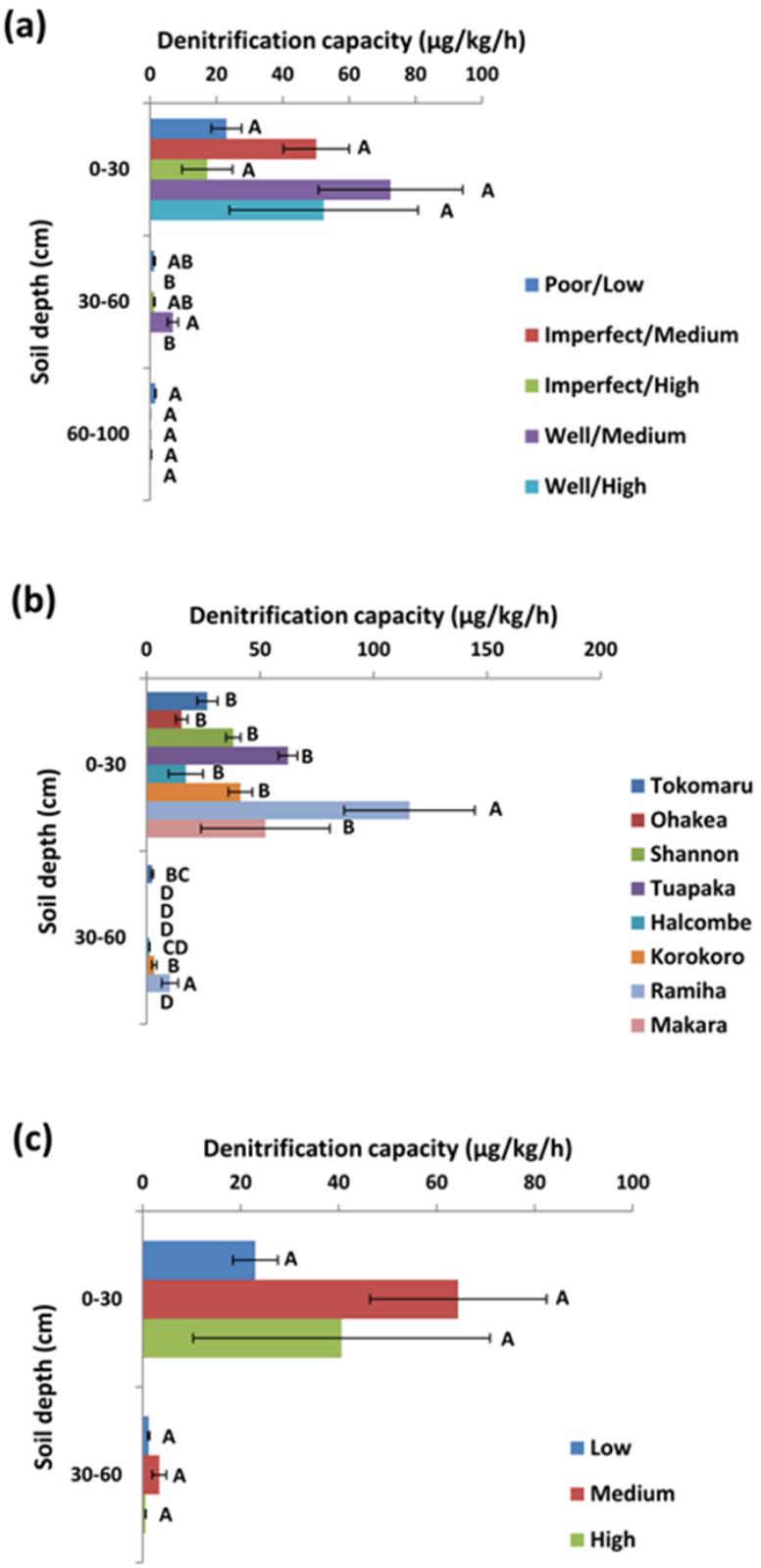

Figure 3.3: Denitrification capacity of (a) combined soil drainage and slope classes, (b) soil types, and (c) slope classes. *Different letters denote significant difference between treatments for a particular soil depth ($p \leq 0.05$). Error bars are standard error of the mean ($n = 5$). The 60-100 cm soil depth was omitted in (b) and (c) because minimal denitrification capacity was observed within this soil layer.*

3.3.4 Variations in properties of soil/water extracts

Nitrate concentration generally decreased with increasing soil depth for all the treatments, except for the Imperfect/High treatment (Halcombe soil) which had higher nitrate concentrations at lower soil depths (Figure 3.4a). The nitrate concentration of the Well/High treatment (Makara soil) was significantly ($p \leq 0.05$) lower than that of the other treatments at the surface 30 cm soil depth.

The mean pH of the Poor/Low treatment ranged from 6.50-6.74 and was significantly ($p \leq 0.05$) higher than the pH of the other treatments (except Imperfect/High), along the entire profile (Figure 3.4b).

There were no significant ($p \leq 0.05$) differences in the soil moisture content of the treatments at the surface 30 cm depth (Figure 3.4c). Further down the profile (30-60 cm), however, the moisture content of the Well/Medium treatment was significantly higher than that of the other treatments.

Similarly, there were no significant ($p \leq 0.05$) differences in the water-extractable concentrations of Al, Fe, and Mn of the treatments at the surface 30 cm soil depth (Figure 3.5). The water-extractable concentrations of Al and Fe increased with increasing soil depth on the Imperfect/High and Well/Medium treatments (and to some extent on the Poor/Low treatment).

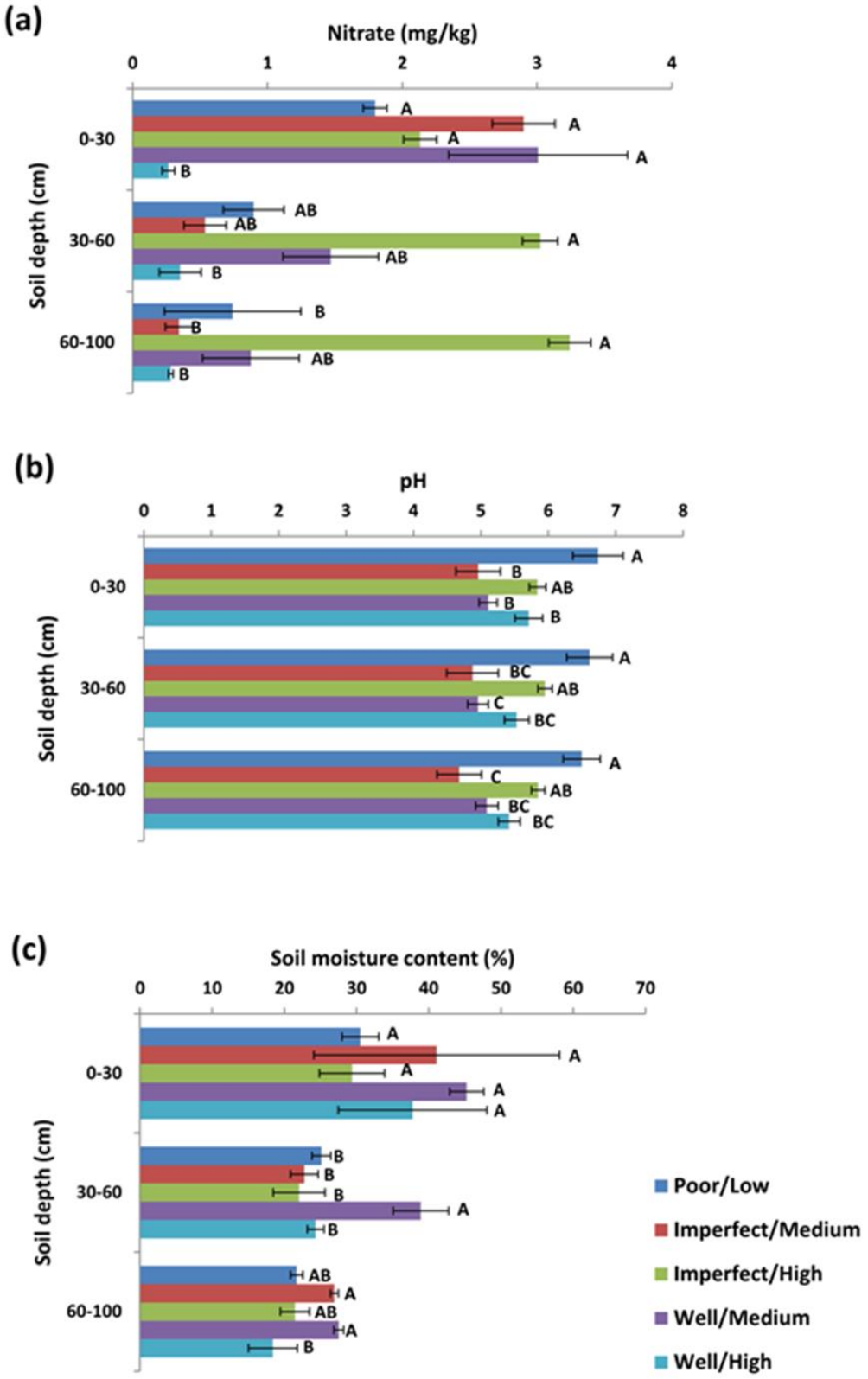

Figure 3.4: Variations in (a) nitrate concentration, (b) pH, and (c) gravimetric soil moisture content as influenced by soil drainage and slope classes. *Different letters denote significant difference between treatments for a particular soil depth ($p \leq 0.05$). Error bars are standard error of the mean ($n = 5$).*

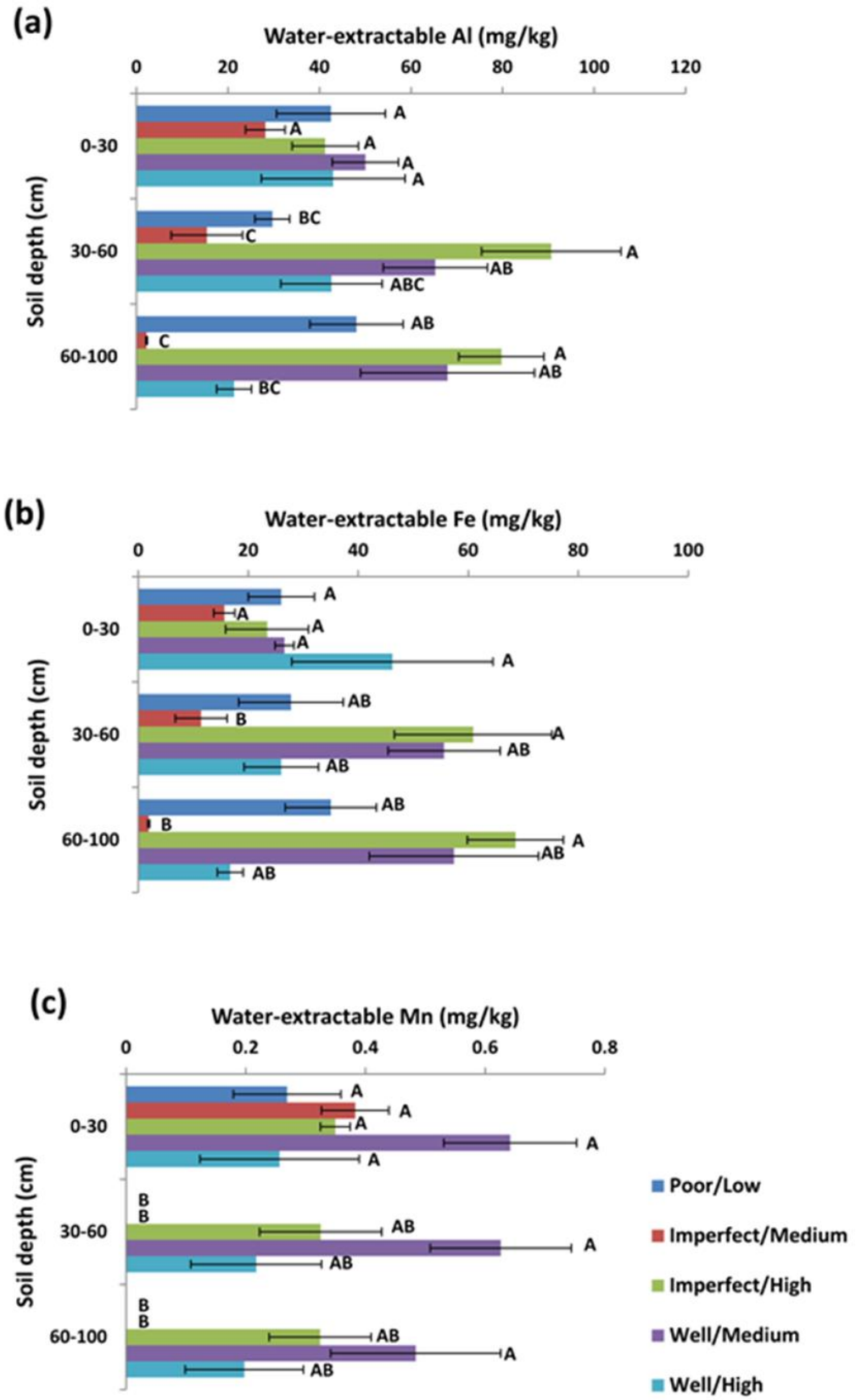

Figure 3.5: Variations in water-extractable (a) Al, (b) Fe, and (c) Mn as influenced by soil drainage and slope classes. *Different letters denote significant difference between treatments for a particular soil depth ($p \leq 0.05$). Error bars are standard error of the mean ($n = 5$).*

3.3.5 Relationship between denitrification capacity and properties of soil/water extracts

Significant ($p \leq 0.05$) positive correlation existed between denitrification capacity and the following parameters: soil moisture content, DOC and water-extractable Mn (Table 3.4). Compared to the other parameters, soil moisture had the strongest relationship (r = 0.86; $p < 0.001$) with denitrification capacity. There was no significant ($p \leq 0.05$) correlation between denitrification capacity and pH. Negative and non-significant ($p \leq 0.05$) correlations existed between denitrification capacity and three other parameters, i.e. nitrate, water-extractable Al and Fe. Multiple regression analysis with the best subsets option showed that soil moisture content and DOC were the best predictors of the denitrification capacity of the soil (adjusted $R^2 = 74\%$; $p < 0.001$).

Table 3.4: Correlation and regression values between denitrification capacity and soil/water-extract properties

Correlation results	**Soil moisture content**	**DOC concentration**	**pH**	**Nitrate**	**Water-extractable Al**	**Water-extractable Fe**	**Water-extractable Mn**
R	0.863	0.591	0.082	–0.107	–0.136	–0.167	0.440
p-value	*< 0.001*	*0.003*	*0.709*	*0.626*	*0.537*	*0.445*	*0.036*
Regression equation:	Denitrification capacity = –60.5 + 2.4***soil moisture content*** + 0.1***DOC concentration*** (adjusted $R^2 = 74\%$; *p-value < 0.001*)						

n = 23

3.4 Discussion

The soil type and slope of the farm are intricately linked (i.e. specific soil types occur on specific slope, e.g. Tokomaru soil occurred only on low slope), hence the effect of slope on denitrification was masked in this study, as evidenced by the absence of significant differences in the denitrification capacity of the slope classes. On the other hand, the effect of soil type on denitrification was dominant, mainly due to the soil parent material/composition and not the drainage class *per se*. For instance, a number of studies have reported higher denitrification occurring on poorly-drained soils compared to well-drained soils primarily due to higher moisture retention in the poorly-drained soils which creates anoxic conditions that favour denitrifiers (Gambrell *et al.*, 1975; Schnabel and Stout, 1994; Morales *et al.*, 2015a). In the present study, however, higher

denitrification occurred in the well-drained soils (Well/Medium treatment only) compared to the poorly-drained soils. This is mainly because the Well/Medium treatment contained the Ramiha soil which has a high content of short-range order constituents such as allophane and thus a higher capacity to store C (Dahlgren *et al.*, 2004). The total C (and to some extent the DOC) of the Ramiha soil was 2-3 times higher than that of the other soil types (Table 3.3). This explains why it had a higher denitrification capacity compared to the other soil types. It should be noted that nitrate concentrations of this soil at depth were not significantly larger than those in the other soils studied. Overall, the results imply that the Ramiha soil (and other soils with similar properties) plays an important ecosystem function in terms of nitrate attenuation in landscapes and this should be considered if N loss restrictions are made for hill country farms. It is worth mentioning that denitrification was minimal within the 60-100 cm depth layer of the soils, despite the presence of DOC at this depth, indicating that other factors such as a decrease in the availability of denitrifying microbes and/or other nutrients could have limited denitrification at this soil depth.

Soil sampling was carried out during spring, i.e. when most soils were wet, hence the absence of significant differences in soil moisture within the surface 30 cm of the soil. However, multiple regression analysis showed that soil moisture content was a good predictor of the soil denitrification capacity in this study. This relationship between soil moisture and denitrification highlights the important influence seepage wetlands and hillside seeps (which are prevalent in hill country farms) could have on nitrate attenuation within hill country landscapes and therefore calls for further research to improve understanding of the contribution of seepage wetlands and hillside seeps (soil moisture) to denitrification within hill country farms.

The soil parent material/composition may also explain why greater amounts of water-extractable Al and Fe were observed at depth in the Well/Medium and Imperfect/High treatments, since the Ramiha and Halcombe soils contain tephra deposits. The Aokautere Ash found at depth in the Tokomaru soil may have also contributed to elevated concentrations of water-extractable Al and Fe at depth in the Poor/Low treatment. This higher Fe concentration at depth could indicate that anaerobic organisms (that might support denitrification) are present below the topsoil. It also implies that

reduced Fe could serve as a source of electrons for denitrification in these soils. However, no significant correlation (r = –0.167; *p-value* = 0.445) existed between denitrification capacity and water-extractable Fe in this study. The reason for this lack of significant relationship is not clear, since the highest denitrification capacity was observed in the soil which had tephra as an important component of its parent material (i.e. the Ramiha soil).

Soil nitrate concentration increased with increasing soil depth in the Imperfect/High treatment (Halcombe soil) probably due to its varying parent material which gave rise to varying soil textures ranging from silt loam to sandy and gravelly loams that aid the downward movement of water on this soil (Pollok and McLaughlin, 1986). Given that the Halcombe soil has a low denitrification capacity, nitrate could easily be leached to groundwater on this soil. However, more detailed research would be required to verify this assumption.

It is worth mentioning that the high pH of the Poor/Low treatment is most likely due to the soil management practices on this area of the farm, i.e. the area is usually sown to crops for spring/summer feeding of animals and hence has received higher inputs of lime (and fertiliser) compared to other areas of the farm. Furthermore, the impaired drainage of this treatment may explain, to some extent, its higher pH as there is a smaller loss of base cations. The fact that it is at the bottom of the hill (low slope) might also contribute to the accumulation of base cations in this treatment. Although some studies have shown that complete denitrification is favoured by neutral to basic soil pH (Koskinen and Keeney, 1982; Čuhel *et al.*, 2010), the absence of a significant correlation (r = 0.082; *p-value* = 0.709) between pH and denitrification capacity indicates that pH cannot be used to predict the denitrification capacity of the soils in this study.

The denitrification rates obtained in the current study are lower than those obtained in a similar study (no added C source in DEA assay) on a New Zealand dairy farm (Luo *et al.*, 1998). This is mainly because dairy farms are more intensively managed and thus contain more substrate for denitrification compared to hill country farms. Comparing the present study with most other New Zealand pastoral studies (on both hill country

and dairy farms) would be misleading since most of these studies involved the addition of an artificial C source in the DEA assay (Jha *et al.*, 2012; Rivas *et al.*, 2014a). However the notable reduction in denitrification with increasing soil depth obtained in this study, compares well with the findings of these New Zealand studies.

3.5 Conclusion

Greater denitrification capacity was observed in the well-drained medium slope treatment mainly due to its higher DOC concentration (and moisture content) resulting from the presence of the Ramiha soil. This soil had the highest soil organic C concentrations which is associated with the presence of short-range order constituents. Nitrate concentrations at depth in this soil were also similar to those of the other soils. This indicates that the Ramiha soil could play an important role in nitrate attenuation in farms where they occur and thus should be accounted for if N loss restrictions are introduced for hill country landscapes. However, despite the relatively large amount of DOC found within the 60-100 cm depth layer of the Ramiha soil, the denitrification capacity of this depth layer was negligible. This negligible denitrification could be due to nutrient or microbe limitations, although further investigation would be required to confirm this assumption.

In general, compared to slope, soil type had a greater effect on denitrification in this study, as the effect of slope was masked by the unique pattern of soil distribution within the farm. More detailed research is needed to understand the effect of soil type (with regard to parent material/composition) on denitrification/nitrate leaching within the farm, especially on the Halcombe soil where higher nitrate concentrations were observed at depth.

CHAPTER 4:
A comparison of the nitrate attenuation capacity of hill country wet and dry areas as influenced by dissolved organic carbon concentration and chemistry

Research highlights

- The dissolved organic carbon (DOC) concentration in the surface 30 cm soil depth was in the order: seepage wetland > hillside seep > dry area.
- The denitrification capacity of the seepage wetland within the 0-30 and 30-60 cm soil depths was 7 and 69 times greater, respectively, than that of the dry area.
- The higher moisture and concentration of readily-decomposable (e.g. lower molecular weight) DOC in the seepage wetland soil most likely contributed to its higher denitrification capacity.

4.1 Introduction

Pastoral hill country landscapes occupy more than 60% of New Zealand's agricultural area (Hoogendoorn *et al.*, 2011b), and contribute substantially to the country's export earnings (Morris, 2013). These landscapes contain important naturally occurring wet areas which have the capacity to attenuate excess nitrate before it reaches receiving waters (Clarkson *et al.*, 2013; Rutherford *et al.*, 2018). These natural wet areas include "seepage wetlands" which are flat, boggy areas (approximately < 5000 m^2 in size) that are close to streams and remain wet all year round, and "hillside seeps" which are hillside areas where ground water discharges at the surface and creates wet areas that are usually < 10 m^2 in size.

Hill country seepage wetlands are particularly unique because of their unconsolidated soils and the virtual absence of a channelised flow (Rutherford *et al.*, 2018). These wetlands are usually grazed and thus are sometimes drained by farmers to reduce the risks of animals getting mired (Tanner *et al.*, 2015). In addition, because of their relatively small size, they are rarely identified in wetland inventories, thus there is limited research understanding of the ecosystem services they provide (Uuemaa *et al.*, 2018).

The capacity of seepage wetlands to effectively attenuate nitrate depends on a number of factors such as flow characteristics, concentration of nutrient inputs, soil types, vegetation, and climatic conditions (International Water Association, 2000). Although several mechanisms such as plant uptake, microbial nitrogen (N) immobilisation, dissimilatory nitrate reduction to dinitrogen/nitrous oxide (denitrification) and ammonium (DNRA) are responsible for attenuating nitrate in wetland systems (Matheson *et al.*, 2003; Zaman *et al.*, 2008), denitrification has been identified as the dominant nitrate attenuation process (Hoffmann *et al.*, 2000).

Dissolved organic carbon (DOC) is an important requirement for denitrification in soil-water systems (Saggar *et al.*, 2013). It is abundant in wetlands because their anoxic nature slows down organic matter decomposition which contributes to the accumulation of organic carbon (C) (Nahlik and Fennessy, 2016). Even though DOC may become adsorbed to or precipitated as organo-mineral complexes with iron (Fe), at the redox interfaces of wetlands (Riedel *et al.*, 2013), part of it could become mobilised during the reductive dissociation of Fe oxy-hydroxides under anoxic conditions (Chin *et al.*, 1998; Fiedler and Kalbitz, 2003). It is worth mentioning that organic C accumulation in wetlands also makes them potential sources of methane (Bridgham *et al.*, 2013). However, methane emission from pastoral wetlands is considered insignificant compared to the ecosystem role they play in attenuating nutrients for improved water quality (Wilcock *et al.*, 2008).

Although it has been generally assumed that DOC concentration and denitrification capacity will be higher in seepage wetlands compared to drier areas, no study has measured and quantified this on pastoral hill country farms. A number of studies have investigated how changes in climatic conditions and flow characteristics affect nitrate attenuation in pastoral wetlands (Tanner *et al.*, 2005; Zaman *et al.*, 2008; Uuemaa *et al.*, 2018). However, the specific contribution of DOC concentration and chemistry to seepage wetland denitrification in hill country landscapes has not been well researched. In addition, comparisons between the denitrification capacity of seepage wetlands and adjacent dry areas within hill country farms is absent in literature. An improved understanding of the nitrate attenuation capacity of hill country seepage wetlands (relative to adjacent dry areas), as influenced by soil properties such as DOC, is

necessary to account for and enhance the nitrate attenuation capacity of seepage wetlands. This will result in efficient N management at the farm level. It will also allow hill country farmers a flexible management response to possible regional council nutrient regulations, and ultimately limit the adverse effects of nitrate on water quality.

This study tested the hypothesis that the naturally occurring wet areas in hill country landscapes have higher DOC concentration and denitrification capacity than the surrounding drier areas. It aimed to investigate the role of DOC in the denitrification capacity of pastoral hill country seepage wetlands. The specific objectives of the study were to (i) compare the DOC concentration and chemistry of hill country wet and dry areas; and (ii) determine the differences in the denitrification capacity of these contrasting landscape features, and relate the differences (if any) to soil properties.

4.2 Materials and methods

4.2.1 Study site

The study was carried out at Massey University's Agricultural Experimental Station, Tuapaka, which is a sheep and beef cattle farm located approximately 15 km north-east of Palmerston North, lower North Island, New Zealand (40°21'20.1"S, 175°44'19.6"E) (Figure 4.1). The research area has a humid temperate climate with an annual average rainfall of 1100 mm, and experiences significant soil moisture deficits during summer. The research took place in a 12.75 ha paddock within the farm (Figure 4.1), with slopes ranging from 4 to ~ 35°. This paddock consists of seepage wetlands, hillside seeps, and dry areas (landscape units which do not have any obvious hydrological activity). The dry areas within the paddock are dominated by the Makara soil (New Zealand classification: Typic Orthic Brown Soil), the Korokoro soil (Typic Firm Brown Soil), and the Ramiha soil (Acidic Allophanic Brown Soil). These soils have previously been described by Pollok and McLaughlin (1986). The wet areas (seepage wetland and hillside seep) are dominated by a Gley soil developed from a colluvium of the Makara soil and an alluvium of the Ramiha/Korokoro soils. The vegetation of the paddock is primarily comprised of long-term (> 20 years) browntop (*Agrostis capillaries* L.) and perennial ryegrass (*Lolium perenne* L.), with rushes (*Juncus edgariae* L.) occurring in the wet areas.

4.2.2 Experimental design and sample collection

The experiment was carried out in two phases, both occurring in November 2017 (spring). The landscape features and sampling strategy used for each phase is summarised in Table 4.1. The first phase of the study was designed to determine the degree of "within treatment" variability in the DOC concentration of the selected hill country landscape features (treatments). This information would aid in the design of a subsequent (second phase) more detailed sampling of the landscape features. Three landscape features/treatments (seepage wetland, hillside seep, and dry area – belonging to the Makara soil) were examined in the first phase of the study. Five replicate soil samples were collected from within an area of 1 × 1 m^2 for each treatment (Figure 4.1), using a soil corer of ~ 4 cm in diameter. Each replicate was composed of three soil depths (0-30, 30-60 and 60-100 cm). The samples were kept cool and transported to the laboratory where they were stored at < 4°C and analysed for DOC concentration within three days of sample collection.

Based on the degree of DOC variability obtained in the first phase, the second phase of the study involved more intensive sampling of the three dominant dry area soils (Makara, Korokoro and Ramiha) and the seepage wetland soil. Three different sites (1×1 m^2 area for each site) were sampled for each soil type (Figure 4.1). Soil was sampled again from depths of 0-30, 30-60 and 60-100 cm. A different number of replicates, six (for each seepage wetland site) and twelve (for each dry area site) were collected in the second phase of the study. This was due to the higher 'within site' variability in DOC concentration of the dry area soil sampled in the first phase of the study. The hillside seep was not sampled in the second phase of the study because of the measured similarity in its topsoil DOC concentration to that of the dry area, as observed during the first phase of the study (see the Results section). All soil samples were kept cool and analysed for DOC concentration as with the previous phase.

Comparisons for all other soil properties, including denitrification capacity, were made only between the seepage wetland and the Makara dry area soil (which was closest to the seepage wetland – Figure 4.1) because the different dry area soils within this paddock had comparable DOC concentrations (Table 4.2), which was contrary to what was observed in Chapter 3, where representative soil samples were collected from the

whole farm. For these comparisons, three soil cores were randomly selected from each sampled site of the seepage wetland and Makara dry area soil (from the second phase of the study).

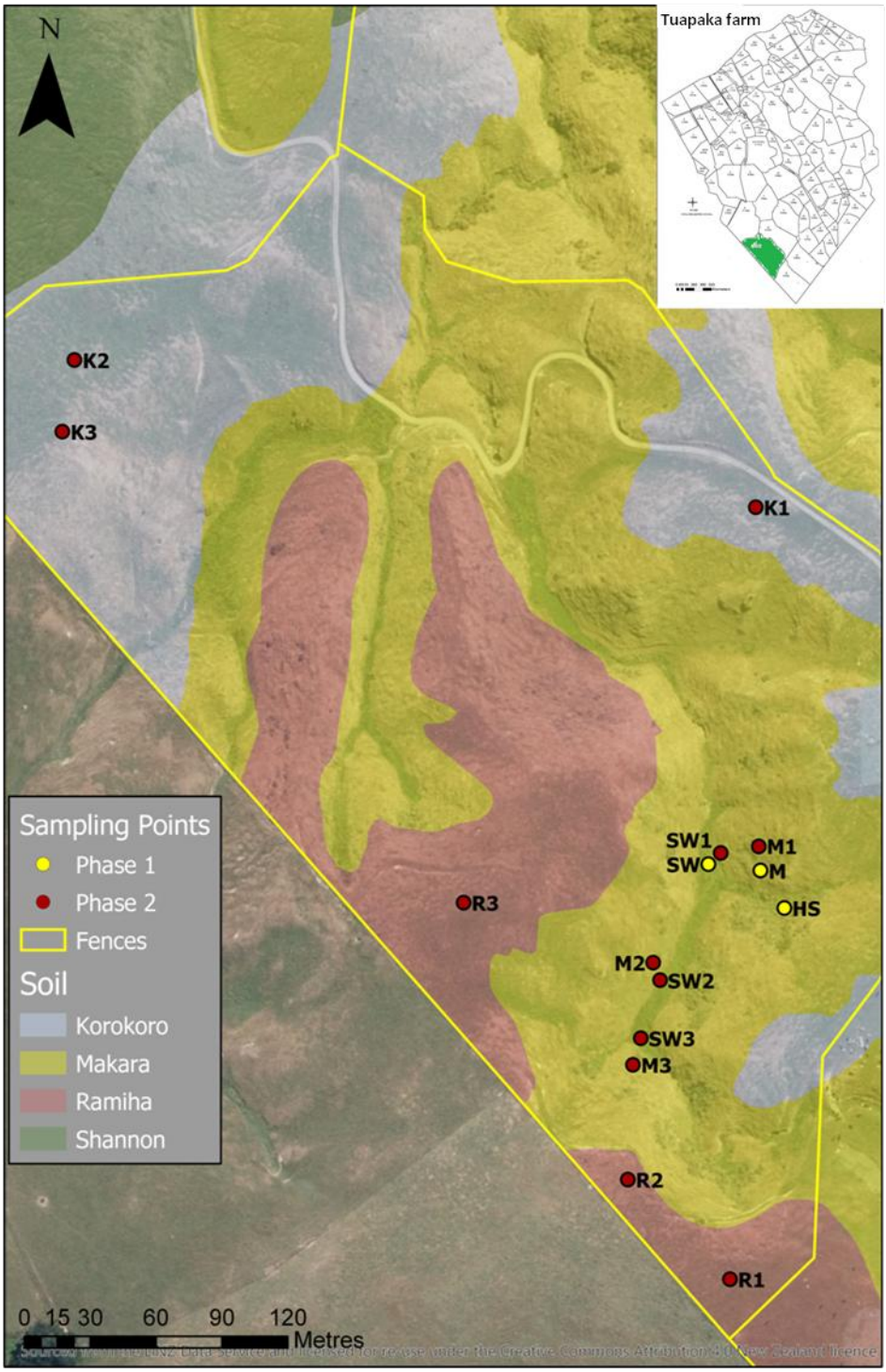

Figure 4.1: Map showing location of the sampled paddock within the farm (green area in insert) and sampling points within the paddock. *K: Korokoro dry area; M: Makara dry area; R: Ramiha dry area; SW: seepage wetland; HS: hillside seep. Numbers represent the different sites for the seepage wetland and each dry area.*

Table 4.1: Summary of sampling strategy for both phases of the experiment

Landscape feature	Soil type	Number of sampling sites (site size = 1 x 1m^2 area)	Number of [δ]replicate samples per site	Number of observations per landscape feature
Phase one				
Seepage wetland	[β]Gley soil	1	5	5
Hillside seep	[β]Gley soil	1	5	5
Dry area	Makara	1	5	5
Phase two				
Seepage wetland	[β]Gley soil	3	6	18
Dry area	Makara	3	12	36
Dry area	Korokoro	3	12	36
Dry area	Ramiha	3	12	36

[δ]Each replicate was composed of 3 soil depths (0-30, 30-60 and 60-100 cm), during each phase of the experiment; [β]a soil developed from a colluvium of Makara soil and an alluvium of Ramiha/Korokoro soils.

4.2.3 Laboratory analyses

Soil sample homogeneity was obtained by manually mixing each sample thoroughly before extraction. Ten grams of the homogenised sample was extracted with 25 mL of deionised water (1:2.5 wt/v), by shaking (for 1 h) in a 50 mL extraction tube, on a rotatory shaker at room temperature. The agitated sample was subsequently centrifuged at 5000 rpm for 2 h (the centrifugation process separated more particulate organic matter than a 0.22 and 0.45 µm filter, as showed on a sub-set of 10 samples). Thereafter, the centrifuged sample was gently decanted and the extract was stored at < 4°C for subsequent analyses.

DOC concentration of the extract was determined with the dry combustion method (section 2.7.3), using a total organic carbon–TOC analyser (Shimadzu TOC-L). In order to have an idea of the nature/complexity of the DOC present in the landscape features, DOC chemistry was assessed using three different indices, namely (i) sUVa index: absorbance measured at 254 nm divided by DOC concentration (mg L^{-1}), (ii) E2/E3 index: ratio of absorbance measured at 250 nm and 365 nm, and (iii) E4/E6 index: ratio of absorbance measured at 465 nm and 665 nm. The sUVa index has been reported to be positively correlated with molecular weight and conjugated unsaturated C systems, such as those in aromatic molecules, while E2/E3 and E4/E6 indices have been reported

to be negatively correlated with molecular weight and aromaticity (Peuravuori and Pihlaja, 1997; Weishaar *et al.*, 2003; Wallage *et al.*, 2006). These indices were determined with a Cintra 202 UV/VIS spectrometer (GBC Scientific Equipment SDS 720) over a spectrum range of 200 to 700 nm.

Denitrification capacity was determined via the denitrification enzyme activity (DEA) assay in the laboratory. This was accomplished with the acetylene inhibition method, using the vacuum pouch incubation technique described by Rivas *et al.* (2014a) with some modifications as follows: twenty grams of fresh soil (dry weight equivalent) was weighed into a polyethylene pouch (10 × 28.5 cm) fitted with a luer-lock valve. Thereafter, the pouch was heat-sealed and vacuumed with a syringe via the luer-lock valve. The vacuumed pouch was then flushed with 50 mL of dinitrogen (N_2), after which 20 mL of acetylene, 20 mL of DEA solution (containing 50 µg NO_3^--N g^{-1} dry soil and 10 mg L^{-1} chloramphenicol) and 180 mL of N_2 were consecutively inserted inside the pouch via the luer-lock valve. Acetylene was added to the solution to arrest the denitrification reaction at the nitrous oxide (N_2O) stage as it is easier to sample and measure N_2O concentrations than N_2, because of low levels of N_2O in the atmosphere. Chloramphenicol served as an enzyme inhibitor to prevent *de novo* synthesis of enzymes during incubation (Dendooven *et al.*, 1994). The pouch/sample was subsequently incubated in the dark at 20°C, on a rotary shaker (160 rpm). Gas samples (25 mL) were collected from the pouch at 2-hour intervals, i.e. 0 (initial gas sample before incubation), 2, 4 and 6 h of incubation. Each gas sample was compressed into a 12 mL vac-vial for subsequent N_2O analysis with a Shimadzu Gas Chromatograph (GC) 17 A (Japan) equipped with a ^{63}Ni electron capture detector, and operating at a column and detector temperature of 55 and 330°C, respectively. The concentration of N_2O in the gas sample was obtained by plotting a calibration curve of N_2O standard gases, which were obtained from serial dilutions of a standard N_2O gas (100 mg L^{-1}). Thereafter, the mass of N_2O in the pouch headspace was calculated as follows:

Mass of N_2O (µg) = concentration of N_2O (µg L^{-1}) from GC

× volume of gas in pouch (L)

× 0.544 Bunsen absorption coefficient

Denitrification capacity was then calculated from the slope of N_2O mass and incubation time, divided by the mass of soil.

Nitrate and ammonium concentrations were determined by continuous flow analysis (Technicon® AutoAnalyser II). The concentrations of water-extractable (total) aluminium (Al), Fe and manganese (Mn) were determined with a 4200 Microwave Plasma-Atomic Emission Spectrometer–MP-AES (Agilent Technologies). The pH and redox potential (E_h) of the saturated soil paste were measured with standard pH and E_h meters (Meter Lab® and Eutech Instruments, respectively).

Electrical conductivity (EC) was determined with a standard EC meter (Hanna Instruments) on a soil/solution (deionised water) ratio of 1:2.5 wt/v. Acid ammonium oxalate extraction was performed on the soil samples as described by Blakemore *et al.* (1987), in order to determine the concentrations of Al, Fe and silicon (Si) in short-range order soil constituents, and Al and Fe in organo-metal complexes. The concentrations of these elements were read with a 4200 MP-AES (Agilent Technologies). The gravimetric soil moisture content was also determined and thus nutrient concentrations were converted to the oven-dry weight of soil.

The laboratory analyses described above were carried out using appropriate quality control protocols, including the use of sample duplicates, blank, reference and spiked samples.

4.2.4 Statistical analyses

Analysis of Variance (ANOVA) with Tukey comparison procedure (p = 0.05) was performed on measured parameters to detect significant differences in treatment means. The relationship between denitrification capacity and other soil properties was compared with Pearson correlation technique (p = 0.10). All statistical analyses were performed with Minitab software (17.2.1 Minitab, Inc.).

4.3 Results

4.3.1 Variations in soil DOC concentration

Phase one: The DOC concentration of the seepage wetland was significantly ($p \leq 0.05$) higher than that of the hillside seep and dry area (Figure 4.2). The DOC concentration of the hill side seep and dry area were similar within the surface 30 cm soil depth, but further down the profile, DOC concentrations were significantly ($p \leq 0.05$) higher in the hillside seep.

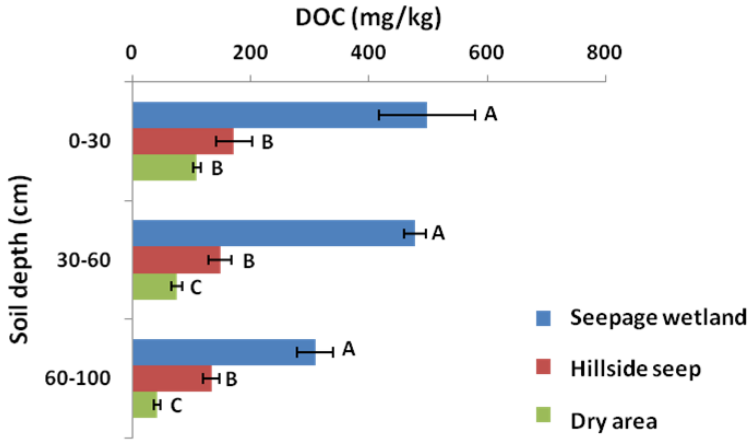

Figure 4.2: DOC concentration of wet areas and Makara dry area at various soil depths. *Different letters indicate significant ($p \leq 0.05$) difference between treatments for a particular soil depth. Error bars are standard error of the mean ($n = 5$). Number of sites examined for each treatment = 1.*

Phase two: The "within site" variability in DOC concentration was generally higher in the dry area soils compared to the seepage wetland soil (Table 4.2). For example, within the surface 30 cm soil depth, coefficient of variation (CV) was 18 - 38% in the dry area soils, but was < 15% in the seepage wetland soil. Conversely, compared to the soils in the dry areas, the seepage wetland soil had the highest "between site" variability in DOC concentration in the surface 30 cm depth (CV was ≤ 21% in the dry areas vs > 50 % in the seepage wetland) (Table 4.2). This "between site" variation was significant ($p \leq 0.05$) for each of the sampled soil types, except the Ramiha soil. When all the soil types were compared, the soils of the different dry areas had similar DOC concentrations (especially in the surface 60 cm depth), and these were significantly ($p \leq 0.05$) lower than that of the seepage wetland soil. Thus, more attention was paid on comparing differences in only the soils of the seepage wetland and Makara dry area, as it was closest to the seepage wetland.

Table 4.2: Variations in DOC concentration (mg kg^{-1}) of soils in the seepage wetland and dry areas within the farm paddock

	Seepage wetland			Makara dry area			Korokoro dry area			Ramiha dry area		
Soil depth (cm)	***Grand mean***	***Within site SD***	****Between site SD***	***Grand mean***	***Within site SD***	****Between site SD***	***Grand mean***	***Within site SD***	****Between site SD***	***Grand mean***	***Within site SD***	***Between site SD***
0-30	389.8^{A}	56.8 (14.6)	219.5 (56.3)	128.3^{B}	22.4 (17.5)	24. 4 (19.0)	82.1^{B}	24.7 (30.1)	17.0 (20.7)	93.8^{B}	36.0 (38.4)	16.8 (17.9)
30-60	313.8^{A}	57.2 (18.2)	72.2 (23.0)	70.4^{B}	25.6 (36.4)	25.1 (35.7)	48.9^{B}	24.0 (49.1)	44.6 (91.2)	61.2^{B}	36.9 (60.3)	12.7 (20.8)
60-100	311.0^{A}	90.9 (29.2)	47.3 (15.2)	41.7^{B}	8.7 (20.9)	13.0 (31.2)	24.6^{BC}	14.2 (57.7)	29.9 (121.5)	16.9^{C}	20.0 (118.3)	4.2 (24.9)

Grand mean: mean of all the replicates from the three sites; SD: standard deviation; * indicates "between site" variation was significant ($p \leq 0.05$) for all the soil depths considered; different letters indicate significant ($p \leq 0.05$) difference between treatments (i.e. seepage wetland and dry areas), for a particular soil depth; values in bracket are the coefficient of variation (CV) in percentage. Number of sites examined for each treatment = 3. Number of observations (n) per site = 6 (for seepage wetland) and 12 (for dry area).

Table 4.3 shows the key soil chemical properties of the seepage wetland and Makara dry area at different soil depths. The seepage wetland soil had lower amounts of short-range order constituents, especially at depth, compared to the Makara dry area soil, while the Makara dry area soil displayed proto-andic properties, i.e. 12 g kg^{-1} < Al_o + ½Fe_o < 20 g kg^{-1} (IUSS Working Group WRB, 2015). The seepage wetland soil had a greater EC (1:2.5 aqueous soil suspension) than the Makara dry area soil.

Table 4.3: Electrical conductivity (EC) and properties associated with short-range order constituents in the seepage wetland and Makara dry area soils

Treatment	Soil depth (cm)	EC (µS cm^{-1})	Al_o (g kg^{-1})	Fe_o (g kg^{-1})	Si_o (g kg^{-1})	Al_o + ½ Fe_o (g kg^{-1})
Seepage wetland	0-30	151.63	6.15	8.61	1.52	10.46
	30-60	103.37	4.18	8.99	1.23	8.68
	60-100	95.73	4.15	7.56	2.16	7.93
Dry area	0-30	25.71	7.75	6.88	6.39	11.19
	30-60	17.76	9.27	8.79	5.54	13.67
	60-100	14.27	6.27	7.78	7.71	10.16

Subscript o: acid ammonium oxalate extracts.

4.3.2 Variations in soil DOC chemistry

Molecular weight and the presence of conjugated unsaturated C compounds (i.e. aromatic C) in DOC increased with increasing soil depth (Table 4.4). All DOC chemistry indices suggest a smaller molecular size and, most likely, less aromatic C in the seepage wetland soil, these differences being significant ($p \leq 0.05$) for sUVa and E2/E3.

Table 4.4: DOC chemistry indices of the seepage wetland and Makara dry area soils

Soil depth (cm)	Treatment	sUVa	E2/E3	E4/E6
0-30	Seepage wetland	0.02^{B} ± 0.0008	3.30^{A} ± 0.08	3.45^{A} ± 0.17
	Dry area	0.05^{A} ± 0.0057	3.18^{A} ± 0.15	3.31^{A} ± 0.29
30-60	Seepage wetland	0.02^{B} ± 0.0007	3.34^{A} ± 0.52	3.69^{A} ± 0.17
	Dry area	0.09^{A} ± 0.0010	2.93^{B} ± 0.11	3.25^{A} ± 0.30
60-100	Seepage wetland	0.03^{B} ± 0.0028	3.44^{A} ± 0.07	3.80^{A} ± 0.37
	Dry area	0.12^{A} ± 0.0227	2.86^{B} ± 0.09	3.07^{A} ± 0.21

Values are means ± standard error of the mean (n = 9); different letters indicate significant ($p \leq 0.05$) difference between treatments for a particular index and soil depth.

4.3.3 Denitrification capacity and properties of the water-extracted soil

Both the seepage wetland and dry area had decreasing denitrification capacity with increasing soil depth (Figure 4.3a). The denitrification capacity of the seepage wetland within the topsoil (0-30 cm depth) and subsoil (30-60 cm depth) layers was 7 and 69 times higher, respectively, than that of the dry area. Further down the soil profile (60-100 cm), however, there were no significant ($p \leq 0.05$) differences in the denitrification capacity of the seepage wetland and dry area soils. In addition, the denitrification capacity of the seepage wetland decreased by 94% compared to that in the surface 30 cm depth.

The nitrate concentrations of the seepage wetland and dry area soils were similar; however, the seepage wetland had significantly ($p \leq 0.05$) higher ammonium concentration compared to the dry area (Figures 4.3b and c). Significant ($p \leq 0.05$) differences were also absent in the water-extractable Al, Fe and Mn concentrations of the two soil types considered (Figures 4.3d-f). However, water-extractable Al concentrations were consistently higher in the dry area soil, while water-extractable Mn concentrations were consistently higher in the seepage wetland soil.

The mean pH values of the seepage wetland vs dry area within the 0-30, 30-60 and 60-100 cm soil depth were, 6.1 vs 6.0, 6.3 vs 5.7 and 6.6 vs 5.6, respectively (Figure 4.4a). Significant ($p \leq 0.05$) differences in pH of the two soils occurred only within the 30-100 cm depth. No significant ($p \leq 0.05$) difference was observed in the E_h of the seepage wetland and dry area soils (Figure 4.4b). However, consistently lower values were observed in the seepage wetland soil.

The "between site" variation in the moisture content of the seepage wetland ranged from 14 to 44%, and soil moisture was significantly ($p \leq 0.05$) higher in the seepage wetland compared to the dry area (Figure 4.4c).

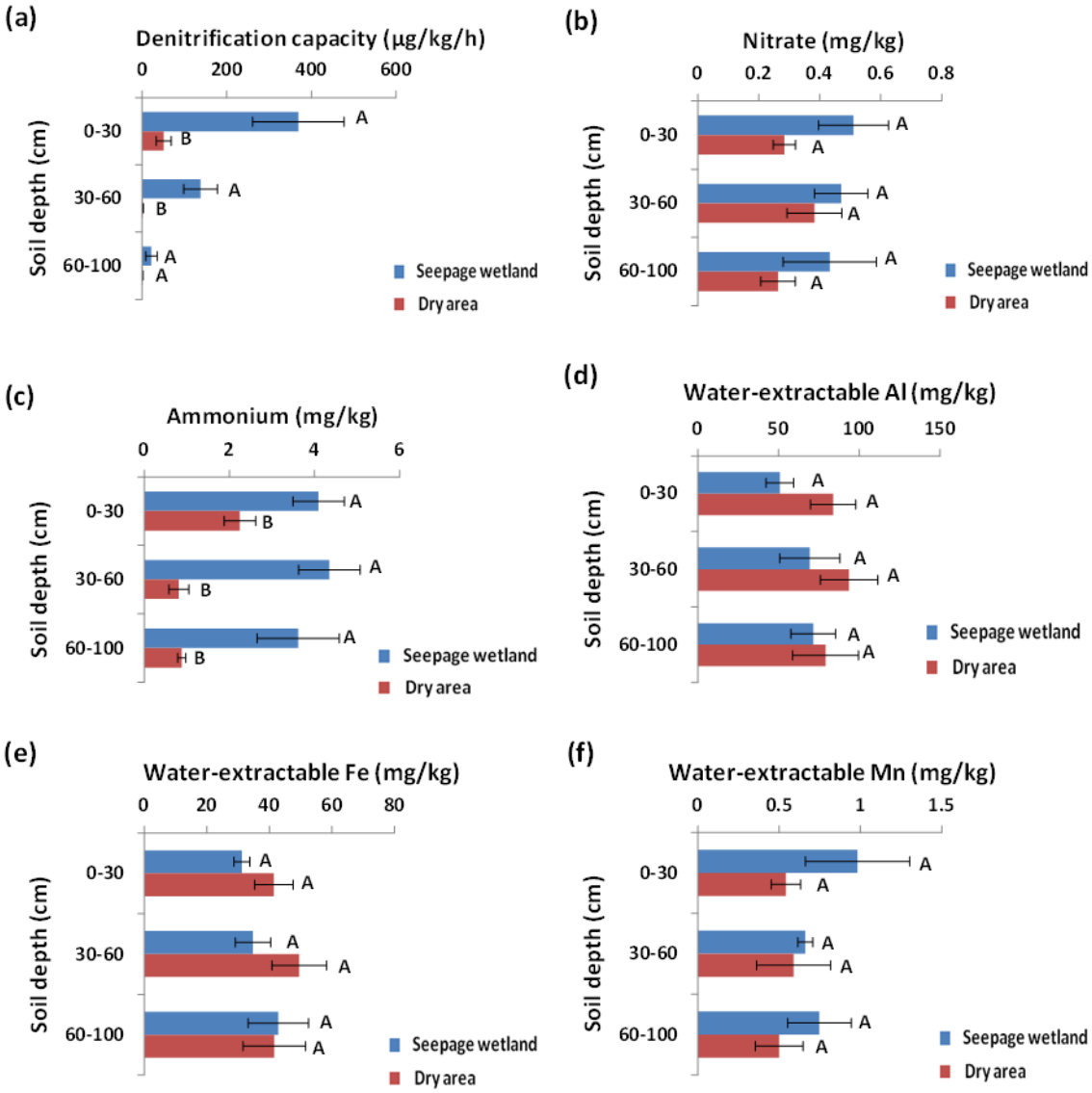

Figure 4.3: Variations in (a) denitrification capacity, and concentrations of (b) nitrate, (c) ammonium, (d) water-extractable Al, (e) water-extractable Fe, and (f) water-extractable Mn of the seepage wetland and Makara dry area. *Different letters indicate significant ($p \leq 0.05$) difference between treatments for a particular soil depth. Error bars are standard error of the mean ($n = 9$).*

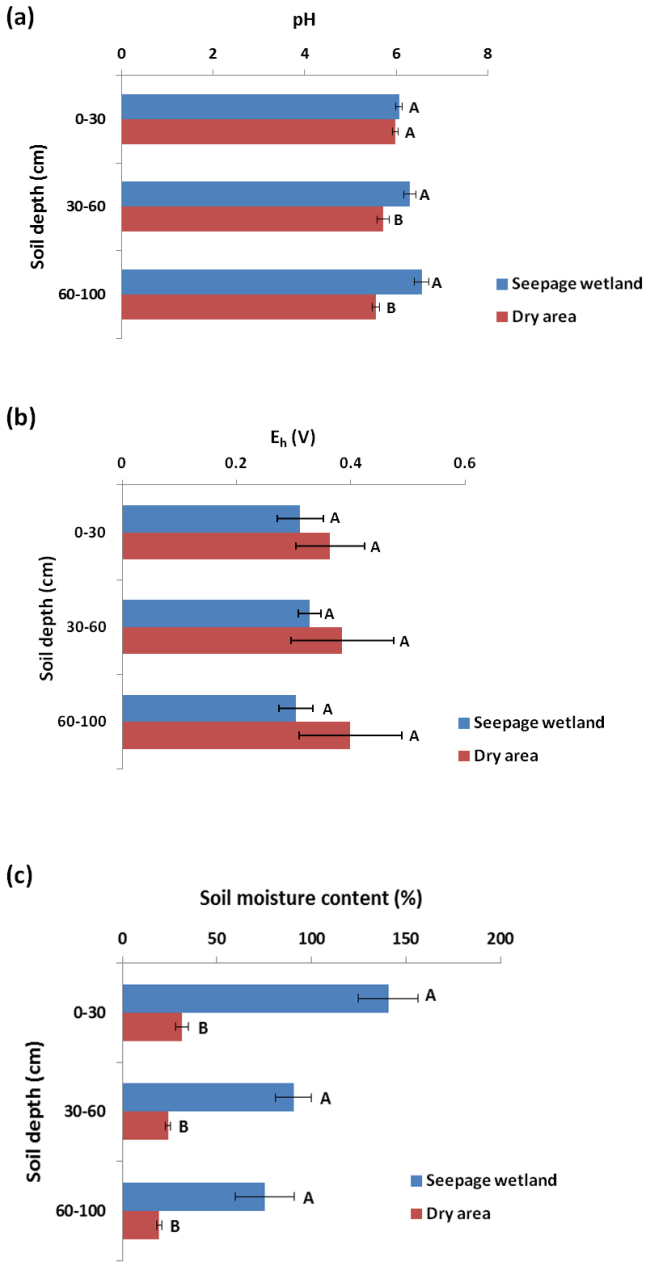

Figure 4.4: Variations in (a) pH (b) E_h, and (c) gravimetric soil moisture content of the seepage wetland and Makara dry area. *Different letters indicate significant ($p \leq 0.05$) difference between treatments for a particular soil depth. Error bars are standard error of the mean ($n = 9$).*

Pearson correlation analysis showed significant ($p \leq 0.10$) correlations between denitrification capacity and the following soil properties of the seepage wetland and Makara dry area: DOC concentration and chemistry, soil moisture, ammonium, water-extractable Al and Mn (Table 4.5). These correlations were positive for DOC concentration, soil moisture, ammonium and water-extractable Mn, and negative for DOC chemistry (abundance of conjugated unsaturated C compounds and large molecular size) and water-extractable Al.

Table 4.5: Pearson correlation analysis between denitrification capacity and some measured soil properties of the seepage wetland and Makara dry area

Correlation results	**DOC concentration**	**DOC chemistry (sUVa)**	**Soil moisture**	**pH**	**E_h**	**Nitrate**	**Ammonium**	**Water-extractable Al**	**Water-extractable Fe**	**Water-extractable Mn**
r	0.273	–0.332	0.513	0.111	–0.126	–0.075	0.267	–0.353	–0.230	0.544
p-value	*0.070*	*0.026*	*0.001*	*0.467*	*0.409*	*0.624*	*0.076*	*0.017*	*0.129*	*0.001*

n = 45

4.4 Discussion

4.4.1 Denitrification capacity as a function of soil moisture and DOC

The higher soil moisture of the seepage wetland strongly influenced its denitrification capacity, since moisture supports denitrification by creating anoxic conditions and increasing the availability of DOC and nitrate in soils (Luo *et al.*, 1999b; Saggar *et al.*, 2013). The relatively high variation (14-44%) in soil moisture content observed at the seepage wetland sites, in addition to the spatial variation in deposition of dung and urine, may have contributed to the high "between site" variation in DOC concentration recorded at the seepage wetland sites, as vegetation was the same at all the sampled sites. This implies that spatially different seepage wetland sites within a farm might require unique management practices (that may involve maintaining or improving their water-logged conditions) to maintain or enhance their ability to denitrify and hence reduce the transport of nitrate from pastoral hill country farms to receiving waters. Fencing (rather than draining) of seepage wetlands to prevent/limit stock access is one of such practices that would maximise the nitrate attenuation capacity of these important pastoral hill country landscape features. Given that seepage wetlands are flat areas, fencing them to limit stock access would not reduce nutrient input substantially, as they receive runoff and hence nutrient input from the surrounding farm catchment.

In addition to soil moisture, DOC concentration and chemistry were two parameters that significantly influenced the denitrification capacity of the soils in this study. The higher concentration of DOC in the seepage wetland compared to the hillside seep and the Makara dry area was attributed to limited organic matter decomposition in the seepage wetlands which contributed to the accumulation of organic C (Nahlik and Fennessy, 2016). Furthermore, limited adsorption of dissolved organic matter in the seepage wetland soil, due to the lower amounts of short-range order constituents, could have also contributed to its higher DOC concentration. The DOC in the seepage wetland was shown to have lower molecular weight and conjugated unsaturated C compounds (lower sUVa). Therefore, since a lower sUVa has been associated with increased bioavailability (Austnes *et al.*, 2010), the observed results suggest that a high proportion of DOC present in the seepage wetland soil was readily available to support denitrification in the soil-water system.

The reduction in the denitrification capacity of the seepage wetland with increasing soil depth is expected because substrate (DOC) availability also decreased with increasing depth. However, it is worth mentioning that with increasing soil depth, there is typically a reduction in vertical mixing of water (which promotes the transport of nitrate to denitrifying microbes), hence this may hinder denitrification at depth, even in the presence of substrate (Rutherford *et al.*, 2018). Thus, even though laboratory incubation measurements of denitrification (DEA assays) in soil may show that denitrification occur at depth, actual field measurements may produce different results for seepage wetlands.

Most studies on denitrification in wetlands involve the addition of an artificial C source in the DEA assay (Cooke *et al.*, 1990; Zaman *et al.*, 2008; Gardner and White, 2010; Genthner *et al.*, 2013), thus are not comparable to this study. However, a comparison with soil sampled from a New Zealand dairy farm under the same experimental conditions, i.e. no added C source in DEA assay on soils sampled during spring (Luo, 1996; 1998), showed that the denitrification rate of the hill country seepage wetland soil examined in the current study was ~ 3.5 times higher than that in the poorly-drained soil of the dairy farm (369 vs ~104 $\mu g^{-1} kg^{-1} h^{-1}$). This result is interesting as dairy farms are more intensively managed and thus have denser pasture and higher stocking rate. Therefore, they are likely to have more DOC from plant materials, as well as from dung and urine deposition (Williams and Haynes, 1990; Ghani *et al.*, 2011). Soil compaction associated with higher stocking rate could also lead to the release of C occluded within soil aggregates (Barnett *et al.*, 2014). Conversely, when comparisons were made between the dry area in the current study and the dairy farm, the reverse was the case, i.e. lower denitrification rates were observed in the dry area soil compared to the soil in the dairy farm (51 $\mu g^{-1} kg^{-1} h^{-1}$ vs ~104 $\mu g^{-1} kg^{-1} h^{-1}$). These comparisons highlight the potential contribution of hill country seepage wetlands to nitrate attenuation for improved water quality. In order for this knowledge to be applied to an on-farm context, adequate mapping of hill country farms would be required to identify where seepage wetlands occur, relative to the drier areas. More information (*in situ* farm measurements) would also be needed, including stream flow and nitrate concentrations into and out of the seepage wetland.

4.4.2 Other possible factors affecting nitrate attenuation

The significant correlation between denitrification capacity and water-extractable Mn suggests that Mn could play an important role in nitrate reduction in the studied soils. Studies have shown that under anoxic conditions, nitrate could act as an oxidant of Mn^{2+} resulting in a significant reduction of nitrate to N_2 (Luther *et al.*, 1997; Pyzola, 2013). More research is needed to understand this avenue of nitrate reduction, especially in anoxic soils of seepage wetlands.

The higher pH of the seepage wetland soil compared to that of the dry area soil is related to the fact that reducing conditions tend towards neutrality because reducing reactions consume protons. On the other hand, oxidative reactions (e.g. nitrification), which are generators of acidity, are impaired under anoxic conditions. In addition, these seepage wetland sites receive all runoff from the surrounding areas within the farm and thus a higher proportion of lime (aerially applied every few years for farm management purposes) may end up washing into the seepage wetland. Given that hydromorphic conditions also affect weathering, leaching, degree of base saturation, and changes in the speciation of metals in the soil (Grybos *et al.*, 2007), it is likely that the more neutral condition observed in the seepage wetland slowed the weathering of primary minerals and decreased the solubility of Al in aqueous solution (Sparks, 2003). This may explain the lower water-extractable Al concentration of the seepage wetland soil compared to the dry area soil. In addition, the higher ionic strength of the seepage wetland soil (as inferred from EC measurements) may have resulted from reduced leaching; thus, favouring crystallisation as opposed to the formation of short-range order compounds (Sparks, 2003). However, despite the reducing conditions observed in the seepage wetland soil, there was no significant correlation between denitrification capacity and water-extractable Fe in this study. This suggests that Fe^{2+} may not be a source of electrons for denitrification on the sampled soils.

The ammonium concentration of the seepage wetland was higher than that of the dry area, and this is, to some extent, attributed to weak nitrification, given that nitrifiers are strict aerobes. This higher ammonium concentration of the seepage wetland could also suggest the occurrence of DNRA, since this is favoured by strictly anoxic condition (in some sites/soil cores which were saturated), high pH (compared to the dry area), and

large amounts of readily-oxidisable organic matter (Cooke *et al.*, 2008). The labile C (DOC)/nitrate ratio of the seepage wetland was > 600 and thus clearly exceeded the suggested threshold of > 12 for significant DNRA occurrence in soil (Yin *et al.*, 1998; Rütting *et al.*, 2011). However, further research is needed to establish the sources and processes of elevated levels of ammonium in the seepage wetlands, and also to ascertain the contribution of DNRA (and other processes such as plant uptake) to nitrate attenuation in hill country seepage wetlands.

4.5 Conclusion

This study has demonstrated that seepage wetlands in pastoral hill country landscapes have a high capacity to attenuate nitrate, compared to surrounding dry areas, and hence can play an important ecosystem role of decreasing the amount of nitrate reaching receiving waters – a role that should be valued and enhanced. Therefore, this important role of hill country seepage wetland should be accounted for if N loss restrictions are introduced to hill country farms in the future.

The large amounts of readily-available DOC and moisture in the seepage wetland soil were important factors that most likely enhanced its denitrification capacity. However, due to spatial variations in soil moisture and deposition of animal dung and urine, various seepage wetlands within a farm may differ in their denitrification capacity and thus unique management practices may be required to maintain/enhance the nitrate attenuation capacity of these seepage wetlands. It is important that strategies that preserve hill country seepage wetlands, such as fencing to prevent/limit stock access, be promoted and adopted on hill country farms rather than the draining of these areas which reduces moisture and substrate availability for denitrification.

In order to aid in the proper understanding and management of hill country seepage wetlands for targeted and effective water quality management, further research is needed in the following areas: (i) factors that influence the spatial variation in DOC concentration and denitrification capacity across seepage wetland sites, (ii) planned seasonal *in situ* measurements of the denitrification capacity of hill country seepage wetlands, (iii) other pathways of nitrate attenuation besides denitrification (e.g. Mn

oxidation, DNRA, and plant uptake) in these seepage wetlands, and (iv) monitoring of streamflow and nitrate/ammonium concentrations into and out of the seepage wetland areas.

CHAPTER 5:
Short-term effect of forage crop establishment on the dissolved organic carbon dynamics in a pastoral hill country soil

Research highlights

- The agrochemicals used for clearing out pasture before forage crop establishment increased the dissolved organic carbon (DOC) concentration of only the top 5 cm soil depth on days 1and 6 after application.
- Proposed mechanisms for the increase in DOC concentration include direct carbon (C) contribution from the agrochemicals, displacement of adsorbed organic molecules, and/or decomposition of root necromass.
- The agrochemicals also enhanced nitrogen (N) mineralisation within the top 5 cm soil depth possibly due to the decomposition of the agrochemicals themselves.

Peer-reviewed publication from this chapter:
Chibuike, G., Burkitt, L., Camps-Arbestain, M., Bishop, P., Bretherton, M. & Singh, R. (2019). Effect of forage crop establishment on dissolved organic carbon dynamics and leaching in a hill country soil. *Soil Use and Management*. https://doi.org/10.1111/sum.12497

5.1 Introduction

Hill country landscapes, which support sheep and beef cattle production, occupy more than 60% of New Zealand's agricultural area (Hoogendoorn *et al.*, 2011a). Due to the reduced growth of pasture during winter (June to August) compared to spring/early summer (September to December), New Zealand hill country farmers often replace perennial pasture with forage crops on selected areas to supplement animal feed production. Increased agricultural intensification on New Zealand's hill country farms is also anticipated as the Government's Business Growth Agenda aims to significantly increase the value of Primary Industry exports by 2025 (MPI, 2018). Therefore, practices that increase animal feed production (such as the replacement of perennial pasture with forage crops) are being rapidly adopted in New Zealand (Houlbrooke *et al.*, 2009; Burkitt *et al.*, 2017). However, agricultural intensification in hill country with

its unique features and complexity (steep slopes, fragile soils, and presence of grazing animals) could result in increased losses of nutrients to water bodies, thus significantly reducing water quality (Ledgard and Hughes, 2012). Runoff and leaching of farm-sourced nutrients (particularly nitrate) in water bodies is an issue of increasing concern both in New Zealand and abroad (Puckett, 1995; Davies-Colley, 2013).

Denitrification of nitrate to dinitrogen (N_2) is the most important nitrate attenuation process occurring in the subsurface environment (Rivett *et al.*, 2008), and dissolved organic carbon (DOC) has been identified as the most important factor influencing denitrification in the subsurface environment (Peterson *et al.*, 2013). Leaching of DOC and its availability for subsurface denitrification is influenced by various edaphic, environmental, and management factors (Kalbitz *et al.*, 2000). For instance, land use change from pasture to cropping could decrease the amount of DOC bioavailable for subsurface denitrification, though this could vary with soil type (Ghani *et al.*, 2007). The composition/complexity of DOC could also be altered by practices which increase the presence of more complex forms of carbon (C), and this may reduce the amount of DOC available for denitrification (Engelhaupt and Bianchi, 2001). Understanding how the replacement of perennial pasture with forage crop influences DOC dynamics and subsurface denitrification in hill country is important in order to recommend adequate management options that reduce nitrate loss and its impact on water quality.

The aerial spray and surface sowing technique (comparable to herbicide use and "no tillage" system) is a common method of crop establishment in steep, uncultivable hill country in New Zealand. This technique allows large areas of hill country to be converted from perennial pasture to forage crop within a short time frame. However, the effect of this particular method of crop establishment on soil DOC dynamics is yet to be studied, despite the importance of this factor on denitrification for improved water quality. A recent study on a flat dairy pasture soil (McNally *et al.*, 2017), where C input to soil upon herbicide application during pasture renewal (involving light tillage) was investigated, reported a rapid turnover of root material during the first 11 days of the study, compared to the non-herbicide treatment. The present study, therefore, tested the hypothesis that the application of herbicides (and other agrochemicals) prior to the establishment of a forage crop on a hill country soil, would lead to a short-term (12

days) change in the quantity and quality of DOC, a key substrate which influences denitrification in the soil profile.

The objective of the study was to examine whether the concentration and chemistry of soil DOC were affected by the agrochemicals used for clearing out pasture before crop establishment.

5.2 Materials and methods

5.2.1 Study site

The study was carried out on Massey University's Agricultural Experiment Station, Tuapaka, a hill country farm used for beef cattle and sheep production. It is situated approximately 15 km north-east of Palmerston North, New Zealand (40°21'20.1"S, 175°44'19.6"E). The research area has a humid temperate climate and is about 320 m above sea level. It has an average annual rainfall of 1100 mm, with predominantly dry summers (Massey University, 2016).

The soil in the study site was developed from a colluvium of Ramiha silt loam soil (New Zealand classification: Allophanic Brown soil), with minor incorporation of Makara steepland soil (Orthic Brown soil). The soil is described as Typic Eutrudept in the USDA Soil Taxonomy (Soil Survey Staff, 2014).

5.2.2 Experimental design and sample collection

The two treatments considered were (a) cropping, and (b) pasture, each replicated four times. They were arranged in a Randomised Complete Block Design (RCBD), with a plot size of 4 × 4 m^2 area for each replicate. These plots were fenced out to prevent animal access during the experiment. Historically, all the plots contained long-term (>20 years) browntop (*Agrostis capillaries* L.) and perennial ryegrass (*Lolium perenne* L.). In November 2015, the plots to be cropped were sprayed-out using a mixture of selected agrochemicals including: two herbicides, glyphosate (active ingredient) at 4 L ha^{-1} and dicamba (active ingredient) at 400 mL ha^{-1}; an insecticide, diazinon (active

ingredient) at 400 mL ha^{-1}; and a penetrant, organomodified polydimethyl siloxane (active ingredient) which was added to the mixture at 250 mL ha^{-1} to improve the rapid uptake of the applied herbicides by the pasture.

Four days after the application of agrochemicals, swede (*Brassica napobrassica* Mill.) was sown onto the surface of the sprayed plots at 2.5 kg ha^{-1}. The seeds were broadcasted by hand and then pushed through the pasture thatch using one pass of a tractor, driven over the plots. A fertiliser formulation (NPKS, 18:20:0:1) and a "slug and snail" bait, methiocarb (active ingredient) were applied at the time of sowing at a rate of 250 kg ha^{-1} and 5 kg ha^{-1}, respectively. The fertiliser and "slug and snail" bait were also applied to the pasture plots at the same application rates and hence are not part of the selected agrochemicals referred to in this study.

Three replicate soil cores (core diameter ~ 4 cm) were collected from each plot at depths of 0-5, 5-10, 10-20, 20-30, 30-40, 40-60, 60-80, and 80-100 cm during four sampling periods, i.e. on day 0 (immediately prior to the application of agrochemicals) and days 1, 6, and 12 thereafter. Recent urine patches from grazing animals were avoided during sampling by measuring the conductivity of each sampling point with a portable conductivity meter, before sample collection. Soil samples were kept cool and transported to the laboratory where they were stored at < 4°C and analysed for DOC within five days of sampling.

5.2.3 Calculation of soil water balance

On a daily basis, total rainfall, average air temperature, average relative humidity, total solar radiation, and total wind run were monitored at the experimental site. This climatic data, in addition to depth of root zone (400 mm), slope (15°) and aspect (north-facing), were used as input parameters to model the soil water balance at the site using the FAO56 version of Penman-Monteith equation (Allen *et al.*, 1998), with slope and aspect corrections for incoming solar radiation (Revfeim *et al.*, 1982). This showed that in November 2015, 90% of rainfall was lost through evapotranspiration, while 10% was lost via drainage, i.e. percolation (Figure 5.1). Drainage only reached a depth of 40 cm in November 2015 due to the amount of precipitation (65 mm) over this period, thus

comparisons between the two treatments focused on the surface 40 cm soil depth. At depths below 40 cm, data from both treatments were collated and the corresponding mean values were only considered when trends over time were evaluated.

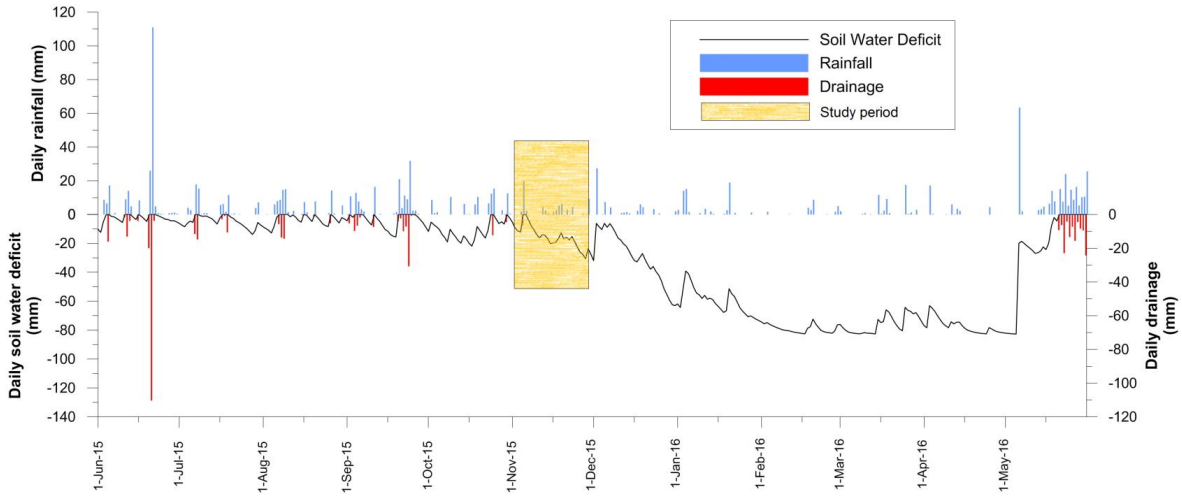

Figure 5.1: Soil water balance at the study site from 2015 to 2016

5.2.4 Laboratory analyses

Soil homogeneity was achieved by passing field-moist soils through a 4 mm sieve. Ten grams of the sieved soil was then shaken for 1 h with 25 mL of deionised water (1: 2.5 wt/v) in a 50 mL extraction tube on a rotatory shaker, at room temperature. The agitated sample was subsequently centrifuged at 5000 rpm (20°C) for 10 min and filtered with Whatman No 41 filter paper. Thereafter, the sample was centrifuged at 5000 rpm (20°C) for 2 h. The second centrifugation was shown to separate most of the particulate organic matter from the extract, compared to a 0.22 µm filter (as determined in a subset of 10 samples). The centrifuged sample (water extract) was decanted and used for subsequent analyses.

DOC concentration in the extract was determined with the dichromate semi-micro method described by Hejzlar and Kopacek (1990), with some modifications as follows: 1 mL of the centrifuged sample was mixed with 1 mL of digestion solution (900 mg L^{-1} $K_2Cr_2O_7$, 7.2 g L^{-1} $KCr(SO_4)_2.12H_2O$ and 46 g L^{-1} $HgSO_4$ in 37.3% m/v H_2SO_4) and 5 mL of $AgSO_4$ in H_2SO_4 solution in a 16 x 150 mm test-tube fitted with a heat resistant cap. This mixture was digested in a thermostatically controlled block at 150°C for 2 h.

After digestion and subsequent cooling at room temperature, 4 mL of deionised water was added to the tube, mixed thoroughly, and allowed to cool before the absorbance was read using a Philips PU 8625 UV/VIS spectrophotometer (Biolab Scientific Limited), at a wavelength of 455 nm. A calibration curve was plotted using potassium hydrogen phthalate (KHP) as the standard, and DOC concentration in the extract was then calculated from this curve.

DOC chemistry of the extract was assessed with four indices: (i) sUVa: absorbance measured at 254 nm divided by DOC concentration (mg L^{-1}); (ii) E2/E3: ratio of absorbance measured at 250 nm and 365 nm; (iii) E4/E6 index: ratio of absorbance measured at 465 nm and 665 nm; and (iv) DOC/DON: ratio of DOC and dissolved organic nitrogen. The DOC/DON and sUVa indices have been reported to positively correlate with conjugated unsaturated C systems (such as those in aromatic molecules) and molecular weight, while E2/E3 and E4/E6 have been reported to negatively correlate with aromaticity and molecular weight (Peuravuori and Pihlaja, 1997; Weishaar *et al.*, 2003; Wallage *et al.*, 2006; Austnes *et al.*, 2010). The absorbance at different wavelengths was determined with a Cintra 202 UV/VIS spectrometer (GBC Scientific Equipment SDS 720) over a spectrum range of 200 to 800 nm. DON was calculated as the difference between total dissolved nitrogen (TDN) and nitrate (ammonium concentrations were < 0.1mg kg^{-1} and thus considered negligible). TDN was measured by the persulphate digestion method as described by Hill (2006). Nitrate in the extract was determined by continuous flow analysis (Technicon® AutoAnalyser II).

The pH of the extract was measured with a standard pH meter (Meter Lab®), while the concentration of water-extractable (total) aluminium (Al), iron (Fe) and manganese (Mn) was determined using a 4200 MP-AES (Agilent Technologies). Total C and nitrogen (N) in the soil samples were determined using the elementar® (vario MICRO cube). Sodium pyrophosphate and acid ammonium oxalate extractions of Fe and Al (and also silicon–Si in ammonium oxalate) were carried out as described by Blakemore *et al.* (1987), and Fe, Al, and Si concentrations were determined using the above-mentioned MP-AES. The concentration of organic C in the sodium pyrophosphate extracts was determined using a modification of the semi-automated dichromate method

described by O'Dell (1993), in which the absorbance (of the digested samples) was read with a spectrophotometer, at a wavelength of 660 nm, using potassium hydrogen phthalate (KHP) standards to produce a calibration curve. The gravimetric soil moisture content was also determined and nutrient concentrations were corrected to the oven-dry weight of soil.

All laboratory analyses were carried out using appropriate quality control protocols, including the use of sample duplicates, blank, reference and spiked samples.

5.2.5 Statistical analyses

Analysis of Variance (ANOVA) was performed to detect significant differences between treatment means. This analysis was performed using General Linear Model (GLM) taking into account the repeated measures design of the experiment. Comparisons were made using the Tukey comparison procedure, at 95% confidence level. The relationship between DOC concentration and measured soil parameters was determined using the Pearson correlation technique. All analyses were performed using Minitab statistical software (17.2.1 Minitab, Inc.).

5.3 Results

5.3.1 Soil characterisation

The characterisation of the soil at day 0, i.e. immediately prior to the application of agrochemicals (Table 5.1) shows that it is slightly acidic (6.1 - 6.5), with a high content of organic C (~ 160 g kg^{-1} [105 t ha^{-1}] and 43 g kg^{-1} [123 t ha^{-1}] in the top 30 cm and 30-100 cm soil depth, respectively). The C/N ratio ranges between 9.3 and 11.6. As expected, total C, N and sodium pyrophosphate-extractable C (C_p) tend to decrease with soil depth, with C_p being ~ 1/3 of total C at the surface, with an increasing trend of this ratio down the soil profile. The Al_p/C_p molar ratio also increases with depth, from 0.1 to > 0.3. In addition, the Al_p/Al_o molar ratio is generally > 0.6, while the $Al_o + ½Fe_o$ of the topsoil is ≤ 12 g kg^{-1}.

Prior to the application of agrochemicals, there were generally no significant ($p \leq 0.05$) differences within plots for most of the soil properties (Table 5.1).

Table 5.1: Selected soil chemical properties

Soil depth (cm)	pH$^{\delta}$	Total C (g/kg)	Total N (g/kg)	C_p (g/kg)	Al_p (g/kg)	Fe_p (g/kg)	Al_o (g/kg)	Fe_o (g/kg)	Si_o (g/kg)	Total C/Total N	C_p/Total C	Al_p/C_p	$(Al_p+Fe_p)/C_p$	Al_p/Al_o	$Al_o+½Fe_o$ (g/kg)
Cropping															
0-5	6.4	62.7	5.4	17.5	4.4	5.6	5.1	5.4	1.0	11.6	0.3	0.1	0.2	0.9	7.8
5-10	6.3	47.8	4.3	15.7	4.7	6.1	4.5	5.3	0.6	11.1	0.3	0.1	0.2	1.0	7.2
10-20	6.2	33.5	3.1	11.7	4.6	6.1	3.5	4.5	0.5	10.8	0.3	0.2	0.3	1.3	5.8
20-30	6.3	22.9	2.2	10.1	3.9	5.3	4.5	5.2	0.4	10.4	0.4	0.1	0.3	0.9	7.1
30-40	6.3	16.2	1.5	7.4	3.3	3.8	4.4	5.7	0.6	10.8	0.5	0.2	0.3	0.8	7.3
40-60	6.3	11.6	1.1	6.5	2.9	3.3*	5.2	5.4	1.3	10.5	0.6	0.2	0.3	0.6	7.9
60-80	6.3	8.1	0.8	5.2	2.7	3.4	7.4	5.0	3.3	10.1	0.6	0.2	0.4	0.4	9.9
80-100	6.2	6.6	0.6	3.1	3.4	4.3	5.9	5.0	2.4	11.0	0.5	0.5	0.8	0.6	8.4
Pasture															
0-5	6.5	56.5	5.1	15.5	3.4	4.3	6.9	6.3	1.9	11.1	0.3	0.1	0.2	0.5	10.1
5-10	6.3	44.1	3.9	13.8	4.1	4.9	3.8	5.0	0.3	11.3	0.3	0.1	0.2	1.1	6.3
10-20	6.2	31.4	3.0	11.5	4.3	5.1	4.2	4.4	0.3	10.5	0.4	0.2	0.3	1.0	6.4
20-30	6.2	21.2	2.1	9.3	4.7	5.7	4.7	5.5	0.4	10.1	0.4	0.2	0.4	1.0	7.5
30-40	6.1	15.3	1.5	6.9	4.8	6.0	5.2	5.1	0.4	10.2	0.5	0.3	0.5	0.9	7.8
40-60	6.2	10.4	1.1	6.0	4.1	5.7	5.7	5.6	0.7	9.5	0.6	0.3	0.5	0.7	8.5
60-80	6.3	8.4	0.9	5.5	3.6	4.4	6.6	5.8	1.1	9.3	0.7	0.3	0.5	0.5	9.5
80-100	6.2	8.8	0.9	4.5	3.3	3.8	7.9	7.0	2.2	9.8	0.5	0.3	0.5	0.4	11.4

δ: pH of water extracts on day 0 (before the application of agrochemicals); subscript p: sodium pyrophosphate extracts; subscript o: acid ammonium oxalate extracts; Al_p/C_p, $(Al_p+Fe_p)/C_p$ and Al_p/Al_o: molar ratios; * indicates significant ($p \leq 0.05$) difference between land uses/treatments for a particular soil depth (n = 12).

5.3.2 Variations in DOC concentration and chemistry

DOC concentration generally decreased with soil depth for both treatments (Figure 5.2). However, it did not correlate well with both total C and C_p (r = 0.4, and p = 0.06, for both parameters). In the surface 30 cm depth, DOC contributed to ~ 0.2% of total C, whereas within 30-100 cm depth, its contribution increased to ~ 0.5% of total C.

On days 1 and 6, the agrochemical treatment (cropping) had a significantly ($p < 0.05$) higher DOC concentration in the surface 5 cm (~ 20 mg kg^{-1} greater on both days). At lower soil depths, the effect of the agrochemicals was not obvious, with no significant differences between treatments. Significant ($p < 0.05$) differences in sampling periods were also observed at different soil depths, with day 1 having the highest mean DOC concentration in most cases.

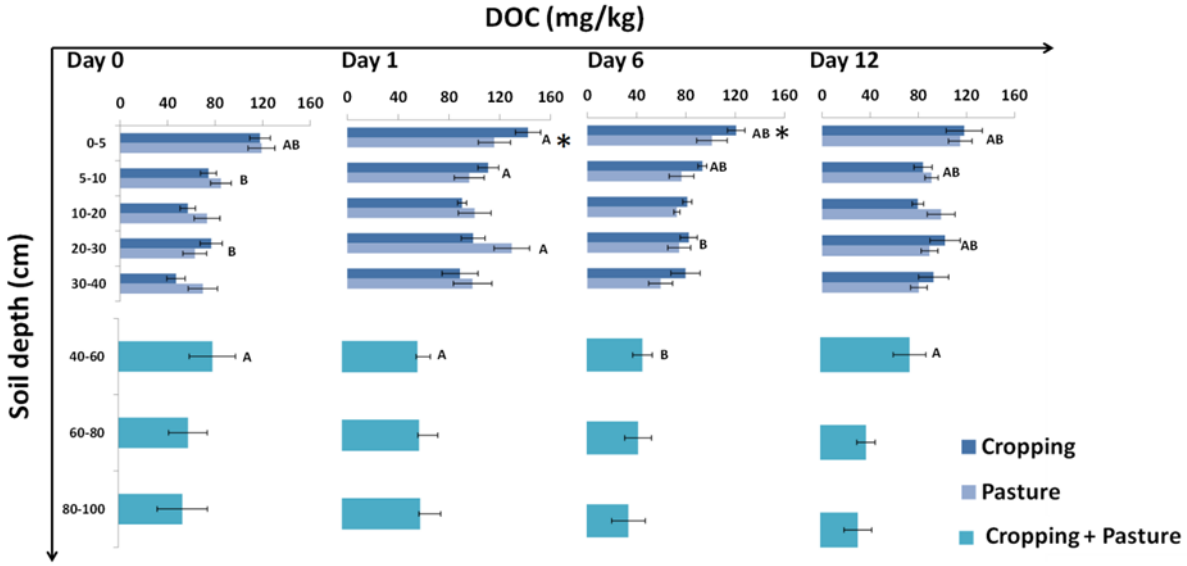

Figure 5.2: Changes in DOC concentration of the cropping (agrochemical) and pasture (non-agrochemical) treatments. *Day 0: before the application of agrochemicals; Day 1, 6 and 12: days after the application of agrochemicals; * indicates significant difference between land uses/treatments; different letters indicate significant difference between sampling periods for a particular soil depth ($p \leq 0.05$). Error bars are standard error of the mean (n = 12).*

No significant ($p \leq 0.05$) difference was observed between the agrochemical and non-agrochemical treatments for all the indices of DOC chemistry considered (Table 5.2). However, some trends, i.e. increasing presence of conjugated unsaturated C compounds (aromatic C) with depth were detected for sUVa and E2/E3 in both treatments.

Table 5.2: DOC quality parameters showing variations within depth of soil water percolation

Soil depth (cm)	sUVa				E2/E3				E4/E6				DOC/DON			
	Day 0	Day 1	Day 6	Day 12	Day 0	Day 1	Day 6	Day 12	Day 0	Day 1	Day 6	Day 12	Day 0	Day 1	Day 6	Day 12
Cropping																
0-5	0.003(35)^{C}	0.003(64)^{B}	0.002(20)^{C}	0.002(27)^{B}	4.4(15)^{A}	5.7(39)^{A}	6.1(23)^{AB}	5.9(10)^{AB}	3.1(59)^{AB}	1.0(110)^{A}	0.7(17)^{A}	1.1(94)^{A}	10.2(46)^{A}	18.4(72)^{A}	10.6(46)^{A}	10.6(42)^{A}
5-10	0.003(54)^{C}	0.003(27)^{B}	0.003(18)^{C}	0.003(22)^{B}	4.5(13)^{A}	5.9(18)^{A}	7.1(27)^{A}	6.0(26)^{A}	2.6(48)^{B}	1.0(147)^{A}	0.7(11)^{A}	1.1(140)^{A}	8.6(45)^{A}	14.4(56)^{A}	10.5(29)^{A}	13.6(19)^{A}
10-20	0.007(50)^{B}	0.004(62)^{B}	0.004(21)^{B}	0.004(45)^{AB}	4.0(10)^{AB}	4.9(32)^{A}	5.8(23)^{AB}	5.4(28)^{AB}	3.8(46)^{AB}	1.7(158)^{A}	0.6(23)^{A}	1.2(173)^{A}	8.7(57)^{A}	12.1(29)^{A}	10.3(35)^{A}	13.7(20)^{A}
20-30	0.008(46)^{B}	0.005(51)^{AB}	0.006(26)^{B}	0.005(42)^{AB}	3.9(13)^{AB}	4.8(20)^{A}	4.4(19)^{B}	4.2(10)^{B}	4.1(45)^{A}	0.4(38)^{B}	0.4(42)^{A}	2.0(171)^{A}	9.5(58)^{A}	15.9(52)^{A}	12.4(28)^{A}	17.0(39)^{A}
30-40	0.014(81)^{A}	0.007(41)^{AB}	0.010(28)^{A}	0.007(43)^{AB}	3.7(10)^{AB}	4.3(19)^{A}	4.4(20)^{B}	4.0(12)^{B}	4.6(49)^{A}	2.2(162)^{A}	0.4(60)^{A}	3.5(154)^{A}	7.2(63)^{A}	15.5(39)^{A}	12.7(37)^{A}	17.0(19)^{A}
Pasture																
0-5	0.003(41)^{B}	0.003(50)^{B}	0.002(35)^{B}	0.003(144)^{B}	4.6(11)^{AB}	6.1(38)^{AB}	7.6(23)^{A}	5.4(36)^{A}	4.0(50)^{A}	1.7(118)^{A}	0.7(35)^{A}	1.1(112)^{A}	13.2(42)^{A}	11.2(23)^{A}	11.1(48)^{A}	13.8(60)^{A}
5-10	0.004(63)^{B}	0.003(39)^{B}	0.003(33)^{B}	0.003(13)^{B}	4.9(17)^{AB}	7.1(24)^{A}	6.9(24)^{AB}	5.4(13)^{AB}	3.3(37)^{AB}	0.5(67)^{A}	0.7(15)^{A}	0.7(169)^{AB}	16.1(31)^{A}	11.5(31)^{A}	8.3(48)^{A}	12.5(20)^{A}
10-20	0.007(54)^{AB}	0.004(50)^{B}	0.005(44)^{AB}	0.004(50)^{AB}	4.3(14)^{AB}	5.3(21)^{AB}	5.4(24)^{ABC}	4.4(16)^{ABC}	4.4(45)^{A}	0.5(86)^{A}	0.6(36)^{A}	0.8(142)^{AB}	12.9(28)^{A}	13.7(46)^{A}	8.2(21)^{A}	17.2(47)^{A}
20-30	0.011(57)^{A}	0.007(65)^{AB}	0.010(41)^{A}	0.005(50)^{AB}	3.8(8)^{B}	4.3(24)^{B}	4.5(18)^{C}	4.1(9)^{BC}	4.2(71)^{A}	0.5(84)^{A}	0.5(30)^{A}	2.5(137)^{A}	13.1(12)^{A}	16.7(36)^{A}	8.8(30)^{A}	15.8(22)^{A}
30-40	0.011(81)^{A}	0.010(48)^{AB}	0.012(60)^{A}	0.010(51)^{A}	3.8(13)^{B}	4.3(14)^{B}	4.3(16)^{C}	3.9(9)^{C}	4.0(54)^{A}	0.2(77)^{A}	0.5(58)^{A}	4.2(137)^{A}	10.0(40)^{A}	12.1(49)^{A}	9.8(38)^{A}	19.3(63)^{A}

sUVa: ratio of absorbance at 254 nm and DOC concentration; E2: absorbance at 250 nm; E3: absorbance at 365 nm; E4: absorbance at 465 nm; E6: absorbance at 665 nm. sUVa and DOC/DON are directly related to molecular weight and conjugated unsaturated C systems (such as those in aromatic molecules), while E2/E3 and E4/E6 are inversely related to molecular weight and aromaticity. Day 0: before the application of agrochemicals; Day 1, 6 and 12: days after the application of agrochemicals. Different letters denote significant difference ($p \leq 0.05$) in soil depths for each land use/treatment. No significant difference between treatments was observed. Values in bracket are the coefficient of variation in percentage (n = 12).

Gravimetric soil moisture generally decreased with increasing soil depth (Table 5.3). There was no significant ($p \leq 0.05$) difference in the gravimetric soil moisture content of the agrochemical (cropping) and non-agrochemical treatments (pasture) during all the sampling period (and at all the soil depth) considered. The different sampling periods also had similar gravimetric soil moisture content.

Table 5.3: Changes in gravimetric soil moisture content

Soil depth (cm)	Land use (Treatment)	Day 0	Day 1	Day 6	Day 12
0-5	C	53.77 ± 0.01	54.05 ± 0.02	59.84 ± 0.02	59.40 ± 0.02
	P	51.12 ± 0.02	54.40 ± 0.02	55.22 ± 0.02	54.59 ± 0.02
5-10	C	43.50 ± 0.01	46.93 ± 0.04	46.00 ± 0.01	47.07 ± 0.02
	P	40.80 ± 0.01	43.30 ± 0.01	45.82 ± 0.02	43.57 ± 0.01
10-20	C	35.71 ± 0.01	35.70 ± 0.01	36.47 ± 0.01	37.35 ± 0.01
	P	32.71 ± 0.01	35.69 ± 0.01	38.65 ± 0.02	36.70 ± 0.02
20-30	C	31.93 ± 0.01	32.55 ± 0.01	32.27 ± 0.01	32.70 ± 0.01
	P	30.58 ± 0.01	33.10 ± 0.03	32.06 ± 0.01	31.69 ± 0.01
30-40	C	30.56 ± 0.01	31.52 ± 0.01	30.04 ± 0.01	31.30 ± 0.01
	P	30.12 ± 0.01	31.21 ± 0.01	31.05 ± 0.01	30.67 ± 0.01
40-60	C + P	31.82 ± 0.01	32.27 ± 0.01	31.93 ± 0.01	32.23 ± 0.01
60-80	C + P	33.74 ± 0.02	35.17 ± 0.02	35.31 ± 0.02	36.59 ± 0.03
80-100	C + P	32.92 ± 0.03	36.15 ± 0.02	37.43 ± 0.03	34.74 ± 0.03

Day 0: before the application of agrochemicals; Day 1, 6 and 12: days after the application of agrochemicals; C: cropping; P: pasture. Numbers represent means ± standard error of the mean (n = 12). No significant ($p \leq 0.05$) difference was observed between land uses/treatments, and between sampling periods, for a particular soil depth.

5.3.3 Changes in properties of water-extracted soil

After the application of agrochemicals, there was little change in the pH of the water-extracted soil (Table 5.4) with significant ($p \leq 0.05$) differences between treatments being observed only within the 5-10 cm depth on day 1. In this instance, the

agrochemical treatment (cropping) showed significantly higher mean pH than the non-agrochemical treatment, i.e. pasture (6.4 vs. 6.2, respectively).

Table 5.4: Changes in pH of water-extracted soil

Soil depth (cm)	Land use (Treatment)	Day 0	Day 1	Day 6	Day 12
0-5	C	6.4 ± 0.03	6.6 ± 0.06	6.5 ± 0.06	6.4 ± 0.07
	P	6.5 ± 0.05	6.5 ± 0.07	6.5 ± 0.07	6.5 ± 0.06
5-10	C	6.3 ± 0.04	6.4 ± 0.05*	6.4 ± 0.04	6.3 ± 0.06
	P	6.3 ± 0.05	6.2 ± 0.05	6.3 ± 0.04	6.4 ± 0.05
10-20	C	6.2 ± 0.04	6.3 ± 0.03	6.3 ± 0.05	6.2 ± 0.04
	P	6.2 ± 0.06	6.2 ± 0.05	6.3 ± 0.05	6.3 ± 0.04
20-30	C	6.3 ± 0.05	6.3 ± 0.03	6.2 ± 0.04	6.3 ± 0.04
	P	6.2 ± 0.05	6.1 ± 0.05	6.2 ± 0.03	6.3 ± 0.05
30-40	C	6.3 ± 0.05	6.2 ± 0.05	6.3 ± 0.04	6.1 ± 0.05
	P	6.1 ± 0.08	6.1 ± 0.05	6.1 ± 0.06	6.1 ± 0.06
40-60	C + P	6.3 ± 0.05	6.2 ± 0.06	6.3 ± 0.06	6.2 ± 0.06
60-80	C + P	6.3 ± 0.07	6.2 ± 0.07	6.3 ± 0.07	6.2 ± 0.07
80-100	C + P	6.2 ± 0.07	6.1 ± 0.06	6.2 ± 0.08	6.2 ± 0.06

Day 0: before the application of agrochemicals; Day 1, 6 and 12: days after the application of agrochemicals; C: cropping; P: pasture. Numbers represent means ± standard error of the mean (n = 12). * denotes significant difference ($p \leq 0.05$) between land uses/treatments for a particular soil depth. No significant difference was observed between sampling periods.

The nitrate concentration of the water-extracted soil ranged from 0.1 to 11 mg kg^{-1} (Figure 5.3). Its concentration in the surface 5 cm depth for both treatments progressively increased on days 6 and 12, and significant ($p \leq 0.05$) differences between treatments were only detected within this soil depth, with the cropping treatment showing higher concentrations of nitrate than the pasture treatment especially on day 12 (11 vs 4 mg kg^{-1}, respectively). However, there was also a marked decline in the concentration of nitrate with increasing soil depth, for both treatments on days 6 and 12 after the application of agrochemicals.

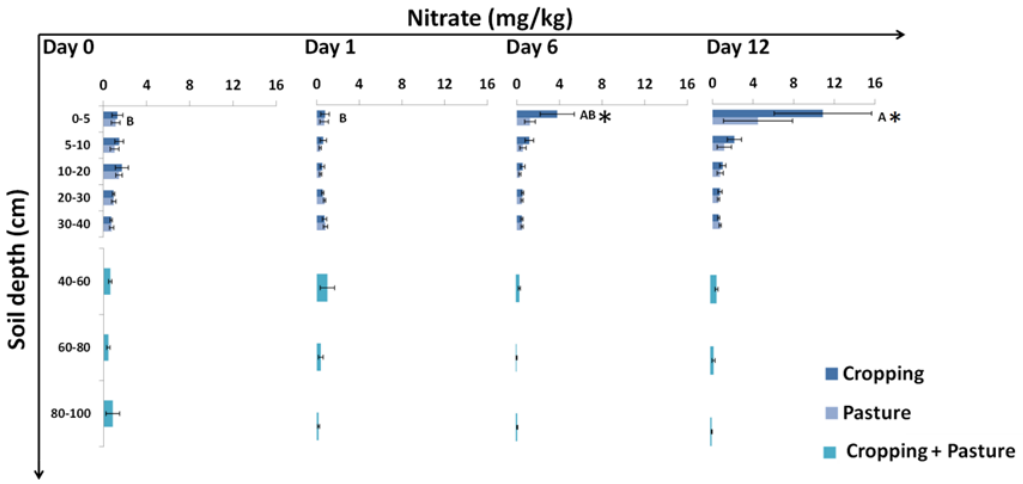

Figure 5.3: Changes in nitrate concentration of the water-extracted soil from the cropping (agrochemical) and pasture (non-agrochemical) treatments. *Day 0: before the application of agrochemicals; Day 1, 6 and 12: days after the application of agrochemicals; * indicates significant difference between land uses/treatments; different letters indicate significant difference between sampling periods for a particular soil depth ($p \leq 0.05$). Error bars are standard error of the mean (n = 12).*

The [δ]DOC/nitrate molar ratio of the cropping treatment was significantly ($p \leq 0.05$) higher than that of the pasture treatment within the surface 5 cm depth on day 1 (Figure 5.4). Within this soil depth (0-5 cm), significant ($p \leq 0.05$) differences in the DOC/nitrate molar ratio of the sampling periods were also observed, with day 1 showing higher mean DOC/nitrate molar ratio compared to the other sampling periods.

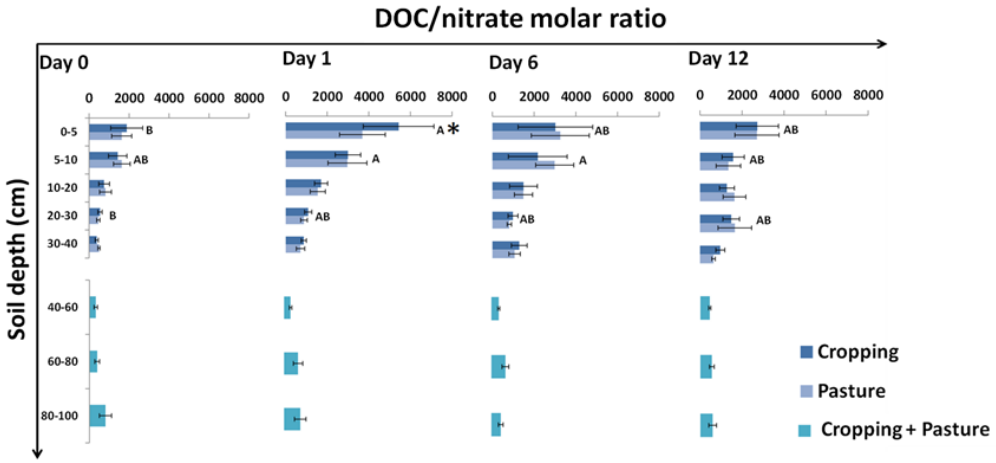

Figure 5.4: Changes in DOC/nitrate molar ratio of the water-extracted soil from the cropping (agrochemical) and pasture (non-agrochemical) treatments. *Day 0: before the application of agrochemicals; Day 1, 6 and 12: days after the application of agrochemicals; * indicates significant difference between land uses/treatments; different letters indicate significant difference between sampling periods for a particular soil depth ($p \leq 0.05$). Error bars are standard error of the mean (n = 12).*

[δ]The DOC/nitrate molar ratio is an index used to assess a system's ability to reduce nitrate via denitrification (Xu *et al.*, 2015). A higher DOC/nitrate molar ratio suggests a higher denitrification capacity.

Water-extractable Al and Fe generally increased with increasing depth and peaked within the 30-60 cm depth (from ≤ 30 mg kg^{-1} in the surface 10 cm depth to > 60 mg kg^{-1} within the 30-60 cm depth), before decreasing with deeper soil depths ≤ 30 mg kg^{-1} within the 80-100 cm depth) (Figures 5.5 and 5.6). Significant differences between treatments were recorded on day 6 in the top 5 cm (for water-extractable Al and Fe) and also 10 cm (for water-extractable Al), with the cropping treatment having higher values compared to the pasture treatment.

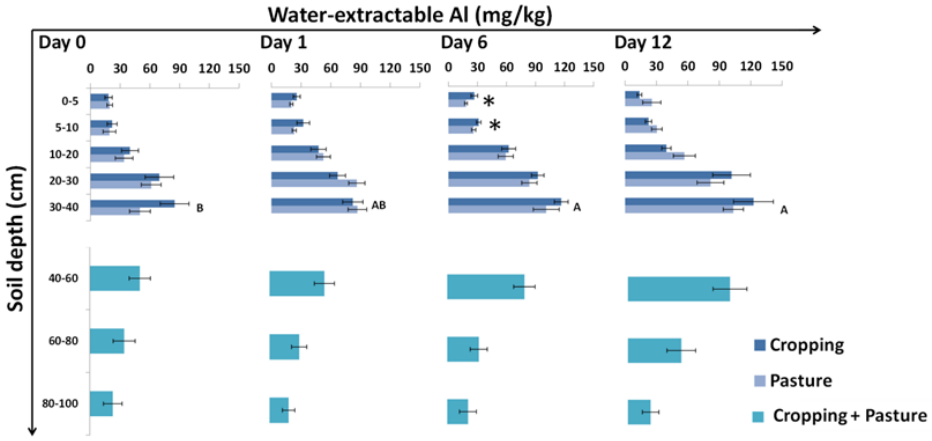

Figure 5.5: Changes in water-extractable Al concentration of the cropping (agrochemical) and pasture (non-agrochemical) treatments. *Day 0: before the application of agrochemicals; Day 1, 6 and 12: days after the application of agrochemicals; * indicates significant difference between land uses/treatments; different letters indicate significant difference between sampling periods for a particular soil depth (p ≤ 0.05). Error bars are standard error of the mean (n = 12).*

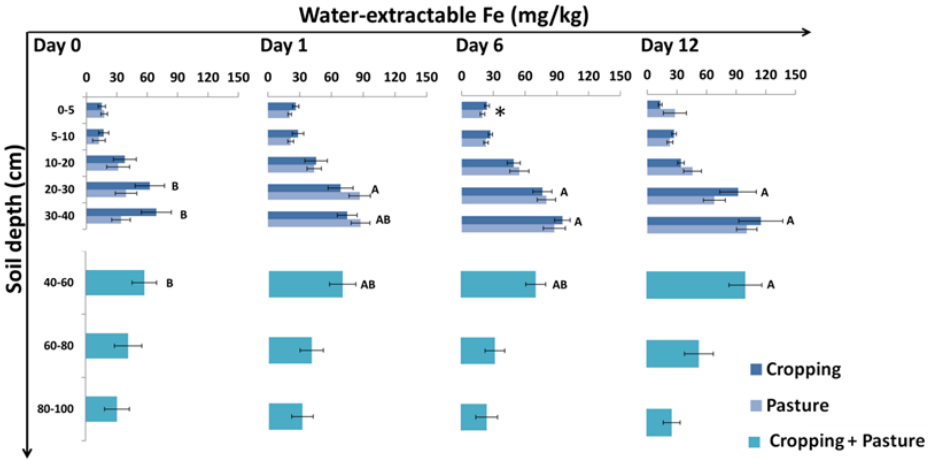

Figure 5.6: Changes in water-extractable Fe concentration of the cropping (agrochemical) and pasture (non-agrochemical) treatments. *Day 0: before the application of agrochemicals; Day 1, 6 and 12: days after the application of agrochemicals; * indicates significant difference between land uses/treatments; different letters indicate significant difference between sampling periods for a particular soil depth (p ≤ 0.05). Error bars are standard error of the mean (n = 12).*

Compared to water-extractable Al and Fe, the amount of water-extractable Mn was generally low, ranging from 0.1 to 1.6 mg kg^{-1} (Figure 5.7). However, as with water-extractable Al and Fe on day 6, water-extractable Mn concentration in the 0-5 cm (and 10-20 cm) depth under cropping was significantly ($p \leq 0.05$) higher than that under pasture.

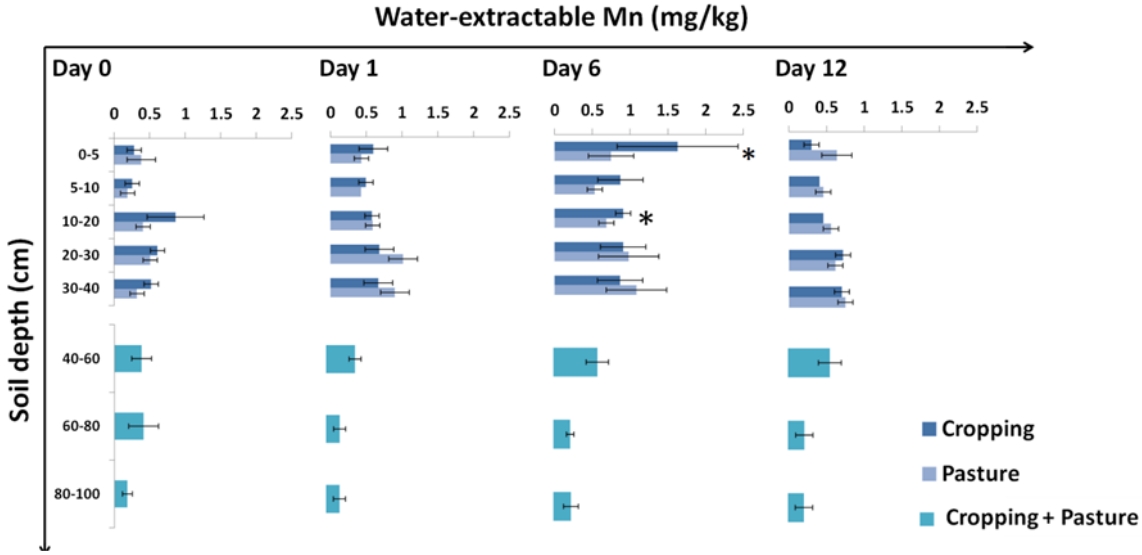

Figure 5.7: Changes in water-extractable Mn concentration of the cropping (agrochemical) and pasture (non-agrochemical) treatments. *Day 0: before the application of agrochemicals; Day 1, 6 and 12: days after the application of agrochemicals; * indicates significant difference between land uses/treatments ($p \leq 0.05$). No significant difference between sampling periods. Error bars are standard error of the mean (n = 12).*

5.3.4 Relationship between DOC concentration and properties of water-extracted soil

Significant ($p \leq 0.05$) negative correlations were found between DOC concentration and pH on day 1 and 12, though these correlations were very weak (r = 0.2) (Table 5.5). Conversely, significant ($p \leq 0.05$) positive correlations were observed between DOC concentration and the following properties: nitrate, water-extractable Al, Fe, and Mn. These correlations were weak for nitrate ($r \leq 0.3$), and moderately strong for water-extractable Al, Fe and Mn (r generally ranged from 0.4 to 0.6).

Table 5.5: Pearson correlation coefficients (r) and *p-values* for correlations between DOC concentration and selected properties of the water-extracted soil of both the cropping and pasture treatments

Sampling Period	Correlation result	pH	Nitrate	Water-extractable Al	Water-extractable Fe	Water-extractable Mn
Day 0	r	−0.115	0.323	0.546	0.519	0.401
(n=190)	*p-value*	*0.116*	*0.001*	*0.001*	*0.001*	*0.001*
Day 1	r	−0.210	0.142	0.451	0.434	0.379
(n=192)	*p-value*	*0.003*	*0.050*	*0.001*	*0.001*	*0.001*
Day 6	r	−0.102	0.220	0.570	0.522	0.421
(n=192)	*p-value*	*0.159*	*0.002*	*0.001*	*0.001*	*0.001*
Day 12	r	−0.203	0.089	0.617	0.640	0.698
(n=192)	*p-value*	*0.005*	*0.221*	*0.001*	*0.001*	*0.001*
ALL	r	−0.165	0.186	0.521	0.515	0.441
(n=766)	*p-value*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*

Day 0: before the application of agrochemicals; Days 1, 6, and 12: days after the application of agrochemicals; ALL: includes all sampling periods.

5.4 Discussion

5.4.1 Description of soil properties

The soil in the study area does not have proto-andic properties, as the $Al_o + ½Fe_o$ of the topsoil is < 12 g kg^{-1} (IUSS Working Group WRB, 2015). Its C/N ratio (9.3-11.6) reflects the dominance of decomposed organic matter, while its Al_p/Al_o molar ratio (generally > 0.6) indicates the predominance of Al ions complexed with organic matter, compared to the presence of short-range ordered Al inorganic constituents. In addition, the increase in its Al_p/C_p molar ratio with increasing soil depth (from 0.1 to > 0.3) reflects the possible increasing presence of Al hydroxide polymers to which organic ligands are adsorbed with depth – as opposed to Al precipitated with organic ligands (Camps Arbestain *et al.*, 2003), although further information would be needed to support this.

The absence of significant differences within plots for most soil properties (observed prior to the application of agrochemicals) suggests that any change occurring during the study period would unlikely be attributable to any intrinsic variability across the site.

However, burrowing activities of earthworms, expressed as distinct tunnels of dark coloured soils, were found in some soil cores; thus earthworms may play an important role in the localised spatial distribution of C within the soil profile (Shuster *et al.*, 2001).

5.4.2 Changes in the chemical composition of water-extracted topsoil after the application of agrochemicals

Although spraying an existing pasture sward with agrochemicals is expected to result in C input to soil as pasture roots die and decompose, the timing and degree of this effect is unknown. In the present short-term study, significant differences in DOC concentrations between the non-agrochemical and agrochemical treatments were only detected in the surface 5 cm depth on days 1 and 6 (increasing by ~ 20 mg kg^{-1} i.e. ca. 14 kg ha^{-1}). These differences were paralleled by significant increases in water-extractable Al, Fe and Mn on day 6, but not on day 1. Different mechanisms are hereby proposed to have influenced DOC on these two days and these are described below.

The additional DOC detected in the agrochemical treatment on day 1 is unlikely to be as a result of decomposing plant litter because, although plant growth stops within hours of herbicide application, perennial plant cells begin to show signs of lysis within 3-20 days of herbicide application (Grossmann, 2010; Henderson *et al.*, 2010). In the present study, signs of lysis (complete dying of the roots) were detected four days after herbicide application. Conversely, the agrochemicals could have, to some extent, directly contributed to DOC, since the organic C input from glyphosate, diazinon and dicamba were 14.4, 5.7 and 3.8 kg ha^{-1}, respectively, totalling 23.9 kg ha^{-1}. Given that the DOC concentration of the agrochemical treatment was ca. 58 kg ha^{-1} in the surface 5 cm depth on day 1, it is also possible that glyphosate and diazinon, via their phosphate functional group, could have become adsorbed to soil reactive surfaces (Arienzo *et al.*, 1994; Gimsing and Borggaard, 2002), and displaced adsorbed organic molecules, hence indirectly contributing to an increase in DOC. Similarly, the occurrence of ligand exchange of hydroxyl at surface sites with the phosphate group could have occurred, and this might explain the slight increase in the pH of the topsoil on day 1.

On day 6, the significantly greater DOC concentration in the agrochemical treatment (50 kg ha^{-1}) compared to the non-agrochemical treatment (36.5 kg ha^{-1}) could be attributable to the C input from root necromass and the rhizosphere microbial biomass involved with root decomposition. Although, McNally *et al.* (2017) working with New Zealand pastoral systems and using $\delta^{13}C$ as a tracer, detected a rapid turnover of root material occurring between days 4 and 11 after glyphosate application (and attributed this to the contribution of dying roots), changes in DOC concentration were not measured. It is worth mentioning that differences in DOC concentration between treatments may have been mitigated in the present study, as fertiliser (NPKS, 18:20:0:1) was applied on day 4 (after the application of the agrochemicals), potentially accelerating the decomposition of DOC. However, the increase in the amount of water-extractable Fe and Mn suggests the existence of more reduced conditions in the agrochemical treatment that may have resulted from the decaying root necromass, consistent with the above hypothesis. The significant increase in the amount of water-extractable Al (pH of water-extracted soil = 6.5) is consistent with the mobilisation of this cation through complexation with DOC. While both treatments received fertiliser, nitrate concentrations (in the surface 5 cm soil depth) were significantly higher in the agrochemical treatment on days 6 and 12. It may be possible that this increase in nitrate concentration over time is related to the release of N originally present in glyphosate and diazinon. Glyphosate, and to some extent diazinon, have also been reported to increase N mineralisation in soil (Haney *et al.*, 2002; Cycoń *et al.*, 2010).

In general, it is possible that the increased DOC concentration resulting from the agrochemicals may have increased the denitrification capacity of the topsoil, as suggested by the significantly higher DOC/nitrate molar ratio of the agrochemical treatment on day 1; however, further research is needed to verify this assumption.

5.4.3 Changes in the chemical composition of water-extracted subsoil

The absence of significant differences in the DOC concentration of the cropping (agrochemical) and pasture (non-agrochemical) treatments below the topsoil suggests that the applied agrochemicals did not contribute to any changes in DOC concentration of the subsoil in this study. This observation is not surprising because the high soil

adsorptivity/low leachability of glyphosate and diazinon have previously been documented (Sprankle *et al.*, 1975; Arienzo *et al.*, 1994). Dicamba on the other hand is known to be highly leachable in the soil (Hill *et al.*, 2000); however, the minimal drainage/percolation recorded during the study period, as depicted in the soil water balance would have restricted the movement of this agrochemical.

The increase in water-extractable Fe concentration within the 30-60 cm soil depth of both treatments, suggests the existence of more reducing conditions in that soil layer, which could potentially support denitrification. Concentrations of water-extractable Fe showed a moderately strong correlation ($r \geq 0.5$; $p = 0.001$) with DOC concentration. This suggests the release of DOC previously associated with oxidised Fe, as previously reported by Hall and Silver (2013).

5.4.4 DOC chemistry of the water-extracted soil

DOC chemistry was unlikely to have been altered by the application of agrochemicals in the present study, as inferred from the absence of significance difference in the indices of DOC chemistry measured for both treatments. The increasing presence of conjugated unsaturated C compounds (i.e. aromatic C) with depth observed in both treatments is consistent with previous studies (Ussiri and Johnson, 2003; Gangloff *et al.*, 2014) and is associated with the existence of older forms of organic C within the subsoil (Angst *et al.*, 2016).

5.5 Conclusion

The agrochemicals used for clearing out pasture before crop establishment did not influence the DOC concentration of the subsoil, mainly due to the limited mobility of DOC under the experimental conditions studied. The effect of the agrochemicals on DOC dynamics was most obvious in the top 5 cm of the soil, with higher amounts of DOC detected in the agrochemical treatment (cropping) compared to the non-agrochemical treatment (pasture). Different mechanisms were proposed to be responsible for the higher DOC of the agrochemical treatment on days 1 and 6 following the application of agrochemicals. These mechanisms were associated with: (i)

a direct contribution of C from the agrochemical molecules, (ii) an indirect C contribution through displacement of adsorbed organic molecules, and (iii) the decomposition of root necromass.

The agrochemicals did not significantly affect DOC chemistry in this study. However, the agrochemicals, particularly glyphosate and diazinon, appeared to have enhanced N mineralisation, as inferred from the increased nitrate concentration in the topsoil, some of which could have originated from the decomposition of the agrochemicals themselves. Seasonal monitoring of the treatments will improve research understanding of how the magnitude of N mineralisation resulting from the establishment of a forage crop in hill country, compares to nitrate losses from pasture and/or cropping management. This will enable definitive conclusions to be drawn regarding the effect of forage crop establishment and other related factors (such as climate) on the DOC dynamics and potential for nitrate leaching from pastoral hill country soils.

CHAPTER 6:
Temporal variations in the dissolved organic carbon concentration and denitrification capacity of a hill country soil after forage crop establishment

Research highlights

- Within a one-year time frame, soil dissolved organic carbon (DOC) concentration and denitrification capacity were generally not influenced by the establishment of a brassica forage crop using the surface sowing technique.
- An increase in rainfall and soil moisture, after periods of soil water deficit, increased the DOC concentration of the soil.
- Soil denitrification capacity decreased by more than 50% following an increase in soil pH, suggesting the immobilisation of metal ions (cofactors) supporting denitrification enzymes.

6.1 Introduction

Similar to other countries with a temperate climate, the growth rate of winter pasture in New Zealand farms is lower (typically 5-6 times lower) than that in late spring/early summer (White *et al.*, 2010). Thus, in order to increase animal feed production during this period of low pasture growth, a common practice of New Zealand hill country farmers is to replace perennial pasture with winter forage crops on selected paddocks to supplement existing perennial pasture.

In order to meet New Zealand Government's Business Growth Agenda of significantly increasing the value of Primary Industry exports by 2025 (MPI, 2018), agricultural intensification is anticipated in these hill country farms. Therefore, the conversion from perennial pasture to forage cropping is now becoming more popular on New Zealand hill country landscapes (Houlbrooke *et al.*, 2009; Fraser *et al.*, 2016; Burkitt *et al.*, 2017). Such changes in land use could result in an immediate increase in topsoil dissolved organic carbon (DOC) concentration via the direct and indirect effect of the agrochemicals used for clearing out pasture (Chapter 5). However, little is known of

how such modification of soil DOC concentration varies with respect to weather seasonality, and the effect on subsurface denitrification.

Denitrification below the root zone is an important nitrate attenuation process that limits the availability of nitrate in soil-water systems (Rivett *et al.*, 2008). Thus, it helps to improve the quality of water leaving agricultural landscapes and entering water bodies. Denitrification studies are of utmost importance because the presence of farm-sourced nutrients (particularly nitrate) in water bodies is an issue of increasing concern both in New Zealand and abroad (Puckett, 1995; Davies-Colley, 2013). It has been reported that amongst the factors necessary for denitrification to occur (anoxic environment, electron acceptor–nitrate, electron donor–DOC, and denitrifying bacteria), DOC is the most limiting factor, especially below the root zone (Peterson *et al.*, 2013). Therefore, practices that affect changes in DOC leaching and availability will most likely affect denitrification below the root zone. Understanding the impact of the change from perennial pasture to forage cropping on the DOC concentration and denitrification capacity of hill country soils is therefore critical.

The concentration of DOC in soil changes with weather seasonality. For instance, higher DOC concentrations have been observed during periods of warmer temperature and increased soil moisture, mainly after rainfall events (Christ and David, 1996; Don and Schulze, 2008). The rewetting of dried soils is known to increase soil DOC concentration (Lundquist *et al.*, 1999; Denef *et al.*, 2001). Lundquist *et al.* (1999) attributed this increase in DOC concentration to the (i) decrease in the utilisation of DOC by soil microbes during the dry period, (ii) increase in microbial biomass turnover and condensation of microbial products induced by rewetting, and (iii) disruption of soil structure which releases adsorbed carbon (C) in the form of DOC. The temporal variations in soil DOC concentration in New Zealand hill country, and the effect on subsoil denitrification, have not yet been researched, especially following a change from perennial pasture to forage cropping. Hence, there is a knowledge gap on how these changes may affect nitrate attenuation for improved water quality outcomes.

Due to the risk and difficulty of using machinery on sloping landscapes, cultivation is seldom practiced on steep hill country slopes > 20°; rather, surface sowing is the

common method of crop establishment. This method is unique in comparison to the practice of no-tillage, as seed is placed on the soil surface (usually via helicopter) without any drilling. This technique has allowed increasing areas of hill country to be converted from long-term pasture to forage crop over short periods of time. There is limited research understanding of the effect of this method of crop establishment on the DOC concentration and subsoil denitrification capacity of hill country landscapes, despite the important influence these factors have on the concentration of nitrate in receiving waters.

This study tested the hypothesis that land use change from pasture to forage cropping influences soil DOC concentration and denitrification capacity, within a one-year period. The objective of this study was to investigate the temporal changes in the DOC concentration and denitrification capacity of a hill country soil following a change from perennial pasture to forage cropping, using the surface sowing technique.

6.2 Materials and methods

6.2.1 Site description

This experiment was carried out on Massey University's Agricultural Experiment Station, Tuapaka, a hill country farm used for sheep and beef cattle production. It is located approximately 15 km north-east of Palmerston North, New Zealand (40°21'20.1"S, 175°44'19.6"E). The study area, which is approximately 320 m above sea level, has a humid temperate climate, with an average annual rainfall of 1100 mm and predominantly dry summers (Massey University, 2016).

The soil at the research site was developed from a colluvium of Ramiha silt loam soil (New Zealand classification: Allophanic Brown soil), with minor incorporation of Makara steepland soil (Orthic Brown soil). In the USDA Soil Taxonomy, the soil is described as Typic Eutrudept (Soil Survey Staff, 2014).

6.2.2 Experimental design and sample collection

The two treatments monitored were (i) cropping, and (ii) pasture. Each was replicated four times (plot size of 4 × 4 m for each replicate) and arranged in a Randomised Complete Block Design (RCBD). Historically, both treatments were comprised of long-term (> 20 years) browntop (*Agrostis capillaries* L.) and perennial ryegrass (*Lolium perenne* L.). The cropping treatment was established by spraying out established pasture with a mixture of selected agrochemicals, namely glyphosate (active ingredient) at 4 L ha^{-1}, dicamba (active ingredient) at 400 mL ha^{-1}, diazinon (active ingredient) at 400 mL ha^{-1}, and organomodified polydimethyl siloxane (active ingredient) at 250 mL ha^{-1}. Thereafter, swedes (*Brassica napobrassica* Mill.) were sown at a rate of 2.5 kg ha^{-1} using a surface sowing technique, i.e. broadcasting by hand and passing a tractor (once) over the seeds to push them into the soil with the tractor tyres. The pasture treatment served as the control and so was left in its historic state. Both treatments received a fertiliser formulation (Cropmaster DAP, 18:20:0:1) at the time of treatment establishment – November 2015 (250 kg ha^{-1}) and also four months after treatment establishment (150 kg ha^{-1}).

Three replicate soil cores (core diameter ~ 4 cm) were collected from each plot at depths of 0-5, 5-10, 10-20, 20-30, 30-40, 40-60, 60-80, and 80-100 cm on four different dates (sampling periods), i.e. 9 November 2015 (spring), 24 May 2016 (autumn), 9 August 2016 (winter), and 22 November 2016 (spring). Sampling took place on these dates in the expectation that the soil conditions on these days were generally representative of the long-term average for the season within which sampling took place. Soil samples were not collected during summer because dry and hard soil conditions made soil sampling impractical. Samples were stored below 4°C and analysed for DOC concentration and denitrification capacity within five days of sample collection. Additional soil/water-extract properties were also determined as described below.

6.2.3 Laboratory analyses

Soil sample homogeneity was obtained by manually mixing each sample thoroughly before extraction. DOC in the soil samples was extracted at room temperature by

shaking 10 g of fresh soil sample with 25 mL of deionised water (1:2.5 wt/v) in a 50 mL extraction tube, on a rotatory shaker for 1 h. Thereafter the agitated samples were centrifuged at 5000 rpm for 10 min and filtered with Whatman No. 41 filter paper. The filtered samples were subsequently centrifuged at 5000 rpm for 2 h (a sub-set of 10 samples was used to determine that this second centrifugation was better at removing particulate organic matter compared to filtration with 0.22 and 0.45 µm filters). The centrifuged samples were decanted and the supernatants (extracts) analysed for DOC concentration using a modification of the semi-automated dichromate method described by O'Dell (1993), whereby the absorbance was read with a spectrophotometer at a wavelength of 660 nm, using potassium hydrogen phthalate (KHP) standards to produce a calibration curve.

The extracts were also analysed for pH (with a standard pH meter), nitrate (by continuous flow analysis), total aluminium-Al, iron-Fe and manganese-Mn (with a Microwave Plasma-Atomic Emission Spectrometer–MP-AES). The gravimetric soil moisture content was determined on each soil sample (thus, nutrient concentrations were converted to the oven-dry weight of soil), and the obtained values were converted to volumetric soil moisture.

Denitrification enzyme activity (DEA), via the acetylene inhibition method, was used to determine the denitrification capacity of the soil in the laboratory. This was achieved via the vacuum pouch incubation technique (Rivas *et al.*, 2014a) as follows: 20 g of fresh soil (dry weight equivalent) was placed inside a polyethylene pouch (10 × 28.5 cm) fitted with a luer-lock valve. The pouch was subsequently heat-sealed and vacuumed with a syringe via the luer-lock valve. Thereafter, the pouch was flushed with 50 mL of dinitrogen (N_2), after which 20 mL of acetylene, 20 mL of DEA solution (containing 50 µg NO_3^--N g^{-1} dry soil and 10 mg L^{-1} chloramphenicol) and 180 mL of N_2 were consecutively added into the pouch. Acetylene terminates the denitrification reaction at the nitrous oxide (N_2O) stage, as it is much easier to sample and measure N_2O concentrations compared to N_2, which is ubiquitous in the atmosphere. Chloramphenicol served as an enzyme inhibitor to prevent *de novo* synthesis of enzymes during incubation (Dendooven *et al.*, 1994). The content of the pouch was subsequently incubated at 20°C in the dark, on a rotary shaker (160 rpm). Gas samples

were collected from the pouch at 0 (before incubation), 2, 4 and 6 h of incubation. The N_2O concentration in the gas samples was analysed with a Shimadzu Gas Chromatograph (GC) 17 A (Japan) equipped with a ^{63}Ni electron capture detector, and operating at a column and detector temperature of 55 and 330°C, respectively. The concentration of N_2O in the gas sample was obtained by plotting a calibration curve of N_2O standard gases, which were obtained from serial dilutions of a standard N_2O gas (100 mg L^{-1}). Thereafter, the mass of N_2O in the pouch headspace was calculated as follows:

Mass of N_2O (µg) = concentration of N_2O (µg L^{-1}) from GC

× volume of gas in pouch (L)

× 0.544 Bunsen absorption coefficient

Denitrification capacity was subsequently calculated from the slope of N_2O mass and incubation time, divided by the mass of soil.

The laboratory analyses described above were carried out using appropriate quality control protocols, including the use of sample duplicates, blank, reference and spiked samples.

6.2.4 Meteorological measurements and soil water balance

Soil temperature sensors (HortPlus MicroLoggers, Model Z), buried at four soil depths (5, 10, 30, and 60 cm), were used to monitor the daily soil temperature at the site (from May to November 2016). Other meteorological data collected on a daily basis were total rainfall, total solar radiation, total wind run, average air temperature and relative humidity. These meteorological data, in addition to some site-specific information (slope, aspect and depth of root zone), were used as input parameters to model the daily soil water balance for the site. The version of the Penman-Monteith equation suggested by Allen *et al.* (1998) was used to estimate the reference crop evaporation, while equations suggested by Revfeim *et al.* (1982) were used to estimate the effect of slope and aspect on incoming solar radiation at the site.

6.2.5 Statistical analyses

Analysis of Variance (ANOVA), with Tukey comparison procedure (p = 0.05), was performed on the measured data to detect significant differences between treatment means. This analysis was performed using General Linear Model (GLM) to account for the repeated measures design of the experiment. The Pearson correlation technique was used to determine the relationship between denitrification capacity and other soil/water-extract parameters. All analyses were performed using Minitab statistical software (17.2.1 Minitab, Inc.).

6.3 Results

Selected soil chemical analyses (Table 5.1), which were measured prior to establishing the treatments, indicated that there were generally no significant ($p \leq 0.05$) differences in soil properties within plots before treatment establishment. This suggests that any change observed during the study period is unlikely to be attributed to any inherent variability across the site.

6.3.1 Variations in daily rainfall, daily soil temperature, measured soil moisture, and modelled soil water balance

There was no rainfall event on 9 November 2015, and on the three days prior to this sampling date (Figure 6.1a). The highest rainfall event (~ 20 mm) before this date occurred on 4 November 2015. Soil temperature was not measured during this sampling period (November 2015). Approximately 15 mm of rainfall occurred on 24 May 2016, with significant amounts of rainfall events occurring prior to this date (Figure 6.1b). Average daily soil temperature to a depth of 60 cm ranged from 11 to 12°C on this sampling day (24 May 2016). No rainfall event occurred on 9 August 2016; however, 26-45 mm of rainfall occurred 3-5 days prior to this sampling date (Figure 6.1c). Average daily soil temperature to a depth of 60 cm ranged from 5 to 8°C on 9 August 2016. There was also no rainfall event on 22 November 2016; however, rainfall occurred consecutively, few days prior to this date (Figure 6.1d). Average daily soil temperature to a depth of 60 cm ranged from 13 to 14°C on this sampling day (22 November 2016).

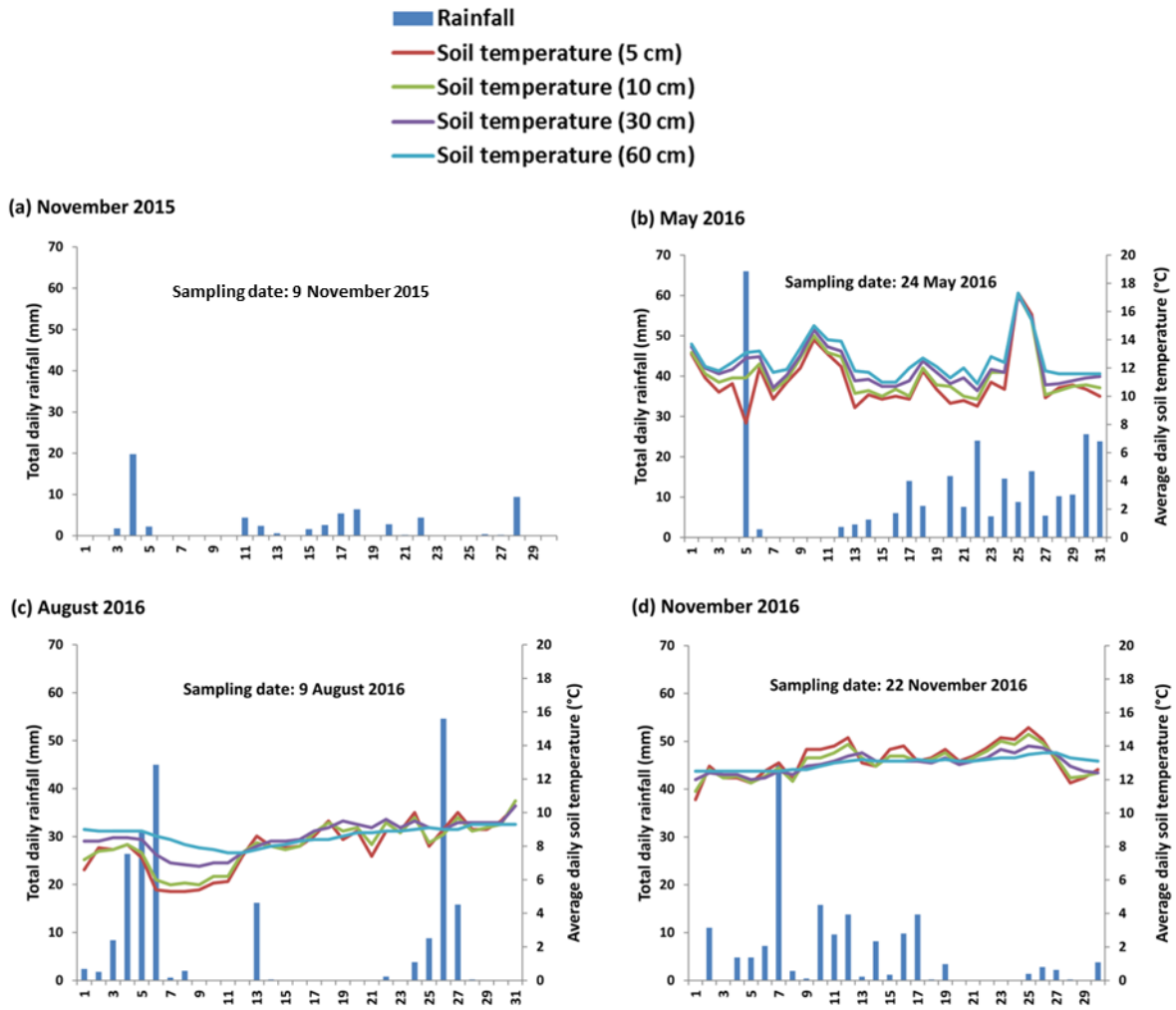

Figure 6.1: Total daily rainfall and average daily soil temperature during the months of soil sampling

Consistently lower mean volumetric soil moisture contents were measured on 9 November 2015 within the surface 40 cm soil depth (Figure 6.2). This corresponded with the lower rainfall events which occurred before this sampling date, compared to the other sampling dates. There were no significant ($p \leq 0.05$) differences in soil moisture content between the pasture and cropping treatments. Similarly, the mean values of water-filled pore space (WFPS) measured on 9 November 2015 were significantly ($p \leq 0.05$) lower than that measured during the other sampling dates (Table 6.1). The pasture and cropping treatments also had similar mean values of WFPS during all the sampling dates.

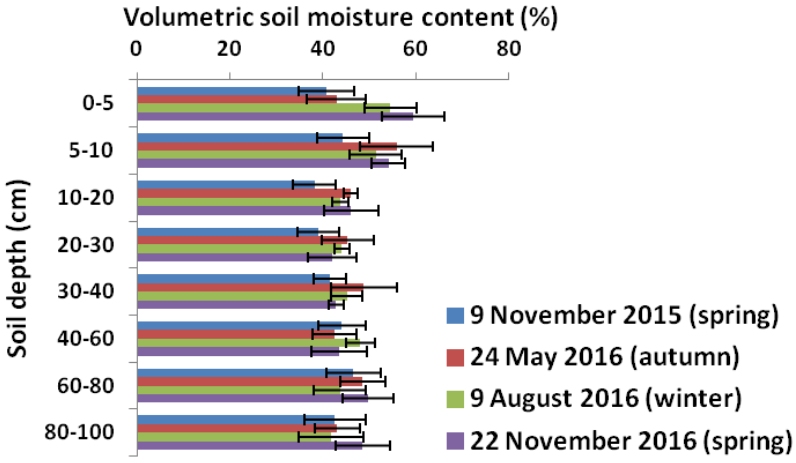

Figure 6.2: Volumetric soil moisture content during the four sampling dates. *Error bars are standard error of the mean (n = 24).*

Table 6.1: Changes in water-filled pore space (%) of the cropping and pasture treatments at different soil depths

Soil depth (cm)	9 November 2015 (spring)	24 May 2016 (autumn)	9 August 2016 (winter)	22 November 2016 (spring)
Cropping				
0-5	77.88 ± 1.42 ^{B}	91.54 ± 1.62 ^{A}	97.45 ± 1.64 ^{A}	97.32 ± 1.76 ^{A}
5-10	71.57 ± 1.72 ^{B}	92.68 ± 1.91 ^{A}	80.61 ± 1.54 ^{AB}	86.27 ± 2.64 ^{A}
10-20	61.71 ± 1.57 ^{B}	92.82 ± 1.98 ^{A}	76.04 ± 1.58 ^{AB}	87.33 ± 1.64 ^{A}
20-30	61.82 ± 1.42 ^{B}	90.23 ± 1.32 ^{A}	85.59 ± 1.34 ^{A}	73.99 ± 1.88 ^{AB}
30-40	63.07 ± 1.65 ^{B}	90.08 ± 1.25 ^{A}	83.52 ± 1.54 ^{A}	74.70 ± 1.55 ^{AB}
40-60	69.89 ± 1.73 ^{B}	95.05 ± 1.08 ^{A}	80.31 ± 1.17 ^{A}	75.99 ± 2.70 ^{A}
60-80	67.78 ± 4.07 ^{B}	91.48 ± 2.90 ^{A}	91.41 ± 3.97 ^{A}	94.25 ± 3.66 ^{A}
80-100	64.92 ± 4.07 ^{B}	94.35 ± 5.43 ^{A}	97.48 ± 4.98 ^{A}	95.37 ± 6.17 ^{A}
Pasture				
0-5	71.06 ± 2.24 ^{B}	94.36 ± 2.00 ^{A}	94.07 ± 1.25 ^{A}	94.46 ± 1.94 ^{A}
5-10	64.92 ± 1.60 ^{B}	91.53 ± 1.87 ^{A}	80.54 ± 2.76 ^{AB}	88.21 ± 2.14 ^{A}
10-20	57.56 ± 2.14 ^{B}	88.49 ± 3.34 ^{A}	79.16 ± 2.69 ^{A}	89.13 ± 1.59 ^{A}
20-30	59.80 ± 2.01 ^{B}	92.53 ± 1.69 ^{A}	82.02 ± 3.43 ^{A}	76.75 ± 2.68 ^{A}
30-40	60.81 ± 1.33 ^{B}	96.57 ± 3.22 ^{A}	86.97 ± 1.86 ^{A}	82.30 ± 1.54 ^{A}
40-60	67.19 ± 3.27 ^{B}	97.23 ± 2.32 ^{A}	82.61 ± 2.88 ^{A}	73.95 ± 3.64 ^{B}
60-80	75.60 ± 3.76 ^{AB}	92.52 ± 3.23 ^{A}	85.55 ± 3.34 ^{A}	90.63 ± 3.53 ^{A}
80-100	69.28 ± 4.25 ^{B}	91.42 ± 5.20 ^{A}	85.92 ± 6.02 ^{A}	91.61 ± 5.16 ^{A}

Numbers represent means ± standard error of the mean (n = 12); no significant difference between treatments for a particular soil depth; different letters indicate significant difference between sampling dates for a particular soil depth ($p \leq 0.05$).

The daily soil water balance modelled for the root zone at the site predicted significant soil water deficits from early November 2015 to mid-May 2016 (Figure 6.3). The model

also predicted minimal soil water deficits from mid-May 2016 to mid-November 2016, with significant drainage events during this period.

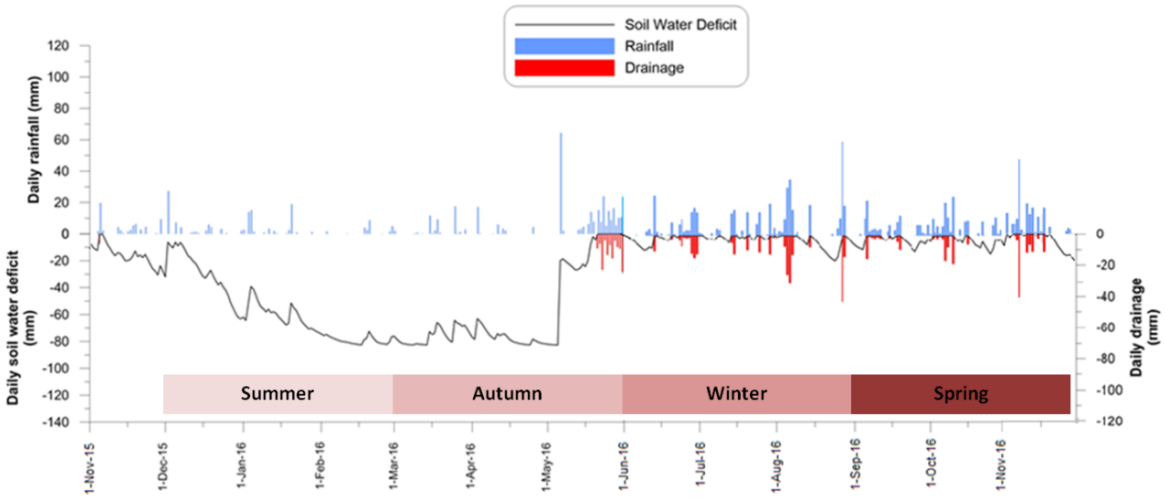

Figure 6.3: Daily soil water balance at the experimental site from November 2015 to November 2016

6.3.2 Variations in DOC concentration and denitrification capacity of the soil profile

DOC concentration decreased with increasing soil depth (Figure 6.4a). The highest ($p \leq 0.05$) DOC concentrations, regardless of land uses (treatments), were measured on 9 August (winter) and 22 November (spring) 2016, especially within the 0-5 cm soil depth. The DOC concentrations (of both treatments) measured on 22 November 2016 were significantly ($p \leq 0.05$) higher than that of 9 November 2015, within the 0-40 cm soil depth. Significant ($p \leq 0.05$) differences between land uses were observed only within the 0-30 cm depth, with the cropped treatment having a higher DOC concentration (within 5 to 30 cm depth) on 24 May and 9 August 2016. However, after a year of crop establishment (22 November 2016), the pasture treatment had significantly ($p \leq 0.05$) higher DOC concentration within the 0-5 cm soil layer.

Denitrification capacity also decreased with increasing soil depth (Figure 6.4b). Lower denitrification capacities were observed on 9 August 2016 compared to 24 May and 22 November 2016, with > 50% reductions observed in the top 5 cm depth on 9 August 2016. Significant ($p \leq 0.05$) differences between land uses were not observed, except

within the 10-20 cm soil depth on 24 May 2016 (245 µg kg^{-1} h^{-1} under pasture vs 119 µg kg^{-1} h^{-1} under cropping).

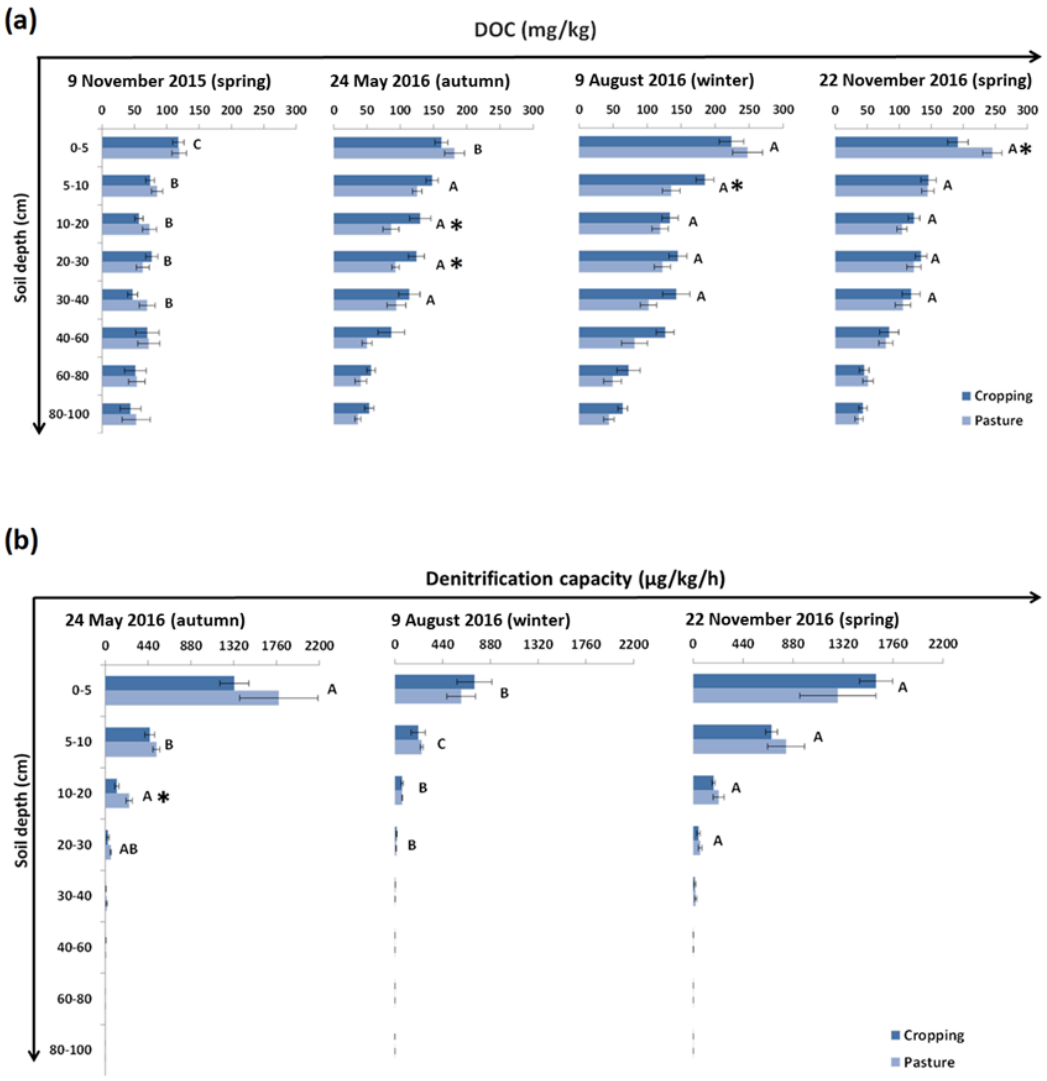

Figure 6.4: Changes in (a) DOC concentration, and (b) denitrification capacity within the soil profile. * *indicates significant difference between land uses; different letters indicate significant difference between sampling dates for a particular soil depth ($p \leq 0.05$). Error bars are standard error of the mean (n = 12).*

6.3.3 Variations in properties of the water-extracted soil

Nitrate concentration decreased with increasing soil depth (Figure 6.5a). Mean nitrate concentrations in the surface 20 cm depth were consistently higher on 24 May 2016 (autumn), though significant ($p \leq 0.05$) differences between sampling days were not observed at this depth. Significant differences were, however, observed within the 40-60 cm soil layer, where higher mean values (> 0.7 mg kg^{-1}) were observed on 9 November 2015 and 22 November 2016 compared to mean values of 0.1-0.5 mg kg^{-1}

observed on 24 May 2016 and 9 August 2016. Significant ($p \leq 0.05$) differences between land uses only occurred within the surface 20 cm depth on 24 May 2016, with the cropped treatment having > 55% greater nitrate concentration compared to the pasture treatment.

The pH of the water extract ranged from 5.6 to 6.9, with no significant ($p \leq 0.05$) difference between the land uses (Figure 6.5b). The pH values measured on 22 November 2016 (5.6-6.1) were significantly different ($p \leq 0.05$) from that of 9 November 2015 (6.1-6.5). Higher pH values (6.8 and 6.9) were found within the 0-5 cm of both land uses on 24 May and 9 August 2016.

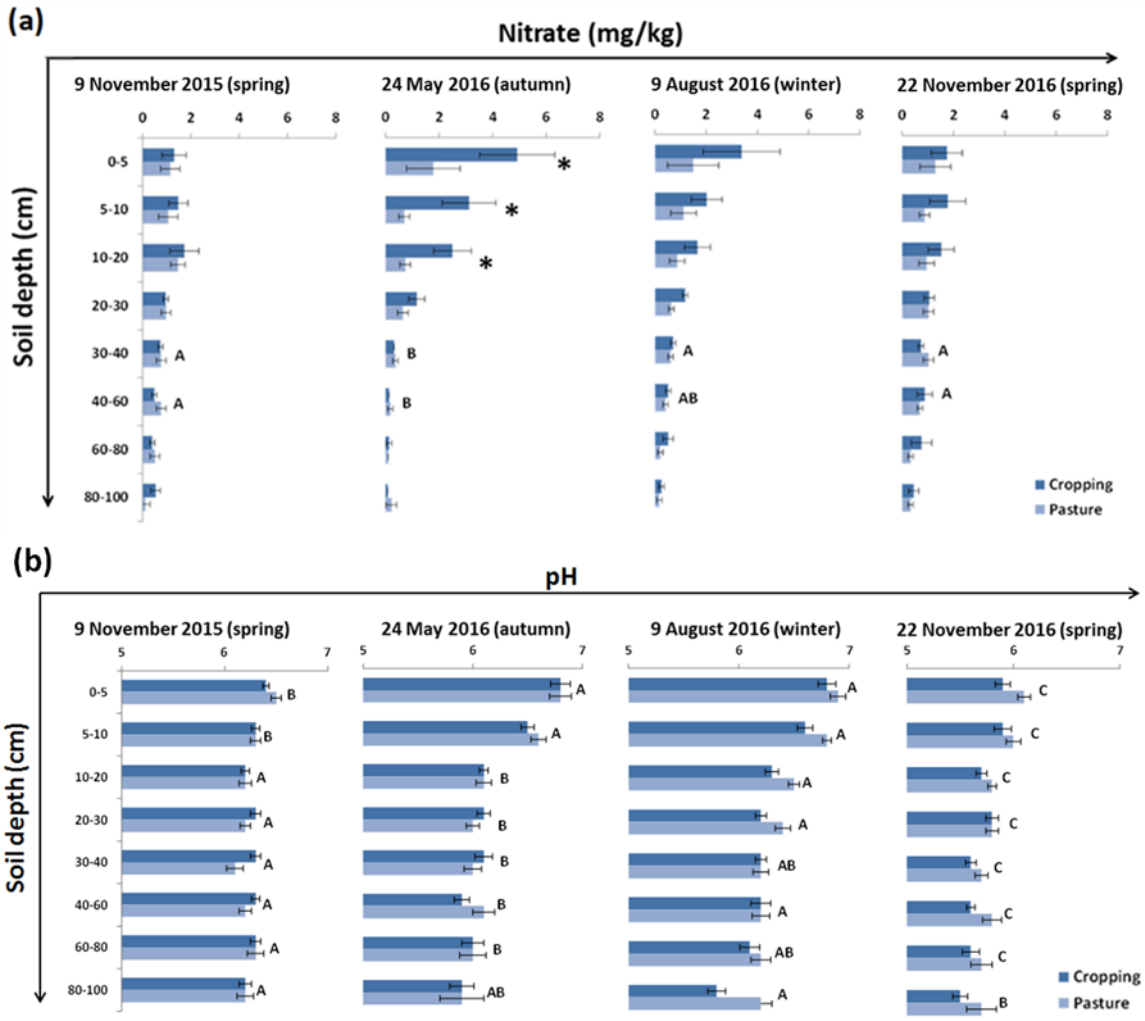

Figure 6.5: Changes in (a) nitrate concentration, and (b) pH of the water-extracted soil. ** indicates significant difference between land uses; different letters indicate significant difference between sampling dates for a particular soil depth ($p \leq 0.05$). Error bars are standard error of the mean (n = 12).*

Water-extractable Al and Fe concentrations generally increased with increasing depth, with greater concentrations occurring within the 30-60 cm depth (from ≤ 30 mg kg^{-1} within the 0-10 cm depth to 50-80 mg kg^{-1} and 40-60 mg kg^{-1} within the 30-60 cm depth for water-extractable Al and Fe, respectively) before decreasing to ≤ 30 mg kg^{-1} within the 80-100 cm depth (Figures 6.6a and b). The concentrations of water-extractable Al and Fe recorded on 9 November 2015 were significantly ($p \leq 0.05$) higher than those recorded for all the sampling dates in 2016, especially at depth (60-80 cm for water-extractable Al, and 40-80 cm for water-extractable Fe). Significant ($p \leq 0.05$) differences between land uses were observed in the surface 60 cm depth, with the pasture treatment having higher concentrations of these elements.

The concentration of water-extractable Mn was below 1 mg kg^{-1} throughout the study (Figure 6.6c). Similar to water-extractable Al and Fe, significantly ($p \leq 0.05$) higher water-extractable Mn concentrations were recorded on 9 November 2015 (spring) compared to values observed during all the sampling periods in the following year, especially within the 40-80 cm depth. A significantly ($p \leq 0.05$) higher water-extractable Mn concentration was observed in the pasture treatment on 24 May 2016 (autumn), within the 30-40 cm depth.

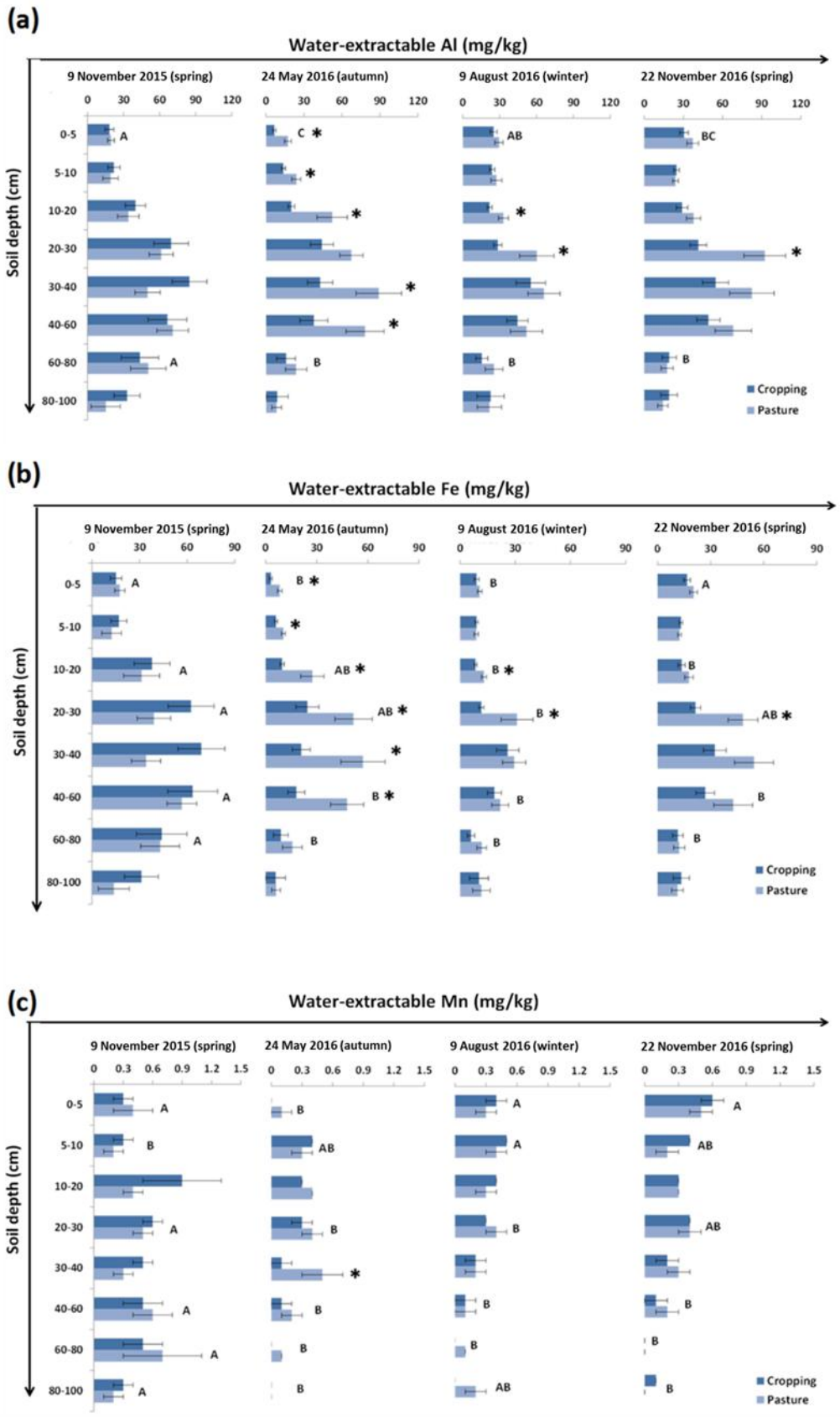

Figure 6.6: Changes in water-extractable (a) Al, (b) Fe, and (c) Mn within the soil profile. * *indicates significant difference between land uses; different letters indicate significant difference between sampling dates for a particular soil depth ($p \leq 0.05$). Error bars are standard error of the mean (n = 12).*

6.3.4 Relationship between denitrification capacity and properties of soil/water-extracts

Table 6.2 shows that significant ($p \leq 0.05$) positive correlations existed between denitrification and four measured parameters: volumetric soil moisture, WFPS, DOC concentration, pH and nitrate, for the three sampling dates considered (except on 9 August 2016, when no significant correlation was observed for nitrate). These correlations were strong (r > 0.8) for volumetric soil moisture and WFPS, and moderately strong (r ≥ 0.50 - 0.67) for DOC concentration. Moderately strong correlations (r > 0.6) were also observed between denitrification capacity and pH on 24 May and 9 August 2016.

The correlations of denitrification capacity with water-extractable Al and Fe were negative and weak (r = −0.2) across all the sampling dates, while those with water-extractable Mn were positive and weak on 22 November 2016 (r = 0.36, $p \leq 0.05$). The relationship between denitrification capacity and soil temperature did not follow a consistent pattern, i.e. both positive and negative relationships were observed.

Table 6.2: Pearson correlation coefficients (r) and *p-values* for correlations between denitrification capacity and soil/water-extract properties of both the cropping and pasture treatments

Sampling dates	Pearson corr. result	Vol. soil moisture content	WFPS	Soil temp.	DOC conc.	pH	Nitrate	Water-extractable Al	Water-extractable Fe	Water-extractable Mn
24 May 2016 (n = 55)	r	0.876	0.892	−0.365	0.589	0.741	0.427	−0.208	−0.234	−0.168
	p-value	*0.001*	*0.001*	*0.003*	*0.001*	*0.001*	*0.001*	*0.063*	*0.043*	*0.110*
9 August 2016 (n = 47)	r	0.863	0.874	−0.647	0.501	0.662	0.176	−0.237	−0.255	0.068
	p-value	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.119*	*0.050*	*0.042*	*0.325*
22 November 2016 (n = 60)	r	0.864	0.881	0.408	0.665	0.290	0.233	−0.198	−0.194	0.357
	p-value	*0.001*	*0.001*	*0.001*	*0.001*	*0.012*	*0.037*	*0.065*	*0.069*	*0.003*
ALL (n = 162)	r	0.812	0.825	0.118	0.540	0.421	0.308	−0.179	−0.192	0.033
	p-value	*0.001*	*0.001*	*0.068*	*0.001*	*0.001*	*0.001*	*0.011*	*0.007*	*0.340*

WFPS: water-filled pore space; ALL: includes all sampling dates.

6.4 Discussion

The cropped treatment had greater amounts of nitrate within the 0-20 cm soil depth on 24 May 2016, i.e. two months after fertiliser application. This suggests that the pasture treatment utilised more of the applied fertiliser. However, nitrate concentrations in the cropped treatment decreased with increasing depth to similar concentrations to that of the pasture treatment, suggesting that cropping did not constitute a risk for nitrate leaching at this time and for this soil. It is possible that some of the nitrate in the cropped treatment could have been denitrified since its DOC concentration was greater than that of pasture in this soil layer (10-30 cm). The consistently higher mean nitrate concentrations observed within the surface 20 cm depth of the cropped treatment on 24 May 2016 could also be due to nitrate accumulation which occurred over summer, when there is a reduction in soil water (Figure 6.3) and hence limited leaching, and suppressed plant growth and uptake of N. This trend was, however, not observed in the pasture treatment, hence the absence of significant differences in the nitrate concentration of the sampling dates within the surface 20 cm soil depth. The significantly higher nitrate concentration observed on 9 August 2016 (compared to 24 May 2016), within the 30-40 cm soil depth, is likely to be associated with a major drainage event (> 30 mm) which occurred on 6 August 2016 (Figure 6.2); thus, highlighting the impact of drainage on nitrate leaching.

An inconsistent pattern was observed in the topsoil DOC concentration of both treatments in this study. The significantly higher DOC concentration observed within the 10-30 cm depth of the cropped treatment on 24 May 2016 could be because the swede bulbs were expanding and displacing soil during this period. Thus, they could have also indirectly promoted organic matter mineralisation through the disruption of soil structure (Cochrane and Aylmore, 1994). The cropped treatment also had a higher DOC concentration within the 5-10 cm soil depth on 9 August 2016. However, this trend changed on 22 November 2016 – one year after crop establishment, when the pasture treatment had a higher DOC concentration within the surface 5 cm soil depth. This lack of a distinct pattern in the topsoil DOC concentration, coupled with the absence of significant differences in subsoil DOC concentration of both treatments suggest that land use change from pasture to forage cropping had negligible impact on the DOC concentration of this soil within the duration of this study.

The significantly lower DOC concentrations measured (in both treatments) on 9 November 2015 is associated with the limited rainfall which occurred before this date (Figure 6.1). This limited rainfall resulted in a decrease in volumetric soil moisture and WFPS (and hence DOC mobility/availability) compared to the other sampling dates, when significant rainfall events occurred before and/or during sampling. Therefore, this suggests that the soil water deficits predicted for this soil from early November 2015 to mid-May 2016 (Figure 6.3) negatively impacted on soil DOC concentration. However, as the study progressed, the rewetting of the soil by more frequent and heavier rainfall events most likely increased soil DOC concentration. This observation is consistent with previous studies which reported increasing soil DOC concentrations after the rewetting of dried soils due to several mechanisms, including the increase in microbial biomass turnover and condensation of microbial products induced by rewetting (Lundquist *et al.*, 1999; Denef *et al.*, 2001).

Similar denitrification capacities were observed in both treatments during this one-year experiment, (except on 24 May 2016, within the 10-20 cm depth), suggesting that land use change from pasture to forage cropping did not influence the denitrification capacity of the soil. Some trends were, however, observed in the temporal denitrification capacity of the soil, regardless of treatments. For instance, lower mean denitrification capacities were observed on 9 August 2016–winter (< 800 μg kg^{-1} h^{-1}) compared to the other sampling periods (> 1200 μg kg^{-1} h^{-1}). This lower denitrification capacity is not related to DOC supply, since the DOC concentrations measured on 9 August 2016 were generally not lower than that of the other sampling dates in 2016. It is possible that the higher pH of the soil profile on 9 August 2016 (relative to the other sampling dates in 2016) could have negatively impacted on denitrification via the immobilisation of metal ions (such as copper) which are cofactors for denitrification enzymes (Saggar *et al.*, 2013). However, further investigation would be required to confirm this assumption.

The significant positive correlations recorded between denitrification capacity and soil moisture parameters (volumetric soil moisture content and WFPS) highlight the importance of soil moisture in the denitrification process. Soil moisture promotes denitrification mainly by decreasing soil oxygen availability via the occupation of pore

spaces (Saggar *et al.*, 2013). Substrate mobility and hence availability for denitrification can also be improved by higher soil moisture content (Luo *et al.*, 1999a).

It is worth mentioning that negligible denitrification capacities were observed below 30 cm depth for both treatments during all the sampling periods, despite the presence of DOC. It is not clear whether these low denitrification capacities were due to the decreased nitrate (or other nutrients) concentration or the absence of denitrifying microbes. This is an area that requires further research.

Concentrations of water-extracted Fe increased with increasing depth, and its correlation with denitrification capacity on 24 May and 9 August 2016, were also significant, though weak ($r < -0.3$; *p-value* = 0.04). Studies have shown that Fe promotes denitrification through Fe^{2+} oxidation, especially under anoxic and near neutral soil conditions (Melton *et al.*, 2014; Wang *et al.*, 2016). Therefore, this suggests that the high Fe^{2+} (the stable species in water-extracts at room temperature) concentration found within the 30-60 cm depth of the soil profile (especially in the pasture soil) has the potential to enhance subsoil denitrification. In addition, although the concentrations of water-extractable Mn (Mn^{2+}) were > 1 mg kg^{-1} in the present study, a significant correlation ($r < 0.4$; *p-value* = 0.003) was observed between denitrification capacity and water-extractable Mn on 22 November 2016, suggesting a possible contribution of this element to denitrification. Based on these observations, further investigation on the contributions of both Fe and Mn to denitrification is required for this soil.

6.5 Conclusion

Land use change from pasture to forage cropping, using a surface sowing technique with no cultivation, did not affect the subsurface DOC concentration and denitrification capacity of the well-drained colluvial soil during this one-year experiment. The establishment of a brassica forage crop, however, resulted in an initial greater nitrate concentration in the topsoil (0-20 cm) compared to pasture, possibly due to poor N utilisation by the growing brassica forage crop. This increased nitrate concentration did not present a risk for nitrate leaching, as lower nitrate concentrations, similar to the

pasture system, were observed at lower soil profile depths in the cropped system. An increase in rainfall and soil moisture, after periods of soil water deficit, increased the DOC concentration of the studied soil. The significant positive correlations observed between soil moisture parameters (WFPS and volumetric soil moisture content) and denitrification capacity in this study highlight the important contribution of soil moisture to denitrification.

This study has demonstrated that the annual establishment of a brassica forage crop to increase animal feed production did not have any significant impact on the nitrate attenuation capacity of the studied hill country soil. In addition, there is no evidence that this practice increased the risk of nitrate leaching compared to the pasture treatment. Given that forage crop establishment typically occurs in annual cycles in most New Zealand hill country farms, longer-term observations (> 1 year) will not be pragmatic. However, increasing the number of sampling days within each season would allow for a more thorough understanding of the seasonal changes in DOC concentration and denitrification capacity as influenced by forage crop establishment. Since the current study was carried out on a small plot scale (plot size of 4 × 4 m for four replicates), larger scale experiments, incorporating more than one forage crop and soil, would be necessary to further examine the effect of forage crop establishment on nitrate attenuation in New Zealand hill country landscapes. Other areas for further research on the sampled soil are the negligible denitrification capacities of the subsoil, despite the presence of DOC, as well as the contribution of Fe and Mn to nitrate attenuation.

CHAPTER 7:
Monitoring the leaching of dissolved organic carbon in a hill country soil

Research highlights

- Leaching of plant-residue-derived DOC was limited below the surface 20 cm soil depth, even under high water flux conditions.
- This was attributed to the rapid turnover of exogenous DOC within the topsoil.
- Hence, the relatively large amounts of DOC present in the subsoil layers of the studied soil may be associated with buried soils.

7.1 Introduction

Studies have shown that the supply of dissolved organic carbon (DOC) is a major factor limiting denitrification below the topsoil (Yeomans *et al.*, 1992; Luo *et al.*, 1998). The quality of DOC also affects its bioavailability for denitrification below the root zone. For instance, a large portion of organic carbon–C (and by extension DOC) found in the subsoil tends to be more recalcitrant compared to C arising from the topsoil (Whitmore *et al.*, 2015; Angst *et al.*, 2016). This implies that DOC in the subsoil may not be readily available for denitrification, and this could negatively impact on the amount of nitrate leaching into receiving waters.

Pasture soils tend to have high amounts of DOC at the surface, mainly due to the accumulation of animal waste and the dense rhizosphere of improved pastoral species (Williams and Haynes, 1990). Certain practices in pastoral hill country farms, such as the replacement of perennial pasture with forage crops (via the surface sowing technique) which involves the use of agrochemicals, could also lead to a short-term increase in topsoil DOC concentration (Chapter 5). It is important to ascertain if DOC arising from such practices eventually becomes available in the subsoil, as this would improve understanding of the potential impact of these practices on the amount of nitrate entering ground and surface water.

A laboratory experiment undertaken by Peterson *et al.* (2013) reported that DOC which was extracted from the topsoil enhanced denitrification in the subsoil environment. This supports previous research (Schwesig *et al.*, 2003; Kaiser and Kalbitz, 2012) which noted that, although subsoil DOC is rich in nutrients (nitrogen–N and phosphorus–P) and carbohydrates , it does not seem to be used as an energy and nutrient source because it is the leftover of decomposition and thus is more recalcitrant. This further highlights why it is necessary to establish if topsoil DOC, originating from recent organic matter input, leaches down the soil profile in amounts that could potentially result in significant increases in subsoil denitrification under field conditions.

Kaiser and Kalbitz (2012) proposed a conceptual model that accounts for the vertical movement of DOC with soil water. This model assumes a temporal immobilisation of DOC in the upper soil compartments, followed by microbial processing, before the release of the altered compounds into soil water. The model links the transport of DOC within the soil profile to the composition and radiocarbon age of organic matter within the subsoil, and shows that recent plant-derived compounds decrease with soil depth, while aged/microbially processed plant-derived compounds increase with depth. However, Kaiser and Kalbitz (2012) noted that the model only applies to mineral soils, where transport processes are controlled by percolating water. It is critical to ascertain if this model applies to a soil on the complex terrain of New Zealand hill country, bearing in mind that the leaching of DOC down the soil profile is influenced by several factors such as the type and amount of organic matter, soil microbes, pH, temperature, and wet-dry cycles (Kalbitz *et al.*, 2000). In most cases, however, the effect of any one of these factors is not mutually exclusive from that of the others, though the effect of a particular factor may be more dominant, depending on the soil and prevailing environmental conditions.

There is limited information on the leaching of DOC from the fragile soils of pastoral hill country, especially after the alteration of topsoil DOC concentration through practices that increase plant residue such as forage crop establishment (via surface sowing technique), which is increasingly being adopted in New Zealand hill country farms.

Based on the aforementioned knowledge gap, this study tested the hypothesis that a substantial amount of DOC found within the subsoil arises from the leaching of recently added DOC from the topsoil. The objective of this study was to investigate if DOC originating from an artificial C source (maize silage) added to the soil surface, leaches down the soil profile under field conditions. Extrapolations were then made on how the results would influence subsoil denitrification for improved water quality.

7.2 Materials and methods

7.2.1 Study site

The study was carried out at Massey University's Agricultural Experiment Station – Tuapaka, a sheep and beef cattle hill country farm located approximately 15 km north-east of Palmerston North, New Zealand (40°21'20.1"S, 175°44'19.6"E). The study area is approximately 320 m above sea level and has a humid temperate climate, with an average annual rainfall of 1100 mm and predominantly dry summers.

The soil in the study area was developed from a colluvium of Ramiha silt loam soil (New Zealand classification: Allophanic Brown soil), with minor incorporation of Makara steepland soil (Orthic Brown soil). The soil is best described as a Typic Eutrudept in the USDA Soil Taxonomy (Soil Survey Staff, 2014). The parent material of the Ramiha silt loam soil is formed from a mixture of loess and volcanic ash, and contains allophane as the dominant clay constituent. The Makara steepland soil is formed essentially from greywacke (Pollok and McLaughlin, 1986).

7.2.2 Experimental design and sample collection

Two treatments were compared in this experiment, the + C source treatment which contained maize silage, and the control treatment (bare soil). Infiltration rings (56 cm in diameter and 14 cm high), six replicates for each treatment, were arranged in a Randomised Complete Block Design (RCBD) by inserting the rings along a straight line across a uniform slope (15°). In order to prevent DOC input from existing pasture species, the site was cleared of existing pasture by covering with plastic for two weeks prior to the commencement of the experiment. Subsequent pasture growth during the

study was removed by hand. Suction probes (ceramic cups) were inserted at three soil depths (0-20, 20-40 and 40-60 cm) within each ring (Figures 7.1 and 7.2) and these were allowed to stabilise within the soil for one month before maize silage (13 t ha^{-1}, dried and ground to < 2 mm, C/N = 30) was evenly distributed on the surface of the + C source rings only.

To ensure the active flushing of DOC (from maize silage) down the soil profile (60 cm), measured soil physical properties (section 7.2.3) were used to determine the pore volume and hence the irrigation to be applied to the infiltration rings (see section 7.3.1). The irrigation events occurred on a weekly basis from 6 June to 25 July 2017. An irrigation event also occurred before treatment application (23 May 2017) in order to obtain the background nutrient concentrations. Each irrigation event involved applying 85 mm (equivalent depth) of stream water to each infiltration ring. The DOC concentration (7 mg L^{-1}) of the stream water was determined prior to irrigation (and corrections were made during DOC calculation). To avoid preferential flow and ensure that the applied water seeped evenly throughout the area of the rings, a plastic grid (35 × 35 cm) was pinned onto the soil surface within each ring (Figure 7.2).

Soil water samples were collected two days after each irrigation event, from the three soil depths via the suction probes. Prior to sample collection, the probes were flushed with dinitrogen (N_2), to avoid oxic conditions. The collected soil water samples were transported to the laboratory for DOC analysis, which was carried out within 24 h of sample collection. The rest of the samples were stored below 4°C for subsequent analyses. An initial pilot study with a bromide tracer (potassium bromide – 7000 mg applied to the surface of the infiltration rings, irrigated with 85 mm of water and sampled two days after irrigation), performed before the application of maize silage, confirmed the assumption that water moved vertically down the soil profile (60 cm) through the infiltration rings.

At the end of the experiment, soil samples were collected from four depths within the effective pasture root zone (0-7.5, 7.5-15.0, 15.0-22.5, 22.5-40.0 cm) in each of the infiltration rings, and these soils were analysed for total C and N, and sodium pyrophosphate-extractable C fraction.

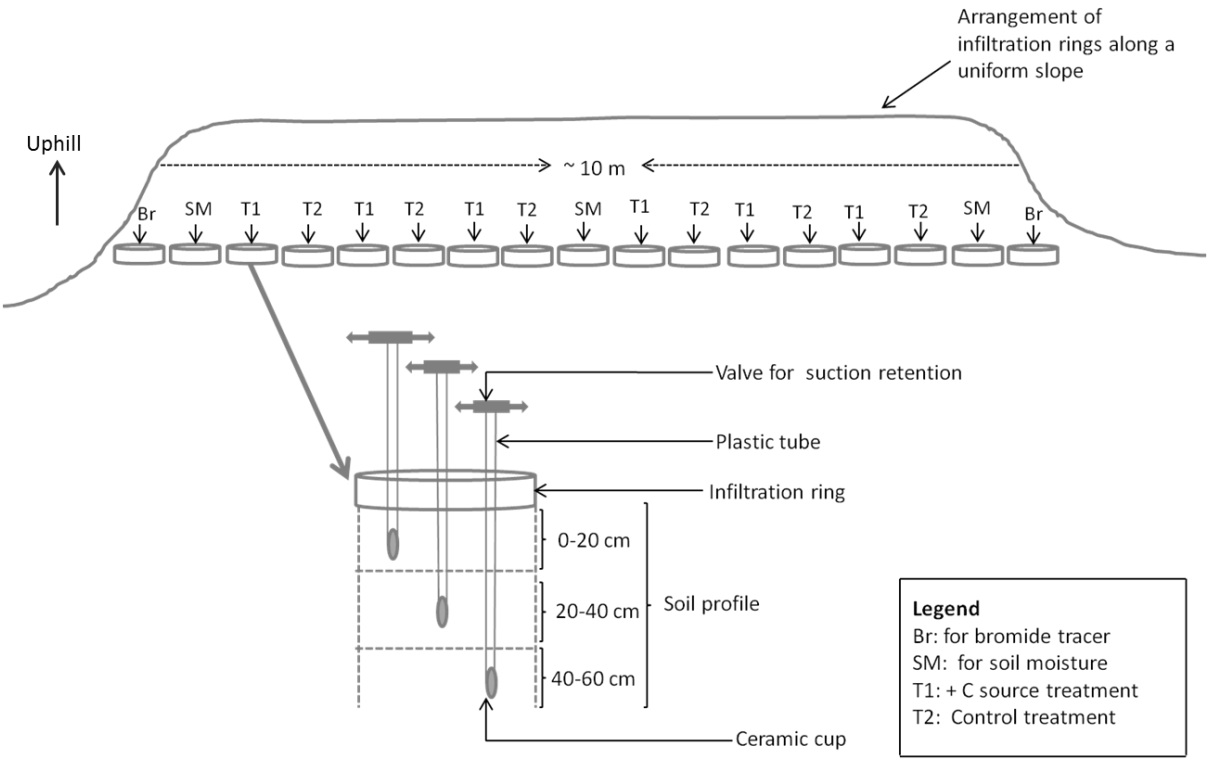

Figure 7.1: Schematic of experimental design

Figure 7.2: Arrangement of infiltration rings/suction probes in the field (insert shows a close-up photo of the suction probes and plastic grid within an infiltration ring)

7.2.3 Meteorological measurements, soil water balance and physical properties

The daily soil temperature of the site was measured with soil temperature sensors (HortPlus MicroLoggers, Model Z), which were buried at four soil depths (5, 15, 30, and 50 cm) close to the infiltration rings. Other meteorological site data collected on a daily basis were total rainfall, total solar radiation and total wind run, as well as average air temperature and relative humidity. These meteorological data, in addition to some site specific information (slope, aspect and depth of root zone), were used to model the soil water balance for the root zone at the site using the FAO56 version of Penman-Monteith equation (Allen *et al.*, 1998) with slope and aspect corrections applied to incoming solar radiation as described by Revfeim *et al.* (1982).

The gravimetric soil moisture content (from three depths: 0-20, 20-40, 40-60 cm) was measured on additional infiltration rings arranged alongside the experimental rings (Figure 7.1). Other soil physical properties determined on soil cores collected from the experimental site include: bulk density, field capacity, wilt point, and saturated hydraulic conductivity. These soil properties were determined as described by Klute (1986). In brief, bulk density was calculated after measuring the oven-dry (105°C for 16 h) weight of a known volume of soil. Field capacity and wilt point were measured by using a pressure plate to exert a suction of −0.33 and −15 atm, respectively to saturated soil samples, after which soil moisture was determined gravimetrically. Saturated hydraulic conductivity (Ksat) was determined via the intact core technique that involved measuring the volume of water flowing through saturated soil cores over a period of time.

7.2.4 Chemical analyses

The soil water samples were passed through a 0.45 µm filter and analysed for DOC concentration using a modified version of the semi-automated dichromate method described by O'Dell (1993) as follows: 10 mL of extract, 1.5 mL of digestion solution (5.1 g $K_2Cr_2O_7$ + 84 mL conc. H_2SO_4 + 16.7 g $HgSO_4$ + 500 mL deionised water) and 10 mL of catalyst solution (5 g $AgSO_4$ + 500 mL conc. H_2SO_4) were consecutively added into a 100 mL digestion tube. The solution was subsequently mixed with a vortex mixer and placed on a thermostatically controlled (150°C) digestion block for 2 h. After

digestion, the solution was allowed to cool to room temperature, made up to 25 mL with deionised water and mixed with a vortex mixer. Thereafter, the absorbance of the sample solution was read with a spectrophotometer (Philips PU 8625 UV/VIS, Biolab Scientific Ltd.) at a wavelength of 420 nm. The DOC concentration was subsequently obtained by plotting a calibration curve, using potassium hydrogen phthalate (KHP) as a standard.

In order to determine the fraction of organic C from the maize silage as opposed to that from the soil profile, the isotopic fractionation of organic C in soil water was carried out. To achieve this, the organic $\delta^{13}C$ isotope of the soil water (sampled on weeks 1 and 2 after treatment application) was determined with a modified OI Analytical model 1030 wet total organic carbon (TOC) analyser, with a model 1051 autosampler interfaced to a Thermo Finnigan DeltaPlus XP isotope ratio mass spectrometer (IRMS) for analysis by continuous flow. In addition, the isotopic composition of maize silage was determined by combustion on the elementar® (vario MICRO cube), followed by "trap and purge" separation and on-line analysis by continuous-flow with an IRMS coupled with a ConFlo III interface. The portion (F) of maize silage-derived C in DOC of the + C source treatment was determined based on the natural abundance isotope technique using a two-compartment isotopic mixing model (Blagodatskaya *et al.*, 2011) as follows:

$$F = (\delta^{13}C_t - \delta^{13}C_3) / (\delta^{13}C_4 - \delta^{13}C_3)$$

where $\delta^{13}C_t$ is the $\delta^{13}C$ value of the DOC under maize silage (+ C source treatment); $\delta^{13}C_3$ is the $\delta^{13}C$ value of the corresponding DOC in the control treatment; and $\delta^{13}C_4$ is the $\delta^{13}C$ value of maize silage.

Other analyses carried out on the soil water samples include nitrate concentration via the continuous flow analyser (Technicon® AutoAnalyser II), pH and redox potential (E_h) using standard pH and E_h meters (Meter Lab® and Eutech Instruments, respectively). Total dissolved iron (Fe) and manganese (Mn) concentrations were measured with a 4200 Microwave Plasma-Atomic Emission Spectrometer – MP-AES (Agilent Technologies). Bulk soil samples were analysed for total C and N content using the elementar® (vario MICRO cube). The sodium pyrophosphate-extractable C

fraction was determined as described by Blakemore *et al.* (1987), and the concentration of organic C in the sodium pyrophosphate extracts was determined with the semi-automated dichromate method as described above.

The analyses described above were carried out using appropriate quality control protocols, including the use of sample duplicates, blank, reference and spiked samples.

7.2.5 Statistical analyses

In order to detect significant differences between treatments, analysis of variance (ANOVA) was performed on the data, using General Linear Model (GLM) to account for the repeated measures design of the experiment. The Tukey comparison procedure was used for treatment comparison at 95% confidence level. The strength of the relationship between DOC and other measured parameters was determined with the Pearson correlation technique. All analyses were carried out on Minitab software (17.2.1 Minitab, Inc.).

7.3 Results

7.3.1 Daily rainfall, soil temperature, physical properties and water balance

The highest total daily rainfall occurred in week 6 (Figure 7.3), with 61 mm occurring one day after sample collection i.e. 14 July 2017, and 20 mm occurring on the sampling day. The lowest average daily soil temperature (4.2°C) also occurred in week 6 (on the day of sample collection i.e. 13 July 2017).

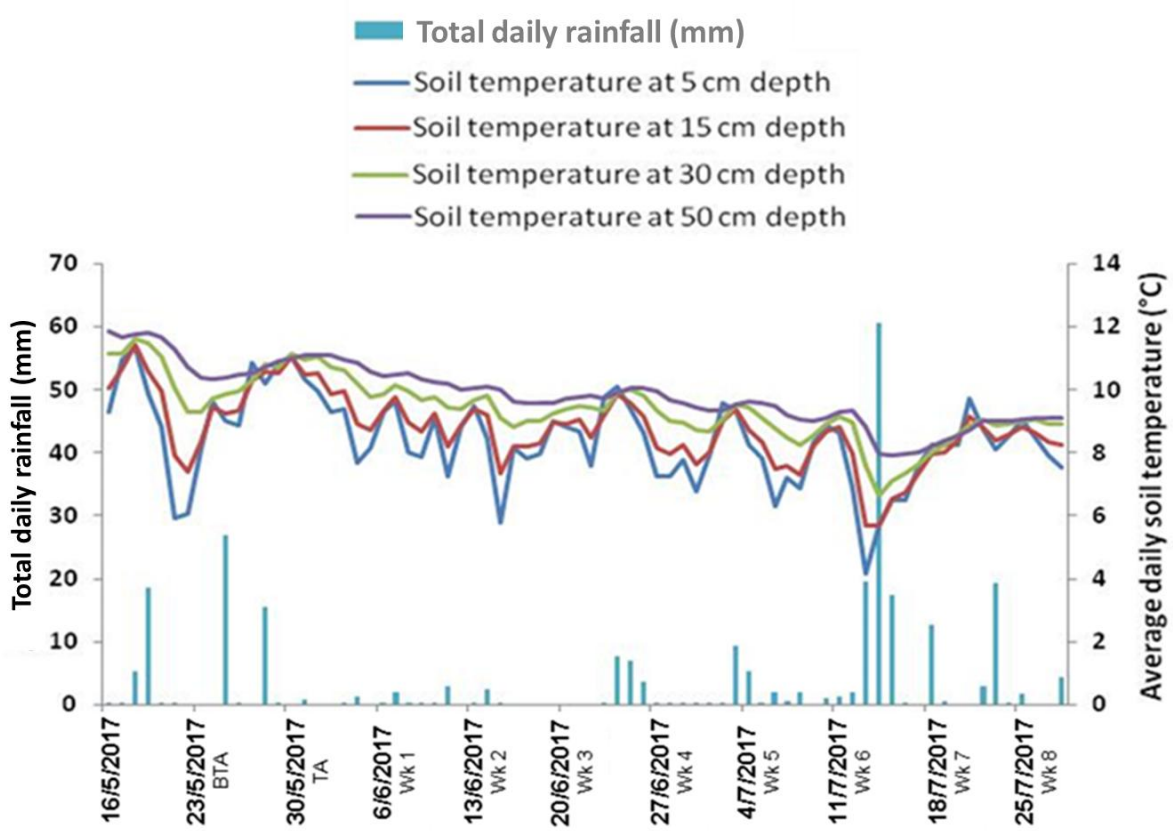

Figure 7.3: Total daily rainfall and average daily soil temperature during the study period. *BTA: before treatment application; TA: treatment application; Wk 1-8: weeks after treatment application. Eighty-five millimetres of water (irrigation) was applied before treatment application and on each of the dates after treatment application (data not presented in the graph).*

Table 7.1 shows that the accumulated 'total' pore volume down to 600 mm depth is 21.2 L for a cylinder with a diameter of 560 mm (which is representative of the infiltration rings used in the present study). This pore volume was obtained by subtracting wilt point from field capacity. Therefore, it is an approximation of the true pore volume because field capacity does not measure the water held in large macropores, as this water drains rapidly – at the rate determined by the saturated hydraulic conductivity (Ksat) of the soil (Table 7.2) – until field capacity is reached. Similarly, the measurement of wilt point does not account for the extremely small micropores containing water. This water is held very strongly within these pores and takes little part in the 'pore flushing' process as water drains via matrix flow through the soil profile.

Based on the calculations in Table 7.1, one pore volumes equals 86.1 mm. The original intent of the experiment was to add two pore volumes over 2 months (i.e. 172 mm) to flush the DOC (from maize silage) down the soil profile (60 cm). However, due to an error of unit conversion in a previous calculation, 7.9 pore volumes were added over 2 months (i.e. 681.9 mm). This was applied as eight weekly applications (i.e. 85.2 mm per week) from 6 June to 25 July 2017 (the weeks after treatment application).

Table 7.1: Calculated pore volume for specific soil depths

Depth (mm)	Bulk density (ρ) (g cm^{-3})	Wilt point (mm)	Field capacity (mm)	Pore volume (mm)	Pore volume (L) (560 mm diameter ring)
0 – 50	0.74 ± 0.05	22.9 ± 1.3	34.0 ± 1.1	11.1	2.7
50 – 100	0.94 ± 0.06	20.5 ± 1.3	29.1 ± 0.7	8.6	2.1
100 – 200	1.04 ± 0.11	36.2 ± 1.5	55.2 ± 2.6	19.0	4.7
200 – 300	1.14 ± 0.06	36.6 ± 3.1	51.1 ± 2.2	14.5	3.6
300 – 400	1.23 ± 0.08	38.0 ± 2.7	49.8 ± 2.0	11.8	2.9
400 – 600	1.30 ± 0.07	78.0 ± 3.0	99.1 ± 3.3	21.1	5.2
600 – 800	1.39 ± 0.09	69.5 ± 8.6	94.6 ± 5.3	25.1	6.2
800 - 1000	1.21 ± 0.18	69.8 ± 8.7	102.8 ± 13.0	33.0	8.1

Bulk density and field capacity values were averaged over four cores down to 600 mm depth, over three cores for 600 – 800 mm depth, and over two cores for 800 – 1000 mm depth; wilt point values were averaged over four cores; solid soil particle density was assumed to be 2.65 g cm^{-3}; error values are standard errors of the mean; pore volume for each depth was calculated by subtracting wilt point from field capacity.

Table 7.2: Saturated hydraulic conductivity (Ksat) of the three soil depths examined in the experiment

Profile ID	Soil depth (cm)	Ksat (mm/h)
I	0-20	23.50
	20-40	1.29
	40-60	0.00
II	0-20	26.01
	20-40	1.85
	40-60	0.00

The calculation of a soil water balance could not be used to predict daily soil moisture status at the soil depths examined in the present study. This is because the infiltration rings (height = 140 mm) penetrated only the top 70 mm of the soil (the purpose of these rings was to localise the application of irrigation water and leaching of DOC from maize silage). At this soil depth (70 mm), there is a less likelihood of lateral loss of the applied water. However, since 85 mm of water was applied to the infiltration rings during each irrigation event (nine events), all the pores within the depth of the rings will quickly become saturated and lateral movement of water is likely to be induced from beneath the rings to the surrounding drier soil matrix. Therefore, even though the bromide tracer pilot study indicated that water moved vertically down the profile through the infiltration ring, the results may well have been satisfactory with the first application of 85 mm of water (before the application of maize silage), but the subsequent weekly applications will have quickly saturated the soil and encouraged lateral flow. This lateral spread is due to the marked decrease in saturated hydraulic conductivity with depth (Table 7.2) as well as the hydrostatic head (~ 70 mm) associated with the irrigation event. The quantity of this lateral flow is unknown; however, it is likely to be significant, especially below the surface 300 mm depth, where hydraulic conductivity (both saturated and unsaturated) is diminished.

7.3.2 Variations in DOC concentration of soil water after the application of maize silage

Soil water DOC concentration of the + C source (maize silage) and control treatments decreased with depth (38-58% reduction compared to the surface 20 cm depth) (Figure 7.4). Differences in DOC concentration of both treatments were significant ($p \leq 0.05$) only within the surface 20 cm soil depth in week 1 after treatment application (> 58% difference between treatments). Thereafter, there was a notable reduction in the DOC concentration of the + C source treatment (> 50% reduction in week 2), and no significant difference was observed between both treatments for the rest of the experiment (weeks 2-8).

The estimate of maize-silage-derived C in DOC of the + C source treatment, measured within the surface 20 cm soil depth in week 1 (23%) was not significantly ($p \leq 0.05$)

different from that in week 2 (14%) (Figure 7.4). A negligible amount of this DOC (≤ 5%) was found within the 20-60 cm soil depth in weeks 1 and 2 after treatment application.

When differences in the DOC concentration of the sampling dates were compared, no specific trend was observed for both treatments (Figure 7.4).

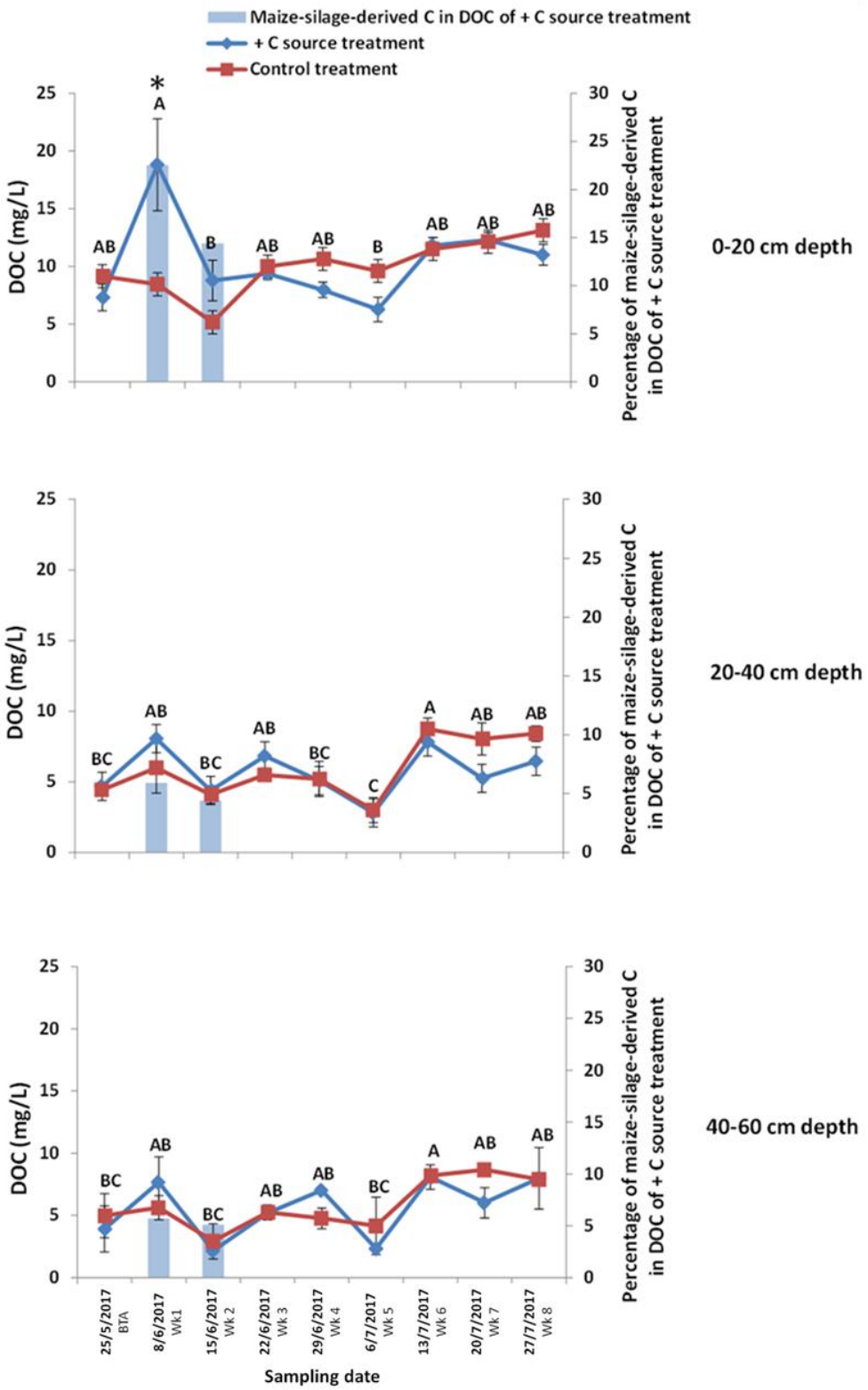

Figure 7.4: Weekly variations in DOC concentration of + C source and control treatments at different soil depths, and percentage of maize-silage-derived C in DOC of + C source treatment on the first two weeks after treatment application. *BTA: before treatment application; Wk 1-8: weeks after treatment application; * represents significant difference between treatments; different letters represent significant difference ($p \leq 0.05$) between sampling dates for a particular soil depth. Error bars are standard error of the mean ($n = 6$).*

7.3.3 Variations in properties of soil water

The nitrate concentrations of both the + C source and control treatments were generally higher within the 40-60 cm soil depth compared to the upper soil layer (0-40 cm), in weeks 2 to 4 after treatment application and irrigation (Figure 7.5). There was a significant ($p \leq 0.05$) decrease in the nitrate concentration of both treatments with increase in sampling time, at all the soil depth examined. The + C source treatment had consistently lower mean nitrate concentration within the 0-40 cm soil depth; however, significant ($p \leq 0.05$) differences were observed only within the top 20 cm depth, in weeks 3 and 4 after treatment application.

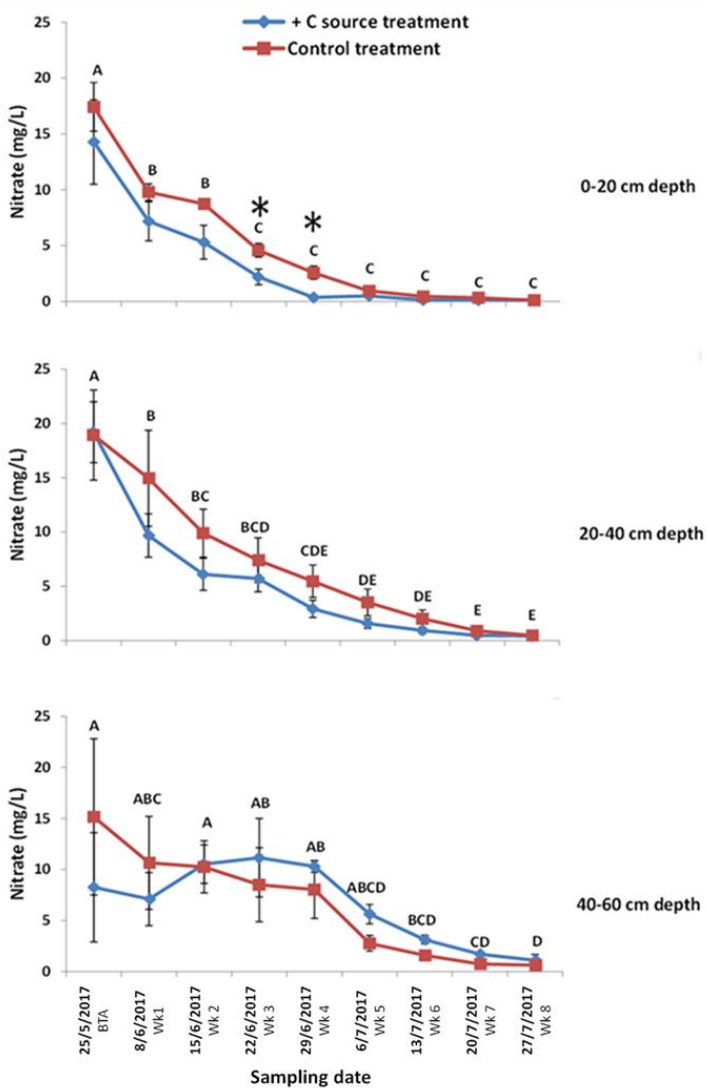

Figure 7.5: Weekly variations in nitrate concentration of + C source and control treatments at different soil depths. *BTA: before treatment application; Wk 1-8: weeks after treatment application; * represents significant difference between treatments; different letters represent significant difference ($p \leq 0.05$) between sampling dates for a particular soil depth. Error bars are standard error of the mean (n = 6).*

No specific trend was observed in the dissolved Fe concentration of the sampling dates and treatments for all soil depths considered (Figure 7.6). Within the 0-20 cm depth, the control treatment had a significantly ($p \leq 0.05$) higher dissolved Fe concentration in week 3, whereas within the 40-60 cm depth, significantly ($p \leq 0.05$) higher Fe concentrations were observed on the + C source treatment in weeks 2 and 5, and on the control treatment in week 4.

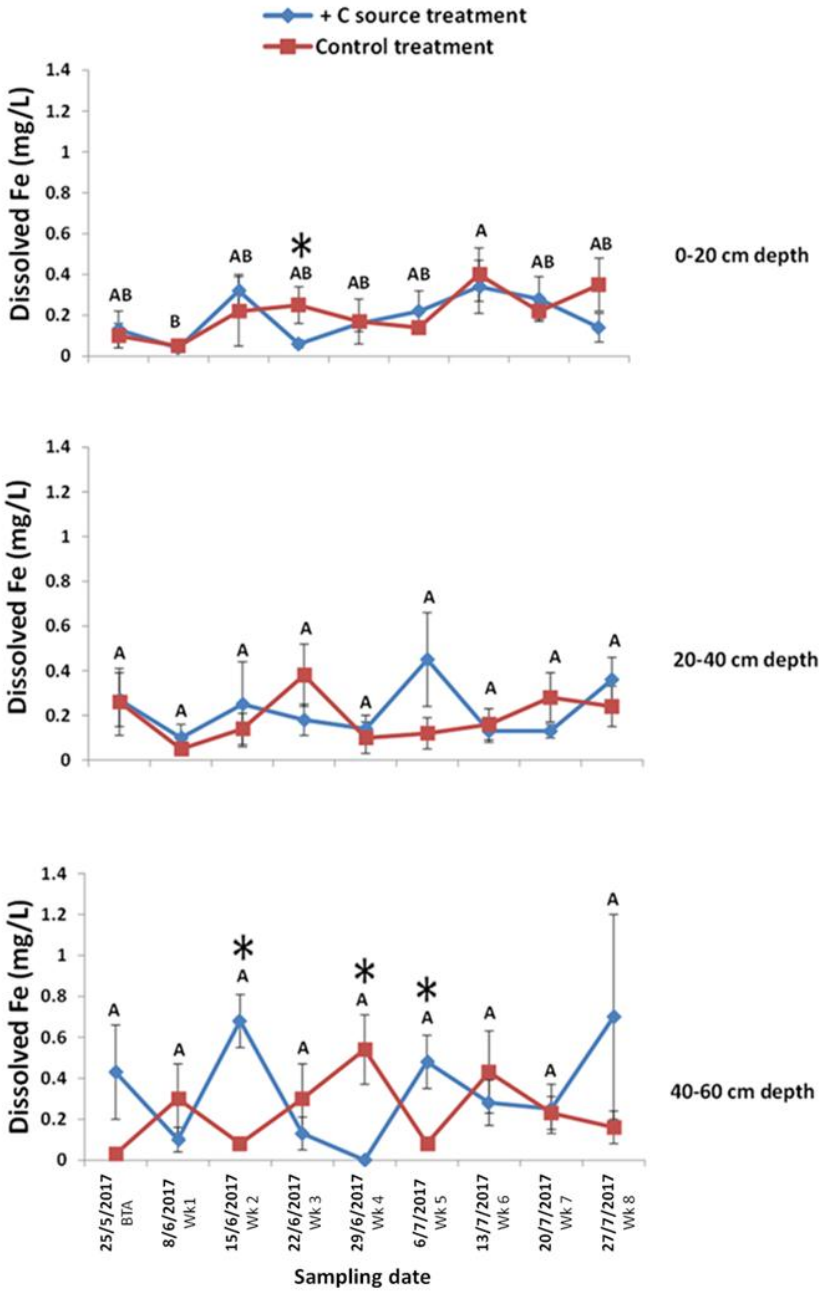

Figure 7.6: Weekly variations in dissolved Fe concentration of + C source and control treatments at different soil depths. *BTA: before treatment application; Wk 1-8: weeks after treatment application; * represents significant difference between treatments; different letters represent significant difference ($p \leq 0.05$) between sampling dates for a particular soil depth. Error bars are standard error of the mean (n = 6).*

There was no significant ($p \leq 0.05$) difference in the pH and E_h of the + C source and control treatments at all the depths considered (Figures 7.7 and 7.8). The pH and E_h range values for both treatments were 6.5-7.6 and 0.39-0.43 V, respectively. There were no trends observed for these parameters across sampling dates and soil depth.

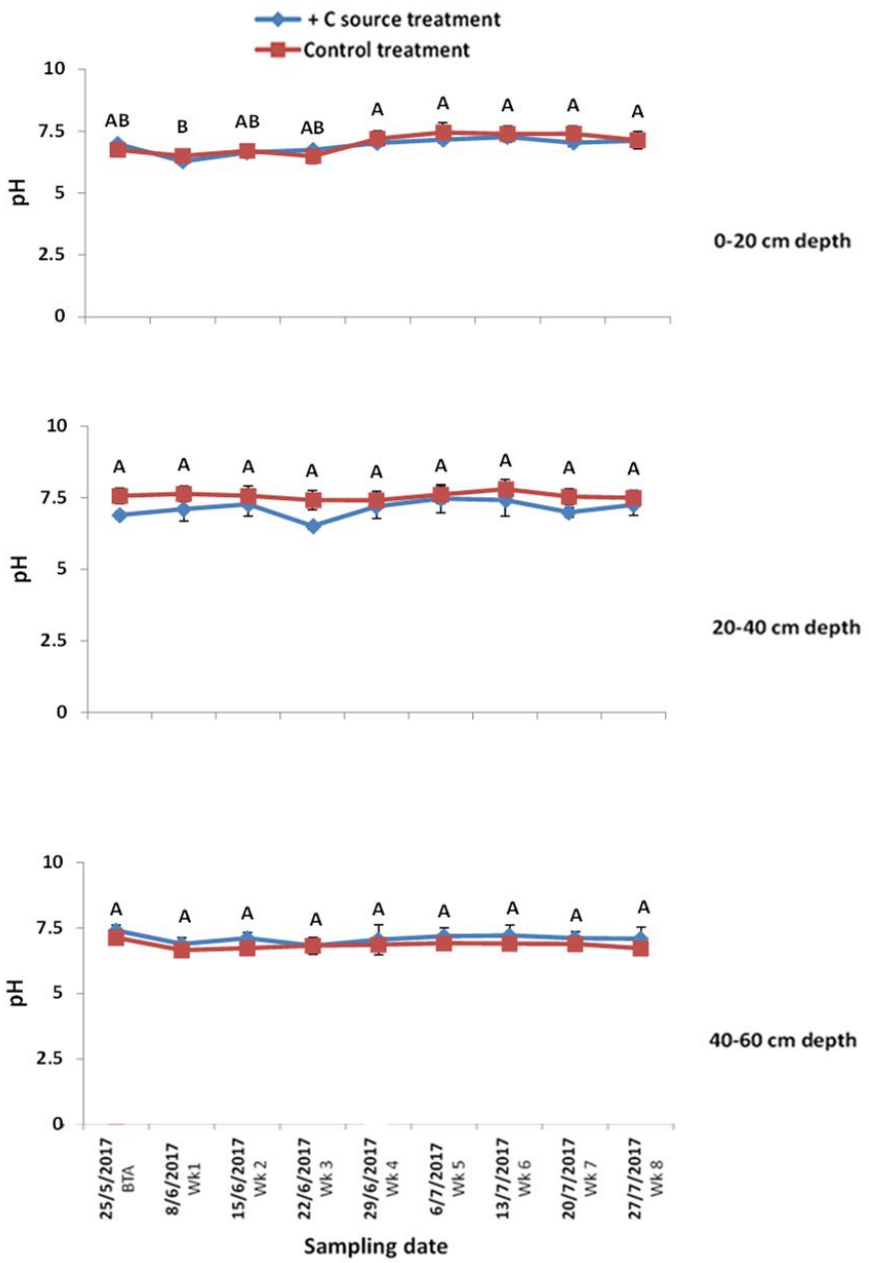

Figure 7.7: Weekly variations in pH of + C source and control treatments at different soil depths. *BTA: before treatment application; Wk 1-8: weeks after treatment application; different letters represent significant difference ($p \leq 0.05$) between sampling dates for a particular soil depth. Error bars are standard error of the mean (n = 6).*

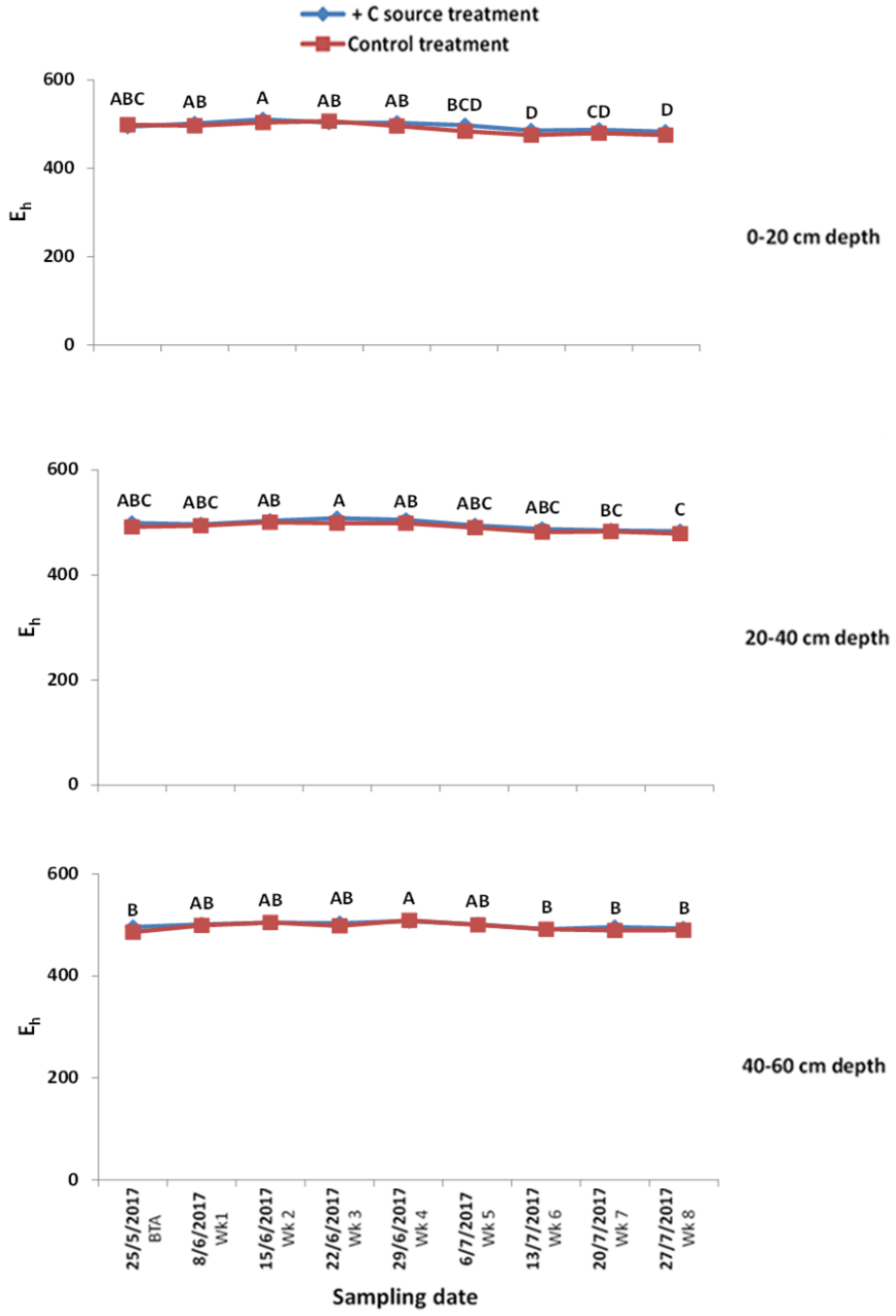

Figure 7.8: Weekly variations in E_h of + C source and control treatments at different soil depths. *BTA: before treatment application; Wk 1-8: weeks after treatment application; different letters represent significant difference ($p \leq 0.05$) between sampling dates for a particular soil depth. Error bars are standard error of the mean (n = 6).*

Dissolved Mn was below the detection limit (0.01 mg L^{-1}) in both the + C source and control treatments. There was no significant ($p \leq 0.05$) difference in the sodium pyrophosphate-extractable C fraction of both treatments (Figure 7.9) – both had a C/N ratio of ~11 at the soil depths considered.

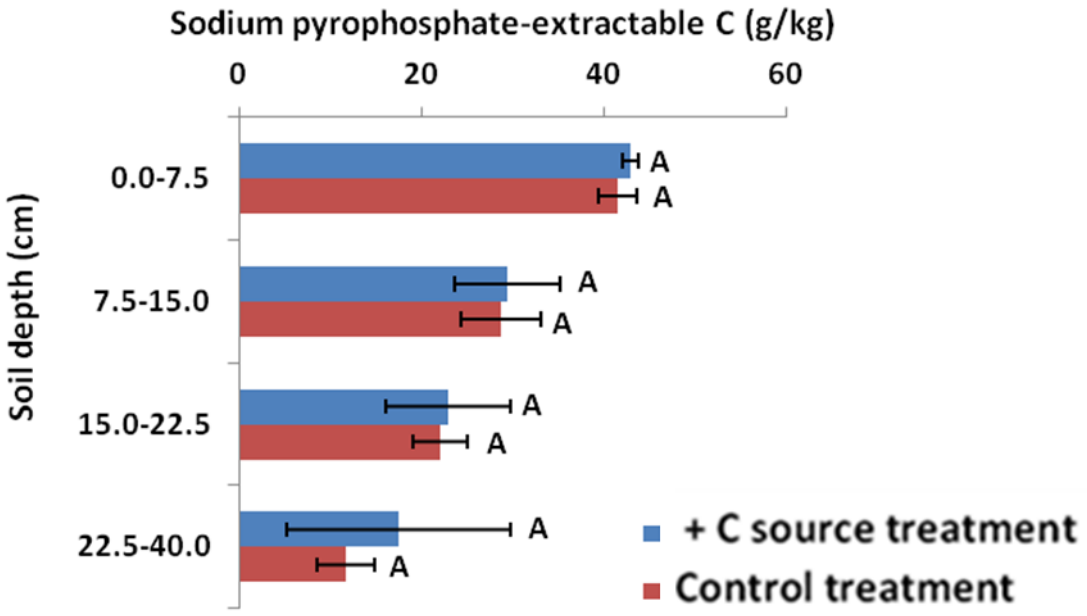

Figure 7.9: Sodium pyrophosphate-extractable C concentration of + C source and control treatments at different soil depths. *Different letters represent significant difference ($p \leq 0.05$) between treatments for a particular soil depth. Error bars are standard error of the mean (n = 6).*

7.3.4 Relationship between DOC concentration and properties of soil water

Significant ($p \leq 0.01$) negative correlations existed between DOC and nitrate in weeks 3, 6, and 8 after treatment application, as well as when all the sampling dates were combined (Table 7.3). The relationships between DOC and other properties (pH, E_h and dissolved Fe) were not consistent, i.e. both positive and negative relationships were observed, and these relationships were mostly insignificant.

Table 7.3: Pearson correlation coefficients (r) between DOC concentration and soil water properties of both the + C source and control treatments

Sampling dates	Nitrate	pH	E_h	Dissolved Fe
25 May 2017 (BTA)	−0.047	−0.424	0.296	−0.492*
8 June 2017 (Week 1)	−0.388	−0.425	0.349	−0.222
15 June 2017 (Week 2)	−0.203	−0.105	0.450*	−0.007
22 June 2017 (Week 3)	−0.521**	−0.333	0.352	−0.230
29 June 2017 (Week 4)	−0.325	0.031	−0.227	−0.252
6 July 2017 (Week 5)	−0.352	0.106	−0.331	−0.032
13 July 2017 (Week 6)	−0.463**	0.215	−0.340	0.396
20 July 2017 (Week 7)	−0.406	−0.108	−0.186	0.076
27 July 2017 (Week 8)	−0.492**	−0.111	−0.228	0.180
ALL (25 May–27 July 2017)	−0.232**	−0.135	−0.151	−0.033

BTA: before treatment application; Weeks 1-8: weeks after treatment application; *significant at 0.05 level; **significant at 0.01 level; n = 27 (BTA and weeks 1-8), and 243 (ALL).

7.4 Discussion

The artificial C source only influenced soil DOC concentration within the 0-20 cm soil depth (mostly within the confines of the infiltration rings), in the first week after treatment application and irrigation. The absence of significant DOC differences between the + C source and control treatments after the first week is unlikely to be as a result of a slow turnover rate of the maize silage, since it was dried and ground to increase its surface area and thus maximise its interaction with soil microbes. It is also unlikely to be due to the high flux of water that was added to the soil after treatment application, since negligible amounts of maize-silage-derived C in DOC (as estimated by the isotopic ^{13}C signature) were measured within the 20-60 cm soil depth compared to the top 20 cm. Conversely, the absence of significant differences after the first week could be attributed to the rapid turnover of exogenous DOC. This result is consistent with that of Zhao *et al.* (2017) who reported that the rate of C mineralisation on a maize stover amended soil peaked on the second day of incubation and decreased with incubation time, compared to the control soil. Similarly, Chen *et al.* (2009) also reported that mineralisation of maize residue was rapid within the first 14 days of

incubation, and subsequent mineralisation was slow. In addition, Wilts *et al.* (2004) noted that over a 29-year period, only 5-43% of C from maize stover enters the soil C pool, while the remaining portion enters the atmosphere. Even though DOC constitutes a small fraction of organic C, it is readily available to soil microbes and thus can be easily mineralised within a short timescale (Gu *et al.*, 2004). Hence, this supports the assumption that DOC from maize silage was rapidly mineralised within the first week in the present study. The near neutral soil pH (6.5-7.6) could have also favoured microbial activities and hence the turnover of DOC in this soil (Yadvinder *et al.*, 2005).

The negligible amount of maize-silage-derived C in DOC found within the subsoil is consistent with the rapid turnover of the added DOC within the topsoil. It is also consistent with the findings of McCarty and Bremner (1992), who reported that the rapid decomposition/mineralisation of plant residue in the topsoil resulted in a reduction in DOC availability for subsoil denitrification. The decomposition of DOC within the topsoil has also been shown to result in the presence of a hardly-decomposable form of DOC (i.e. the leftover of decomposition) in the subsoil (Schwesig *et al.*, 2003; Kaiser and Kalbitz, 2012). Thus, this may explain why previous studies on the same site (Chapters 5 and 6) showed that spraying out pasture (which created decaying plant residue) for forage crop establishment did not result in significant changes in the DOC concentration and denitrification capacity of the subsoil. The relatively large amount of DOC observed below the topsoil in these studies (Chapters 5 and 6) is likely to be more associated with the presence of buried soils caused by the burrowing activities of earthworms (expressed as tunnels of dark coloured soils and small pieces of charred wood from the burning of native bush). In addition, since the study was carried out on a colluvial soil, the geological history of colluvial deposition is likely to result in buried soils of varying maturity. Although buried soils have been shown to enhance denitrification capacity at depth (Barkle *et al.*, 2007; Clague *et al.*, 2013), the older forms of organic C in these soils may not be preferred by soil microbes compared to the newer and more readily available C in the topsoil (Schwesig *et al.*, 2003; Kaiser and Kalbitz, 2012). Hence, this may have contributed to the reduced denitrification capacity observed below the surface 30 cm soil depth (despite the presence of DOC) as reported in Chapter 6. However, further detailed research is required to arrive at a definite conclusion regarding this assumption.

The potential influence of lateral flow on DOC concentration at depth (below the infiltration rings) cannot be disregarded. Thus, it is also possible that some maize-silage-derived C in DOC moved away from the profile without being captured by the suction cups. Lysimeter studies would, therefore, be needed to eliminate this uncertainty while monitoring the leaching of DOC in the soil. In addition, the possibility of C (from the artificial source) retention within the profile was not investigated in this study and should not be overlooked, even though at the near neutral pH measured in this study (probably due to the high flux rate), C sorption is likely to be lower compared to under more acidic conditions (Doetterl *et al.*, 2015). The marked reduction in DOC concentration (of soil water) with depth suggests that the near neutral pH had negligible effect on the displacement of existing C adsorbed on reactive surfaces within the subsoil (Monteith *et al.*, 2007).

It is possible that the slightly higher DOC concentration measured within the 20-60 cm soil depths on 13 July 2017 (week 6) was due to the 20 mm of rainfall that occurred on this day, which (in addition to the 85 mm of irrigation) could have increased DOC leaching. Furthermore, the lower soil temperature (4.2°C at the top 5 cm) measured on this day likely slowed DOC mineralisation (Christ and David, 1996) and thus contributed to the amount of DOC that leached through the soil profile. However, more detailed research would be needed to confirm this assumption.

The significant negative correlations observed between nitrate and DOC concentration suggests that denitrification and/or N immobilisation were causing nitrate concentrations to drop, especially on the + C source treatment which had consistently lower nitrate concentrations (compared to the control treatment) in the surface 40 cm depth. Denitrification could have occurred on the + C source treatment due to the blocking of soil pores by the plant residue or greater carbon dioxide production – both of which enhance anoxic soil conditions. However, microbial measurements would be required to confirm these assumptions. The slight increase in nitrate concentration with depth observed in both treatments also suggests the possible leaching of nitrate on this well-drained soil, given that a considerable amount of water was added to the soil column. This (leaching) may also explain why there was a marked decrease in the

nitrate concentration of both the + C source and control treatments (especially in the surface 20 cm depth) as the study progressed.

7.5 Conclusion

The addition of plant residue to this hill country soil increased its topsoil DOC concentration in the first week after treatment application. Under the experimental conditions of this study, leaching of the plant-residue-derived DOC was limited and this was attributed to a fast turnover of exogenous DOC, although other processes such as lateral flow and the potential retention of C within the soil profile cannot be ignored.

This study suggests that practices such as the spraying out of pasture before forage crop establishment (which increases topsoil DOC concentration) in hill country farms, are unlikely to increase subsoil DOC concentration (for potential denitrification). Therefore, the relatively large amounts of DOC present in the subsoil layers of the studied soil may be associated with buried soils from the burrowing activities of earthworms and colluvial deposition, rather than recent organic materials from the topsoil.

CHAPTER 8: Summary and recommendations

8.1 Summary

Pastoral hill country landscapes occupy more than 60% of New Zealand's agricultural area. Sheep and beef cattle production on these landscapes contributes substantially to the country's export earnings. Due to the unique features and complexity of hill country landscapes (steep slopes, fragile soils, and presence of grazing animals), agricultural production on these landscapes potentially increases the leaching and runoff of nitrate to ground and surface waters, thus negatively impacting on water quality. Furthermore, due to the Government's Business Growth Agenda to significantly increase the value of New Zealand's Primary Industry exports by 2025, agricultural intensification is anticipated on pastoral hill country farms. One of such agricultural intensification practices, which has been widely adopted in hill country, is the replacement of perennial pasture with forage crops for supplementary feed production. It is important to ensure that such practices which increase production also minimise negative impacts on the environment, particularly on water quality, in order to advance the long-term sustainable productivity of hill country landscapes.

A review of literature on nitrate attenuation (Chapter 2) showed that denitrification is an important nitrate attenuation process that results in the removal of nitrate from soil-water systems. Hence, it positively impacts on the quality of water leaving agricultural catchments. A key factor limiting denitrification below the topsoil is the supply/availability of dissolved organic carbon (DOC). Several edaphic and environmental factors affect the availability of DOC for denitrification. Therefore, the unique features of hill country landscapes (soil type, slope, wet areas and land use) have the potential to influence the leaching and availability of DOC for subsurface denitrification. However, the mechanisms by which these landscape features affect the DOC and denitrification capacity of hill country soils is yet to be investigated. Consequently, this research (thesis) was designed to assist in filling this knowledge gap.

The aim of this thesis was to investigate the influence of hill country landscape features on DOC concentration and the potential for nitrate loss via denitrification. In particular, the thesis objectives were to investigate the following:

1. The influence of soil type and slope on the DOC concentration and denitrification capacity of pastoral hill country.
2. The denitrification capacity of hill country wet areas and the role of DOC in nitrate attenuation within these landscape features.
3. The effect of the annual establishment of forage crops (land use change) on DOC dynamics in the root zone, its transport below the root zone, and the impact on soil denitrification capacity.

To achieve these objectives, several experiments were conducted on Massey University's Agricultural Experiment Station (Tuapaka), a sheep and beef cattle hill country farm located approximately 15 km north-east of Palmerston North, New Zealand. The main findings of these experiments are discussed in this chapter.

In general, the factors (examined in this thesis) that influence nitrate attenuation in pastoral hill country are grouped into two categories, namely dry area and wet area features (Figure 8.1). The dry area features are soil type, slope, and land use (influenced by weather variables), while seepage wetland and hillside seep are the wet area features.

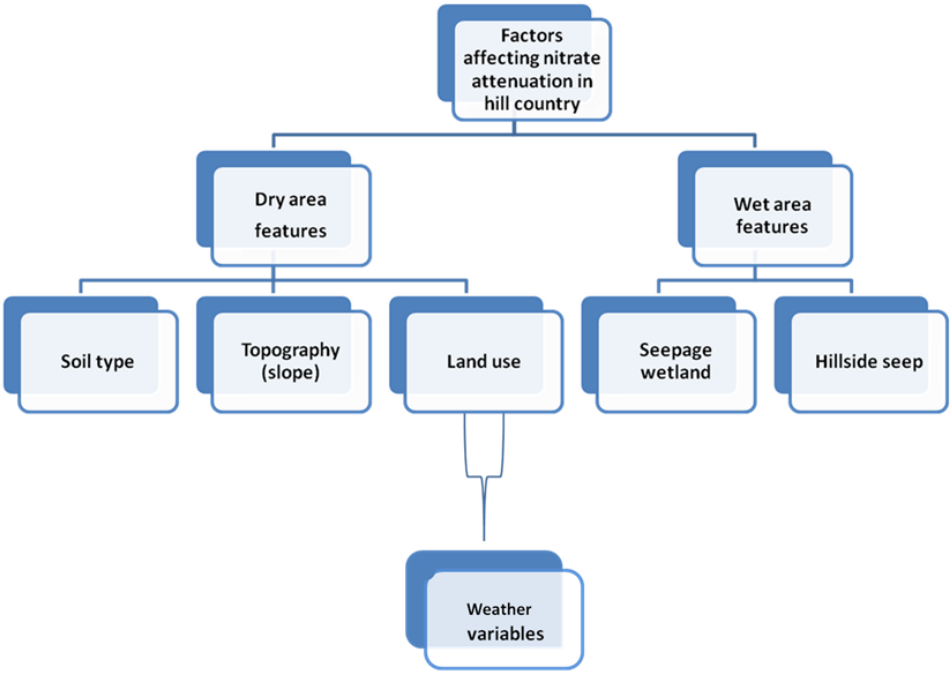

Figure 8.1: Summary of the factors (examined in this thesis) affecting nitrate attenuation in pastoral hill country

The effects of soil type and slope on DOC concentration and denitrification capacity of pastoral hill country soils were assessed (Chapter 3) by sampling fifty locations selected from the lowest to the highest elevation in the farm. These locations were distributed across three slope classes which were comprised of eight different soil types (grouped into three drainage classes). This experiment showed that regardless of slope and soil drainage class, the highest denitrification capacity occurred in the soil with the highest DOC concentration, i.e. the Ramiha soil, which is a well-drained soil on medium slope. It was expected that poorly-drained soils on low slope would have the highest denitrification capacity due to the tendency of these soils to accumulate carbon (C); however, this was not the case in this study.

The high DOC concentration of the Ramiha soil was attributed to its parent material which consists of a high amount of short-range order constituents such as allophane and thus a high capacity to store C. Therefore, it was concluded that the Ramiha soil (and soils with similar characteristics) could play an important role in nitrate attenuation in hill country farms where they occur and thus should be accounted for if nitrogen (N) loss restrictions are introduced in hill country farms in the future. Consequently, farm scale soil mapping of hill country pastoral systems would potentially offer more options to manage nitrate losses from these farms. However, despite the relatively large amount of DOC found within the 60-100 cm depth layer of the Ramiha soil, the denitrification capacity at this depth was minimal. The reason for this minimal denitrification capacity was not established in this experiment, though it was assumed to be related to a decrease in the availability of denitrifying microbes and/or other nutrients.

A forage crop trial was established to investigate the effect of the annual establishment of forage crops (land use change) on DOC dynamics in the root zone, its transport below the root zone, and the impact on the denitrification capacity of a hill country soil. The first phase of this experiment (Chapter 5) aimed at examining the changes in the DOC concentration and chemistry of a hill country soil (Ramiha colluvium), as influenced by the agrochemicals used in clearing out pasture before forage crop establishment using the surface sowing technique. The results of the experiment indicated that the applied agrochemicals increased the DOC concentration of the surface 5 cm soil depth by ~ 20 mg kg^{-1} on days 1 and 6 after application of the agrochemicals. This increase was most probably due to the following mechanisms: (i) direct

contribution of C from the agrochemical/organic molecules; (ii) indirect C contribution via the displacement of adsorbed organic molecules; and (iii) root necromass decomposition. The agrochemicals did not influence the DOC concentration of soil layers below the surface 5 cm depth, and this was likely due to the limited mobility of the agrochemicals under the experimental conditions studied. Soil DOC chemistry was not influenced by the applied agrochemicals in this experiment. The agrochemicals, however, seemed to have enhanced N mineralisation only within the surface 5 cm soil depth. These results imply that the agrochemicals used for clearing out pasture before crop establishment are unlikely to have any impact on nitrate attenuation in the subsoil environment.

The second phase of the forage crop trial (Chapter 6) focused on monitoring temporal changes in the DOC concentration and denitrification capacity of the established treatments (cropping and pasture) over a one-year period. The results of this phase of the experiment showed that, within the duration of the study, soil DOC concentration and denitrification capacity were generally not influenced by the establishment of a brassica forage crop via the surface sowing technique. However, forage crop establishment resulted in an initial increase in the nitrate concentration of the surface 20 cm soil depth (six months after crop establishment). This effect was absent below the surface 20 cm depth, suggesting that there was no evidence that swede cropping increased the risk of nitrate leaching. It is worth noting that this study was carried out on a small plot scale (plot size of 4 × 4 m for each four replicates). Therefore, larger scale experiments may be required to further examine the effect of forage crop establishment on nitrate attenuation in New Zealand hill country farms.

Weather variables, particularly rainfall seemed to have influenced DOC concentration in the second phase of the land use change (forage crop establishment) experiment. An increase in rainfall and soil moisture, after periods of soil water deficit, increased the DOC concentration of the studied soil. Strong correlations were also observed between soil moisture parameters (water-filled pore space–WFPS and volumetric soil moisture content) and denitrification capacity in this phase of the experiment, thus highlighting the important contribution of soil moisture to denitrification (i.e. increased substrate availability).

Similar to what was observed in the Ramiha soil (Chapter 3), negligible denitrification capacities were observed below 30 cm depth in this soil (Ramiha colluvium), even in the presence of relatively large DOC concentrations. The reason for this was not ascertained in this phase (second) of the experiment, but was again assumed to be related a decrease in the availability of denitrifying microbes and/or other nutrients.

Based on the results of the forage crop establishment trial, it was necessary to ascertain if DOC added to the topsoil eventually moves into the subsoil. Therefore, a DOC leaching experiment (Chapter 7) was carried out. This experiment attempted to address the origin of the DOC found in the subsoil. To achieve this, dried and ground maize silage was applied to the surface of infiltration rings which were inserted on a cleared out pasture soil (Ramiha colluvium). These rings were irrigated and sampled (down to 60 cm depth for soil water), via suction probes, on a weekly basis for eight weeks. The results of the study indicated that the addition of plant residue to the topsoil increased DOC concentration within the 0-20 cm soil depth, in the first week (only) after treatment application. During this period, the amount of maize-silage-derived C in DOC was > 20% in the surface 20 cm soil depth, and ≤ 5% further down the profile. The negligible amount of maize-silage-derived C in DOC found in the subsoil was attributed to the rapid turnover of exogenous DOC in the topsoil, though the design of the experiment could have also allowed the lateral flow of DOC away from the suction probes. The available data suggests that the relatively large amount of DOC found within the subsoil (Chapter 6) is likely to be associated with the presence of buried soils arising from earthworm burrowing activities and colluvial deposition.

The effect of hill country wet areas on nitrate attenuation (Chapter 4) was assessed by first comparing the DOC concentration of a seepage wetland, a hillside seep and an adjacent dry area soil, all within a paddock. The results showed that the DOC concentrations of these landscape features were in the order: seepage wetland > hillside seep > dry area. The DOC concentration of the hillside seep and dry area was similar in the surface 30 cm soil depth, but was significantly lower than that of the seepage wetland. A more detailed assessment of the same paddock involved further sampling of the seepage wetland and three dry area soils. This assessment indicated that the different dry area soils had comparable DOC concentrations but these were significantly lower than that of the seepage wetland at all the soil depths considered. The seepage wetland

sites had variable DOC concentrations which were assumed to be attributable to differences in soil moisture and deposition of animal dung and urine. Lower molecular weight DOC was also observed in the seepage wetland compared to the dry area, indicating the presence of a more-bioavailable/readily-decomposable form of DOC in the seepage wetland.

The denitrification capacity of the surface 60 cm soil depth of the seepage wetland was 7-69 times higher than that of the dry area. This was attributable to the large and readily-decomposable DOC found in the seepage wetland. The results of this experiment, therefore, suggest that DOC concentration and chemistry, as well as moisture content, were key factors that significantly enhanced the denitrification capacity of the seepage wetland. The results of the study also suggested that dissimilatory nitrate reduction to ammonium (DNRA) and manganese (Mn^{2+}) oxidation were other factors which could have influenced nitrate attenuation within the seepage wetland. Based on these findings, it is vital to delineate seepage wetlands in hill country farms where they occur, so that they can be appropriately managed for nitrate attenuation purposes. It is important to note that even though this experiment highlighted the important contribution of DOC concentration and chemistry to the denitrification capacity of hill country seepage wetlands, more detailed research involving stream monitoring (discussed in section 8.3) is needed to better understand and enhance the contribution of seepage wetlands to nitrate attenuation for improved water quality in pastoral hill country landscapes.

8.2 Key findings of the thesis

1. Regardless of slope and soil drainage class, denitrification capacity was highest in the soil with the highest DOC concentration (i.e. the Ramiha soil, which has a high content of short-range order constituents such as allophane and thus a high capacity to store C).
2. The agrochemicals used for clearing out pasture before forage crop establishment are unlikely to have any significant impact on DOC concentration and nitrate attenuation in the subsoil environment.
3. Leaching of DOC from plant residue was limited below the surface 20 cm soil depth, even under high water flux conditions.

4. Soil DOC concentration and denitrification capacity were generally not influenced by the establishment of a forage crop within a one-year time frame. Soil moisture and temperature had a greater influence on DOC concentration and denitrification capacity within this period.
5. DOC concentrations in this hill country study were in the following order: seepage wetland > hillside seep > dry area. The hillside seep and dry area had comparable DOC concentrations within the surface 30 cm soil depth.
6. The denitrification capacity in the surface 60 cm of the seepage wetland soil was 7-69 times higher than that of the dry area soil. This was due to the higher soil moisture, and the large and readily-decomposable DOC found in the seepage wetland.

The findings of this thesis provide a novel and valuable contribution to our understanding of denitrification capacity and nitrate attenuation potential in hill country pastoral landscapes. This study has shown that soil types such as Ramiha have unique properties which could enhance nitrate attenuation and should be identified and valued on farms. The thesis also showed that the increasingly common farm practice of hill country forage cropping is unlikely to impact on nitrate attenuation over the short-term and over the longer term, whereas factors such as soil temperature and moisture have a larger influence. Further research found that hill country seepage wetlands have a significantly higher capacity to attenuate nitrate compared to other hill country features, and represent a valuable landscape feature which could be enhanced to improve water quality leaving hill country farms.

This thesis has shown that both the dry area and wet area features in pastoral hill country farms impact on nitrate attenuation capacity in different ways and thus need to be managed accordingly to reduce nitrate loss to receiving waters. One way of managing these farms would be to map them at an appropriate scale to identify areas (such as seepage wetlands and soils with a high content of short-range order constituents e.g. the Ramiha soil) which have a higher potential to accumulate DOC and thus possess a higher nitrate attenuation capacity than surrounding areas. Strategies that maintain/enhance the nitrate attenuation capacity of these high nitrate attenuation areas also need to be promoted/adopted. For instance, seepage wetland areas on farms could

be fenced to limit/manage the risk of grazing animals becoming mired, rather than draining these areas which decreases their nitrate attenuation capacity. Fencing of these seepage wetlands would not reduce nutrient input substantially because the topography of seepage wetlands (flat area) is such that they are able to receive runoff and hence nutrients from the surrounding farm catchment. Such farm-scale management strategies would provide water quality improvements and also help hill country farmers gain environmental credits in a future N loss regulated system.

8.3 Recommendations for future research

The effects of soil type and slope on DOC concentration and denitrification capacity reported in this thesis were based on the combinations of soil drainage and slope classes present in the studied farm. It would be useful if this experiment is replicated in other pastoral hill country farms containing other combinations of drainage and slope classes which were not considered in the present study, as this would help to better quantify and manage the effect of these variables on the DOC concentration and denitrification capacity of pastoral hill country landscapes. The incorporation of other topographical components such as aspect in subsequent hill country experiments of this nature would be useful, as aspect has the potential to influence soil DOC concentration and denitrification capacity through its effect on soil moisture, with drier soil conditions occurring on northern aspects. Farm-scale spatial modelling of DOC concentration in terms of soil type and slope would also aid in effective N management for improved water quality outcomes in hill country farms.

In this thesis, relatively large amounts of DOC were found in the subsoil layers of both the Ramiha soil and its colluvium, yet the denitrification capacities of these subsoil layers were negligible. It is possible that the DOC within these subsoils is from buried soils (hence is not preferred by denitrifying microbes due to the more recalcitrant nature of DOC) as suggested by the results of the DOC leaching experiment. However, there are other reasons which could have been responsible for this, such as the reduced presence or complete absence of denitrifying microbes and/or other nutrients in these soil layers. Therefore, more detailed research is needed to ascertain the exact reason for this low denitrification capacity.

Temporal changes were assumed to be representative of seasonal changes in DOC concentration and denitrification capacity (following forage crop establishment) in this thesis. However, further investigation into the daily variability of DOC concentration and denitrification capacity during each season need to be carried out before such assumptions can be verified. Therefore, it is recommended that subsequent studies of this nature should increase the number of sampling days within each season. In addition, given that the forage crop establishment experiment described in this thesis was conducted on a small plot scale, larger scale experiments (involving larger plot size and replicates, as well as multiple forage crops and soil types) would be necessary to examine the effect of several variables and thus arrive at a more definitive conclusion regarding the effect of forage crop establishment on nitrate attenuation in New Zealand hill country landscapes.

The use of infiltration rings (with the high rate of irrigation) was a major limitation in the DOC leaching experiment because the rings allowed lateral flow of water away from the soil profile at depth and thus might have influenced the results of the study. The use of lysimeters is recommended for future DOC leaching experiments, as they provide a more contained system that will enable more definite conclusions to be made on the leaching of DOC in pastoral hill country soils.

The investigation of the contribution of seepage wetland to nitrate attenuation, carried out in this thesis, is a step forward regarding nitrate management for improved water quality in pastoral hill country. However, more detailed research would be required to adequately manage hill country seepage wetlands to reduce nitrate losses to receiving waters. Such future research should incorporate seasonal *in situ* denitrification measurements with the monitoring of streamflow and nitrate (and ammonium) concentrations into and out of the seepage wetland areas. In addition, this thesis did not ascertain the reason for the spatial variation in DOC concentration across the seepage wetland sites examined (though some assumptions were made). Therefore, it would be necessary to investigate these factors in subsequent seepage wetland studies, as they have the potential to influence denitrification capacity. Such studies should also investigate other pathways of nitrate attenuation such as DNRA, Fe^{2+} and Mn^{2+} oxidation, and plant uptake, which were not investigated in the present study.

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APPENDIX

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary 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:	Grace Chibuike
Name/title of Primary Supervisor:	Dr Lucy Burkitt
Name of Research Output and full reference:	
Chibuike, G., Burkitt, L., Bretherton, M., Campo-Alboslam, M., Singh, R., Bishop, P., Hedley, C. & Rhodes, P. (2019). Dissolved organic carbon concentration and denitrification capacity of a hill country sub-catchment as affected by soil type and slope.	
In which Chapter is the Manuscript /Published work:	Chapter 3
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	90
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
Grace assisted in designing the study and undertook all the field sampling and laboratory analysis. She statistically analysed the data, compiled all graphs and tables and drafted the manuscript independently. Our contribution as co-authors	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Grace Chibuike Digitally signed by Grace Chibuike Date: 2019.04.19 01:17:45 +12'00'
Date:	19/04/2019
Primary Supervisor's Signature:	Lucy Burkitt Digitally signed by Lucy Burkitt Date: 2019.04.19 12:39:44 +12'00'
Date:	19/04/2019

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

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Name of candidate:	Grace Chibuike
Name/title of Primary Supervisor:	Dr Lucy Burkitt
Name of Research Output and full reference:	
Chibuike, G., Burkitt, L., Campo-Arteaga, V., Bishop, P., Beckwith, W. & Singh, R. (2019). Effect of forage crop establishment on dissolved organic carbon dynamics and leaching in a hill country soil. Soil Use and Management. https://doi.org/10.1111	
In which Chapter is the Manuscript /Published work:	Chapter 5
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	90%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
Grace assisted in designing the study and undertook all the field sampling and laboratory analysis. She statistically analysed the data, compiled all graphs and tables and drafted the manuscript independently. Our contribution as co-authors	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Grace Chibuike Digitally signed by Grace Chibuike Date: 2019.04.19 01:38:43 +12'00'
Date:	19/04/2019
Primary Supervisor's Signature:	Lucy Burkitt Digitally signed by Lucy Burkitt Date: 2019.04.19 13:03:05 +12'00'
Date:	19/04/2019

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