An investigation of the spatial distribution of N_2O emissions from sheep grazed hill country pastures in New Zealand

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Abstract

New Zealand's (NZ) greenhouse gas (GHG) profile is unique amongst developed countries as almost 50% of GHG emissions are derived from agriculture. In contrast, agricultural sectors of other developed countries typically contribute <10% to the national total GHG profile. In NZ, agricultural GHG emissions are dominated by methane (CH_4) from enteric fermentation and nitrous oxide (N_2O) from excreta deposition and nitrogen (N) fertiliser application. Nitrous oxide emissions from agricultural soils are largely affected by N inputs and soil moisture conditions, and contribute 33% of agricultural GHG emissions. In pastoral hill country these factors are inherently more variable than in flat land pastures due to topography-driven differences in excretal N returns and in soil moisture. This limits the application of N_2O emission data collected from trials conducted on flat land to hill country situations. The objective of this thesis was to determine the influence of topography and fertiliser N inputs to soil on N_2O emissions in hill country. Small scale trials were conducted to measure these aspects of N cycling.

Three trials were conducted to measure the effect of slope and fertiliser N input on nitrification potential (NP) and N_2O emissions. The results of these short term trials suggested that slope class and fertiliser N rates significantly affected nitrification rates and N_2O emissions in hill country due to differences in N inputs and moisture status, as affected by slope. Both NP and N_2O emissions were highly spatially variable during the measurement periods and the results presented in this thesis suggest that the majority of N_2O emissions in sheep grazed hill country are produced from low slope/stock camping areas. Based on our findings it is recommended that mitigation options to reduce the risk of N loss from sheep grazed hill country should be targeted at low slope/stock campsite areas. Due to the significant relationship between slope class and N_2O emissions, slope class may be a suitable parameter for up-scaling estimates of N_2O emissions from sheep grazed hill country.

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CHAPTER 1 GENERAL INTRODUCTION

1.1 Introduction

By the late 1970's there was growing global recognition that increases in anthropogenic induced greenhouse gas (GHG) emissions were having an impact on the global climate. Through the United Nations (UN), the International Panel on Climate Change (IPCC), which included a collection of climate change experts from around the world, was formed in the late 1980's to investigate this phenomenon. Numerous intergovernmental meetings were held through the 1980's and early 1990's to address the growing concern around the increases in anthropogenic GHG emissions, and the impacts of global climate change (Ministry for the Environment 2010a). As a result of these meetings the Kyoto Protocol was formed in 1997. This is an international agreement to address global climate change. The Protocol's primary aim is to reduce total anthropogenic induced GHG emissions from developed countries (and countries with economies in transition) to 5% below the 1990 level, in an effort to stabilise or delay global climate change (Ministry for the Environment 2010a). The initial assigned total GHG emissions for NZ under the Kyoto Protocol is 61,893 Gg carbon dioxide equivalents (CO_2-e), (Ministry for the Environment 2010b). This amount cannot change during the first commitment period (2008–2012).

1.2 Agricultural greenhouse gas emissions in New Zealand

New Zealand's total GHG profile has changed from being dominated by methane (CH_4) from the agricultural sector (largely derived from enteric fermentation) in 1990, to being dominated by carbon dioxide (CO_2) from the energy sector (largely from electricity generation, heat production, and transport) in 2008 (Table 1.1, Ministry for the Environment 2010b). However, the agriculture sector remains the largest source of total GHG emissions (47% or 34,826 Gg CO_2-e) in NZ. New Zealand's GHG profile is therefore unique amongst developed countries where GHGs derived from the agricultural sector typically contribute <10% to the total GHG profile (Ministry for the Environment 2010b). New Zealand's agricultural GHG emissions are dominated by CH_4 from enteric fermentation, and nitrous oxide

(N_2O) from excreta deposition on pasture as well as increasing rates of nitrogenous fertiliser application (Ministry for the Environment 2010b). Agriculture alone contributed 96% (11,434 Gg CO_2-e) of NZ's total N_2O emissions in 2008 (Ministry for the Environment 2010b).

Table 1.1 New Zealand's greenhouse gas (GHG) emissions by gas in 1990 and 2008; Reproduced from the Ministry for the Environment, 2010b; pg 6

Direct GHG emissions	**1990**	**2008**	**Change from 1990**	**Change from 1990**
		Gg CO_2-e	**Gg CO_2-e**	**%**
CO_2	24,893.3	36,063.2	+11,169.9	+44.9
CH_4	25,456.4	25,816.2	+359.8	+1.4
N_2O	9,778.9	11,913.4	+2,134.6	+21.8
HFCs	NO	812.5	+812.5	NA
PFCs	629.9	38.8	-591.0	-93.8
SF_6	15.2	14.5	-0.7	-4.3
Total	60,773.6	74,658.7	+13,885.1	+22.8

CO_2, CH_4 and N_2O values exclude emissions and removals from LULUCF. The % change for HFCs is not applicable (NA) as production of HFCs in 1990 was not occurring (NO).

Two of the main sources of N_2O emissions in NZ agriculture are from the nitrogen (N) in the excreta (dung and urine) deposited on pasture by grazing stock, and from soils as a result of adding N in the form of synthetic fertiliser (Ministry for the Environment 2010b). Both these sources are considered key categories (key categories comprise 95% of the total emissions for each GHG) and are subject to Tier 2 Level and Trend assessment (Figure 1.1). Tier 2 involves a detailed approach to estimating N_2O emissions and requires country-specific information on livestock characteristics and management practices. The Tier 2 approach is recommended when the data used to develop the IPCC default values (Tier 1) do not correspond well with the country's livestock and management conditions (IPCC 2006).

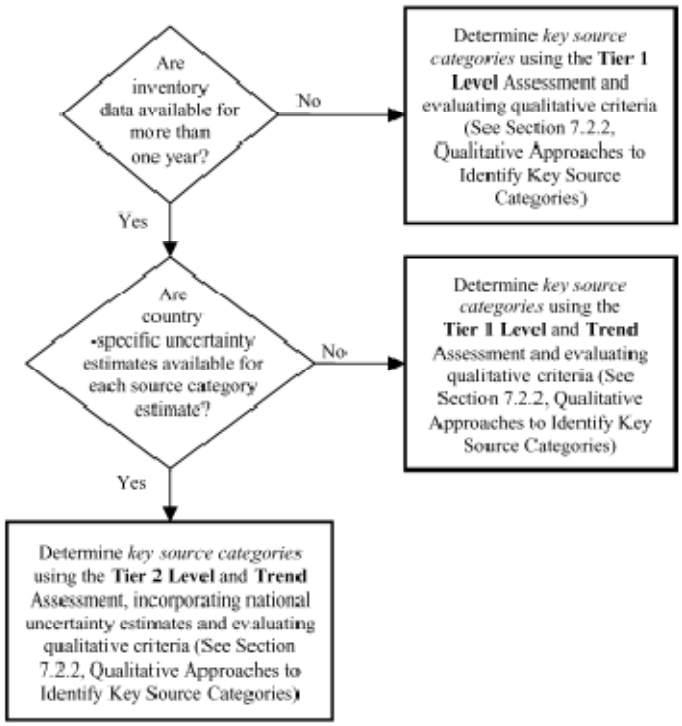

Figure 1.1 International Panel for Climate Change (IPCC) Decision Tree to identify key source categories; Reproduced from the Ministry for the Environment, 2010; pg 266

1.3 Nitrous oxide emission research in New Zealand

Due to the significant contribution agriculture makes to NZ's total N_2O emissions, research to discover economically and environmentally viable mitigation options to decrease N_2O emissions has become a priority within the agricultural sector (Leslie *et al.* 2008). Research into mitigation strategies to reduce N_2O emissions from our unique year round, free grazing practices, as outlined by de Klein and Ledgard (2005), focus around:

- Reducing the amount of excretal N deposited on pasture through diet manipulation.
- Increasing N use efficiency of both fertiliser and excreta N through grazing management and/or use of nitrification inhibitors.
- Managing soils to avoid conditions conducive to increased denitrification activity through drainage improvements and reducing soil compaction.

There is evidence to suggest that N_2O emissions are highly spatially variable in hill country due to the unique combination of topographical, climatic and soil conditions

(Carran *et al.* 1995; Hoogendoorn *et al.* 2008; Parker 2008). For example Carran *et al.* (1995) assumed the constant relationship currently used to estimate total N_2O emissions from excreta and fertiliser N when selecting sites to determine the range of N_2O emissions from NZ North Island grazed pastures. While a steep sloped hill site with low excretal returns chosen to represent the low end of the range, had predictably low N_2O emissions, a flat slope site with high excretal returns chosen to represent the high end of the range, also returned low emissions. Parker (2008) however found that N_2O emissions decreased as slope increased in sheep grazed hill country. Hoogendoorn *et al.* (2008) found that the increases in N_2O emissions measured under increasing rates of N fertiliser in sheep grazed hill country were similar to dairy grazed pastures.

Hill country farming operations cover approximately 3.5 million ha (28% of NZ farmland) and carry 25% and 20% of current total sheep and beef numbers respectively (Carran and Saggar 2004b). Therefore a significant proportion of livestock occupies farm land that differs significantly from most experimental areas used to determine the relationship between N inputs to soil and subsequent N_2O losses to estimate N_2O emissions from farmland in NZ. A better understanding of spatial variation of N cycling and subsequent N_2O emissions in hill country pasture, as influenced by slope, could have a significant impact on the method for calculation of total N_2O emissions from this part of the agricultural sector.

1.4 Thesis outline

The objective of this Thesis was to determine the influence of topography and fertiliser N application rate on nitrification potential (NP) and N_2O emissions in hill country. Chapter 2 identifies some knowledge gaps with respect to N cycling and N_2O emissions in hill country pastures in NZ. Chapter 3 outlines the sites chosen for study and provides the reader with a comprehensive set of environmental data and experimental methodologies to reference when considering the results and data interpretation in the following experimental chapters. Chapters 4 to 6 are experimental chapters and each poses a separate aim and research question based on some of the knowledge gaps identified in the Literature Review (Chapter 2). The experimental chapters each provide a brief introduction to the topic and state the

research questions. The methods used to investigate the question are briefly described (refer to Chapter 3 for full methodology descriptions for all experimental work); the analysis of results, and then a discussion and conclusion are reached based on the results of the investigation. Briefly, Chapter 4 aimed to determine if NPs in hill country soils are affected by fertiliser N application and subsequent increase in excretal N, by comparing NP in sheep grazed plots treated with 0 or 500 kg N fertiliser. Chapter 4 also aimed to determine if NP rates (which contributes to the soil cycling rates of N), are affected by slope due to the pattern of excretal N return. Chapter 5 aimed to determine the impact of increasing rates of fertiliser N on N_2O emissions from sheep grazed hill country paddocks. Chapter 6 aimed to determine the effect of slope class on N_2O emissions by measuring N_2O fluxes from four distinct land classes (campsites 0-12, medium slopes 13-25, steep slopes > 26°, and gullies with drainage areas) to provide quantitative information on the spatial distribution and magnitude of N_2O emissions from sheep grazed hill country soils. An assessment of the relative contribution of these different slope classes to the estimated total paddock N_2O emissions was also made. Chapter 7 summarises the aims, results and conclusions from each experimental chapter. Some exploratory calculations based on the results in this Thesis and speculative commentary is also made in this chapter, which places the results into the context of NZ's national anthropogenic N_2O inventory. The implications for estimating N_2O emissions from hill country and for targeting N_2O mitigation are also discussed. Data from each of the experimental chapters has been nationally or internationally peer reviewed and published, or is currently submitted for journal review. The experimental chapters have been submitted in this Thesis in the journal reviewed format, as far as possible.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

The literature review aims to provide the reader with a summary of the above and below ground flow of nitrogen (N) as affected by N inputs in New Zealand (NZ) pastoral hill country. The review will identify some of the information gaps in the literature on N cycling in NZ grazed hill country and thereby provide the justification for the research questions around N cycling and N_2O emissions investigated in this Thesis.

2.11 New Zealand pastoral hill country

In hill country, N cycling, including N losses, are heavily influenced by the unique topographical, climatic, and soil conditions (Ball *et al.* 1982; Carran *et al.* 1995; Gillingham and During 1973; López *et al.* 2003b). In particular, the camping behaviour of grazing animals induced by varied land slopes and aspects influences excreta return to pastures and subsequent nutrient cycling (Rowarth 1987). This may limit the application of results from flat land N cycling trials to hill country situations.

This important interaction between slope and excreta return is demonstrated by a simplified N annual balance of the major components of the N cycle in a summer dry sheep grazed hill country system (Figure 2.1) which has been adapted from the work by Bowatte (2003). The N cycle in Figure 2.1 provides quantitative estimates of the N flow (kg N/ha.y) between a number of compartments (e.g. soil, plant, animal and atmosphere), for low, medium and steep slopes, respectively. This simplified diagram highlights that low slopes receive significantly more excreta N compared to both medium and steep slopes (432, 50, 9 kg N/ha, respectively). While plant uptake of N is higher on low slopes, than on medium and steep slopes (150, 148, 55 kg N/ha, respectively), the low slopes still gain N overall (282 kg N/ha), while both the medium and steep slopes lose N overall (-98 and -46 kg N/ha, respectively).

A summary of published NZ hill country nutrient and soil physical data is included in Section 2.6. The following sections will review the literature and describe the flow of N through pastoral hill country in the order of N inputs to the system, N transformations and subsequent losses, and finally N uptake and removal via animal products.

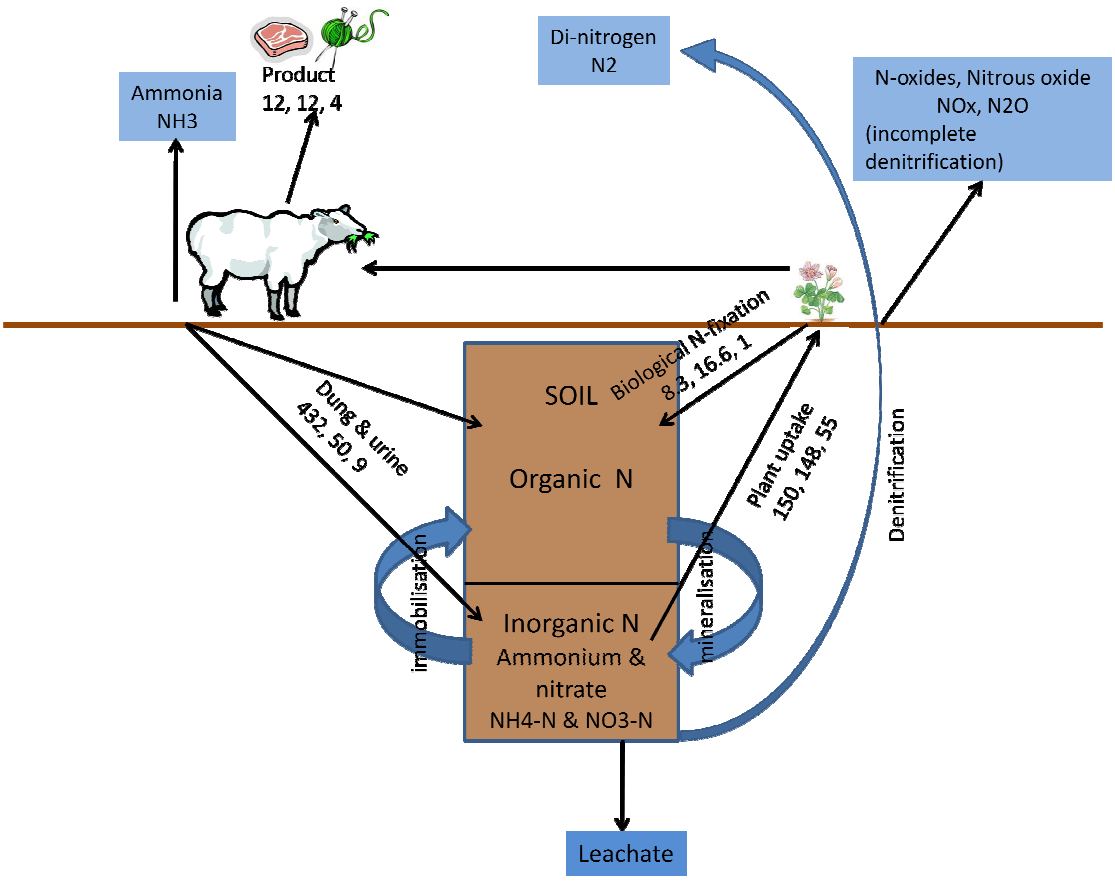

Figure 2.1 Modified N cycle combined with above ground N-balances developed for a notional 1 ha summer dry hill country paddock consisting of 12% low, 46% medium and 42% steep slopes, in New Zealand. The values alongside various N transfers denote the annual quantity of N (kg/ha.y) transferred on low, medium and steep slopes respectively, and are the arithmetic mean values for north and south aspects estimated by Bowatte (2003)

2.2 Nitrogen Inputs

2.21 Excretal N

The largest supply of N to the hill country soil system is via excreta from grazing animals. Sheep excrete approximately 60 to 90% of ingested N, potassium (K), phosphorus (P), sulphur (S), calcium (Ca) and magnesium (Mg) in the form of

dung and urine (Williams and Haynes 1990). Excretion of N in sheep dung is approximately 0.8 g N/100 g DM consumed. The concentration of N in sheep urine is 5 to 15 g N/L urine, with an average of 8 to 10 g N/L urine. The concentration of N in both dung and urine varies and is dependent on the amount of N consumed, the physiological state of the animal and the amount of water consumed (Woodford and Nicol 2004). Typical values for N excretion in urine from sheep and cattle are presented in Table 2.1, and are reproduced from Whitehead (1995).

Urea accounts for, on average between 60 and 90% of the total N in sheep urine, but it can be as low as 24% on a low protein diet. Urine also contains other nitrogenous constituents; hippuric acid, allantoin, uric acid, xanthine, hypoxanthine, creatine and creatinine and their proportions vary considerably. Urine urea in soil is typically hydrolysed to NH_4^+ within 1 to 7 days, thus rapidly becoming available for volatilisation, plant uptake, nitrification to NO_3^-, immobilisation or fixation onto clay minerals (Haynes 1986).

Table 2.1 Typical values for the excretion of N in urine by dairy cows, steers and sheep. Reproduced from Whitehead, 1995 p 74

	Dairy cows	Steers	Sheep
Urinations per day	8-12	8-12	15-20
Volume per urination (L)	1.5-3.5	1.0-3.0	0.1-0.2
Urine volume per day (L)	10-40	10-30	1-7
Dry matter in urine (g/L)	60-120	60-120	-
N concentration (g/L)	2-20	2-20	5-15
N excreted in urine (g N/day)	80-320	80-240	10-70
N excreted in urine (kg N/y)	30-120	30-90	5-25

Faecal production by sheep varies with the amount and digestibility of feed consumed (Gillingham 1980). The concentration of N in sheep faeces fed on grass or grass-clover is between 1.2 and 4.0% of dry matter, or about 0.2 to 0.5% on a fresh weight basis (Whitehead 1995). Faecal N content from sheep fed on unfertilised hill pasture is at the low end of the range (1.1 to 2.1% of dry weight). Daily output of faecal N is between 10 and 25 g for a 70 kg ewe.

Nutrients in dung are in organic forms and must be incorporated into the soil through physical breakdown by soil macro fauna, rainfall and the invasion of plant roots before being transformed by micro-organisms to inorganic forms available for plant uptake. The rate of decomposition of sheep dung is dependent on climate, particularly rainfall and soil moisture levels and can be completed within 28 or >75 days in wet and dry seasons respectively (Williams and Haynes 1990). Therefore mineralisation rates for the conversion of faecal N and S into inorganic forms is relatively slow compared to N conversions in urine (Williams and Haynes 1990).

Cycling of N via excreta deposition by the grazing animal and the act of grazing itself increases the availability and rate at which N is cycled by stimulating plant growth and subsequent N-uptake (Williams and Haynes 1990). However as outlined previously, grazing and excreta N return patterns are heavily influenced by topographical, climatic, and soil conditions (Ball *et al.* 1982; Carran *et al.* 1995; Gillingham and During 1973; López *et al.* 2003b; Saggar *et al.* 1990). Saggar *et al.* (1990) estimated for an experimental farmlet area (10 – 16 SU/ ha) with a distribution of 31% low, 41% medium and 28% steep slopes that 60, 30 and 10% of dung and 55, 31 and 14% of urine was returned to the low, medium and steep slopes, respectively. Rowarth (1987) had previously reported that faecal deposition did not vary significantly from this pattern, regardless of the proportion of land area represented by each slope class. Later work by Hoogendoorn *et al.* (2008) also found that the proportion of urine and dung N did not significantly affect N_2O emissions within slope classes. Gillingham (1973) estimated for a notional 1 ha hill country paddock that total N input via excreta (dung and urine) was 106.7, 135.7 and 36.3 kg N/ha.y on north facing slopes and 106.7, 85.2, 38.9 kg N/ha.y on south facing slopes for campsites, easy (25º) and steep (45º) slopes, respectively. As the pattern of excreta deposition largely determines N cycling Chapter 4 will include a measure of the distribution of excreta (as indicated by dung counts) in the hill country paddocks studied in this Thesis.

2.22 Biological N Fixation

Most NZ hill soils are naturally low in available N and other deficiencies typically include phosphorus (P), sulphur (S) and molybdenum (Mo) (Lambert *et al.*

1982c; White 1990). In hill country the N deficit has traditionally been addressed with the application of fertiliser P and S and the over sowing of legume species (e.g. *Trifolium repens* L, T. *pratense* L, T. *subterraneum* L and *Lotus pedunculatus*) to augment biological legume N fixation as well as to improve feed quality (Grant and Lambert 1979; Lambert *et al.* 1982a; Walker 1959). The N fixed by legumes is transferred to the soil via stock excreta, root excretion or through the decomposition of dead legume material, and therefore increasing soil mineral N levels available for plant uptake (Ledgard and Steele 1992). In work reported by Lambert *et al.* (1983), N fixation by legumes in North Island (NZ) hill country rose from estimates of 30 kg N/ha.y with no fertiliser P application to 70 and 120 kg N/ha.y in paddocks receiving low (125 kg P/ha.y) and high (630 kg P/ha.y) rates of P fertiliser in the form of single super-phosphate (SSP). As soil N levels via biological N fixation rise, the persistence of high fertility grasses such as *Lolium perenne* increases (Lambert *et al.* 1982a; Ledgard and Brier 1993). Subsequent increases in pasture production follow and clover dominance reduces (Lambert *et al.* 1983). In work reported by Roach *et al.* (1996), annual pasture production was 41 to 53% higher in paddocks receiving 250 kg P/ha.y for 10 years compared to control pastures (0 kg P/ha.y for 10 years). There was also an increase in low fertility grasses such as *Agrostis* spp. in control paddocks.

At adequate P (and S and Mo) levels in hill country leguminous biological N fixation (BNF) and net herbage accumulation (kg DM/ha) is influenced by soil N (Ledgard and Steele 1992), climate and moisture status across seasons (Lambert *et al.* 1982a), as well as by slope and aspect (Ledgard *et al.* 1987). Legume species distribution also varies according to slope and aspect as legume species are adapted to corresponding soil moisture and temperature regimes (Ledgard *et al.* 1987). Ledgard *et al.* (1987) found that the concentration of N in legume communities on different slopes was relatively constant (mean 4.9%, range 4.3 to 6.0%). The proportion of legume N obtained by fixation (P_N) was also relatively constant for all slopes except campsites (mean 82%, range *c.* 72 to 92% excluding campsites). In campsite soils large variations in P_N values reflected fluctuating mineral N levels. High mineral N levels coincided with low P_N values following intensive grazing and subsequent excretal N inputs. Both N concentration and P_N were lowest in summer and were therefore closely related to net herbage accumulation across the seasons. Biological N fixation in Ledgard *et al.* (1987) was therefore estimated by multiplying legume production

(kg DM by 0.049, mean N concentration) and 0.82 (mean P_N value). For campsites the P_N value was reduced to 0.62 as follows:

kg N fixed/ha on slopes = 0.049 x 0.82 x legume DM/ha
kg N fixed/ha on campsites = 0.049 x 0.62 x legume DM/ha

N inputs via biological N fixation on improved summer dry hill country were estimated by Ledgard *et al.* (1987) to be 54, 75 and 75 kg N/ha.y for low, medium and steep slopes, respectively, and are similar to previous estimates by Luscombe and Fletcher (1981) (23, 64 and 63, and 54, 74.5 and 74.5 kg N fixed/ha.y for soils from low, medium and steep slopes in paddocks receiving 50 and 750 kg P/ha.y, respectively). Steep slopes are highly reliant on BNF for N supply due to the transfer of N in herbage away from these slopes via stock excreta. Reduction of BNF and legume production (kg DM/ha) in campsites is common in NZ hill country due mainly to increased levels of inorganic N and competition by grasses at these sites (Ledgard 1987; Luscombe 1981; Whitehead 1995). It should be noted that while legume production (kg legume DM/ha) usually increases with slope, total herbage production (i.e. combined grass and legume total) does not. Total herbage production closely follows grass production, which is higher on campsites, largely due to the increased available N via animal excreta at these sites. The contributions of legumes to total DM (kg DM/ha.y) were measured as 13, 25 and 38% for low, medium and steep slopes by Ledgard *et al.*(1987). Luscombe and Fletcher (1981) estimated legume contribution to total DM to be <2 % in campsites.

One limitation of fertiliser P application is that a subsequent increase in herbage growth is not always immediate. Several large additions of fertiliser P (over 500 kg P/ ha.y) followed by maintenance applications are typically required for soil P levels to be increased sufficiently for increased legume growth and N fixation to increase soil N status and then pasture production and composition (Ball *et al.* 1982; Grant *et al.* 1973; Lambert *et al.* 1982a). On a previously unfertilised central North Island hill soil receiving high fertiliser P loadings (>300 kg cobaltised superphosphate/ ha as well as S and lime), it took 3 to 4 years to obtain a good grass-clover balance (i.e. 20 to 40% clover). It took a further 21 to 22 years for the soil C:N ratio to decrease from 33 to 11 under this fertiliser regime (Walker *et al.* 1959). In work reported by

Lambert *et al.* (1983) significant increases in annual herbage accumulation in paddocks receiving high rates of fertiliser P (630 kg P/ha.y) compared to those receiving low rates (125 kg P/ha.y) did not occur in the first two years after the initial application, however annual herbage accumulation then ranged from 21 to 50% higher on an annual basis for the remaining 4 years of the trial. Following several years of fertiliser P application workers have found that both legume herbage production and legume P efficiency decrease due to competition from grasses as soil N increases (Lambert *et al.* 1982a; Ledgard and Steele 1992). Lambert *et al.* (1982a) found that the efficiency by legumes in using P applied initially increased from 11 kg extra legume DM/kg extra P applied in the first year of P application to 22 in the second year, but it then dropped to 9 kg extra legume DM/kg extra P applied in the last year of a 5 year trial.

Clover sensitivity to soil N status also renders pasture production dependent on optimal grazing management. In hill country extensive/lax grazing practices in large paddocks encourages the concentration of excreta N and P in campsites, therefore significantly impacting on clover performance and soil N status outside of the campsite areas (Ball *et al.* 1982; Williams and Haynes 1990). While fertiliser P application may indirectly increase annual pasture production, it has a negligible effect on the seasonal distribution of growth due to the dependency of legumes on soil water availability and sensitivity to soil N status (Ball *et al.* 1982; Lambert *et al.* 2003).

2.23 Fertiliser N

In contrast to fertiliser P applications pasture growth responses to fertiliser N (typically urea N) are almost immediate and even single applications often have residual effects on herbage production due to the recycling of N through excreta N (Lambert *et al.* 1986; O'Connor 1961c; Sherlock and O'Connor 1973). In North Island hill country low fertiliser N application rates (<50 kg N/ha) yield pasture responses in the range of 10 to 39 kg DM/kg N applied in paddocks of both high and low Olsen P status. This suggests that N response is independent of P fertiliser (Lambert *et al.* 1986; Luscombe 1979; Sherlock and O'Connor 1973). While pasture response is influenced by the timing of application (i.e. in relation to soil moisture

levels and temperature), previous trial results suggest that N response efficiency generally declines once fertiliser N application rates exceed 80 – 100 kg N/ha in hill country (Lambert *et al.* 1986). Response efficiencies were lower in campsites where soil N is already high. In grazed systems residual effects are often further prolonged into second and third seasons due to the cycling of N via animal excreta (Ball *et al.* 1982; Lambert *et al.* 2003). Fertiliser N applications increase initial grass yields significantly (i.e. 40 – 100 % increases in kg DM/ha, in paddocks receiving between 50 to 120 kg N/ha.y) and significantly decrease clover yields (Lambert *et al.* 2003; O'Connor 1961a). Increases in grass production however more than compensate for any depressions in clover production (Lambert *et al.* 2003; O'Connor 1961a).

Fertiliser N has been recognised as a strategic tool for the management of autumn and winter feed in mild moist North Island hill country (Luscombe 1979). Several authors confirm that in the North Island, autumn and winter applications (<50 kg N/ ha) stimulate pasture growth through the traditionally low yielding late winter/early spring period where soil moisture and temperature are not limiting. Low pasture growth rate over the winter/early spring period often limits stock carrying capacity in hill country (Luscombe 1979). The application of fertiliser N may therefore overcome this seasonal deficit to allow intensification to occur. O'Connor (1961a) reported low responses by grasses to fertiliser N applications in winter in South Island hill country trials which may limit intensification of hill country through the use of N fertiliser in this region.

Strategic applications that boost productivity through low yielding seasons allow for significantly higher winter/early spring stocking rates (SU/ha) as well as yielding heavier lambs and more wool per unit (Ball *et al.* 1982; Lambert and Clark 1985; White 1990). Costs involved in the application (i.e. aerial top dressing) of fertiliser N however need to be balanced by high product prices and low fertiliser prices. To maintain pasture quality careful management/redesign of current hill country grazing practices is also required if N is added to the system (Lambert *et al.* 2003; O'Connor 1961b). If the management of stocking rate and rotation does not match the increased pasture growth rates due to increased fertiliser N use, the risk is that pasture may turn rank and will ultimately decrease in pasture quality and quantity.

2.24 Organic N

Hill pastures commonly have a layer of plant litter (A_0 horizon) consisting of partially decomposed organic material mainly derived from herbage neglected by grazing animals (Floate 1970). For the purposes of this Thesis this is regarded as both the attached and unattached material below grazing level at the end of any grazing period and represents an organic N source (Gillingham and During 1973). Bowatte (2003) estimated the annual amount of N in plant litter by deducting the annual animal utilisation from the annual pasture N uptake. As grazing behaviour and therefore pasture utilisation is modified by slope and aspect, N inputs from plant litter will also vary according to slope. Annual pasture utilisation by grazing animals in an intensively managed sheep hill country farming operation on campsites, medium and steep slopes were estimated to be 79.2, 82.8 and 76.2%, respectively (Gillingham 1982). Gillingham (1982) therefore estimated organic N inputs to campsites, medium and steep slopes to be 31.1, 31.3, and 13 and 31.1, 19.7 and 13.5 kg N/ha.y on north and south facing slopes, respectively. Cycling of N in this material will be slower because the N in plant matter is mainly in organic forms which are not immediately available for plant uptake (Williams and Haynes 1990).

It should be noted that N in the form of dung is a significant source of organic N and was discussed in Section 2.21 as a source of excretal N.

2.3 Nitrogen transfers and losses

In a grassland system N moves at different rates and in different forms as influenced by biotic and abiotic processes. These include the processes of volatilisation, mineralisation/immobilisation, nitrification, and denitrification. Each of these processes and the influence of slope on them are outlined in sections below.

2.31 Volatilisation

In grassland soils urea N from animal urine and fertiliser rapidly undergoes hydrolysis by urease, a microbial enzyme widespread in soils, plants and plant litter (Haynes and Williams 1993). Except in cold or dry conditions, urea is hydrolysed within a few days. On soils with a pH <6.3 the product of hydrolysis is NH_4^+:

$CO(NH_2)_2+2H_2O \rightarrow NH_4^+ + NH_3 + HCO_3^-$

This reaction causes localised areas of high pH at the site of hydrolysis. The high pH of animal urine (8.6) increases the rate of hydrolysis because this is the optimum pH for urease activity and urine contains hippuric acid which has a stimulatory effect on urea hydrolysis (Doak 1952; Whitehead 1995). In urine patches soil pH rises rapidly in the first 24 h, as hydrolysis proceeds. A rise in pH of 2.5 to 3.5 units is not uncommon in the surface 0.5 cm soil during this time (Sherlock and Goh 1984). Within a few days following hydrolysis large amounts of NH_4^+ accumulate in the surface (<10 cm) of the soil. Soil NH_4^+ concentrations can commonly reach 100 to 250 µg/g soil at this time (Whitehead 1995). The conversion of soil NH_4^+ ions to NH_3 is reliant on free OH^- near the soil surface, and is the major process regulating the potential loss of NH_3 from soils or the rate of volatilisation.

NH_3 (in atmosphere)
↕
$NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O$ (in soil solution)
(Source; Whitehead 1995)

The NH_4^+ is in equilibrium with NH_3^- in the soil solution. In turn the soil solution NH_3 is in equilibrium with gaseous NH_3. Numerous factors affect the equilibrium between NH_4^+ and NH_3. Volatilisation of NH_3 is favoured by high soil pH, high temperatures and high rates of evapotranspiration from the soil (Saggar *et al.* 2004b). Most workers agree that temperature is particularly important Sherlock and Goh 1984; Vallis *et al.* 1982; Whitehead 1995). In New Zealand, Sherlock and Goh (1984) found mean urine patch volatilisation losses of urine N were 22, 25 and 12% in summer, autumn and winter, respectively. Losses via volatilisation from urine patches are also typically highest from pastures during and immediately after grazing, and an increase in stocking density also increases rates of volatilisation (Jarvis *et al.* 1989).

The pattern of urine deposition, as well as suitable soil conditions (as influenced by slope) for volatilisation will likely affect the spatial and temporal distribution of NH_3 losses from slopes on different aspects in hill country. There is currently no information available to determine NH_3 volatilisation losses from NZ hill country.

2.32 Mineralisation/Immobilisation

The microbially mediated processes of mineralisation and immobilisation occur when organic forms of N in recently added plant or animal residues are converted to inorganic N forms in the soil (Whitehead 1986). Mineralisation occurs as soil microbes excrete enzymes into the soil to convert organic N into mineral forms for their own use. Following the breakdown of organic N into amino acids, soil organisms then cause these amino compounds to be converted into NH_3 (McLaren and Cameron 1996). This process is referred to as 'ammonification' and is carried out by heterotrophic aerobic micro-organisms. Some of the mineral N produced is then assimilated by the microbial mass. The uptake of mineralised N by the microbial biomass is termed immobilisation. Some of the mineral N is released into the soil to be taken up by plants or nitrified to NO_3^- (Whitehead 1995). The two processes may occur at the same time and both are continuous processes in the soil. However, while an increase in mineral N in grassland soils typically represents net mineralisation, a decrease in mineral N does not necessarily indicate net immobilisation. A decrease in mineral N may also be due to volatilisation, plant uptake, leaching and/or denitrification (Whitehead 1986).

Immobilisation is primarily mediated by the amount of available (soluble) C (Bothe *et al.* 2007). An increase in C availability in organic residues may lead to an increase in the soil microbial biomass and therefore the rate of assimilation/immobilisation. As the rate of immobilisation increases the amount of soil available N for pasture growth and nitrification decreases (Bothe *et al.* 2007; Szili-Kovacs *et al.* 2002).

In legume based pastures such as conventional hill country pastures, a major supply of plant available N is dependent on mineralisation. The rate of N mineralisation is a relatively slow process and is influenced by the C: N ratio of plant litter (Marhan and Scheu 2005) and particularly the amount of available N in the residues to be decomposed (Bothe *et al.* 2007; Whitehead 1986). A high C: N ratio will lower mineralisation rates. Grass residues and stock dung, which have a C: N ratio around 30:1 may take several years before the microbial demands for N reach an equilibrium and for the excess N to become available in the soil (Ledgard 2001). The urea N in stock urine however, hydrolyses rapidly and is therefore mineralised to NH_4^+ in a matter of hours to days. The presence of soil macro fauna such as worms greatly

improves the mineralisation rate of organic N via mechanical fragmentation of organic matter in the gut, which conditions residues for microbial decomposition in worm casts (Marhan and Scheu 2005). Worm casts and legume residues (i.e. stolons, roots and herbage), which have a C: N ratio around 15 - 25: 1, can mineralise within a period of a few weeks (Marhan and Scheu 2005; Whitehead 1995). The lower C: N ratio in worm casts is due to the loss of C during respiration by worms, and the excretion of ammonium, urea and/or free amino acids (Whitehead 1986). In NZ Ruz Jerez *et al.* (1988) found that the presence of earth worms resulted in about 50% more soil inorganic N after an 11 week incubation period. Other factors that influence the rate at which residue breakdown and microbial mineralisation occurs are soil moisture (i.e. rainfall, slope and soil texture), and temperature (i.e. season, slope and aspect, (Hawke 2001; Ladd *et al.* 1985; Marhan and Scheu 2005; Rowarth *et al.* 1985).

Given the spatial variation of BNF and C: N ratio in hill country pastures it is anticipated that N mineralisation rates will also be spatially variable. Stock grazing is also known to increase mineralisation rates by decreasing the supply of carbon in plant litter form (Stark and Grellmann 2002) and through modification of the pasture structure (Rossignol *et al.* 2006). Grazing also indirectly increases microbial activity and heterogeneity through increasing the amount of available N in excreta form. In semi extensive sheep and beef hill pastures and retired hill land, Hawke (2001) found that mean soil C: N ratios were significantly lower in campsites compared to steep sites. However in this work, the correlation between soil and plant ^{15}N was inconsistent between each of the 3 campsites sampled and therefore the relationship between slope and N cycling rates is not straightforward.

2.33 Nitrification

The process of nitrification occurs when soil NH_4^+ is oxidised to soil NO_3^-. This process is mediated mainly by two groups of autotrophic bacteria; NH_4^+ oxidisers (predominantly *Nitrosomonas* ssp. in agricultural soils, Haynes, 1986):

$NH_4^+ + 1.5[O_2] \rightarrow NO_2^- + H_2O + 2H^+$
Source: Whitehead 1995

And nitrite (NO_2^-) oxidisers (predominantly *Nitrobacter* spp. in soils, Haynes, 1986):

$2NO_2^- + [O_2] \rightarrow 2NO_3^-$
Source: Whitehead 1995

Some heterotrophic nitrification is known to occur in soils (e.g. bacteria, fungi and actinomycetes) as well as methyltrophic and chemical nitrification (Ferguson *et al.* 2007; Haynes 1986). However autotrophic nitrification is believed to be the main oxidative pathway in agricultural soils and will be the focus of this review (Haynes 1986; Whitehead 1995).

Autotrophic nitrification is particularly sensitive to temperature (Haynes & Williams, 1993). Nitrifiers are active between 5 and 35º C and the rate of activity (i.e. rate of NO_3^- production) increases as temperature increases. The optimal temperature is ~25º C (Subarao *et al.* 2006). Nitrification may also occur very slowly at temperatures below 5º C and is inhibited above 35 º C (Black 1968). Low temperatures inhibit the second stage of NH_4^+ oxidation (NO_2^- to NO_3^-) in particular and NO_2^- may accumulate in the soil (Whitehead 1995). Under warm temperate conditions NO_3^- is often the major form of N present in urine patches (Haynes and Williams 1993).

The rate of nitrification in soil is also highly influenced by soil pH. The optimum range for nitrification is between pH 7.5 and 8, and bacterial growth rates slow considerably below a soil pH of 6 (Prosser 2007; Sarathchandra 1978). Numerous authors report that nitrification rate decreases as soil pH decreases (Haynes 1986; Sarathchandra 1978; Steele *et al.* 1980; Wakelin *et al.* 2009; Whitehead 1995; Young *et al.* 2002) and is inhibited completely below 4.5. As the oxidation of NH_4^+ to NO_3^- is accompanied by a decrease in soil pH (with the production of $2H^+$) this may limit nitrification activity in poorly buffered or acidic soils (Prosser 2007). Wakelin *et al.* (2009) and Kemmit *et al.* (2006) found that liming soils, which is common practice in NZ, increased soil pH, soil microbial biomass, and nitrification rates. Sarathchandra (1978) found that increasing pH in soil incubations also improved nitrification rate. There are examples of nitrification occurring at pH<4.5 by specialised bacteria adapted to local conditions (Prosser 2007; Whitehead 1995); and Sarathchandra (1978) found that high rates of nitrification occurred at low pH (<5.5) in allophanic

soils in NZ. There is also evidence to suggest that nitrification may continue at low pH in the presence of urea whereby urea enters bacterial cells by diffusion and intracellular urea hydrolysis, and oxidation is able to occur independently of extracellular pH in the range 4 to 7.5 (Burford and Bremner 1975).

Nitrification is also inhibited by strongly alkaline soil (Whitehead 1995). Urea N from animal urine and fertiliser undergoing hydrolysis causes localised areas of high pH. The high pH of animal urine (8.6) also increases the rate of hydrolysis which inhibits the oxidation of NO_2^- to a greater extent than NH_4^+ oxidation (Haynes and Williams 1993). Soil DNA profiling from synthetic urine patches suggests that patch effects may not change the total microbial biomass, but change the microbial community structure, and hence the rates of NH_4^+ and/or NO_2^- oxidation (Nunan *et al.* 2006; Rooney and Clipson 2008). When large amounts of ammoniacal N are added to soils, such as in urine patches or when large quantities of fertiliser N are applied, the nitrifier population may therefore be adversely affected initially by the high pH conditions which often results in a lag period of up to several days in the onset of nitrification activity (Sarathchandra 1978; Whitehead 1995; Williams and Haynes 2000), as well as an initial accumulation of NO_2^- (Whitehead 1995). After the initial delay however nitrification rates typically increase (Williams and Haynes 2000).

The optimum soil moisture tension for nitrification activity is between -0.1 and -1.5 MPa (Whitehead 1995), although Alexander (1965) observed that the optimum moisture level is not necessarily the same for nitrifying populations in different soils. As a general rule Alexander (1965) found that nitrification activity is greatest between 50 to 75% of field capacity. Because nitrification is an aerobic process, water logged soils depresses activity due to the lack of O_2 gas diffusion to microsites (Bowatte 2003). Extremely dry soil conditions also inhibit nitrification, however nitrifying bacteria can persist in an inactive state for prolonged periods of time (Haynes and Williams 1993; Whitehead 1995). When dry soils are wetted there is a characteristic flush of mineralisation and nitrification (Haynes 1986).

The time taken to completely oxidise NH_4^+ from urine patches varies from 14 days in ideal conditions (Vallis *et al.* 1982) up to 77 days (Williams and Haynes 2000). The variation in soil moisture (i.e. aeration), substrate distribution, soil pH and

temperature are likely responsible for the spatial and temporal variation of nitrification activity observed in grasslands. Where rates of nitrification are high and there is a surplus supply of NO_3^- to plants there is increased risk of N losses via leaching and denitrification (Fair *et al.* 1994). Nitrate is highly mobile in the soil and is therefore highly susceptible to losses via leaching and may also be used in the process of denitrification (Alexander 1965; Whitehead 1995). Consequently nitrification may be of considerable importance in campsite areas in hill country because large quantities of substrate (i.e. excretal N) combined with favourable soil and climatic conditions in these areas relative to the rest of the paddock may increase the risk of N losses. No accelerated leaching losses were measured however by Sakadevan *et al.* (1993b) from campsite areas, and Lambert *et al.* (1982a) also observed that the North Island hill country soil they worked on was N retentive due to the high C:N ratios.

Due to the importance of nitrification for nitrous oxide (N_2O) emissions, both direct and indirectly through the supply of soil NO_3^- for denitrification, the focus of Chapter 4 will be to determine the nitrification potential, as influenced by slope and N inputs via fertiliser and excreta. It should also be noted that in aerobic conditions N_2O is produced during the process of biological nitrification in soil (Firestone and Davidson 1989; Skiba *et al.* 1993). It is a strictly aerobic process and N_2O is produced merely as a by product of oxidation (Haynes 1986).

2.34 Denitrification

Denitrification is the process by which soil NO_3^- and NO_2^- are reduced to di nitrogen (N_2) and N_2O gas (Firestone and Davidson 1989). Under anaerobic conditions the pathway of NO_3^- reduction during biological denitrification is:

$NO_3^{-}\ ^{(1)}\rightarrow NO_2^{-}\ ^{(2)}\rightarrow NO\ ^{(3)}\rightarrow N_2O\ ^{(4)}\rightarrow N_2$

$^{(1)}$ NO_3 reductase enzyme, $^{(2)}$ NO_2 reductase enzyme, $^{(3)}$ NO reductase enzyme, $^{(4)}$ N_2O reductase enzyme
Source: Haynes *et al*, 1986

Biological denitrification includes both respiratory and non-respiratory denitrification. Non-respiratory denitrification produces N_2O only and is carried out by some groups of bacteria, yeasts, fungi and algae (de Klein 1994). Respiratory denitrification is the

biological reduction of NO_3^- to N_2O and N_2, and is recognised as the largest source of N_2O from soils (Whitehead 1995). It will therefore be the focus of this review.

Denitrification is often described as the last step in the N cycle because it returns inorganic N to the atmosphere as N_2. Incomplete denitrification however results in the emission of N_2O, a greenhouse gas (GHG), to the atmosphere (Figure 2.1). Denitrification of NO_3^- derived from fertiliser and stock excreta N, and the subsequent gaseous losses of N are therefore of economic and environmental interest to agriculturalists and scientists. Deficits found in N balance studies in agriculture have been attributed to losses via denitrification, which represents both an economic loss of N to the atmosphere as well as having a potentially negative environmental impact as a greenhouse gas.

In soils a limited number of bacteria referred to as denitrifiers have the ability to continue to grow using NO_3^- or nitrite (NO_2^-) as electron acceptors in the absence of oxygen (O_2) such as occurs in water-logged soils. Most denitrifiers prefer to use O_2 as their electron acceptor, but possess the ability to use NO_3^- in the absence of O_2 for the same purpose (Fenchel and Blackburn 1979). Therefore it is the air status of the soil and NO_3^- availability that most influence the production of N_2O and N_2. The most commonly encountered species of denitrifiers are *Pseudomonas* spp. and *Alcaligenes* spp. Denitrifiers are ubiquitous throughout most soils and may account for between 1 and 5% of the total heterotrophic soil population (Whitehead 1995). Denitrifiers reduce NO_3^- through the synthesis of reductase enzymes (Fenchel and Blackburn 1979). Some denitrifiers are able to reduce NO_2^- but not NO_3^- (e.g. *A. Odorans*), while others are unable to produce the N_2O reductase enzyme (Tiedje 1982).

Denitrifying bacteria vary in their sensitivity to O_2 and the concentration at which denitrifying enzymes are synthesised and become active (Fenchel and Blackburn 1979). The production of the N_2O reductase enzyme is generally easily inhibited in the presence of even low concentrations of O_2. Therefore as conditions begin to increase aerobicity the ratio of N_2O to N_2 produced increases (Tiedje 1982). It is generally agreed that only small of amounts of NO_2^- are produced during denitrification because the activity of the NO_2 reductase enzyme is higher (Whitehead

1995). Due to O_2 sensitivity anoxia promotes a more complete reduction of soil NO_3^- N, therefore increasing the ratio of N_2 to N_2O emitted from affected soils (Haynes 1986). Suitable anaerobic conditions in soils typically occur when there is an excessive amount of water present following significant rainfall or irrigation. In this situation the rate of the O_2 diffusion through water is 10,000 times slower than through air (Whitehead 1995). Increased denitrification activity is therefore often related to high soil moisture conditions (Florinsky *et al.* 2004; Parsons *et al.* 1991; Pennock *et al.* 1992).

Because soils vary in porosity and texture, they vary in soil water content following a rainfall or irrigation event. Therefore the soil water content at which denitrification becomes appreciable will also differ from soil to soil (Tiedje, 1982). Further, soil drainage is determined by soil structure and texture, which will influence the period during which soil conditions are suitable for denitrification (McTaggart *et al.* 2002). Compaction may therefore influence a soil's vulnerability to prolonged periods of denitrification due to impeded drainage (Bhandral *et al.* 2003) following heavy rainfall or irrigation events. Field and laboratory studies have found that there is often a lag of up to several hours following large rainfall or irrigation events before the rate of denitrification activity increases (Smith and Tiedje 1979). This is likely due to the time taken for water to displace O_2 in pore spaces, deplete O_2 levels in spaces where O_2 cannot diffuse fast enough through water to replenish pore spaces, and for the denitrifier community to synthesise appreciable amounts of the enzymes required to reduce NO_3^- and NO to N_2O or N_2 in measurable quantities once O_2 inhibition has been removed (Smith and Tiedje 1979). Small amounts of denitrification may also occur if soil moisture conditions are low within anaerobic microsites (Tiedje *et al.* 1989). These sites are thought to occur mostly in clay soils and within larger soil aggregates (radius >9 mm, Whitehead 1995). Denitrification may also occur when there is a reduction of O_2 in zones of high microbial activity and respiring plant roots (Parsons *et al.* 1991). There is also molecular evidence to suggest that denitrifiers' community structure and the expression of various denitrifying genes is determined, in the long term, by soil variables such as soil moisture regimes (Wallenstein *et al.* 2006). In hill country, soil moisture conditions are strongly influenced by slope and aspect (Gillingham 1973; Gillingham 1982). For example Florinsky *et al.* (2004) found that denitrification rate in undulating grassland was most affected by the

redistribution of soil moisture and organic matter down slope. Denitrification activity in NZ hill country is therefore expected to be highly spatially variable.

The availability of NO_3^- in the place of O_2 as an electron acceptor will determine denitrification rates (Wallenstein *et al.* 2006). There is typically very little denitrification activity in soils of low NO_3^- concentrations (Haynes 1986). Increasing farming intensity (i.e. increased fertiliser and excreta N inputs), will increase soil NO_3^- concentrations and therefore the potential for increased denitrification rates if other soil conditions are suitable (Skiba and Smith 2000). Urine patches are known to trigger a sharp increase in denitrification activity after a short time lag of a few days before decreasing to background levels several weeks later (de Klein *et al.* 2003). This spike in activity is due to the combined effects of wetting the soil, increases in soluble C due to solubilisation of organic matter, and supplying NO_3^- to the patch (Lovell and Jarvis 1996; Monaghan and Barraclough 1993). As NO_3^- levels increase, further acidifying the soil, the ratio of N_2O:N_2 will typically increase due to the inhibitory effect of low pH on the N_2O reductase enzyme (Haynes 1986).

Regardless of how much NO_3^- is present or the occurrence of anoxia in soil, denitrification in soils requires an energy source in the form of carbon (C) because denitrifiers are heterotrophic (Whitehead 1995). The rate and amount of denitrification is therefore directly related to the amount of available C (Fenchel and Blackburn 1979; Florinsky *et al.* 2004). A more complete reduction of NO_3^- will generally occur with increasing soluble C (Haynes 1986). Carbon availability may also affect denitrification by supplying other microorganisms that require C for growth to indirectly deplete O_2 in soil through increased respiration (Lovell and Jarvis 1996). In grassland soils C generally decreases rapidly with depth and denitrification rates typically follow a similar distribution with depth (Whitehead 1995). Exceptions are peat soils and in soils where a buried profile containing large amounts of organic matter (as in recently cultivated soils) with a perched water table occur (Rolston *et al.* 1978). Denitrification rates in grasslands are generally higher than those in arable soils due to the higher organic matter content in grasslands (Whitehead 1995). Contributions of C from plants will also increase with the age of the crop/pasture, as dead material accumulates and root exudates increase (Whitehead 1995). Denitrification is usually negligible below the root zone as a result of these reasons

(Whitehead 1995). The high C content in dung will also affect denitrification rates and therefore stock camping behaviour is expected to have a large influence on denitrification rates in hill country. Slope will also influence the redistribution of soluble C and therefore the spatial distribution of denitrification activity (Florinsky *et al.* 2004).

The rate of denitrification is positively related to pH in soils, and has an optimum range of pH 7.0 to 8.0 (Haynes 1986). Denitrification is inhibited below pH 6.0 to 5.5, however there is evidence for adaptation to local conditions (Wallenstein *et al.* 2006). Reductase enzymes are sensitive to low pH, particularly the N_2O reductase enzyme (Haynes 1986). As a result, the ratio of N_2O to N_2 increases as soil pH falls (Fenchel and Blackburn 1979).

The rate of denitrification is particularly influenced by temperature and the optimum temperature for denitrification is 25ºC (van der Weerden 1999), however denitrification will occur between 0 and *c.* 75°C. Five degrees Celsius is commonly cited as the lower limit for activity, but some adaptation to local conditions below 5°C is known to occur (Whitehead 1995). The strong seasonal patterns of denitrification rates reflect the associated patterns in soil temperature along with moisture conditions (Haynes 1986; Whitehead 1995).

The way in which soil, climatic and management practices interact to influence denitrification activity and N_2O emissions is represented in Figure 2.2. This figure summarises how denitrification is affected to a greater or lesser extent by primary or proximal soil and environmental factors which are O_2-availability, substrate-N, available soil C, soil temperature and pH. Proximal factors are in turn affected by distal factors. Distal factors are those which impact directly on the regulation of proximal factors and may include rainfall (soil moisture), fertiliser N, excreta N, season and soil type (Figure 2.2).

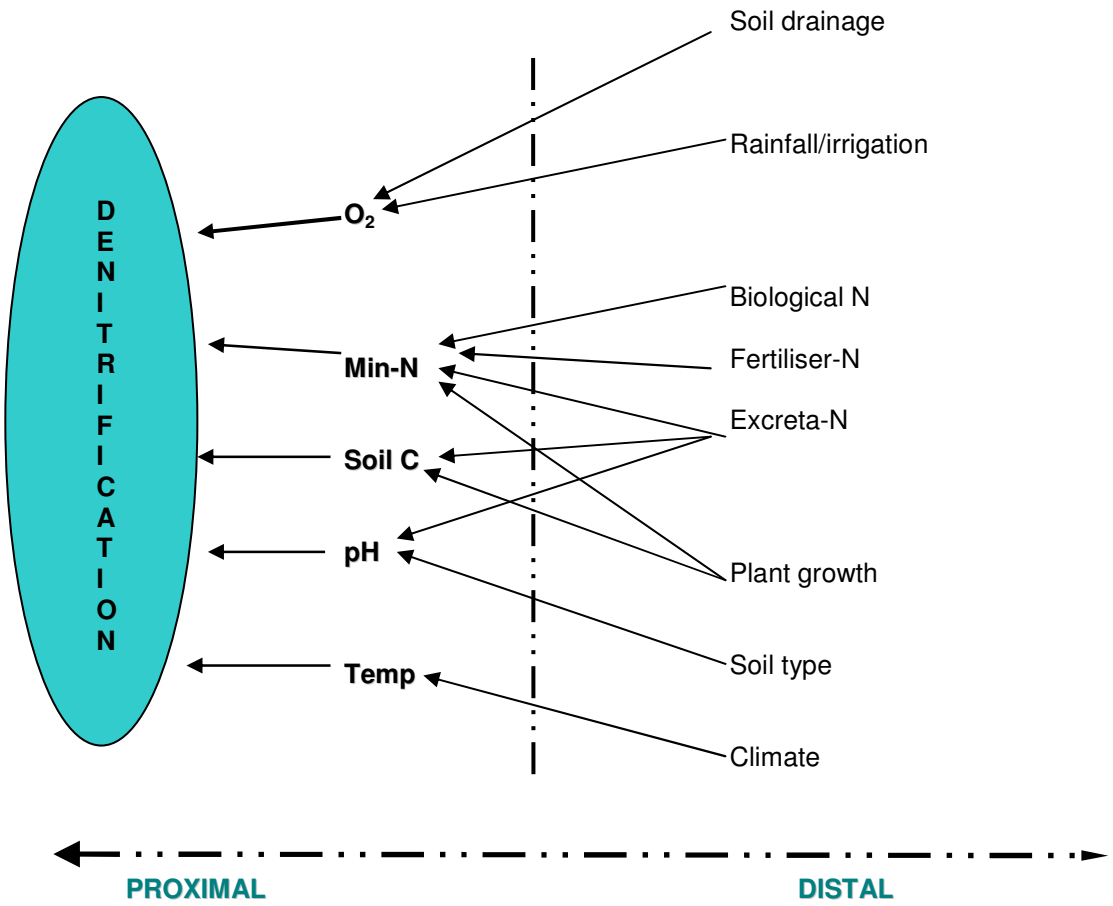

Figure 2.2 Proximal and distal factors affecting biological denitrification. Diagram is modified from de Klein 1994

Although the effects of soil and climate on denitrification activity have been established, soil denitrification rates and N_2O emissions at the soil surface are often poorly related (Florinsky *et al.* 2004; Parsons *et al.* 1991). This is likely due to the numerous factors involved in the synthesis of reductase enzymes (Fenchel and Blackburn 1979), diffusion of N_2O to the atmosphere (Ball *et al.* 1997; Bhandral *et al.* 2007), as well as the complexities of accurately measuring (without artificially influencing) denitrification rates *in situ* or in the laboratory (Yoshinari *et al.* 1977). Denitrification activity is best related to soil moisture in grasslands (Florinsky *et al.* 2004; Parsons *et al.* 1991; Pennock *et al.* 1992), while N_2O emissions are influenced by the position and geometry of slopes within the landscape (Florinsky *et al.* 2004). For example while denitrification rates may be restricted as soil conditions dry out, the increased air filled pore space may augment N_2O diffusion to the soil surface, as well as preventing a more complete reduction to N_2 (Florinsky *et al.* 2004). The

ability to quantify N_2O emissions as accurately as possible is fundamental to determine the contribution N_2O emissions to the GHG profile at the farm, regional, national or global scale. Measurements using variations on a manual or automated closed static chamber method to measure emissions at the soil surface, or micrometeorological stations which can measure N_2O emissions at various heights above the soil surface are therefore commonly employed in preference to lab or field measurements of denitrification activity to quantify N_2O emissions to the atmosphere.

There is a limited amount of data for N_2O emissions from NZ hill country. Recent N_2O measurements in NZ sheep grazed hill country pastures report that mean daily N_2O emission rates (2 to 11 g N_2O/ha.d) increased in response to increased fertiliser N application rate and soil moisture (Hoogendoorn *et al.* 2008). Spatial variation was not investigated by these workers. In Parker (2008), mean N_2O emissions increased in response to rainfall and subsequent increased soil moisture levels, as well as fertiliser N rate in North Island hill country. The author of this work also found that N_2O flux was significantly higher from low slopes compared to steep slopes. Carran *et al.* (1995) found that N_2O emissions from a steep sheep grazed hill country site rarely exceeded 5 g N_2O /ha.d on a steep sloped site, and the stock camping area used in this study rarely exceeded 10 g N_2O /ha.d during a 9 month measurement period. Their results from campsites were therefore noticeably lower than results from the work of Hoogendoorn *et al.* (2008), whose work was relatively short term by comparison. Taking into account the sparse and variable results obtained in NZ hill country for N_2O emissions to date as well as the complex relationship between denitrification activity and N_2O emissions, the focus of experimental Chapters 5 and 6 is therefore to determine the influence of increasing N inputs (via fertiliser and excreta N) and slope on denitrification activity and subsequent N_2O emissions in hill country. These chapters will also investigate the strength of the relationships between N_2O emissions and other soil and climatic variables.

2.4 Nitrogen uptake

2.41 Plant uptake

Plant tissues contain between 1 and 5% N on a dry weight basis (Whitehead 1995). The supply of N to plants is often the main limiting factor to growth rate. Higher plants (including grasses) absorb N as NO_3^- and NH_4^+ ions through the root system (Haynes 1986). Soil NO_3^- tends to be absorbed in preference to NH_4^+, due to the mobility of NO_3^- in soil water and because NH_4^+ is progressively converted to NO_3^- through the process of nitrification (Whitehead 1995). The uptake of N (kg N/ha.y) in managed grass swards is high in comparison to crops and forested soils, and has been estimated to be as high as 500 kg N/ha.y under favourable conditions (Haynes 1986). In situations where N availability is not limiting, daily uptake rates vary according to temperature and soil moisture conditions (i.e. season), growth stage (as governed by season and pasture management), and frequency of grazing or cutting (Whitehead 1995; Haynes 1986). In hill country mean N uptake was estimated to be 150, 148 and 55 kg N/ha from low, medium and steep slopes, respectively by Bowatte (2003), (Figure 2.1). The area assumed to be covered by these slope categories in Figure 2.1 was 12, 46 and 42%, respectively. Therefore the actual uptake on an area basis in Figure 2.1 is:

(0.12*150) + (0.46*148) + (0.42*55) = 109.2 kg N/ha

2.5 Nitrogen removals in animal products

Of the N consumed in herbage by grazing animals, less than 30% is utilised for the production of liveweight gain, milk or wool; the remainder is excreted (Whitehead 1995). At Ballantrae Lambert *et al.* (1982b) calculated that an average of 2.5% of N consumed was removed in animal product (meat and wool) annually. The removal of N in animal product therefore is of little consequence to the N balance by comparison to losses from other parts of the system for this type of farm system.

2.6 Summary

Various aspects of hill country nutrient cycling and productivity have been investigated by numerous workers in NZ. This literature spans three major themes that investigate the effect of fertiliser application rate, slope, aspect and animal behaviour on: 1) Herbage yield and quality (López *et al.* 2003a; López *et al.* 2003b;

Luscombe 1981; Luscombe 1979; Zhang *et al.* 2006), 2) soil nutrient status and cycling (Lambert *et al.* 2000; Letica *et al.* 2006; Saggar *et al.* 1990; Sakadevan *et al.* 1993a; Sakadevan *et al.* 1993b), and 3) animal productivity (Clark and Lambert 1989; Lambert *et al.* 1986; López *et al.* 2003a). There is now a good body of literature to demonstrate that nutrient status and cycling is generally higher in campsites compared to sloped areas due to stock preference for these sites, however nutrient balances appear unique to individual trial situations (Table 2.2). The review however demonstrates that because soil and climatic variables are inherently more variable in hill country than on flat land, knowledge gaps for the influence of slope and aspect on N cycling in hill country remain.

Losses via gaseous N emissions (e.g. NH_3, N oxides and N_2) are particularly poorly understood and quantified. There is currently a small body of work that has measured N_2O losses from NZ sheep grazed hill country pastures (Carran *et al.* 1995; Hoogendoorn *et al.* 2008; Parker 2008). However the influence of land use intensification (i.e. increased fertiliser N use) and the effect of slope, aspect and climate on N_2O emissions in the hill country situation is not yet well understood. The experimental chapters will therefore aim to determine the distribution of excreta N in hill country as influenced by slope, and the effect on subsequent N cycling rates. Given the unique topographical, soil and climatic properties of NZ hill country and the scarcity of data available for N_2O emissions from hill country the major focus of the current Thesis will be to quantify N_2O losses and the effects of increasing fertiliser and excreta N inputs on N_2O emissions. Based on the literature the hypothesis is that N_2O losses will be higher from gully and campsite areas compared to medium and steep slopes, due to the supply of soil NO_3^- and moisture conditions to these areas. The implications of the findings in this Thesis will be discussed in the context of NZ's GHG profile. Mitigation options to reduce N_2O emissions from hill country pastures will also be discussed.

Table 2.2 Summary of soil chemical and physical data for pastoral hill country in NZ

Herbage	Reference	Slope			P
		Low	Medium	High	
Pasture					
Herbage accumulation (kg DM/ha.y)	Lopez *et al.* 2003a, 2003b	9440	8670	8300	*
	Sakadevan *et al.* 1993a	3774	5198		**
	Ledgard *et al.* 1987	14300	8025	4745	
	Luscombe and Fletcher 1981	4200	2250	2200	***
‡Clover net herbage accumulation (kg DM/ha)	Luscombe and Fletcher 1981	144	783	688	***
§Legume net herbage accumulation (kg DM/ha.y)	Roach *et al.* 1996	400	1500	1300	
Legumes (%)	Lambert *et al.* 1986	13	18	24	
Ryegrass (%)	Lambert *et al.* 1986	26	12	4	
Herbage P concentration (%)	Saggar *et al.* 1990	0.30	0.27	0.24	*
Herbage S concentration (%)	Saggar *et al.* 1990	0.24	0.23	0.20	*
§*N fixation (kg N/ha.y)	Bowate *et al.* 2006	1	18	0	
	Ledgard *et al.* 1987	54	75	75	
Plant uptake N (kg/ha.y)	Sakadevan *et al.* 1993a	113	155		**
Soil physical					
Bulk density (mg/m^3)	Lambert *et al.* 2000	0.86	0.89	0.95	***
	Carran *et al.* 1995	0.81		0.67	
	§Sakadevan *et al.* 1993b	0.83	0.87		
Field capacity (g/g)	Carran *et al.* 1995	63		60	
Texture (% clay:silt:sand)	Lambert *et al.* 2000	19:28:53	18:28:54	17:26:57	***
Soil chemical					
Olsen P (mg/kg dry soil)	Lambert *et al.* 2000	16.3	12.3	12.8	***
	Hawke 2001	30	17		*
	Carran *et al.* 1995	40		5	

Total P (mg/kg dry soil)	Hawke 2001	3600	3060		ns
	Lambert *et al.* 2000	759	625	569	***
¥	Gillingham 1980	5075	4562	4095	
Total soil P (kg/ha)	Saggar *et al.* 1990	801	724	671	
Inorganic P (mg/kg dry soil)	Lambert *et al.* 2000	218	172	179	***
Organic P (mg/kg dry soil)	Lambert *et al.* 2000	540	452	390	***
¥Net P balance (kg P/ha)	Gillingham 1980	22.6	-4	-3.7	
Organic C (%)	Lambert *et al.* 2000	5.9	5.4	4.6	***
	Ledgard *et al.* 1987	15.1	7.6	8.3	
Total C (%)	Carran *et al.* 1995	6.5		4.5	
	§Sakadevan *et al.* 1993b	6.0	5.0		
Net C mineralisation (mg/kg dry soil)	§Sakadevan *et al.* 1993b	2131	2328		
NH_4-N (mg/kg)	§Sakadevan *et al.* 1993b	18.6	15.0		
NO_3-N (mg/kg)	§Sakadevan *et al.* 1993b	82	37		
Total N (%)	Hawke 2001	6.5	4.8		***
	Lambert *et al.* 2000	0.5	0.4	0.3	***
	Carran *et al.* 1995	0.55		0.35	
	§Sakadevan *et al.* 1993b	6.0	4.3		
	Ledgard *et al.* 1987	1.0	0.5	0.4	
§*Ammonia volatilisation (kg N/y)	Bowate *et al.* 2006	15.5	7	1	
Net N mineralisation (mg/kg.y)	§Sakadevan *et al.* 1993b	53	30		
§*Leaching (kg N/y)	Bowate *et al.* 2006	14.5	0	0	
N_2O emissions (kg N/ha.y)	Carran *et al.* 1995	<1.0		0.5	
C:N	Hawke 2001	11.1	12.5		**
	Lambert *et al.* 2000	12.8	13.9	15.0	***
	§Sakadevan *et al.* 1993b	9.95	11.7		
	Ledgard *et al.* 1987	15.8	16.7	18.9	
Sulphate S (mg/kg)	Lambert *et al.* 2000	6.5	5.2	4.9	NS
	§Sakadevan *et al.* 1993b	21.1	15.8		
Organic S (mg/kg)	Lambert *et al.* 2000	476	391	297	***
Total S (g/kg)	§Sakadevan *et al.* 1993b	0.77	0.55		
Total soil S (kg/ha)	Saggar *et al.* 1990	626	641	524	

Net S mineralisation (mg/kg.y)	§Sakadevan *et al.* 1993b	18.7	13.5		
Plant uptake S (kg/ha)	Sakadevan *et al.* 1993a	9.8	13.5		**
Exchangeable Ca (mg/kg dry soil)	Lambert *et al.* 2000	6.9	6.1	6.2	*
Exchangeable Mg (mg/kg dry soil)	Lambert *et al.* 2000	31.3	25.5	25.9	***
Exchangeable K (mg.kg dry soil)	Lambert *et al.* 2000	12.2	9.2	7.0	***
Anion storage capacity (%)	Lambert *et al.* 2000	25.5	26.9	24.2	NS
pH	Lambert *et al.* 2000	5.4	5.4	5.4	
	Carran *et al.* 1995	5.0		4.9	
	§Sakadevan *et al.* 1993b	5.7	5.9		
	Ledgard *et al.* 1987	5.3	5.3	5.2	
Other					
Annual grazing probability	Lopez *et al.* 2003a	0.441	0.332	0.197	***

§ Values for each slope category are means based on combined high and low fertility treatments
¥ Values for each slope category are the means based on combined north and south slope aspects
* P< 0.5, ** P< 0.01, *** P< 0.001, NS not statistically significant

CHAPTER 3 METHODS AND MATERIALS

3.1 Introduction

All of the experimentation described in this Thesis was conducted within the "Wise N Use Trial", which was established in 2004 on two AgResearch hill country farm blocks at Invermay, Mosgiel and at Ballantrae, Woodville (Figure 3.1). This chapter therefore includes a description of the "Wise N Use" project designed and run by AgResearch Limited (Wise N use 2007) and funded by a range of agencies: central government (MAF, Sustainable Farming Fund and the Foundation for Research, Science and Technology), levy payer (Meat and Wool New Zealand), consortium (Pastoral Greenhouse Gas Research Consortium) and fertiliser industry (FertResearch, Ravensdown Fertiliser Cooperative, and Balance Agri-nutrients). Soil classifications and descriptions of some soil characteristics (pH, Organic C, total N, C:N ratio, Olsen P, bulk density, and pore size distribution), as well as soil profile descriptions and climate data are included in this chapter to provide the reader with context and reference material when considering the results and discussion sections of this Thesis.

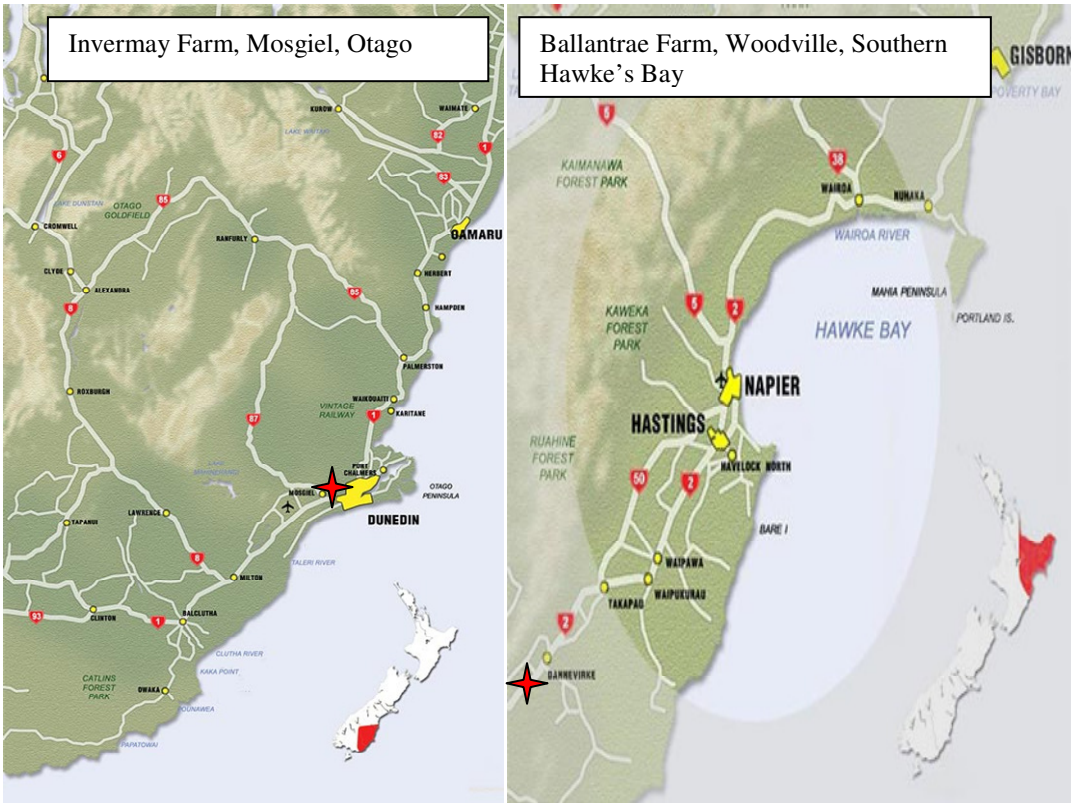

Figure 3.1 Location of Invermay and Ballantrae Research Farms (AgResearch Limited) indicated by the red stars

3.2 The Wise N use trial

The objective of the Wise N Use Trial was to determine best management practices for fertiliser nitrogen (urea N) use (i.e. commercially and environmentally sustainable fertiliser N application rates) through both experimental work and at commercial demonstration farms in a range of hill farming situations in NZ.
Field trials in combination with focus farm demonstrations were established to encourage and demonstrate practices to enhance long term farm profitability and minimise the impact of fertiliser N on NO_3^- leaching in hill country. The field trials were conducted from 2004 to 2007 and 2004 to 2008 on Invermay and Ballantrae Research Farms, respectively. The experiment consisted of paddock scale trials to investigate the effect of increasing application rates of N fertiliser on pasture performance, stocking rate and some aspects of environmental performance. The fertiliser treatments were in a randomised block design with N fertiliser treatment given by 0, 100, 200, 300, 400, and 500 kg N/ha.y from September 2004 to December 2007 at Invermay. At Ballantrae the fertiliser treatments were in a randomised

incomplete block design with N fertiliser treatment given by 0, 100, 200, 300, 400, 500, and 750 kg N/ha.y from September 2004 to June 2008 (Appendix 1). The times and rates of fertiliser N applications for both sites are described in Table 3.1. The experimental blocking structure used in the Wise N Use Trial was adhered to for all experimental purposes in the current Thesis. Various pasture, soil and animal measurements were recorded for the duration of the trial and some of this information was used in this Thesis (e.g. for soil descriptions and to estimate stock rates (SU/ha.y)). Nitrous oxide emissions were also measured during the spring seasons of 2005, 2006, and 2007 and the data was regularly compared to the results in this Thesis.

Table 3.1 Timing and replication of N treatments to Wise N Use Trial paddocks at Invermay and Ballantrae

kg N/ha.y Invermay	Sept	Oct	Nov	Dec	Feb	Mar	Apr	May	Replicates
0									3
100	50					50			3
200	50		50			50	50		3
300	50	50	50	50	50		50		3
400	50	50	50	50	50	50	50	50	3
500	63	63	63	63	63	63	63	63	3
Total									**18**
Ballantrae	**Sept**	**Nov**	**Jan**	**Mar**	**May**	**July**			
0									3
100				50		50			2
200	50			50	50	50			3
300	50	50	50	50	50	50			2
400	67	67	67	67	67	67			3
500	83	83	83.	83	83	83.			2
750	125	125	125	125	125	125			2
Total									**17**

3.3 Site description

3.31 Invermay Agricultural Research Farm. AgResearch Limited

Invermay Research Farm is located near Mosgiel in the Otago region of NZ. The experimental site is situated *c.* 90 – 150 m above sea level (a.s.l, NZMS 260 I44), on rolling land (*c.* 8-15 º, Figure 3.2). In the Wise N Use trial, paddocks were blocked according to pasture age; block 1 >20 y, block 2 *c.* 10 y, and block 3 <3 y old pastures and ranged in size from 0.7 to 1.05 ha (Appendix 1). The trial began in June 2004 and ran until June 2007.

Figure 3.2 Photographs of some of the experimental hill country paddocks on Invermay Farm

Paddocks were rotationally grazed with breeding ewes and hoggets during which time pasture mass between 2,500 and 3,000 kg DM/ha was grazed down to a residual target between 1,000 and 1,800 kg DM/ha. From lambing to lamb weaning, pastures were set stocked and maintained between 1,500 and 2,500 kg DM/ha using a put and take system to alter grazing pressure as required. Grazing stock normally moved from low through to high N application paddocks and were held off for 24 h if moving from high to low N application paddocks (Stevens *et al.* 2008).

The site supported a mixture of high fertility responsive grasses (HFRG, *Lolium perenne*, *Anthoxanthum odoratum*, and *Poa* Spp.) and white clover (*Trifolium repens*). Weedy species were <5 % of pasture composition (Stevens *et al.* 2008). Mean annual herbage yield for the control sites (0 kg N/ha.y) over the trial period was approximately 12,680 kg DM/ha.y and the mean N response rate was 21 kg DM/kg N applied with a response range of 4 to 32 kg DM/kg N applied (Stevens *et al.* 2008).

The farm block is described as summer dry, rolling country (Stevens *et al.* 2008). The low winter soil temperatures (4.6 - 5 °C) and low summer rainfall combined with high potential evapotranspiration rates would not be expected to be conducive for denitrification activity in the soils during these seasons (Table 3.2). Highest N_2O emissions may therefore be expected to occur during spring and autumn when soil moisture and soil temperatures are most suitable for denitrification activity at this site.

Table 3.2 Annual and seasonal climate data summary for Mosgiel from Otago Regional Council (2006). Data are the median values for the period 1970 – 2001

	Spring median	**Summer median**	**Autumn median**	**Winter median**	**Annual median**
Rainfall mm	201-250	181-200	201-250	201-250	801-900
Soil temperature °C 10 cm depth	11-1-11.5	13.6-14.0	6.1-6.5	4.6-5.0	9.1-9.5
Potential evapotranspiration mm	¥171-180	¥161-170	¥66-70	¥91-95	n/a
Solar radiation MJ/ m^2. d	19-1-20.0	17.1-18.0	5.1-6.0	7.1-8.0	12.1-13.0

¥ Seasonal median values are from one month of each season only for the period 1970 - 2001

The trial was situated on predominantly Warepa silt loam, a strongly mottled phase soil (Campbell 1977), and is classified as a Mottled Fragic Pallic Soil (Hewitt 1998). The parent material is loess. The ranges for the soil parameters measured at this site

reported in Table 3.3 were similar to those measured for the Warepa silt loam reported by Campbell (1977), McDowell *et al.* (2004), Otago Regional Council (2006), and Stevens *et al.* (2008).

Table 3.3 Mean physical and biochemical properties of soils in trial paddocks at Invermay Research Farm (0-75 mm depth) with sed. Ranges are reported in parenthesis

	pH	Organic C %	Total N %	C:N ration	Olsen P mg/kg soil	Bulk density g/cm^3	Pore size distribution %		
							<30 µm	30-300 µm	>300 µm
	6.2 (5.7-6.9)	[§]4.89 (4.87-5.1)	[§].41 (.38-.44)	11.3	[§]26 (22-29)	0.90 (0.5–1.0)	50.1	8.1	8.0
SED	**0.05**					**0.01**	**0.58**	**0.42**	**0.66**

[§]Values are from year 1 of the Wise N Use Trial for the Invermay site *in* Stevens *et al.* (2008)

Warepa soils are imperfectly drained due to perching of water on a dense fragipan, evidenced by mottling, making them susceptible to prolonged denitrification activity in times of high rainfall. Soils have a 15 to 25 cm deep topsoil that has moderately developed structure and good root distribution. Subsoil structure is moderate to weak in the upper subsoil, abruptly changing in the lower subsoil to the massive structure of the fragipan at *c.* 60 cm depth, which provides a barrier to root development (Table 3.4). Identified phases that experimental plots were situated on include Warepa deep silt loam, rolling (Wr0zR), Warepa moderately deep silt loam, rolling (Wr1zR) and Warepa deep silt loam, hilly (Wr0zH). Some characteristics of these soil phases are given in Table 3.4. The impeded drainage characteristics and structural vulnerability of this soil type (Table 3.4) renders it vulnerable to increased and/or prolonged denitrification activity and therefore gaseous N_2O emissions in wet conditions.

Table 3.4 Attributes of the Warepa Soil phases Wr0zR, Wr1zR and Wr0zH within the Wise N Use Trial area described by Otago Regional Council (2006)

Soil phases			
	Wr0zR	**Wr1zR**	**Wr0zH**
Slopes	Rolling (8-15º)	Rolling (8-15º)	Hilly (16-25º)
Potential rooting depth	40-80 cm	30-60 cm	40-80 cm
Rooting barrier	Pan	Pan	Pan
Topsoil stoniness	Stoneless	Stoneless	Stoneless
Soil texture	Silt loam	Silt loam	Silt loam
Drainage class	Imperfectly drained	Imperfectly drained	Imperfectly drained
Permeability profile	Moderate over slow	Moderate over slow	Moderate over slow
Water logging vulnerability – irrigated	High	High	High
Structural vulnerability	High	High	High
Drought vulnerability – not irrigated	Moderate	Moderate	Moderate

A detailed soil profile of the Warepa silt loam is described by (McIntosh 1985):

A 0-15 cm very dark greyish brown (10YR 3/2) silt loam; friable; strongly developed fine nut with crumb and granular structure; many roots and bracken rhizomes; indistinct boundary,

A/B 15-26 cm light yellowish brown (2.5Y 6/4) clay loam with profuse mottles of greyish brown (2.5Y 5/2) and dark greyish brown (10YR 4/2); Mn/ Fe

concretions; friable; strongly developed nut and blocky structure; many roots; indistinct boundary,

Bg1 26-47 cm greyish brown (2.5Y 5/3) clay loam with mottles of yellowish brown (10YR 5/6); moderately developed prismatic structure; few roots; indistinct boundary,

Bg2 47-80 cm yellowish brown (10YR 5/6) silty clay loam with net gammate veins, light brownish grey (2.5Y 6/2); firm; strongly developed coarse blocky structure and platy fracture; few roots; diffuse boundary,

Cx 80-100+ cm brownish yellow (10YR 6/6) clay; extremely firm; strongly developed coarse prismatic structure; prisms separated by white (2.5Y 8/2) gammations

These are Typic Fragiudalf soils in the USDA taxonomy (McDowell *et al.* 2004).

3.32 Ballantrae Agricultural Research Farm. AgResearch Limited

Ballantrae Research Farm is located near Woodville in the Hawke's Bay region of NZ (NZMS sheet 2 1988 Ltd Rev). The experimental site is situated *c.* 200 to 350 a.s.l. on steep (*c.* 5 - 45º, Figure 3.3) heavily dissected hill country (Zhang *et al.* 2006). The experimental block design was unbalanced as treatment levels 0, 200, and 400 kg N/ha.y were replicated in all three blocks and the 100, 300, 500, and 750 kg N/ha.y treatments were replicated in two of the three blocks only (hence 17 paddocks in total). Paddocks ranged in size from 0.2 to 0.5 ha and were blocked according to proximity to each other. The trial began in June 2004 and ended in June 2008.

Figure 3.3 Photographs of some of the experimental hill country paddocks on Ballantrae Farm in the North Island

Paddocks were rotationally grazed year round by hoggets and dry two tooths as herbage mass reached approximately 2,000 kg DM/ha in autumn, winter and early spring, and 2,500 kg DM/ha in late spring and summer. Stock numbers were adjusted as appropriate to graze paddocks down to a residual herbage mass of approximately 1,000 kg DM/ha within a 3 to 4 day grazing period.

At the start of the trial the site supported a mixture of 53 % HFRG (*Lolium perenne*, *Anthoxanthum odoratum*, and *Poa* Spp.) and 41 % low fertility tolerant grasses (LFTG, *Agrostis* Spp., *Cynosurus cristatus* and *Festuca rubra*), which moved towards a higher but not significantly different proportion of HFRG by the end of the trial period. Legume contribution (*Trifolium* Spp.) remained low (<5 %) for the duration of the trial (Stevens *et al.* 2008). Mean annual net herbage accumulation from the control sites (0 kg N/ha.y) over the trial period was approximately 10,658 kg DM/ha.y. Mean response to fertiliser N was 7 kg DM/kg N applied with a response range of 3 to 20 kg DM/kg N applied.

The farm block is described as summer moist country (Lambert *et al.* 1983). The relatively mild winter soil temperatures and high seasonal rainfall totals at this site are conducive to year round denitrification activity (Table 3.5). The high rates of potential evapotranspiration in summer, and the rapid uptake of water by plants during spring however may result in soil moisture deficits over prolonged periods at these times. Highest N_2O emissions may therefore be expected to occur during autumn when water losses are smaller and soil temperatures are still warm enough to support denitrification activity.

Table 3.5 Annual and seasonal climate data summary for Ballantrae from Tait *et al.* and Tait and Woods (2006; 2007) with s.e.m in parenthesis, for the period 1975 - 2008

	Spring median	Summer median	Autumn median	Winter median	Annual median
Rainfall mm	322 (13.2)	260 (17.1)	290 (11.5)	358 (17.6)	1209 (40.7)
Soil temperature °C 10 cm depth	11.3 (0.1)	16.8 (0.2)	12.4 (0.1)	6.7 (0.1)	11.8 (0.1)
Potential evapotranspiration mm	239 (2.5)	373.3 (3.9)	158.8 (2.3)	58.3 (1.6)	n/a
Solar radiation MJ/ m^2. d	15.4 (0.1)	19.4 (0.2)	11.7 (0.2)	7.7 (0.3)	13.7 (0.1)

Experimental sites were situated on predominantly Wilford hill silt loam, which is a Mottled Argillic Pallic Soil (Hoogendoorn *et al.* 2008). The parent material is bluish grey mudstone of the Waitotaran age (late Tertiary). The ranges for the soil parameters measured in Table 3.6 at this site were similar to values measured at Ballantrae and reported by Lambert *et al,* (2000). The minimum value measured for soil pH in Table 3.6 may inhibit denitrification activity, although there is evidence for adaptation to local soil conditions by denitrifiers (Haynes 1986).

Table 3.6 Mean physical and biochemical properties of soils in trial paddocks at Ballantrae Research Farm (0-75 mm depth) with sed. Ranges are reported in parenthesis

	pH	Organic C %	Total N %	C:N ratio	Olsen P mg/kg soil	Bulk density g/cm^3	Pore size distribution		
							<30 µm	30-300 µm	>300 µm
	5.7 (4.7-6.6)	5.0 (3.5-7.8)	0.4 (0.3-0.7)	12.5 (10.7-15.6)	[§]25 (18-35)	0.97 (0.78-1.11)	n/d	n/d	n/d
SED	**0.08**	**0.12**	**0.02**	**0.27**		**0.02**			

[§]values are from year 1 of the Wise N Use Trial for the Ballantrae site *in* Stevens *et al.* (2008)
n/d: no data

Wilford hill soils are classed as weakly gleyed, weakly to moderately leached intergrades between central yellow-brown earths and yellow-grey earths (mottled argillic pallic soils). Soils were formed from Tertiary sandstone, siltstone, and mudstone but with some loess influence in areas. The mottling is a sign of impeded drainage which makes this soil vulnerable to increased and/or prolonged denitrification activity at times of heavy rainfall. These soils are characterised by heavy textured firm subsoils with a weakly developed prismatic structure breaking to blocky structure. A profile from a weathered cutting on the Saddle Road on a northerly facing slope of 25° is given by Cowie (1983) below:

A1 10-15 cm brown silt loam; friable; moderately developed fine and medium nutty structure; abundant roots; distinct, irregular boundary with much worm mixing,

B1 13 cm brownish yellow heavy silt loam; firm; weakly developed coarse prismic structure breaking to moderately developed medium blocky structure; some roots; indistinct boundary,

B2 25 cm pale yellow brown clay loam; very firm to slightly hard; weakly developed medium prismic breaking to moderately developed coarse blocky structure; many faint pale grey and yellowish brown mottles; darker brown coatings on prism faces; few roots indistinct boundary. (This horizon stands out as a pan in cuttings)

C1 on grey silty mudstone, very firm; some yellowish mottles in top few inches; strongly developed blocky structure

Subsoil textures range from silty clay loam to clay loam and the numbers of mottles in the subsoil is variable. On shady faces subsoils are more friable, browner in colour and less mottled. These soils are Andic Distrochrepts, Typic Distrochrepts, and Typic Eutrochrepts in the USDA classification (Lambert *et al.* 1983).

3.4 Experimental methods

In the following three experimental chapters soil samples for physical and chemical analyses were routinely removed from directly beneath gas chambers or

within defined plot areas. The frequency and replication for soil sampling varied between trials and is described in more detail within each chapter. The basic principles and terms used throughout the following chapters for all measurements are defined in this section to allow readers easy reference.

3.33Soil physical measurements

There are three basic phases that make a soil and the total mass of soil (M_t) is the sum of those three phases; the mass of solids (M_s) plus the mass of water (M_w) and the mass of air (M_a), (McLaren and Cameron 1996). The total volume of the three phases (V_t) is the sum of the volume of solids (V_s) plus the volume of water (V_w) and air (V_a).

Bulk density (ρ_b):

$$\rho_b = \frac{M_s}{(V_s+V_w+V_a)}$$

where ρ_b is soil dry bulk density (g/cm^3), M_s is the mass of solids, V_s is the volume of solids, V_w is the volume of water, and V_a is the volume of air. Soil dry ρb is the ratio of the mass of dry soil to the total volume of soil (i.e. solids plus pores, not just solids), expressed in g/cm^3. Soil ρb is often used to describe the level of soil compaction and/or drainage. A soil with a low drainage capacity may be vulnerable to extended wet conditions following rainfall and therefore increase the risk of N_2O emissions from denitrification activity. Bulk density is also required for calculations to estimate soil nutrient concentrations (e.g. kg N/ha). Bulk density measurements in this Thesis were made according to the method of Drewry *et al* (1999), by inserting aluminium rings of a known volume (50 mm depth x 100 mm diameter, ~384 cm^3, internal volume) vertically into the soil until flush with the surface and carefully removing them to collect intact soil cores (Figure 3.4). Soil cores were wrapped in plastic bags and transported back to the laboratory and refrigerated until being prepared for measurements in the laboratory. In the laboratory soil cores were trimmed with a sharp knife to ensure that the upper core surface had all remaining herbage removed (so far as possible without damaging the soil surface), and that the lower core surface was flush with the ring. Cores were weighed while field moist. Cores were then dried at ~105° C for at least 48 h and re-weighed.

Figure 3.4 Aluminium ring (50 mm depth x 100 mm diameter, ~384 cm^3) inserted part-way into the soil to take a bulk density and pore size distribution sample.

Porosity (ε):

$$\varepsilon = \frac{(V_w + V_a)}{V_t}$$

where ε is soil porosity, V_w is the volume of water, V_a the volume of air, and V_t is the sum of the volume of solids (V_s) plus V_w and V_a. Porosity is expressed in this Thesis as the percentage ratio (%v/v) of the volume of pores to the V_t, and is negatively related to ρ_b. Porosity is a useful soil descriptor and another indicator of the level of soil compaction or drainage.

Gravimetric water content (θ_m):

$$\theta_m = \frac{M_w}{M_s} * 100$$

where θ_m is the gravimetric (i.e. the weight) content of water, M_w is the mass of water and M_s is the mass of solids. Gravimetric water content is the ratio of the M_w to M_s, expressed as a percentage. A percentage >100% indicates soil saturation. Gravimetric soil moisture content (GSM) was used as an index for *in situ* soil moisture conditions in this Thesis and for calculating nutrient concentrations measured on fresh samples to an oven dry weight basis.

Volumetric water content (θ_v):

$$\theta_v = \frac{V_w}{V_t}$$

where θ_v is the soil volumetric content of water, V_w is the volume of water and V_t is the sum of the volume of solids (V_s) plus the V_w and air (V_a). Volumetric water content is the ratio of V_w to V_t, and is often expressed as a percentage. Volumetric water content is used to describe soil moisture conditions on a volumetric basis.

Water filled pore space (WFPS):

$$WFPS = \frac{V_w}{\varepsilon}$$

where WFPS is the soil water filled pore space, V_w is the volume of water, and ε is total porosity. Soil WFPS is the ratio of the V_w to ε and is often expressed as a percentage. Soil WFPS is an indication of the soil oxygen status; a high soil WFPS indicates low oxygen status because the majority of pores are filled with water and the soil is therefore vulnerable to increased denitrification activity.

Pore size distribution (PSD):

Pore size distribution is used to describe the volumetric ratio of soil pore size classes (<30 µm micropores, and >30 µm macropores) relative to ε. Soil PSD provides detailed information about drainage capacity; a high percentage of micropores will take longer to drain compared to a soil with a high number of macropores. Soil PSD was determined according to the method of Drewery *et al.* (1999); after the fresh soils were prepared and weighed for ρ_b (see method described previously), a voil was attached to the base of each core with rubber bands. A viscous slurry of gypsum and water (~1.75 $CaSO_4$: 1.00 H_2O) was spread over the top of each core, allowed to set (~2 h), then 'peeled' off to reveal an unsmeared soil surface. The trimmed and flush bases of each core provided a continuous surface contact when placed on the tension table. Cores were weighed (field moist) before and after 'peeling'. Cores were then immersed up to the top of the aluminium ring in a formaldehyde bath (~4%) to remove any earthworms that were present and potentially blocking pores, drained,

immersed in tap water, and drained again. The rinsed cores were then equilibrated to -1 kPa and -10 kPa on tension tables for ~3 days each then re-weighed, to measure pores >30 µm and <30 µm diameters, respectively (Drewry *et al.* 1999). Macroporosity was described by the volumetric percentage of pores >30 µm. Microporosity was described as ε minus the percentage volume of pores >30 µm. Measurements to determine PSD were used to determine Field Capacity (FC), which is the equivalent to macroporosity (i.e. >30 µm).

3.34 Nitrification potential

Because N is often the limiting nutrient in plant growth it is inevitable that differences in the rates of mineralisation, immobilisation and nitrification heavily influences plant growth and subsequent animal productivity, as well as N losses. Simplified; under controlled conditions net rates of mineralisation and/or nitrification can be determined from the change in the size of the soil inorganic N pools (NH_4^+- and NO_3^--N) over time. Gross rates of nitrification can also be measured using isotopic techniques (^{14}N and ^{15}N); however such methods are often prohibitively expensive. There are numerous methods for estimating net production of inorganic-N (Table 3.7), of which the soil slurry method was selected to measure nitrification potential in this Thesis. Method selection was heavily influenced by cost and the effort required to fulfil the objectives in the pilot study (Chapter 4). Nonetheless this method provides a good index of the size of the NH_4^+ oxidiser community in soils from different hill country land classes. The nitrification potential method used in this Thesis is described and discussed below.

Table 3.7 Summary of the relative quantity and types of information provided on selected soil N-cycling processes by various methods, relative to the effort required for use. Modified from Hart *et al* (1994)

		Information type				
Method	**Use**	**Mineralisation**	**Immobilisation**	**Nitrification**	**Cost/effort**	**Information**
^{15}N natural abundance	F/L	Gross	Gross	Gross	Very H	Potentially H
^{15}N tracer	F/L	Gross or net	Gross or net	Gross or net	H	H
Isotope-dilution $^{15}NH_4^+$	F/L	Gross	Gross NH_4^+ consumption	No	H	H
Isotope-dilution $^{15}NO_3$	F/L	No	Gross NO_3^- consumption	Gross	M	H
Resin cores	F	Net	Net	Net	M	M
Buried bags	F	Net	Net	Net	M	M
Closed top tubes	F	Net	Net	Net	M	M
Aerobic incubation	L	Net	Net	Net	M	L/M
***Soil-slurry nitrification**	L	No	No	Potential	M	M

F: field, L: laboratory, H: high, M: moderate, *: method used in this Thesis

Nitrification potential assesses the maximum rate of nitrification (Vmax) or potential nitrification activity. In this method net nitrification potential is calculated from the net change in NO_3^- pool size over an incubation period. To obtain the data reported in Chapter 4, in the field within each subplot, six soil cores (25 x 75 mm) were removed from plots (Figure 3.5a) and bulked, stored chilled, transported back to the laboratory and refrigerated immediately. The following day the bulked samples were sieved (2 mm, Figure 3.5b) to remove obvious roots and split into duplicate samples. In 250 mL Schott flasks 15 g samples of fresh soil were adjusted with respect to water content (deionised), NH_4^+ concentration (1.5 mM NH_4^+), P availability (1 mM PO^{3-}_4), and aeration (Hart *et al.* 1994). Foil-sealed flasks were placed on a shaker table (~180 rpm) at ~25°C for 24 h (Figure 3.5c). Each flask was sampled 4 times (2, 4, 22 and 24 h) during the incubation period using an automated pipette to transfer 10 mL of slurry into 50 mL centrifuge tubes (Figure 3.5d). Pipette tips were replaced after each sample to avoid sample contamination. Samples were 'spun' at 3,500 rpm for 4 mins before 5 mL of the clear supernatant was removed by auto-pipette. The 5 mL samples were stored in 35 mL plastic bottles and frozen until analysed.

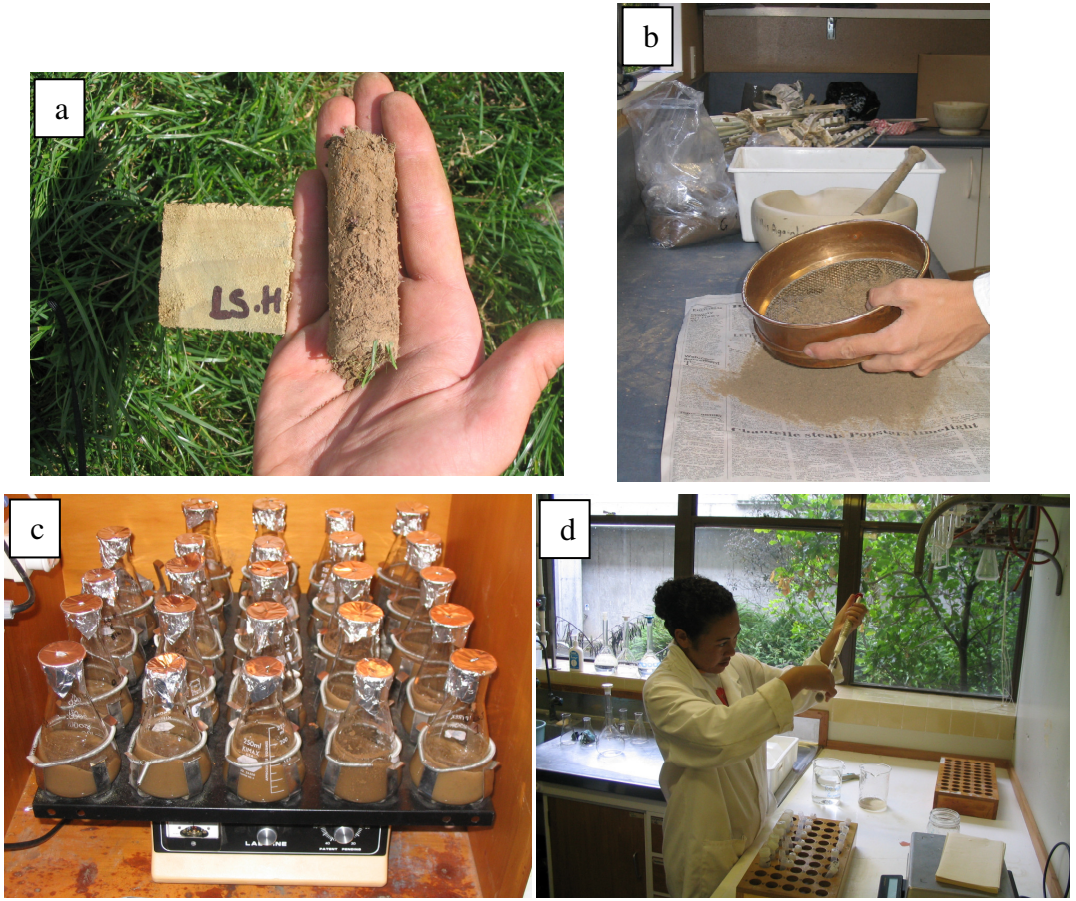

Figure 3.5 Sample processing methods involved in the soil slurry method: a) soil cores removed from field plots, b) bulked samples sieved (2 mm), c) soil slurries incubated at 25°C and shaken on an orbital shaker (~180 rpm), d) slurry samples taken from slurries for further processing at 2, 4, 22 and 24 h.

Samples were analysed colorimetrically on a Technicon Auto-Analyser (AA) at Massey University; based on the method of Blakemore (1987). Soil nitrate concentration was measured as a red azo dye. The AA was configured for analysis as in Figure 3.6, with a heating bath at ~28°C and filters in the colorimeter (520 nm). Standards were run at the beginning of the run through the colorimeter flowcell and the recorder was set to read 90-95% absorption, using the standard calibration control on the colorimeter. Standard curves were prepared (i.e. µg NO_3^-/ mL vs. absorbance).

The solution concentrations for NP were determined by calculating the rate of NO_3^- production (mg NO_3-N/kg dry soil.h) by linear regression of solution concentrations, versus sample time (2, 4, 22, and 24 h as in Hart *et al* (1994)):

$V_{max} = C_i \text{ vs} T_i$

where V_{max} is the maximum rate of nitrification or potential nitrification activity (mg N/kg.h), C_i is the NO_3^- concentration (ppm) in sample *i*….*n*, T*i* is the sample time at hour *i*…..*n*.

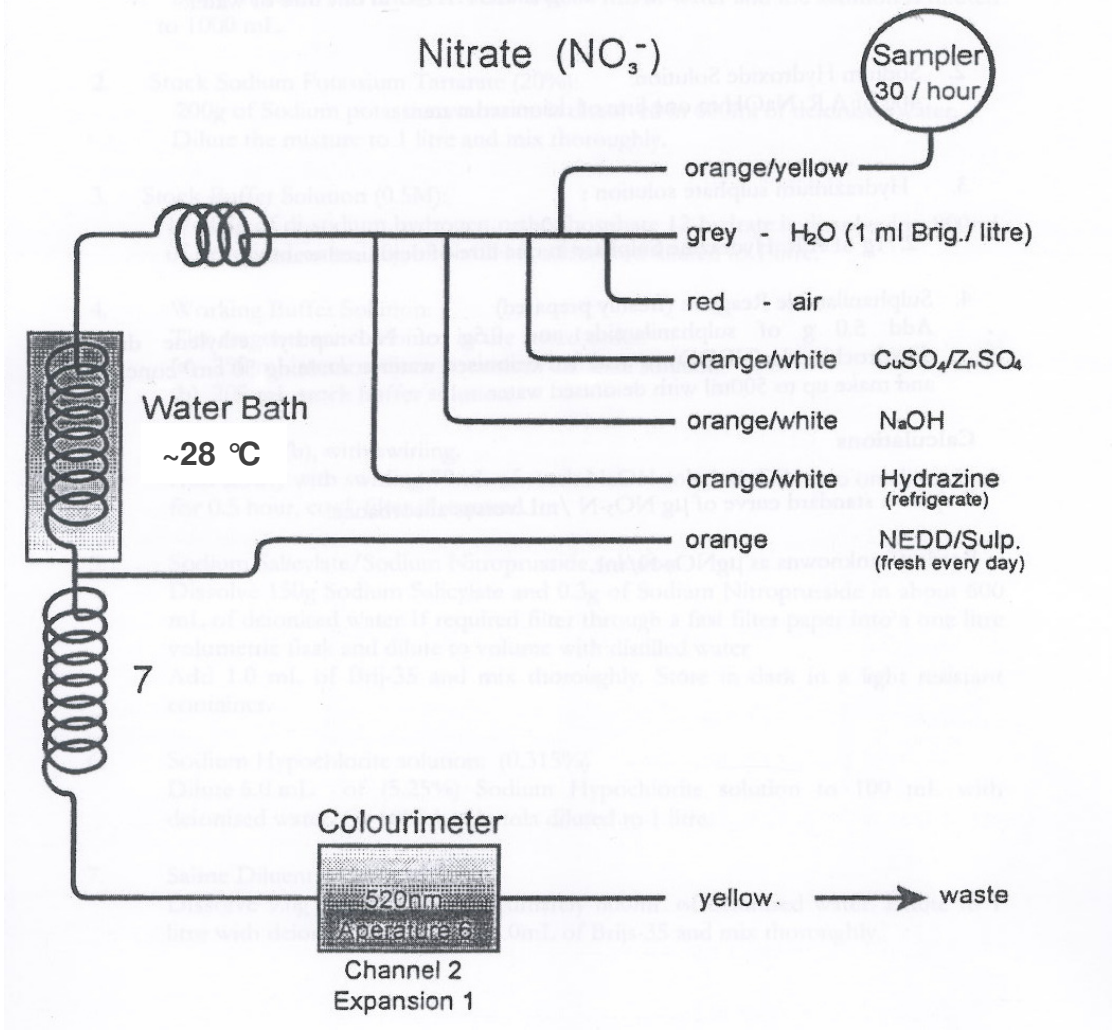

Figure 3.6 Schematic diagram of the Auto-Analyser configuration for the determination of nitrate concentration (ppm). Sourced from Massey University (1998).

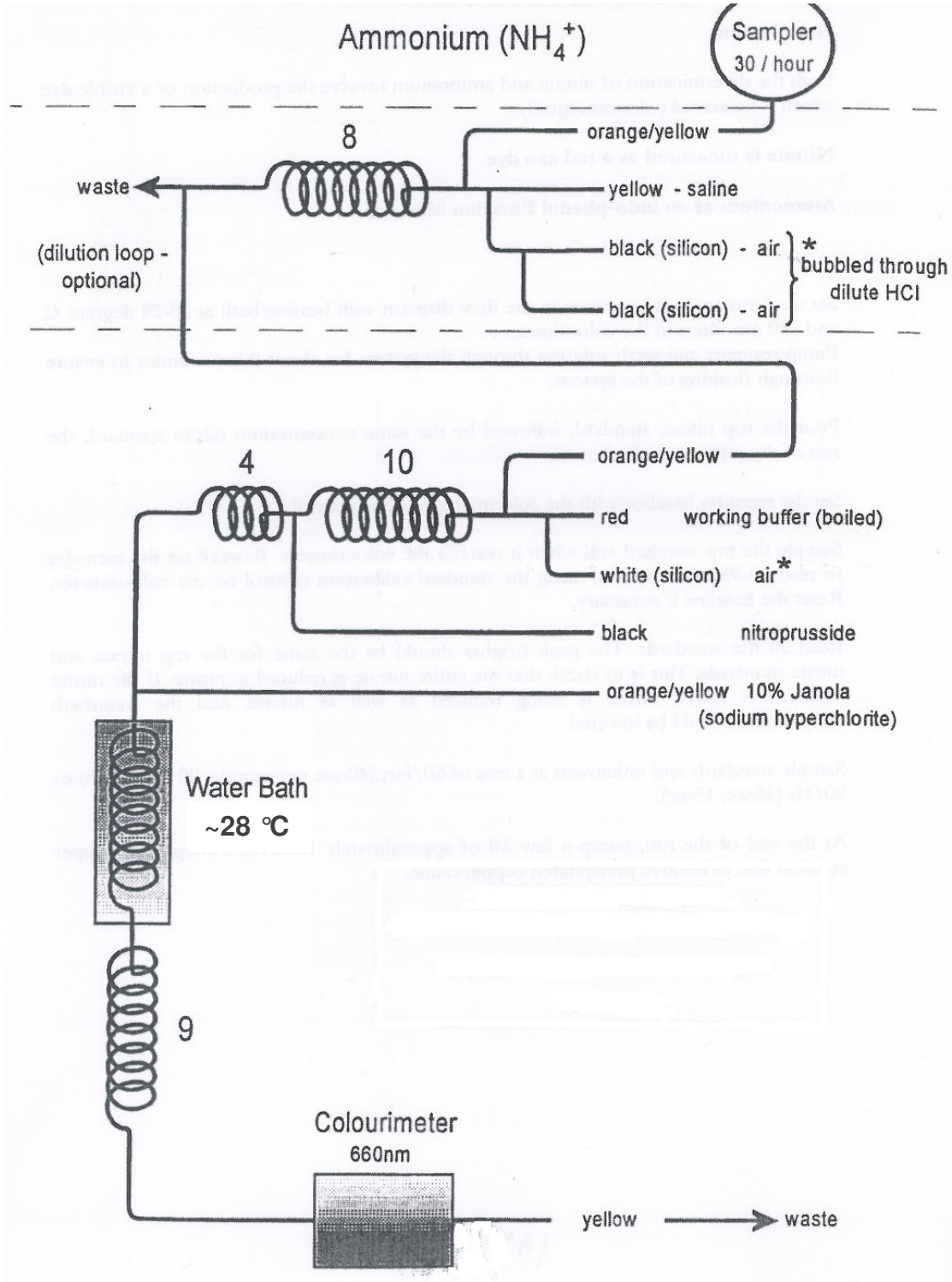

Figure 3.7 Schematic diagram of the Auto-Analyser configuration for the determination of soil ammonium concentration (ppm). Sourced from Massey University (1998).

3.35Mineral nitrogen

The soil mineral N results reported in Chapters 4, 5 and 6 were determined by colorimetric analysis on the Massey University AA based on the method from Blakemore (1987). Soil NH_4^+ was measured as an indo-phenol Prussian blue dye and the AA configuration is described in Figure 3.7. Soil cores (25 x 75 mm) were removed from the field as described in the previous section. Bulked samples were sieved (2 mm) and analysed separately for soil NO_3^- and NH_4^+ by extracting 4 g field moist soil in 30 ml 2M KCl (Hatch *et al.* 2000) for ~1 hr. Soil samples were then filtered through Whatman no. 42 filter papers. Filtered samples were frozen until the day of analysis. Two blanks were included for each set of extractions. The amount of mineral N in soil samples was determined by:

$$N_s = N_c * \frac{(0.1+Mw*0.001)}{(M_d*0.001)}$$

where N_s is mineral N level in the sample (mg N/kg soil), N_c is N concentration of the extract (ppm), M_w is the mass water in the soil sample (g), and M_d is the mass of dry soil in the sample (g). The concentrations were assumed to represent soil depth 0-10 cm depth using this equation.

3.36 Soil pH

Soil pH was measured from a sub sample of 10 g of field moist soil in 30 mL deionised water that was shaken, covered and left to stand overnight. Soil pH was measured using a pre-calibrated pH meter (Hanna instruments 9812). Calibration was performed using buffer solutions of pH 4 and 7 (Fisher Scientific, general purpose grade).

3.37 Nitrous oxide gas

Field sampling technique:
The static chamber methods employed in Chapters 5 for the fertiliser N rate trial and Chapter 6 for the slope class trial (de Klein *et al.* 2003 and Saggar *et al.* 2004a,

respectively) are based on the most commonly used measurement technique for measuring N_2O fluxes from agricultural soils. The main advantages of this technique are that it is relatively inexpensive, versatile in the field, and the technology is easy to adopt. Static chambers are commonly used to investigate within-field heterogeneity. The method is also used to measure single-factor effects on trace gases (N_2O, methane (CH_4, and carbon dioxide CO_2). The static chamber methods are outlined in Table 3.8 and discussed below.

Table 3.8 Static chamber designs used for N_2O field measurements

	de Klein *et al* 2003	**Saggar *et al* 2004a**
Chamber materials	Stainless steel	PVC plastic
Chamber size	250 mm diameter x 130 mm high	250 mm diameter x 200 mm high
Base and collar	NO	YES
Venting	NO	NO
Venting on closing chambers	Yes (2 x 20 mm port)	Yes (1x ~6 mm port)
Seals	Soil to chamber, rubber sub-seals in stainless steel ports	Soil to collar, collar to screw-on hatch lid with rubber O ring
Insulation	Polystyrene covered with self adhesive aluminium foil	NO
Insertion depth	30 mm	15 mm
Deployment	3 x 3 fertiliser N treatments; 4 replicate plots in each treatment = 36 chambers	2 x 4 slope class treatments; 3 replicate plots in each treatment = 24 chambers

Installation	30 min prior to sampling	24 h prior to sampling
Sample times	2 samples: 0 and 30 min (T_0 and T_{30})	2 samples: 0 and 60 min (T_0 and T_{60})
Sample frequency	Weekly and following significant rainfall (>5 mm)	Daily for 2-3 days before and after rainfall
Trial duration	10 weeks	17 days

For the fertiliser N rate trial (Chapter 5) stainless steel gas chambers with 2 open sample ports (Figure 3.8) were inserted into the soil on each sampling day between 1100 and 1500 h. Stainless steel is a robust, light-excluding, non-reactive material and is therefore commonly employed for measuring and analysing N_2O concentrations. The dimensions of the chambers used ensured that they were practical to move and also minimised the risk of inadequate soil/chamber sealing around its circumference. The height allowed adequate mixing of the headspace when taking samples so as to avoid the need to take samples from several different ports in each chamber to ensure representative samples were taken (RT Venterea *pers comm.*). The number of replicates within treatments was adequate to capture spatial variability due to grazing activity at the paddock scale (R.J. Littlejohn *pers comm.*). Replication was also dictated by the cost associated with sample analysis, availability of sampling equipment, as well the cost and the availability of trained gas samplers that would be required to cover large areas within set time periods if the number of replicates were increased. Chambers were not vented, however the chamber ports are open when inserting the chamber into the soil to avoid pressure build up within the chamber headspace at this time. Vents are installed on chambers to prevent potential pressure differentials occurring between the headspace and the outside environment during chamber deployment time (30 min with open ports and 30 min with closed ports, 1 hr total on each sampling occasion), which may influence the rate of gas flux from the affected soil surface (Conen and Smith 1998). However the most notable potential error associated with venting is known as the "venturi effect"; where a partial vacuum of the chamber headspace during high wind conditions may occur, thereby affecting the N_2O concentration in the chamber headspace (Conen and Smith 1998). Given the relatively short deployment time (1 hr total installation time, Table 3.8), the latter was believed to be the greater risk and source for error for the current method and therefore venting was not necessary. The production of N_2O gas in the soil is temperature dependent, therefore gas chambers were insulated to reduce temperature variation within the chamber headspace during placement (Parkin and Venterea 2010). The average daily soil temperature was assumed to occur between 1200 and 1300 h (de Klein *et al.* 2003), therefore sampling times were arranged as close as possible to this time period. Due to the distance between gas chambers in the field and the number of replicate samples, it was not possible to complete sampling of all replicates within the 1 h period. Soil (50 mm depth) and chamber headspace

temperature (ºC) were therefore recorded at the start and completion of each sampling occasion to give an average chamber and soil temperature for each sampling occasion.

Chamber insertion depth needs to be adequate to ensure an adequate seal during the measurement period. Long term installations require deeper insertion depths, therefore insertion depths vary between studies. Insertion depth should also vary according to soil type, with porous soils and high wind conditions requiring deeper insertion (Conen and Smith 1998). Because current measurements were made on heavy finely textured soils (Pallic silt loam), the insertion depth (30 mm, Table 3.8) was assumed adequate. Static chambers may be installed in the field several hours or days before measurements are taken to allow soil conditions to equilibrate. The effects of stock grazing and trampling activity however were expected to be greater than the effect of base insertion in this trial. Following insertion, gas chambers were therefore left with ports open for 30 minutes to allow time for any N_2O to disperse due to soil disturbance and/or increased respiration. Thereafter the chamber ports were sealed using rubber suba-seals and headspace gas samples were collected at 0 and 30 min (T_0 and T_{30}) after sealing. Precise times were recorded for each sample for the calculation of N_2O increase over time (mg N_2O-N/m^2.h). Due to the relatively short measurement period ($T_0 - T_{30}$) and the heavy soils used for measurements, N_2O fluxes were calculated according to the method of de Klein *et al* (2003). These workers found a linear increase in N_2O concentration over 3 samples during the same enclosure period on this soil type, therefore the decision was to take 2 headspace samples only. The cost of sample analysis was also a restricting factor in this trial.

For the slope class trial (Chapter 6), stock were excluded and PVC chamber collars were inserted into the soil on the day prior to gas sampling and left to equilibrate for 24 h. PVC plastic has similar qualities to stainless steel and is also commonly employed for measuring N_2O concentrations. On the sampling day between 1200 and 1300 h modified PVC 'sewer hatches' were fastened to the top of each collar. The hatch rims had an internal locking system with a greased rubber O-ring that formed an air tight seal. The size of the chambers was practical in the steep dissected terrain that chambers were deployed in, as well as having sufficient headspace for sampling. The number of replicates within treatments was heavily dictated by the number of field staff available to sample and the time taken to negotiate the terrain on sampling days

(also see discussion above). However the experimental design was balanced and fulfilled minimum requirements for spatial analysis (R.J. Littlejohn *pers comm.*). The chambers were not vented, nor insulated. Soil (50 mm depth) and chamber headspace temperature (ºC) were recorded at the start and completion of each sampling occasion to give an average chamber and soil temperature over the measuring period.

Sample collection and storage:

An accurate estimate of N_2O gas concentration in air samples requires accurate sampling and storage techniques to be employed to ensure sample integrity. The sample collection and storage procedures in this Thesis were based on methods by de Klein *et al.* (2003) and Saggar *et al.* (2004a), in the fertiliser N rate and slope class trials, respectively. The procedures are outlined in Table 3.9 and discussed below.

Table 3.9 Sample collection and storage for N_2O gas samples

	de Klein *et al* 2003	**Saggar *et al* 2004a**
Collection vessel	Polypropylene 25 mL syringe	Polypropylene 60 mL syringe
Storage vessel	6 mL septum sealed glass exetainer	Polypropylene 60 mL syringe in the field, samples then transferred to 12 mL septum sealed glass exetainer in Lab
Septum	Rubber in silicone	Rubber in silicone
Vial evacuation	NO	YES
Over-pressure	YES	YES
Storage time	Up to 3 months	Up to 1 month
Storage temperature	Refrigerated (5°C) at field laboratory, then room temperature (18°C) in insulted containers at analytical laboratory	Room temperature (20°C) at analytical laboratory

In the fertiliser N rate trial (Chapter 5), gas chamber headspace samples were taken using a syringe to slowly flush 25 mL of headspace air backwards and forwards 4 times via a glass exetainer (Labco Ltd, UK), to ensure effective mixing of the headspace with the syringe (Figure 3.8). On the fourth flush the air was expelled back into the headspace until 5 mL remained, after which valve 1 was closed trapping the remaining air sample in the syringe and exetainer. The remaining 5 mL of air from the syringe was pushed into the exetainer almost doubling the air volume within, resulting in over-pressure. To check if the sample was over-pressurised and that no leaks had occurred within the tubing and stop-cock system, the syringe handle was released, allowing the syringe handle to 'bounce-back'. If the syringe handle 'bounced' the air was pushed back into the exetainer and valve 2 was closed. Pressurising sample exetainers in this fashion also ensured that sample integrity was able to be checked immediately prior to analysis (described below). Once a successful air sample was obtained, the exetainer was detached from the sampling device and placed in a wooden transport block. The syringe was then flushed 2-3 times with ambient air to expel any remaining N_2O molecules from the previous sample from the syringe. Background samples were taken in the same fashion from outside the chamber (i.e. the needle from valve 1 was exposed to ambient air rather than chamber headspace air) at the start and conclusion of each gas sampling occasion. It was assumed that the increase in N_2O concentration within the chamber headspace was linear based on the results of de Klein *et al* (2003), for poorly drained silt loam soils, therefore 2 samples (T_0 and T_{30}) were sufficient. Sample frequency was also restricted by the cost involved in sample analysis.

Samples were transported back to the laboratory and transferred to polystyrene boxes and refrigerated for up to 3 weeks until being sent away for analysis. Samples arrived at the analytical laboratory within 24 h of sending, and stored at room temperature within insulated containers to maintain a constant temperature, until they were analysed. Samples were stored in this fashion for up to 2 months before being analysed.

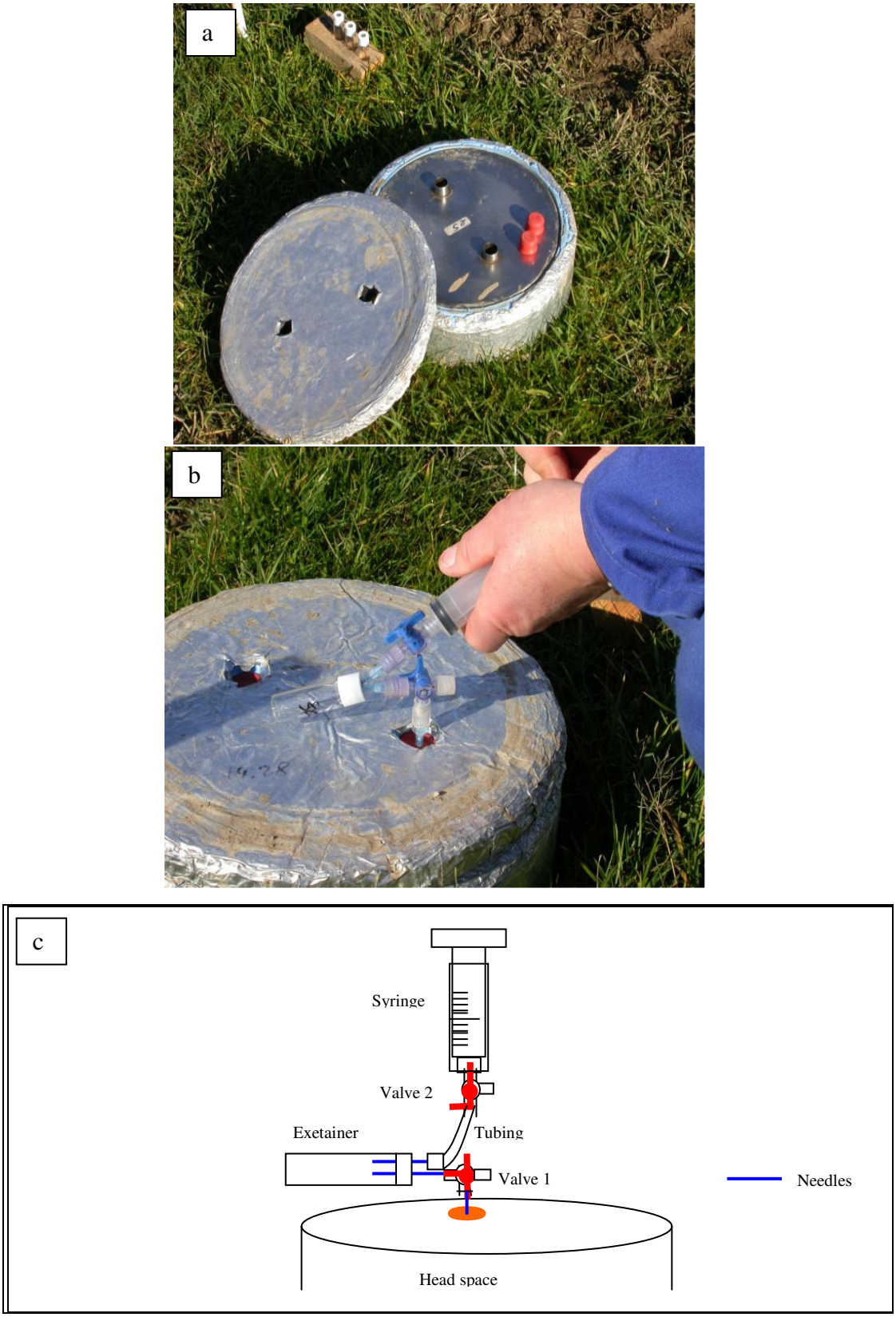

Figure 3.8 Gas chamber configuration in the field for the fertiliser N trial (Chapter 6): a) 2 open sample ports, with 2 rubber suba seals to seal ports after 30 mins, b) Stop cock, sample vial, and syringe configuration, c) schematic diagram of static N_2O chamber sampling configuration. Sourced from de Klein *et al.* 2003.

In the slope class trial (Chapter 6), two gas samples were taken with 60 mL polypropylene syringes at T_0 and T_{60}. Syringes were connected by a 3 way stop cock and an approximately 10 cm length of tubing to the chamber (Figure 3.9). Samples were collected by first flushing the syringe twice with ambient air, outside the chamber headspace. The syringe was then attached to the tubing and slowly flushed 4 times. After the third pump the air from the headspace was then drawn into the syringe a fourth and final time and the stop cock was closed to the chamber, keeping the headspace sample within the syringe. This procedure was repeated using a separate syringe for each chamber. The closed syringes (containing gas samples) were placed carefully in a polyethylene bag. Ambient air samples were taken from outside the chamber at the start and conclusion of each gas sampling occasion.

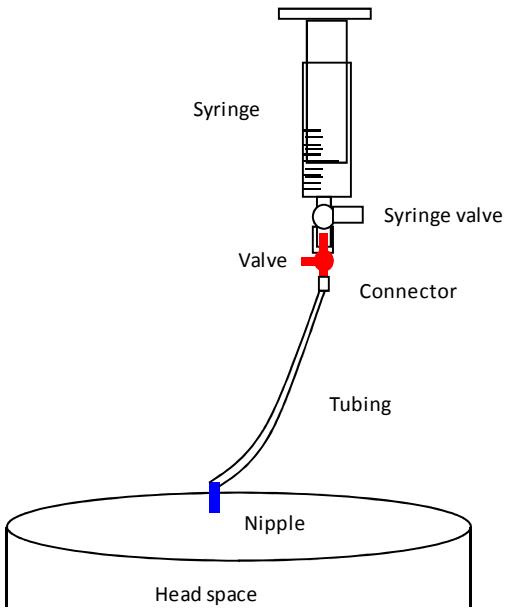

Figure 3.9 Schematic diagram of the gas chamber headspace sampling configuration for the method of Saggar *et al* 2004a.

Nitrous oxide gas losses within polypropylene syringes have been reported to be 16% after 24 h (Rochette and Bertrand 2003). Gas samples were therefore transported back to the laboratory and injected within ~6 hours of sampling into pre-evacuated labelled septum-sealed exetainers. The exetainers were previously evacuated at -80 Pa for 2 min on a manifold. Approximately 25 mL of the gas sample was forced into a 12 mL exetainer, resulting in an over pressure within an exetainer. Exetainers were stored at room temperature (~20° C) and analysed within 1 month. The sample storage method was previously evaluated by Hedley *et al.* (2006) in Table 3.10.

Table 3.10 Storage efficacy of exetainers over a three month trial period. Source: Hedley *et al* (2006)

Days of storage	[a]Percent recovery		
	CH_4	**CO_2**	**N_2O**
30	99.6 (0.62)	99.9 (1.09)	99.2 (0.38)
60	101.2 (0.75)	95.7 (2.22)	96.9 (0.87)
90	100.8 (0.72)	99.7 (1.62)	96.4 (1.17)

[a]Calculated as percent of original concentrations. Numbers in brackets are CV%. Between 5-10 determinations for each set.

Analytical technique:

Sample analysis by gas chromatograph (GC) is currently the most common method to determine N_2O gas concentrations in air samples. Inadequate maintenance or protection of a GCs analytical componentry to air and water contaminants can be a potential source for error and/or analytical drift. Documentation of analytical procedures is likely the best way to evaluate and correct for potential error sources. Regular calibration of GCs is also commonly emphasised by scientists and operators. In the current Thesis, gas samples taken in the field were sent to commercial laboratories for sample analysis (Feed tech and Landcare Research for the fertiliser N rate and slope class trials, respectively), and therefore were processed according to prescribed methods. The analytical specifications and procedures for both commercial operations are detailed in Table 3.11 and discussed below.

Table 3.11 Sample analysis specifications of N_2O gas samples at Feedtech and Landcare Research, Palmerston North

	Feed tech, Palmerston North	**Landcare research, Palmerston North**
Analysis	Laboratory; Hewlett Packard 5890 Series II Gas chromatograph	Laboratory; Shimadzu GC 17A Gas chromatograph
Sample volume	1 mL	1 mL
Sample loop	Manual injection	Autosampler; Modified Gilson 222XL
Flow rate	45 mL/min	45 mL/min
Carrier gas	N_2	N_2 (99.99% purity, Hedley *et al* 2006)
Pre-column	Stainless steel 0.3 m, OD= 0.003	Stainless steel 0.91 m, OD= 0.003 m 60°C
Analytical column	Stainless steel OD= 0.003	Stainless steel 3.66 m, OD 0.003 m 60°C
Column pack material	Porapak 80/100 mesh	Porapak 80/100 mesh
Methaniser	NO	YES, 350°C
Flame ionization detector	NO	YES, 250°C
Electron capture detector (ECD)	350°C	315°C
Retention time	1.75 min	3.9 min
Back flush	NO	YES

In the Feed tech laboratory immediately before analysis the over-pressurised exetainers were brought back to ambient air pressure by immersing one end of a double-ended hypodermic needle in water while the other end was inserted through the exetainer septa. This method resulted in a brief flow of bubbles in water as the excess sample air escaped, bringing samples back to ambient air pressure while avoiding sample contamination. The absence of bubbles would indicate sample leakage and these samples were therefore discarded from analysis in case of contamination.

At Landcare Research carbon dioxide (CO_2) and methane (CH_4) were also analysed by the GC, therefore gas samples were passed through a methanizer at 350° C, where CO_2 was reduced to CH_4 in a catalytic Ni column. Samples then entered into a flame ionization detector (FID, 250° C) after 2.0 and 3.2 min, respectively. After 3.6 min, a four-port valve diverted the gas stream to the electron capture detector (ECD, 315° C, 10% CH_4 in argon (Ar) at a flow rate of 5 mL/min), where N_2O retention time was 3.9 min. A back-flush cycle between 5 and 6 min completed one run (Figure 3.10). Back-flushing continued while the sample loop refilled with the next sample, before a valve switch directed gas sample flow to the FID. Shimadzu GC Solution 2.21 SU1 software was used to integrate peak data, produce calibration curves and calculate sample concentrations.

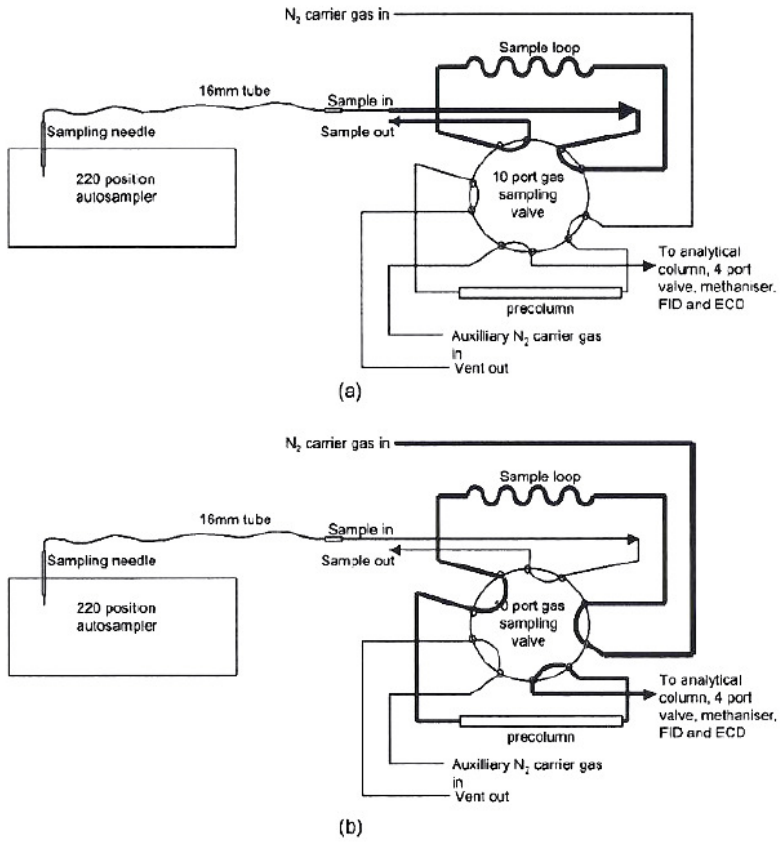

Figure 3.10 Configuration of the Gilson 222XL autosampler and Shimadzu GC-17A Gas Chromatograph (GC) at Landcare Research, a) exetainers are pierced and sampled by a needle in the autosampler where gas flows to the injection port of the GC to fill the 1 mL sample loop. The pre-column is in back-flush mode, b) once the sample loop is full the 10-port gas sampling valve switches and directs the gas sample into the pre- and analytical-columns and onto the flame ionization detector (FID) via the methanizer or the electron capture detector (ECD) to be analysed. Source: Hedley *et al* 2006

Analytical precision:

Instrument drift is a common source of error that may occur during the course of an analytical run as well over the course of a day, as temperature and humidity changes during operation impact machinery. Additionally, lower concentrations of N_2O gas in air samples require a higher level of sensitivity, where small changes (in ppb) become difficult to detect. The procedures used by Feed tech and Landcare Research to determine instrument accuracy and data reproducibility are summarised in Table 3.12 and discussed below.

Table 3.12 Analytical precision of the Gas chromatographs at Feedtech and Landcare Research, Palmerston North

	Feed tech, Palmerston North	**Landcare Research, Palmerston North**
Standards	Gas standard of known N_2O concentration (10 ppm)	Range of known N_2O concentrations (Beta Gas Standards)
Calibration	2 point calibration using standard (10 ppm)	Range of gas concentrations known to cover the analytical range to determine calibration curve (uncertainty at 95% confidence interval)
Standard check frequency	Every 10-15 samples	Every ~30 samples
Precision (CV%)	[a]7	[b]0.74
Minimum detection limit (MDL ppb)	N/A	[b]7.4

[a]Data from Kelliher (2008 $N = 22$) *unpublished*

[b]Data from Hedley *et al* (2006)

N/A: not applicable

At Feed tech a gas standard of a known N_2O concentration (10 ppm) was used as a calibration standard. Duplicate sample standards were run every ~30 samples and samples were run in the order of numbers, as opposed to times (i.e. all T_0 first, then T_{30}). No further calibration or reproducibility data was supplied to customers by Feed tech. In response to this situation, analyses to determine the coefficient of variation of the Feed tech GC were later undertaken by Kelliher (*unpublished,* Table 3.12). In addition, background samples taken during the fertiliser N trial (Chapter 5) were used to assess the GC detection accuracy at the time of sampling. Nine background samples were taken on 7 separate sample occasions (N= 63). The average background concentration (ppm) was 0.32 with an average standard deviation (SD) of 0.05 across the 7 sampling occasions. The average coefficient of variation around the background N_2O concentration for this trial (see Chapter 5) was 17.17%.

At Landcare Research Beta gas standards of known N_2O concentrations were used to assess the precision and calibrate the GC every 10-15 samples. Calibration curves produced by Hedley *et al* (2006) using Beta standards that covered the range of gas sample concentrations and uncertainty at the 95% confidence interval were: ±2% for concentrations >0.1%, ±3% for concentrations between 0.01 and 0.1%, and ±5% <0.01%. When running samples, a beta standard selected to be within the range of the gas samples, was run every ~30 samples to monitor accuracy. The method to determine MDL for N_2O gas was based on a single concentration and is therefore best related to samples of similar concentrations (Hedley *et al.* 2006). It is possible that samples of significantly higher or lower concentrations may have varying MDLs. However the procedure is simple and effective, and commonly used by the U.S Environmental Protection Agency and International Accreditation New Zealand (IANZ, Table 3.13). These workers also determined the flux detection limit (FDL) based on the air samples, at atmospheric pressure and 15° C, with a chamber height of 10 cm over a 1 h sampling period:

FDL= 2*MDL

where FDL is the flux detection limit (mg N_2O-N/m^2.h) for the Landcare Research GC tested under the analytical conditions described above and MDL is 7.4 ppb (Table 3.13), to account for the two measurements taken to determine the flux.

Table 3.13 Precision and method detection limit of gas chromatographic analysis of nitrous oxide (N_2O). Source: Hedley *et al* 2006

Parameter	N_2O (ppb)
Run 1	350.1
Run 2	350.6
Run 3	352.3
Run 4	350.6
Run 5	351.9
Run 6	354.9
Run 7	357.1
Run 8	349.3
Run 9	354.4
Run 10	355.3
n	10
Mean	352.6
SD	2.61
%CV	0.74
[¥]MDL	7.4

[¥]MDL: method detection limit, SD x t (t=2.821, n=10)

Data analysis:

Nitrous oxide fluxes are calculated from the rate of change/increase in gas concentration over time. Such increases may be linear or non-linear. Assuming a sound chamber design curvi-linear concentration increases are linked to a build up of N_2O concentration in the chamber headspace over time which creates a diffusion gradient, or to horizontal gas movement in the soil (Parkin and Venterea 2010). Based on the data from de Klein *et al* (2003) a linear increase was assumed. In the formula N_2O concentrations were corrected for variations between sampling and analytical temperatures, as well as for the ratio of cover volume to surface area using:

$$N_2O \text{ flux} = \frac{\delta N_2O}{\delta t} * \frac{M}{Vm} * \frac{V}{A}$$

where N_2O flux is the hourly emission (mg N_2O-N/m^2.h), δN_2O is the increase in headspace N_2O during the enclosure period (ppm), δt is the enclosure period (h), M is the molar weight of N in N_2O (g/mol), Vm is the molar volume of gas at the sampling temperature (L/mol), V is the headspace volume (m^3), and A is the area covered (m^2).

The hourly N_2O fluxes were then scaled up (g N_2O/ha.d) by:

N_2O emission = N_2O flux*24*10000/1000

where N_2O emission is the daily emission (g N_2O-N/ha.d) , N_2O flux is mg N_2O-N/m^2.h.

To estimate total losses of N_2O over a measurement period the hourly fluxes were integrated using the trapezoid method over time by:

total $N_2O = \Sigma(d_i+d_{i+1})*1000*d_b/2$

Where total N_2O is the total emissions (kg N_2O-N/ha) over the measurement period, d_i is g N_2O-N/ha.d on day 1…..n, d_b is the number of days between d_i and d_{i+1}.

3.38 Denitrification enzyme activity

Short term measurements of denitrification enzyme activity (DEA) provide information about the potential denitrification activity (PDA) of a soil. This method involves anaerobic incubation of optimised (KNO_3, glucose-C in deionised water) soil samples in the presence of acetylene (C_2H_2, 0.1-10%) to prevent the conversion of N_2O to N_2 (Yoshinari *et al.* 1977). This procedure therefore allows for the total product of denitrification activity to be estimated by measuring the rate of N_2O production over a short period. The addition of chloramphenicol to soil assays was to inhibit *'de novo'* synthesis of reductive enzymes associated with the process of denitrification (Dendooven and Anderson 1994), so as to measure the potential enzyme activity at the time of sampling only. In Chapter 5 DEA measurements took place from soils in the 500N treatment paddocks 14 months after the N_2O flux measurements were made (December 2007), however the stock and fertiliser management had remained the same over this period. The method and assumptions of the DEA procedure used in this Thesis were based on the descriptions in (Dendooven and Anderson 1994; Luo *et al.* 1996; Smith and Tiedje 1979; Yoshinari *et al.* 1977):

In the field three soil cores (75 mm depth x 25 mm width) were collected and bulked from six newly established plots (1 m^2). Three plots were on Low slope areas (LS, 0 - 12º) and 3 plots on Medium slope areas (MS, 13 - 25 º), within each of the 500 N paddocks. Bulked soil samples were stored in chilly bins and transported back to the laboratory where they were refrigerated immediately. The following day, soil

samples were sieved (6 mm) and obvious roots removed and samples were homogenised. Conditions for DEA were optimised by adding 20 mL of glucose nitrate solution (0.2 g glucose, 0.1 g KNO_3 and 0.125 g chloramphenicol/L deionised water) to a sub-sample of 10 g soil (fresh weight) in 500 mL glass Scott flasks and sealed with rubber stoppers. Flasks were flushed for 2 minutes with N_2 gas (alpha standard, 99.9% purity) using a hypodermic needle, before 10 mL purified acetylene (C_2H_2, 98%, instrument grade BOC Standard SM3) was added to inhibit the reduction of N_2O to di-nitrogen (N_2). A second needle was inserted through the stoppers to allow the air pressure to equilibrate within flasks when flushing with N_2 or when adding 10 mL C_2H_2. Assays were then incubated at approximately 25 °C. A 12 mL headspace sample was removed using the syringe technique described for field measurements at at T_{20}, T_{40} and T_{60}.

The headspace samples were stored in 6 mL sealed glass vials and were therefore over pressurised, as described previously. All samples were analysed for N_2O following the Feed tech method, described above. The reported rates of N_2O production were determined from linear interpolation of N_2O concentrations as for field measurements:

$$N_2O \text{ flux} = \frac{\delta N_2O}{\delta t} * \frac{M}{Vm} * \frac{V}{A}$$

where N_2O flux is the hourly emission rate (mg N_2O-N/m^2.h), δN_2O is the increase in headspace N_2O during the enclosure period (ppm), δt is the enclosure period (h), M is the molar weight of N in N_2O (g/mol), Vm is the molar volume of gas at the sampling temperature (L/mol), V is the headspace volume (m^3), and A is the area covered (m^2).

CHAPTER 4 SPATIAL DISTRIBUTION AND RATE OF POTENTIAL NITRIFICATION ACTIVITY IN TWO HILL COUNTRY PASTURES

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Some minor changes have been made to the text of the original paper to improve linkage in the Thesis.

Abstract

The purpose of this study was to conduct a preliminary investigation into the effect of increasing fertiliser and excretal nitrogen (N) inputs on the spatial distribution and rate of potential nitrification activity in hill country pasture land at two sites, Invermay and Ballantrae. High nitrification rates could potentially limit N efficiency by increasing N losses through leaching and denitrification. Nitrification potentials (NP) were measured in campsites and medium slopes of hill country soils receiving 0 kg N and 500 kg N/ha.y over the previous 18 months. Nitrification potential was determined by calculating the rate of nitrate (NO_3^-) production (mg NO_3-N/kg soil.h) by linear regression of soil solution concentrations, versus time. Nitrification potential was significantly higher at Invermay than at Ballantrae, which was likely due to a significantly lower soil pH at Ballantrae. At Invermay, NP increased with fertiliser N application rate and in camp site soils. The fertiliser N effect was not observed at Ballantrae. However, soil NO_3^- and NP was significantly greater in soils from camp sites than for soils from medium slopes. Best management practices for fertiliser N application in hill country should make allowances for these factors to maximise farm efficiency and profitability.

Keywords: hill country, nitrification potential, nitrogen fertiliser, stock behaviour, excreta N, mineral N, New Zealand

4.1 Introduction

Conventional hill country pasture management has largely relied on superphosphate, sulphur, or lime applications (Ball *et al.* 1982). Such soil amendments aid establishment and continued survival of legume species (primarily *Trifolium repens*) that supply hill soils with mineral nitrogen (N), (Morton *et al.* 1993). However, even if all other major and trace element requirements are met, plant available N will always remain deficient due in part to the transfer of nutrient N from slopes to flat campsites in the excreta of grazing animals (Ball *et al.* 1982). From the early 1980s, fertiliser N trials in hill country have reported annual response efficiencies that range from 7 to 42 kg dry matter (DM)/kg N. Fertiliser N application rates that yielded these responses ranged from 50 to 400 kg N/ha.y, following various split application regimes across seasons (Ball *et al.* 1982; Hoogendoorn 2006; Lambert *et al.* 2003; Stevens 2006). In response to this and to a favourable relationship between the cost of fertiliser N plus application and farm product prices, typical applications rose almost ten-fold from 1996 to 2002 (Hoogendoorn 2006).

To manage the increased pasture production due to increased fertiliser N application, stocking rates are usually increased to profitably utilise extra forage and maintain pasture quality. The increase in stock density results in an increase in excreta N return to soils (Steenvoorden *et al.* 1986; Whitehead 1995). The return of excreta N becomes increasingly non-uniform as topographical (i.e. slope class) variation increases within hill country pastures (Ball *et al.* 1982). Up to 60% of dung and 55% of urine may be deposited on campsite and track areas that occupy only 15 - 31% of a hill country land unit (Saggar *et al.* 2004b; Sakadevan *et al.* 1993a). Consequently a net accumulation of N occurs on flatter camp and track sites, and net depletion occurs on the steeper slopes where animals graze but do not camp (Bowatte 2003; López *et al.* 2003a; Radcliffe 1982). However, there is little quantitative information regarding the fate of fertiliser or excreta N to microbial processes in hill country pastures (Bowatte 2003; Carran *et al.* 1995).

The microbial oxidation (nitrification) of ammonium (NH_4^+) from urine and urea to nitrate (NO_3^-) is of considerable importance to the fate of fertiliser and excretal N, because of the potential to increase NO_3^- pollution through leaching and denitrification (Fair *et al.* 1994). These losses are important from both environmental and farm production standpoints. Nitrifying bacteria most commonly occur where favourable soil and climatic conditions prevail, and tend to be most active (i.e. oxidise NH_4^+ faster) at these sites (Haynes 1986). Once the supply of NO_3^- to plants exceeds demand, excess NO_3^- may be lost to the environment. Trials have demonstrated that significant losses of NO_3^- due to leaching and denitrification occur under urine patches in both high and low N soils (de Klein and van Logtestijn 1994; Di and Cameron 2002; Field *et al.* 1985; Haynes and Williams 1992).

The present study aimed to determine if the nitrification potential (NP) rates in hill country soils are affected by an increase in fertiliser N and subsequent increases in excreta N, by comparing NP in sheep grazed plots treated with 0 or 500 kg N fertiliser. The working hypothesis was that NP would be higher in plots receiving high rates of fertiliser N compared to plots receiving no fertiliser N, due to increased NH_4^+ availability. We also hypothesised that NP would be higher in low slope soils (i.e. campsites), when compared to that measured in medium slope soils due to the regular addition of NH_4^+ via excreta to campsites.

4.2 Methods

4.21 Field sites

The experiment was established on two AgResearch hill country farms, one in the South Island (Invermay, Mosgiel) and one in the North Island (Ballantrae, Woodville).

The Invermay experimental site is situated *c.* 100 to 200 m above sea level (a.s.l) and receives an average annual rainfall of 700 to 750 mm (Otago Regional Council 2006). The predominant soil type is a Warepa silt loam (Mottled Fragic Pallic soil), situated

on rolling land (*c.* 8 to 15º Hewitt 1998). The Ballantrae Research Farm is *c.* 200 to 350 m a.s.l. and receives an average rainfall of 1270 mm annually (summer moist steep hill country). The predominant soil types are classified as Wilford hill soils (Mottled Argillic Pallic soils on slope 5 to >25º).

4.22 Field trial design

In February 2006 nitrification potential (NP) was measured in soils from sheep grazed plots that had received 0 or 500 kg N/ha.y since September 2004, which represented the minimum and maximum range of hill country land use scenarios with respect to fertiliser N application. There were three replicated paddocks for each treatment, except for the 500 kg N treatment at Ballantrae, which was in duplicate paddocks. The last fertiliser application prior to this study was December 2005 (62.5 kg N/ha) on Invermay Farm and January 2006 (83 kg N/ha) on Ballantrae Farm. The current experiment was a split plot design with N treatments as the main plots. Within each plot, six subplots were established: three Low slope subplots (0 to 12 º, LS) and three Medium slope subplots (13 to 25 º, MS). Soil cores were taken within a 5 h period and the number of dung deposits, within a 30 cm radius of the sample site was recorded as an indicator of camping behaviour (1 stool or collection of pellets covering an area approximately 0.1 m^2 was recorded as 1 dung deposit). Within each subplot, six soil cores (25 x 75 mm) were removed and chilled.

In the laboratory the six cores from each subplot were bulked, sieved (2 mm) and then split into duplicate samples. One sample duplicate was used for mineral N extraction (2M KCl, 1:10 extraction ratio), soil pH, and the determination of soil moisture content (Hatch *et al.* 2000). The second duplicate was used for NP measurements (Hart *et al.* 1994).

Nitrification potential assesses the maximum rate of nitrification (Vmax) or potential nitrification activity. Samples were optimised with respect to water content, NH_4^+ concentration (1.5 mM NH_4^+), P availability (1 mM $PO^3{}_4^-$), and aeration (Hart *et al.* 1994). The NPs were determined by calculating the rate of NO_3^- production (mg NO_3-N/kg dry soil.h) by linear regression of solution concentrations, versus sample time (2, 4, 22, and 24 h as in Hart *et al.* (1994)).

4.23 Statistical Methods

Soil mineral N data (NH_4^+ and NO_3^-) were analysed using a gamma-log generalised linear mixed model (Schall 1991). Site description data (pH, soil moisture and dung counts) were analysed by analysis of variance (ANOVA). The block structure was given by location within pair within paddock. The treatment structure was by site, N treatment and slope, and all interactions involved these terms. For the calculation of NP rate, data points that were below detection limits (<0.25 ppm) were deleted and substitute values (between 0 and 0.25) were calculated and used in the ANOVA (Taylor 1973). Nitrification potential data were analysed by ANOVA. The block structure was given by duplicate within location, within pair, within paddock. The treatment structure was by N treatment, and slope, and their interaction. All analyses were done using the statistical package (GenStat 2006).

4.3 Results and Discussion

At Invermay NP was significantly greater at the higher N fertiliser application rate ($P< 0.05$), and in Low slopes (i.e. campsites) compared to Medium slopes ($P< 0.01$, Table 4.1). At Ballantrae, NP was significantly higher in Low slopes than in Medium slopes ($P< 0.01$), however no significant effect of N fertiliser on NP was detected. Overall, NP rates were significantly higher at Invermay than at Ballantrae ($P< 0.01$), with mean NP ranging from 0.65 to 3.45 mg NO_3-N/kg dry soil.h at Invermay, and 0.26 to 1.15 mg NO_3-N/kg soil.h at Ballantrae. Nitrification rates in previous comparable studies (Sarathchandra *et al.* 1984; Steele *et al.* 1980) ranged from <0.02 to 0.47 µg/g soil.h for yellow-brown earths and 0.08 to 0.76 µg/g soil.h for yellow-grey earths. The results in the present study were generally higher than results from previous work. This is likely due to the higher fertiliser N treatment and higher stocking rates (and hence high excretal N) in our study.

Table 4.1 Rate of potential nitrification (mg NO_3-N/kg dry soil.h) in soil incubations from Low and Medium slopes receiving 0 and 500 kg N/ha.y with sem's on Invermay and Ballantrae hill country farms

slope	Nrate	Ballantrae	Invermay
Low	0	1.15	1.46
Low	500	0.94	3.45
Medium	0	0.36	0.65
Medium	500	0.26	2.46
SEM		0.488	0.562
sig(slope)		**	**
sig(Nrate)		ns	*

* P< 0.05
** P< 0.01
ns Not significant

The observed higher NP rates at Invermay compared to Ballantrae may be due to significant differences in soil pH (Ballantrae 5.3 and Invermay 6.1; Table 4.2). Low pH can significantly inhibit soil microbial activity and the rate of soil C and N cycling (Kemmit *et al.* 2006; Sarathchandra 1978). Steele *et al.* (1980) found for similar NZ soils that pH, organic C, total N, and C/N ratio were related to the rate of initial nitrification activity (R^2 = 0.44, excluding soils of pH >7 and yellow-brown loams); and that when the pH was raised, nitrification rates increased. Sarathchandra (1978) also found that an increase of soil pH in acid soils resulted in an improved nitrification rate. Work from Bowatte (2003), Lambert *et al.* 1982 and Sakadevan (1993) also reported delayed or slow rates of nitrification in North Island hill country soils. It is difficult to draw conclusions about the effect of soil pH on NP based on the current data set. However the fact that soil pH and NP were significantly lower at Ballantrae is consistent with previous results, and suggests that soil pH may influence nitrification rates.

Table 4.2 Significant difference in soil pH at Ballantrae compared to Invermay hill country farm trial sites with sem

slope	Nrate	Ballantrae	Invermay
Low	0	5.4	6.1
Low	500	5.4	6.0
Medium	0	5.1	6.1
Medium	500	5.3	6.1
Site mean		5.3	6.1
SEM			0.15
sig(site)			***

*** P< 0.001

The difference in NP observed between slope and fertiliser N treatment was supported by the soil mineral N measurements (Table 4.3). At Invermay, soil NO_3^- concentrations were significantly greater for the 500 kg N treatment for 0 kg N (mean = 68.1 cf. 10.2 mg/kg soil, P< 0.001). Values for NH_4^+ in the 500 kg N were also significantly greater than in the 0 kg N treatment (mean = 13.5 cf. 5.0 mg/kg soil, P< 0.05). At Ballantrae average NO_3^- levels were significantly higher in Low slope compared to Medium slope subplots (78.2 cf. 40.9 mg/kg soil, P< 0.001). Mean soil NH_4^+ levels at Ballantrae demonstrated no significant N treatment effects, ranging from 21.9 and 37.7 mg/kg soil, and were higher than at Invermay.

Table 4.3 Soil mineral N concentrations (mg N/kg dry soil) in Ballantrae and Invermay hill country soils from Low (LS) and Medium (MS) slopes in 0 and 500 kg N treatment plots with sem

			Ballantrae	Invermay
NH_4-N				
	slope	Nrate		
	Low	0	21.9	5.1
	Low	500	37.7	11.1
	Medium	0	22.9	4.9
	Medium	500	32.7	15.8
	SED		17.1	4.8
	sig(slope)		ns	ns
	sig(Nrate)		ns	*
NO_3-N				
	Low	0	60.7	11.5
	Low	500	95.6	70.0
	Medium	0	30.3	8.8
	Medium	500	51.4	66.1
	SEM		37.2	30.1
	sig(slope)		**	ns
	sig(Nrate)		ns	**

* P< 0.05
** P< 0.01
*** P< 0.001
ns Not significant

In hill country, relatively flat land tends to encourage stock camping behaviour and the subsequent deposition of excreta N (Bowatte 2003; Gillingham 1982; Gillingham and During 1973; López *et al.* 2003a; Sakadevan *et al.* 1993a). The nutrient N in the excreta of grazing animals is a major N supplement for plant growth (Gillingham 1982). We observed a significantly greater number of dung deposits on Low slope compared to Medium slope subplots for the combined data set (P< 0.05, Table 4.4). This observation and the findings discussed above suggests that the non-uniform deposition of excreta N at Ballantrae resulted in the development of nitrification 'hotspots' on the micro relief areas, regardless of the rate of fertiliser N in our study.

An interaction between the number of dung deposits and soil pH may also be a possibility, as the significantly higher number of dung deposits on Low slopes compared to Medium slopes sites coincides with higher pH values (Table 4.2 and 4.4). However this interaction was not included in the analysis.

Table 4.4 Average number of dung deposits in Low (LS) and Medium (MS) slopes at Ballantrae and Invermay hill country farms with sem

slope	Nrate	Ballantrae	Invermay
Low	0	2.1	1
Low	500	2.2	2.2
Medium	0	0.8	0.9
Medium	500	1.7	0.3
SEM			0.35
sig(slope)			**

** P< 0.001

The purpose of the present study was to investigate how high inputs of fertiliser and excretal N affected the NP rates in hill country soils at two locations. In particular results demonstrated that NP rate and NO_3^- is higher in campsites (Low slopes) than on Medium slopes at Ballantrae and Invermay, and that these rates increased with N fertiliser application rate at Invermay. The greater NP and NO_3^- in campsites was probably influenced by the fact that a significantly greater amount of excreta, and therefore greater amount of substrate NH_4^+ was available for nitrification within these subplots. Higher rates of nitrification in camp areas and high N treatments have the potential to increase losses through NO_3^- leaching and denitrification. The fact that application of high rates of N fertiliser over the previous 18 months did not increase NP at Ballantrae should be noted, as it suggests that potential losses may be lower at Ballantrae than Invermay.

More work is required to identify the reason for the lower NP at Ballantrae. As indicated earlier, soil pH is one possible reason, but there may be others such as wide C:N ratios in hill soils and thus high N retention, or climatic restrictions as suggested by Bowatte (2003), Lambert *et al.* (1982), Sakadevan *et al.* (1993), Sarathchandra (1978), and Steele *et al.* (1980). Future work should also include specific leachate and nitrous oxide (N_2O) measurements within these sites to indicate threshold NP rates for N losses via leaching and denitrification. Leachate measurements have been made at both sites according to N fertiliser treatment, but not between camp and non-

campsites. Both unpublished Invermay and Ballantrae data sets detected significant increases of NO_3^- in leachate collected from the 500 N fertiliser paddocks compared to 0 N (Hoogendoorn 2006; Stevens 2006).

An understanding of how site-specific conditions in hill country situations may influence nitrification activity and the risk of NO_3^- pollution via leaching and denitrification has the potential to improve the efficiency and profitability of hill country farmland. Nitrification potential in hill country is evidently influenced by both stock and pastoral management practices. Best management practices for fertiliser application in hill country should consider avoiding areas of high NP (i.e. campsites) in an effort to minimise N losses via leaching and denitrification.

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CHAPTER 5 SHORT TERM MEASUREMENT OF N_2O EMISSIONS FROM SHEEP GRAZED PASTURE RECEIVING INCREASING RATES OF FERTILISER NITROGEN IN OTAGO, NEW ZEALAND

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Some minor changes have been made to the text of the original paper to improve linkage in the Thesis.

Abstract

The purpose of this short term study was to investigate the effect of increasing fertiliser nitrogen (N) application rates on nitrous oxide (N_2O) emissions over the late winter/early spring period from sheep grazed pasture in Otago rolling hill country. We measured N_2O gas emissions from plots on a mottled Fragic Pallic hill soil receiving 0, 100 and 500 kg N/ha.y for 2 years. Plots were sampled weekly for 10 weeks over the 2006 winter/spring period using a static chamber method. Increased fertiliser N rate and the attendant increase in stocking rate significantly increased total N_2O emissions ($P< 0.05$). Total N_2O emissions for the measurement period were estimated to be 0.08, 0.13 and 1.36 kg N_2O-N/ha (sem, 0.1, 0.18 and 0.45) for the 0, 100 and 500 N treatments, respectively. Our results suggest that high application rates of fertiliser N (i.e. 500 kg N/ha.y) and attendant increased stocking rates may significantly increase emissions of N_2O even in dry winter/spring conditions in Otago rolling hill country. These results will assist in the development of best management guidelines for reducing N_2O emissions from fertiliser N in hill country.

5.1 Introduction

Approximately 49% of New Zealand's (NZ) greenhouse gases (GHG) are derived from the agricultural sector (Ministry for the Environment 2006). Within this sector nitrous oxide (N_2O) emissions from agricultural soils comprise approximately 33% of carbon dioxide equivalent (CO_2-e) emissions (Ministry for the Environment 2006). Research has demonstrated that increased inorganic nitrogen (N) availability via fertiliser application, animal manure and legume-based biological fixation inputs to managed grasslands all contribute to increases in N_2O gas production from agricultural soils (Abbasi and Adams 2000; Allen *et al.* 1996; Clough *et al.* 2003; Ruz-Jerez *et al.* 1994; Tenuta and Beauchamp 2003). In NZ, fertiliser N use increased six-fold from 51,787 to 308,406 tonnes in the period 1990 to 2005 (Ministry for the Environment 2007). Due to the increased application of fertiliser N to hill land at times when the relationships between product prices and input costs are favourable (Lambert *et al.* 2003), it has become increasingly important to investigate the impact of this increased N fertiliser use on N_2O emissions from grazed hill pastures.

Nitrogen cycling, including N losses, are heavily influenced by the unique topographical, climatic, and soil conditions in hill country (Ball *et al.* 1982; Carran *et al.* 1995; Gillingham and During 1973; López *et al.* 2003b). This limits the application of results of N_2O emission research from flat land trials to hill country. Few field studies have measured N_2O fluxes from NZ hill country pastures, with even fewer quantifying the effect of fertiliser N rate on emissions (Carran *et al.* 1995; Hoogendoorn *et al.* 2008). Our objective therefore was to quantify short term N_2O emissions from sheep grazed rolling hill country paddocks receiving three different rates of fertiliser N (0, 100 and 500 kg N/ha.y), that represent the current practice (0 – 100 kg N/ha) and the upper range of possible land intensification (500 kg N/ha) in future hill country farming. In a previous study at this site, Letica *et al.* (2006) found that the nitrification potential (NP, mg NO_3-N/kg soil.h) and soil nitrate (NO_3^-) levels (mg NO_3-N/kg soil) were significantly increased in the 500 N compared to 100 N treatment plots. Our hypothesis was therefore, that due to the increased NO_3^- availability and increased microbial activity in high N fertilised soils, N_2O emissions would be significantly higher from hill country paddocks receiving high rates of fertiliser N (500 N) compared to those receiving no or a low rate of fertiliser N (0 N and 100 N).

5.2 Methods & materials

5.21 Experimental site

The experiment was established on Invermay research farm in Otago, NZ. The predominant soil type was Warepa Silt Loam, a poorly drained mottled Fragic Pallic soil (Hewitt 1998), on rolling hill country (c. 8 to 15º slope). Some soil physical properties and paddock parameters are given in Table 5.1. Average annual rainfall is 700 to 750 mm (Otago Regional Council 2006). Nitrous oxide flux and soil physical and chemical parameters were measured from August to October 2006 in sheep grazed paddocks (0.80 to 0.95 ha) that for the previous 2 years had received 0, 100 or 500 kg urea N/ha.y, hereafter referred to as the 0 N, 100 N and 500 N treatments. Additionally, in December 2007, denitrification rate using an acetylene inhibition technique (described by Tiedje (1982), and reviewed by Tiedje *et al.* (1989)), was measured in soil cores taken from the 500 N paddocks only. Fertiliser N treatments were in triplicate paddocks in a randomised block design. Fertiliser N as urea had been applied in each of the previous 2 years at rates of approximately 50 kg N/ha in March and September for the 100 N paddocks, and 62.5 kg N/ha in all months except January, June, July and August for the 500 N paddocks. For the gas and soil physical sampling four subplots (4 x 4 m) were marked within each paddock, thereby giving a total of 12 sample sites for each of the three treatments. Subplot areas were divided into a grid with sampling points 1 m apart. New sample points on each grid were selected at random on each sampling occasion so that destructive soil sampling would not affect subsequent gas sampling occasions. Gas and soil measurements were made in late winter/spring 2006 (24 August to 24 October 2006). After analysing the N_2O emissions results, denitrification enzyme activity was measured in summer 2007 (December 17 2007), in the 500N paddocks only.

Table 5.1 Mean pore size distribution (<30, 30 – 300, >300 µm), bulk density (g/cm^3) and WFPS (% with range in parenthesis). Data are the means of 20 samples for pore distribution (0-50 mm depth) and 36 samples for bulk density and WFPS

Treatment	Paddock	Pore size distribution (%)			Bulk density (g/cc)	WFPS (%)
		<30 µm	30-300 µm	>300 µm		
0N	1	52.9	8.5	9.5	0.76	68.3 (83.7-33.8)
	2	50.8	8.6	6.7	0.88	63.2 (78.2-37.1)
	3	48.9	7.0	8.4	0.93	63.2 (78.2-37.1)
100N	1	52.0	7.2	7.6	0.86	70.4 (83.5-45.1)
	2	51.7	8.9	7.2	0.84	62.9 (85.9-35.5)
	3	49.8	7.1	7.7	0.92	70.1 (83.7-34.4)
500N	1	47.7	7.8	8.1	0.95	61.2 (79.0-34.0)
	2	46.3	9.0	11.1	0.87	55.7 (76.3-32.3)
	3	52.2	6.7	4.4	0.95	76.2 (91.2-54.4)

Over the N_2O gas and soil measurement period paddocks were rotationally grazed within treatments with lambing ewes or rams for 3 to 7 day spells to maintain a standing herbage mass of between 1,500 and 2,500 kg DM/ha which was determined by visual assessment and calibrated according to L'Huillier and Thompson (1988). The lambing percentage was 164 - 174% and the resulting stocking intensity was approximately 45, 31 and 47 stock units (SU)/ha for 0, 100 and 500 N paddocks. Additional stock (rams), were also introduced to the rotation at a stocking intensity of 60 to 68 SU/ha for spells of 1 to 6 day periods when pasture covers became too high. Herbage mass was assessed by eye. Rams were held off experimental pastures for at least 1 day on separate unfertilised pastures if going from a high N to a low N paddock to avoid transfer of excreta N from high N to low N treatments. The amount of dry matter consumed (over the measurement period) and the annual pasture intake from each paddock was calculated (Table 5.2) according to the method of Woodford and Nicol (2004). This gives an indication of the intensity of the grazing system in standard stock units per hectare (13 to 24 SU/ha) and the farming system could therefore be characterised as a moderately intensive hill country sheep farming operation. Mean net herbage accumulation rates over the trial period, calculated using an exclusion cage technique (Lambert *et al.* 1983), were 31, 47 and 59 kg DM/ha.d for the 0, 100 and 500 N treatments, respectively.

Table 5.2 Dry matter consumed in the experimental paddocks over the measurement period, total dry matter consumed in the experimental paddocks in 2006 and annualised stocking rates

N rate	Paddock	Area ha	DM consumed over measurement period (kg/ha)	DM consumed Dec '05 - Nov '06 (kg/ha)	SU/ha.y
0	**1**	0.85	1372	10345	17.4
0	**2**	0.8	1200	7962	13.4
0	**3**	0.96	1559	10731	18
100	**1**	0.9	1642	11252	18.9
100	**2**	0.8	1425	9362	15.7
100	**3**	0.9	1525	9812	16.5
500	**1**	0.96	3169	14023	23.6
500	**2**	0.95	3079	13485	22.7
500	**3**	0.85	3084	13068	22

5.22 Nitrous oxide flux

Nitrous oxide emissions were measured using a static chamber method (de Klein *et al.* 2003). Measurements were made weekly for 10 weeks, with provision for additional sampling in the event of significant rainfall (i.e. >5 mm/12 h) to capture

peaks that are likely to occur following rainfall events. However as no significant rainfall events occurred during the measurement period no additional sampling took place. Similarly, sampling was not intensified following fertiliser application because it was thought that the dry soil conditions would preclude major spikes in N_2O emission. The closed static chamber method and calculations to determine N_2O concentrations in samples are described in detail by de Klein *et al.* (2003) and may be referred to in Chapter 3. The analytical method was developed by Feedtech, Palmerston North (Chapter 3). Briefly, between 1100 and 1500 h on each sampling day, insulated stainless steel flux chambers (250 mm diameter x 130 mm depth) with 2 open sample ports were inserted approximately 30 mm into the soil at a randomly selected grid point in each subplot. Chambers were left with ports open for 30 minutes to allow time for any displaced N_2O from soil to disperse. Thereafter the chamber ports were sealed and headspace gas samples were collected at 0 and 30 min (t0 and t30) after sealing. Precise times were recorded for each sample for the calculation of N_2O increase over time (mg N_2O-N/m^2.h). Headspace samples were taken by syringe and an approximately 12 mL sample was stored in a 6 mL sealed glass vial, hence samples were over-pressurised. Background samples were taken in the same fashion from outside the chamber at the start and conclusion of each gas sampling occasion. All samples were analysed on a Hewlett Packard 5890 Series II Gas Chromatograph with N as a carrier gas at a flow rate of 45 mL/min. Samples (1 mL) were manually injected onto a Porapak Q 80/100 stainless steel column connected to an Electron Capture Detector (HP 5890) operating at 350 °C. The coefficient of variation (CV) for background N_2O concentrations was 17% (N= 22, mean 320 ppb, Chapter 3). Hourly N_2O fluxes were calculated by linear interpolation of the 2 samples and integrated over time to calculate mean daily emissions for each sub plot (within paddocks), and total N_2O emissions for the measurement period for each treatment.

5.23 Soil and climatic parameters

On each sampling occasion, two soil cores (75 mm depth x 25 mm width) were collected from directly beneath each gas chamber following gas sampling. The methods for all soil physical and chemical procedures are described in depth in Chapter 3. Briefly, soil cores were sieved (2 mm) and analysed separately for soil

NO_3^- and NH_4^+ by extracting 4 g field moist soil in 30 ml 2M KCl (Hatch *et al.* 2000), and for pH in a suspension of 10 g of field moist soil in 30 mL distilled water. In addition an aluminium ring (50 mm depth x 100 mm diameter) was inserted vertically into the soil to collect intact soil cores for bulk density and soil moisture measurements. Soil cores were weighed before and after drying at 105 °C for 24 h. Pore size distribution (PSD, <30, 30 - 300 and >300 µm diameter), total porosity and soil field capacity were determined from intact samples from sampling occasions 1 to 3, 8 and 9 according to the method of Drewry *et al.* (1999). Water filled pore space (WFPS) was also calculated by dividing volumetric water content by the total porosity. The means for bulk density and PSD, as well as the range for WFPS in each paddock are reported in Table 5.1. Soil (50 mm depth) and chamber atmosphere temperatures (ºC) were recorded at the start and completion of each sampling occasion. Air temperature and rainfall were also recorded at a meteorological station approximately 2 km from the study site (Table 5.3).

Table 5.3 Mean air, soil and chamber temperatures (ºC) on each sampling day. Temperatures are the means of 2 values taken at the start (1100 h) and conclusion (1500 h) of each sampling occasion with the range in parenthesis. Rainfall (mm) values are the totals for the 24 h period of each sampling occasion

	Sampling occasion									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
***Air temp**	10.4 (10.5-10.3)	18.9 (18-19.8)	11.2 (12.3-10)	17.4 (16-18.8)	15.5 (13.9-17.1)	12.7 (11-14.4)	12.5 (11-13.9)	16.7 (15.1-18.3)	10.8 (10.1-11.5)	9.1 (9.2-8.9)
Soil temp (50 mm depth)	5.7 (4–7.3)	8.6 (6.1-11.1)	9.9 (9.1-10.6)	9.2 (7.3-11)	8.9 (8.2-9.5)	10.3 (10.2-10.4)	11.2 (8.9-13.5)	9.9 (7.7-12.1)	11.2 (8-14.3)	11.4 (10.4-12.4)
Chamber temp	12.5 (10.5-14.5)	12.6 (10.5-14.7)	12 (11-12.9)	14.5 (12.9-16)	10.1 (9.1-11)	13.4 (12.8-14)	13.5 (10.7-16.2	12.6 (10.7-14.5)	12.6 (8.4-16.8)	12.6 (10.9-14.2)
***Rainfall mm/24 h**	0.2	0	0	0	0	5.4	0.4	0	0.1	2.7

*** Air temperature and rainfall data was sourced from a meteorological station approx. 2km from experimental paddocks**

5.24 Denitrification activity

The N_2O flux measurements revealed that one of the three 500N paddocks had much higher emissions than the other 500N paddocks. To assess if the higher N_2O emissions in that 500N paddock could be explained by higher microbial activity in that paddock vs. in the other two 500N paddocks, we conducted denitrification enzyme activity (DEA) measurements in all three 500N paddocks. Because previous results (Chapter 4) revealed that nitrification activity was higher on low slopes than on medium slopes in these paddocks, we conducted the DEA measurements on soil taken from both low and medium slopes. The DEA measurements took place 14 months after the N_2O flux measurements were made (December 2007), however the stock and fertiliser management had remained the same over this period. Three soil cores (75 mm depth x 25 mm width) were collected and bulked from six newly established plots (1 m^2). Three plots were on Low slope areas (LS, 0 -12º) and 3 plots on Medium slope areas (MS, 13 - 25 º), within each of the 500 N paddocks. The method for the DEA assay is described in detail by Smith and Tiedje (1979) and Yoshinari *et al.* (1979; 1977). Briefly, conditions for DEA were optimised by adding 20 mL of glucose nitrate solution (0.2 g glucose, 0.1 g KNO_3 and 0.125 g chloramphenicol/L deionised water) to 10 g fresh weight of bulked soil in glass flasks and sealed with rubber stoppers. The addition of chloramphenicol was to inhibit *'de novo'* synthesis of reductive enzymes associated with the process of denitrification (Dendooven and Anderson 1994), so as to measure the potential enzyme activity at the time of sampling only. Flasks were flushed for 2 minutes with purified N_2 gas using a hypodermic needle, before 10 mL purified acetylene (C_2H_2, 98%, instrument grade BOC Standard SM3) was added to inhibit the reduction of N_2O to di-nitrogen (N_2). A second needle was inserted through the stoppers to allow the air pressure to equilibrate within flasks when flushing with N_2 or when adding 10 mL C_2H_2. Assays were then incubated at approximately 25 °C. A 12 mL headspace sample was removed using the syringe technique described above at 20, 40 and 60 min intervals. The headspace samples were stored in 6 mL sealed glass vials and were therefore over pressurised. All samples were analysed for N_2O following the same method as described in the previous section and described in detail in Chapter 3. The reported rates of N_2O production were determined from linear interpolation of N_2O concentrations of gas samples taken at 20, 40 and 60 minutes.

5.25 Statistical analyses

Total N_2O emissions from each plot were analysed by ANOVA, with the block structure given by sub plot within paddock, the treatment structure by N rate, and weighting by the reciprocal of the sum of between and within paddock variances. This was extended to a REML analysis fitting the additional covariates WFPS (% v/v), PSD (%) and soil NO_3-N (mg NO_3-N/kg soil). Denitrification activities in 500 N plots were also analysed by ANOVA, with the block structure given by location within paddock and the treatment structure by slope class within paddock. All analyses were conducted using the statistical package GenStat version 9 (GenStat 2006).

5.3 Results

5.31 Nitrous oxide emissions

Mean plot daily emissions for the measurement period were 9, 22 and 166 g N_2O-N/ha.d for the 0, 100 and 500 N treatments respectively. Increasing the rate of fertiliser N application from 100 to 500 kg N/ha increased emissions by a factor of 10 in this trial (Table 5.4). The integrated total N_2O losses from the 500N treatment (1.36 kg N_2O-N/ha) were significantly higher than the integrated totals from the 0 and 100 N treatments (0.08 and 0.13 kg N_2O-N/ha, $P< 0.05$ Table 5.4).

Table 5.4 Summary of plot N_2O emissions (g N_2O-N/ha.d), mean total N_2O gas losses (kg N_2O-N/ha) with sem, and fertiliser-N induced emissions for the measurement period

N rate (kg N/ha.y)	Fertiliser N applied (kg/ha)		Min (g N_2O-N/ha.d)	Max (g N_2O-N/ha.d)	Plot mean (g N_2O-N/ha.d)	Integrated total loss (kg N_2O-N/ha)	sem	[§]Fertiliser N-induced losses for the measurement period (kg N_2O-N losses/ha)
	30 Aug.	1 Oct.						
0	0	0	-41	180	9	0.08A	0.10	n/a
100	50	0	-27[*]	505	22	0.13A	0.18	0.05
500	62.5	62.5	-31[*]	1276	166	1.36B	0.45	1.28

AB: Total loss values followed by a different letter indicate significant differences ($P< 0.05$)
n/a: Not applicable
[*]N_2O concentrations used for emission calculation were within background variation; mean 320 ppb, CV 17.17%, as discussed in Chapter 3
[§]calculated according to the method of de Klein *et al.* 2003

The largest mean N_2O emissions for all treatments occurred at the beginning of the measurement period (Figure 5.1). Following the first fertiliser application, there was a noticeable increase in N_2O emission for the 500N but not the 100N treatment. No noticeable N_2O response was recorded after the second application of fertiliser N to the 500N treatment.

Nitrous oxide emissions from the 500N treatment were heavily influenced by the high results from paddock 3. Nitrous oxide emissions were also elevated in paddock 1, subplot 1 in the 100 N treatment, which released over four times the amount of N_2O measured in other paddocks in this treatment (Table 5.5).

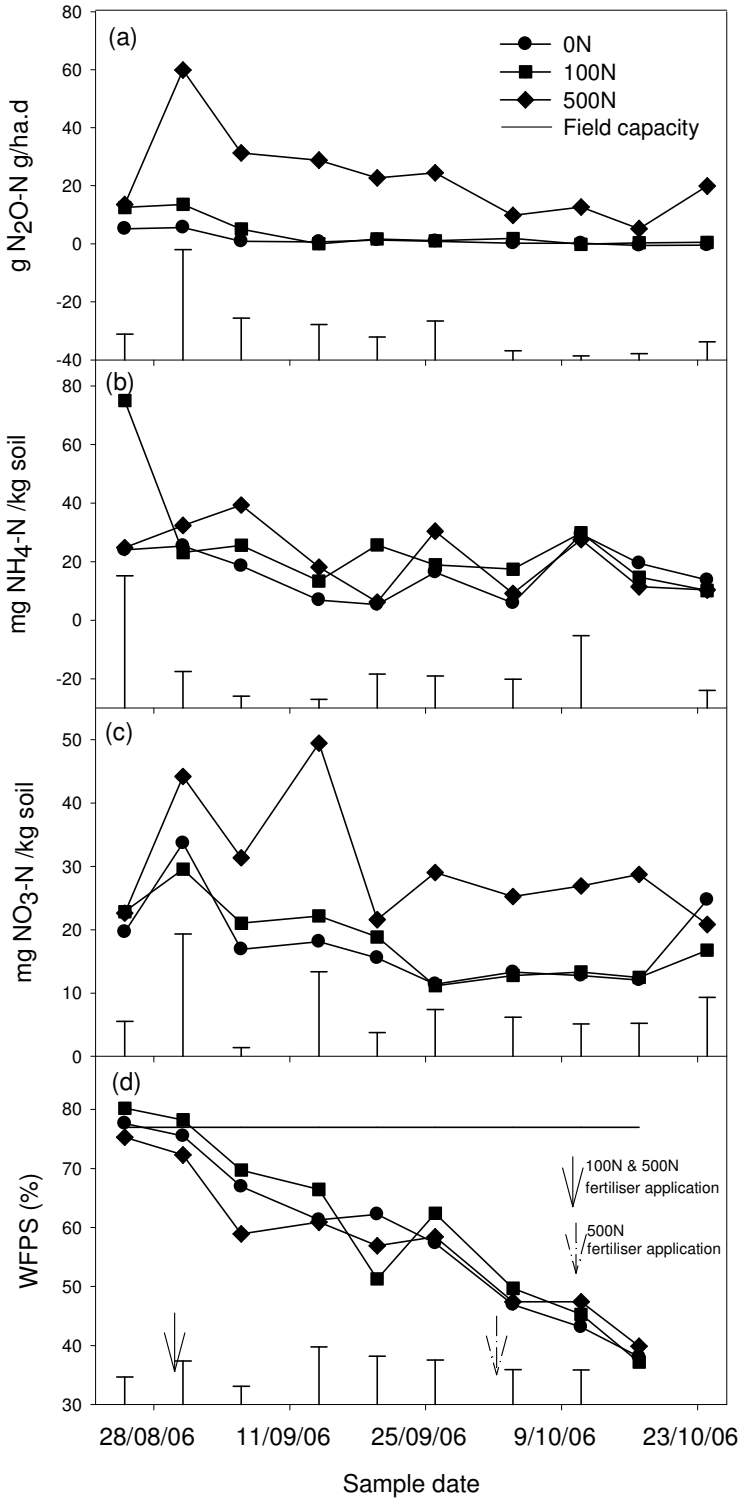

Figure 5.1 Treatment mean a) N_2O emissions (g N_2O-N/ha.d), b) soil NH_4^+ (mg NH_4-N/kg dry soil), c) soil NO_3^- (mg NO_3-N/kg dry soil), and d) WFPS (%) for 0, 100 and 500 N treatments. Bars indicate treatment sem

5.32 Mineral N

Mean soil NH_4^+ levels did not increase following fertiliser applications and there were no significant differences between N treatments over the measurement period. Mean soil NO_3^- levels were consistently and significantly elevated ($P< 0.01$) in the 500N treatment. Soil NO_3^- levels for both N treatments increased following the first fertiliser application but not the second (Figure 5.1). The soil NO_3^- results for the 500N treatment were also influenced by the high values in paddock 3 (Table 5.5).

Table 5.5 Total N_2O gas measured in each subplot (kg N_2O-N/subplot), mean N_2O gas measured for each paddock (kg N_2O-N/ha) over the measurement period and mean soil NH_4^+ and NO_3^- (mg N/kg dry soil), for 0, 100 and 500 N paddocks

N rate	Paddock	Subplot1 kg N_2O-N/subplot	Subplot2 kg N_2O-N/subplot	Subplot3 kg N_2O-N/subplot	Subplot4 kg N_2O-N/subplot	Paddock mean kg N_2O-N/ha	Paddock mean mg NH_4-N/kg dry soil)	Paddock mean mg NO_3-N/kg dry soil)
0	**1**	0.15	0.05	0.17	0.05	0.11	72.6	74.2
	2	0.12	0.13	0.16	0.01	0.11	52.4	47.0
	3	0.09	0.03	0.01	-0.05	0.02	31.4	48.0
100	**1**	1.29	0.44	0.03	-0.06	0.43	63.5	70.6
	2	0.06	0.2	0.13	0.02	0.1	96.7	53.9
	3	-0.06	0.16	0.07	0.04	0.05	46.6	48.3
500	**1**	0.41	0.86	0.65	0.71	0.66	72.8	78.8
	2	0.66	0.7	0.76	0.87	0.75	84.8	99.7
	3	3.63	3.57	2.37	2.54	3.02	54.4	120.2

5.33 Soil physical factors

Mean WFPS for the measurement period ranged from 55.7% in paddock 2 in the 500N treatment up to 76.2% in paddock 3 in the same treatment (Table 5.1). This treatment therefore contained both the wettest and driest of all the experimental paddocks over this period. Soil moisture (WFPS) declined steadily in all treatments as no significant rainfall events (> 5 mm/12 h) occurred during the measurement period (Table 5.3 and Figure 5.1). Mean WFPS for paddock 3 of the 500N and paddocks 1 and 3 of the 100 N treatments were close to field capacity (WFPS 75%) and therefore ideal for denitrification activity at times during the measurement period.

There were no significant differences in pore size distributions or bulk densities between treatments or paddocks. Macro porosity was lowest in paddock 3 of the 500N treatment and highest in paddock 2 of the same treatment (Table 5.1). Conversely micro porosity was lowest in paddock 2 and second highest in paddock 3 of the same treatment. No relationship between bulk density and pore distribution was observed.

All mean soil temperatures (5 cm depth) were >5 ºC, during the measurement period (Table 5.3) and were therefore suitable for denitrification activity (Haynes 1986).

5.34 Soil physical, mineral N and nitrous oxide interactions

Very high N_2O emissions only occurred when both high WFPS and soil NO_3^- levels were also high. However, some low emissions were also measured when both WFPS and NO_3^- levels were high and therefore the relationship between soil NO_3^-, WFPS and N_2O emissions was not consistent (Figure 5.2). A REML analysis of data detected a positive but non significant trend for increasing N_2O emissions with increasing NO_3^- and WFPS. Adding soil pore size distribution (<30, 30-300 and >300 μm) as a co-variate did not improve the results of the ANOVA or the REML.

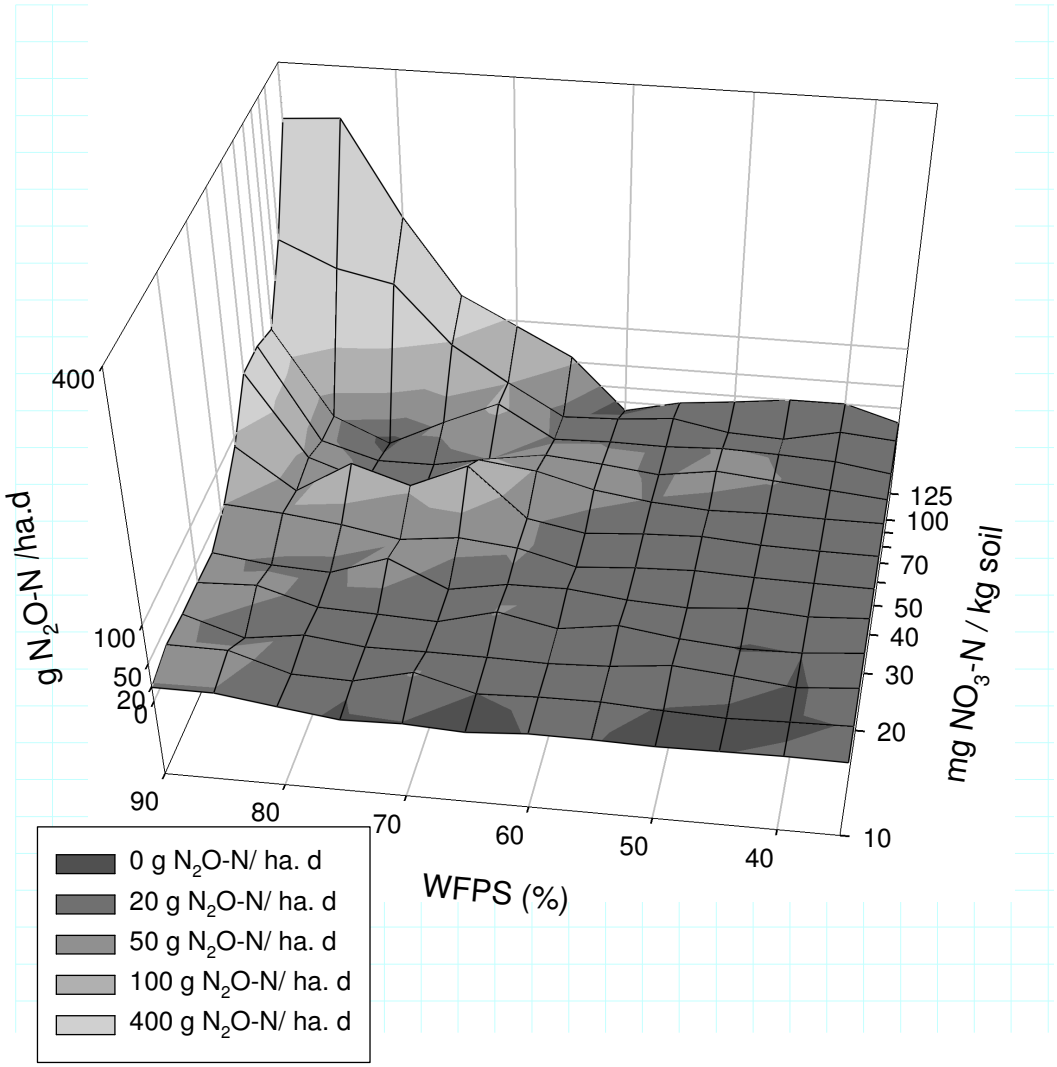

Figure 5.2 Relationship between WFPS (%), soil NO_3^- (mg NO_3-N/kg dry soil) and N_2O emissions (g N_2O-N/ha.d) in rolling sheep-grazed hill country pasture

5.35 Denitrification enzyme activity

Denitrification enzyme activity (DEA) in the 500N paddocks ranged from 0.5 to 3.7 µg N_2O-N/g soil.d. The mean DEA was highest in paddock 1 and lowest in paddock 2 with both slope classes (LS and MS) in paddock 1, and the Low slope in paddock 2 recording one noticeably low DEA result (Table 5.6). The mean DEA in paddock 1 was significantly higher than in the other 500N paddocks ($P< 0.05$, Table 5.6)

Table 5.6 Mean rate of denitrification activity (µg N_2O-N/g soil.d) in soil denitrification enzyme activity (DEA) assays from Low (0-12º) and Medium (13-25º) slopes in 500 N paddocks; ranges are in parenthesis

Paddock	N rate (kg/ha.y)	Slope means (µg N_2O-N/g soil.d)		Paddock means
	500	Low	Medium	
1		3.2 (2.5-3.7)	2.4 (1.6-2.8)	2.8
2		1.5 (0.6-2.0)	1.6 (1.3-1.9)	1.5
3		2.6 (2.1-2.9)	2.1 (2-2.2)	2.3
SED		0.13	0.13	0.39
Significance (slope)		NS	NS	
Significance (paddock)				*
Significance (slope.paddock)				NS

* P< 0.05
NS: not significant

5.4 Discussion

The plot mean daily emission rates of 9 and 22 g N_2O-N/ha.d from the 0 and 100 N treatments were greater than those from sheep grazed pastures reported by Saggar *et al.* (2007b) (7.4 and 3.4 g N_2O-N/ha.d in grazed (16 – 18 SU/ha) and ungrazed plots receiving 80 kg N/ha), and Carran *et al.* (1995) (*c.* ≤5 g N_2O-N/ha.d from land carrying 11 – 16 SU/ha for a 0 N rate). The plot mean daily emissions from the 500N treatment in the current study were also higher than estimates by Ruz-Jerez (1994) for free draining soils with a similar N application rate (*c.* 0 - 50 g N_2O-N/ha.d for a 400 N rate). It should be noted that the high fertiliser rate and attendant high stocking rate in the current study is not typical of hill country operations in NZ and may have increased inorganic N availability via fertiliser application and excreta, which increases N_2O gas production as in Abbasi and Adams (2000), Allen *et al.* (1996) and Tenuta and Beauchamp (2003). Hoogendoorn *et al.* (2008) measured N_2O emissions from the site used in the present study (Invermay) and from a North Island sheep grazed hill country site (Ballantrae), in the spring of 2005 and 2006 from synthetic urine patches and non urine patch areas in paddocks receiving 0, 100, 300, 500 (Invermay), and 750 (Ballantrae) kg N/ha.y during the previous year. Soils at both sites were classed as poorly draining silt loams. These workers found that emissions from non urine patch areas in the high N treatments were greater than emissions from non urine patch 0N areas. This was attributed to increased N in the soil profile due to fertiliser N application and the associated increase in urine and dung return resulting from animals harvesting the increased pasture growth in paddocks receiving high rates of fertiliser N. Estimates of daily plot (g N_2O-N/ha.d)

and total emissions (kg N_2O-N/ha over 42 days) based on the stocking rates and paddock area were also calculated by Hoogendoorn *et al.* (2008) and the results from the 0 N, and 100 N treatments were similar to those in our study. However the plot mean daily estimates from the 500 N treatment at Invermay (99 and 34 g N_2O-N/ha.d in 2005 and 2006, respectively) and the 750 N treatment at Ballantrae (12 and 67 g N_2O-N/ha. d in 2005 and 2006, respectively), were much lower than our results for the 500 N treatment (166 g N_2O-N/ha.d).

Total N_2O emissions from the 500 N treatment were heavily influenced by the high emissions measured in paddock 3. In this paddock the combination of elevated soil NO_3^-, higher microporosity and high WFPS compared to paddocks 1 and 2 (Table 5.1 and Table 5.5) is likely to account for the higher emissions. It is not clear why soil NO_3^- levels were elevated in paddock 3 of the 500N treatment as N inputs (fertiliser and excretal N) were similar within treatments. In the field we observed a higher density of earth worms in paddock 3 compared to all other paddocks which may have influenced the results by increasing mineralisation rates and therefore soil NO_3^- levels, as well as depleting soil O_2 levels through increased microbial activity (Bertora *et al.* 2007; Postma-Blaauw *et al.* 2006). The higher microporosity in paddock 3 may have impeded drainage hence prolonging high WFPS levels, and therefore the period during which the soil was vulnerable to denitrification due to a reduction in oxygen diffusion to anaerobic sites within the soil (Bhandral *et al.* 2003; McTaggart *et al.* 2002). A clear qualitative relationship existed between WFPS, soil NO_3^- and N_2O emissions, as high emissions only occurred when both WFPS and soil NO_3^- were high (>50 mg NO_3-N/kg soil and >70% WFPS, Figure 5.2). However the relationship was not consistent as there were a number of occasions, particularly during the second gas sampling, when both these parameters were high, yet N_2O emissions were very low. This probably explains why the REML analysis did not detect a significant relationship between N_2O emissions, WFPS and soil NO_3^-. Complete denitrification to di-nitrogen (N_2) may have occurred at times when WFPS and soil NO_3^- were high and N_2O emissions were low, however no measurements were made to confirm this occurring. The field notes indicate that high winds occurred on the second sampling occasion which may have compromised the measurements on this occasion by reducing the diffusive flow of N_2O from the soil beneath the chamber as in Matthias *et al.* (1980). It was also observed that the contact between the soil and chamber

became weaker in the dry soil conditions which may have allowed some leakage to occur. Either situation may have caused leakage of the chamber atmosphere. Rochette and Eriksen-Hamel (2008) cautioned that a poor seal increases the risk of sub-surface lateral diffusion of N_2O gas beneath the lower edge of the chamber rim and therefore the underestimation of fluxes by >50%. A greater chamber insertion depth than the 30 mm used in the current experiment may have been required given the soil conditions and weather patterns during the measurement period. Rochette and Eriksen-Hamel (2008) recommend a minimum chamber insertion depth of 50 mm. Sampling technique errors such as these are not likely to be consistent across chambers and may have contributed to the high sem observed during the second sampling occasion (Figure 5.1).

Nitrous oxide emissions are characterised by sharp often short lived increases or spikes as a result of N inputs and/or changes in soil moisture conditions such as following rainfall (Carran *et al.* 1995; Clayton *et al.* 1997; Dobbie and Smith 2001; Monaghan and Barraclough 1993). Very few N_2O 'spikes' occurred over the measurement period in our trial due to the lack of rainfall and the associated decrease in soil moisture (Figure 5.1). Fertiliser applications occurred on August 30 and October 1, with initial sampling days occurring 1 and 3 days following application, respectively (Table 5.4). Spikes that did occur after the first fertiliser application were confined largely to one paddock (paddock 3, 500N treatment, Figure 5.1). After the second fertiliser application no spikes were measured in the 100 or 500N treatment on any of the four sampling occasions that occurred over the following three weeks (Figure 5.1). A spike in N_2O emissions was also observed in paddock 1, subplot 1 in the 100N treatment. This spike heavily influenced the integrated mean emissions for the 100N treatment (Table 5.5). The largest N_2O emissions for all treatments occurred at the beginning of the measurement period when soil moisture was at or near 70% WFPS (Figure 5.1), which was close to field capacity and ideal for denitrification activity (Ruz-Jerez *et al.* 1994). Both WFPS and N_2O emissions steadily declined over the measurement period due to the lack of rainfall and dry soil conditions. Although we had made provisions to increase the sampling frequency to capture emission peaks, due to lack of rainfall we limited our measurement frequency to once a week.

The range in daily emissions for all treatments in our study was larger than those reported by others in NZ (Table 5.4) (Carran *et al.* 1995; Hoogendoorn *et al.* 2008; Ruz-Jerez *et al.* 1994; Saggar *et al.* 2007a), as there were a large number of very low or negative fluxes and a few very high fluxes. Negative fluxes accounted for 21% of the total number of N_2O measurements made over the trial period of 46 days (10 sample days) in late winter/ early spring 2006. The majority of those occurred fairly evenly in the 0 and 100N treatments. Chapuis-Lardy *et al.* (2007) noted that negative fluxes (i.e. N_2O consumption) are frequently recorded in the literature over a range of conditions (temperate, tropical, agricultural and natural systems) but are often dismissed or reported as measurement 'noise' or errors. They suggested that treating apparently negative fluxes in this way may be inappropriate, as in some cases the effect may be both real and important in terms of the estimated global N_2O budget. A range of both denitrifying and nitrifying bacteria have the ability to reduce N_2O to N_2 (Chapuis-Lardy *et al.* 2007) and this process is occurring in soils most of the time. Because the rate of N_2O production normally exceeds its rate of conversion to N_2, the possibility of net N_2O consumption is often overlooked. From the literature surveyed by Chapuis-Lardy *et al.* (2007), net N_2O consumption tends to occur most often when soil NO_3^- is low. Low levels of soil NO_3^- promote net N_2O consumption in two ways. Firstly, the production of N_2O is reduced and secondly, N_2O becomes the favoured electron acceptor for the reduction process because the lower energy option of NO_3^- is not available. Negative N_2O fluxes may also occur when WFPS is high or other soil factors restrict N_2O diffusion to the atmosphere and thereby increase the availability of N_2O for consumption within the soil profile.

There was a lack of information reported by the commercial GC operator (Feed Tech, Palmerston North on the minimum detection limit (MDL) for the analytical procedure that was used in this study. However subsequent work (see Chapter 3) suggested that at the mean background concentration of 320 ppb N_2O, the coefficient of variation (CV) of the analytical procedure was approximately 17%. Using this measure of analytical variability, approximately one third of the negative fluxes calculated were based on concentrations that were within the margin of error and should therefore be treated with caution. An alternative approach is to use the variation in t0 measurements to estimate the errors in the sampling and analysis of N_2O gas in the current study. In theory the t0 measurements should all be similar because the

chambers are open to the atmosphere before the chamber is sealed and the t0 sample is taken immediately. However it is acknowledged that if there are large differences in N_2O emission rates this may impact on the t0 value measurement and lead to an over-estimate of the errors associated with the sampling and analysis of the gas. Based on the measured variability in t0 N_2O concentrations and assuming that the variability associated with sampling and analysis was the same for both t0 and t60 samples, then the calculated FDL for this trial was approximately 0.011 mg N_2O-N/m^2.h. If a FDL of 0.011 mg N_2O-N/m^2.h is assumed, then approximately two thirds of the negative fluxes were within this FDL. Using this approach increased the margin of error to include two thirds of the negative flux data. Although it is not possible to establish whether such fluxes in our study represent true N_2O consumption, or are due to limitations in sampling methodology, field conditions at the time, or detection precision (Rochette and Eriksen-Hamel 2008), we included the negative fluxes when calculating the mean daily emissions and subsequent statistical analyses.

The manual static chamber technique is a common low cost and useful approach for quantifying relative differences between treatments. However as discussed above, the errors associated with this technique (Hutchinson and Livingston 2001; Matthias *et al.* 1980; Rochette and Eriksen-Hamel 2008), and the limited sub sampling within treatment replicates and temporal sampling frequency due to a lack of rainfall, may have meant that some peak events were missed. However given the consistently low soil moisture conditions this is not likely. Nevertheless the data should only be used for relative comparisons between treatments, and not used to estimate monthly or annual fluxes.

Due to the notably higher N_2O emissions from paddock 3 (500N) over the measurement period (Table 5.5), DEA assays of soils from all of the 500N paddocks were conducted to assess if differences in microbial activity could explain this observation. The DEA assay is an estimate of the concentration of denitrifying reductase enzymes in a soil sample at the time of sampling (Dendooven and Anderson 1994). In the assays conditions for denitrification were optimised by saturation of the soil samples with substrate carbon (C) and NO_3^- and removal of oxygen (O_2). Chloramphenicol (an antibiotic) was added to prevent protein synthesis of new reductase enzymes by denitrifying bacteria during the assay (Dendooven and

Anderson 1994). Acetylene was then added (10% of the head space volume) to block N_2O reduction to N_2, the last step in the denitrification process. The rate of N_2O accumulation measured is therefore an indication of the potential for incomplete and complete denitrification activity should soil conditions become favourable at the time of sampling only. The range in DEA rates in the current study was surprisingly narrow (Table 5.6), particularly considering the results for N_2O emissions in the 500N paddocks (Table 5.5) and the known large spatial variability of denitrification activity in grasslands (Jarvis *et al.* 1991; Florinsky *et al.* 2004; Parsons *et al.* 1991; Luo *et al.* 2000). Although replication of DEA assays was low a significant difference in DEA rates was detected between the 500N paddocks in our study. However the average DEA rate in paddock 3 (2.3 ug N_2O-N/g soil.d) was intermediate between paddock 1 (2.8 ug N_2O-N/g soil.d) and paddock 2 (1.5 ug N_2O-N/g soil.d), indicating that higher DEA could not explain the observed higher N_2O emissions in paddock 3 (500N). The low level of DEA assay replication prevented any further statistical tests on the relationship between N_2O emissions and DEA rates.

The DEA analysis was conducted 14 months after N_2O field measurements and may therefore have limited relevance of the DEA data gathered in 2007 to the N_2O field observations discussed here. However stock and fertiliser management did not change within this time frame and there is molecular evidence to suggest that denitrifier community structure and the expression of various denitrifying genes is determined, in the long term, by soil variables such as regional soil mineral N levels and moisture regime (Wallenstein *et al.* 2006).

5.5 Conclusions

In our study we measured N_2O emissions from sheep grazed rolling hill paddocks receiving 0, 100 or 500 kg N/ha.y for 10 weeks in late winter/ early spring. Total N_2O production for the measurement period was significantly higher in the 500 N treatment ($P< 0.05$), increasing by a factor of 10 compared to the 100 N treatment. Soil moisture conditions were below field capacity in most paddocks for the majority of the trial, making conditions unsuitable for high levels of denitrification activity. A large number of low and negative fluxes (i.e. N_2O consumption) were observed in the 0 and 100N treatments and this should be investigated further. However, despite the

relatively dry soil conditions significant N_2O losses were measured from the 500N treatment. This result was heavily influenced by the results from paddock 3 in this treatment. The combination of prolonged relatively elevated WFPS, reduced O_2 diffusion to anaerobic sites, and significantly elevated ($P< 0.01$) soil NO_3^- levels were the likely reasons for the high emissions measured from paddock 3 (500N). In the 500N treatment no positive relationship was detected between N_2O emissions in one year and denitrification activity in soil samples taken 14 months after the gas sampling had occurred. It should be noted that typical fertiliser N applications to NZ hill country rarely exceed 100 kg N/ha.y and therefore these results should be interpreted with caution. However, the results from this study demonstrate that hot spots for N_2O emissions may still exist in overall dry conditions due to stock management and soil structural differences that exist over short distances in rolling hill farming areas. This data is useful for quantifying relative differences between treatments in this study; however the results presented here should not be used to estimate monthly or annual flux rates due to limitations in the sampling technique and intensity.

Based on the current results guidelines for fertiliser N use in rolling hill country pastures should however consider the possibility of significant N_2O gaseous emissions outside of optimal conditions for denitrification activity when high rates of fertiliser N are applied over long periods, and where the attendant increased net herbage accumulation is effectively harvested by grazing animals.

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CHAPTER 6 THE SPATIAL DISTRIBUTION OF N_2O EMISSIONS FROM SHEEP GRAZED HILL COUNTRY IN NEW ZEALAND

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Some minor changes have been made to the text of the original paper to improve linkage in the Thesis. Units (i.e. kg/ha.y) are unchanged from the journal format.

Abstract

Nitrous oxide (N_2O) emissions from soil are largely affected by nitrogen (N) inputs and soil moisture conditions. In pastoral hill country, these factors are inherently more variable than in flat land pastures due to topography-driven differences in excretal N returns and changes in soil moisture. This may limit the application of N_2O emission data collected from trials conducted on flat land trials to hill country situations. A short term study was conducted to measure the effect of topography on N_2O emissions from a poorly drained Mottled Fragic Pallic hill soil. Two paddocks with a history of moderately intensively stocked sheep grazing were classified into Low slopes (Campsites, 0-12°), Medium slopes (13-24°), Steep slopes (>25°) and Gully (ephemeral drainage) areas. Nitrous oxide emissions were measured using a static chamber method before and after rainfall events. A range of soil chemical and physical parameters and slope were also measured at each sampling site. The daily mean and range of N_2O emissions (g N_2O-N/ha.d) decreased in the order; Campsites (27.1, -5.5-180.0) > Gully slopes (5.0, -2.6-34.8) > Medium slopes (3.2, -2.9-6.5) > Steep slopes (0.3, -3.1-1.6). Mean daily log-transformed N_2O losses were

in the order Campsites ($P< 0.001$) > Gully slopes = Medium slopes > Steep slopes ($P< 0.001$). A significant relationship ($P< 0.001$) was detected between log transformed N_2O emissions and slope class. We estimated that 81% of total N_2O emissions measured over the experimental period were produced from Campsites, which represented only 22% of the experimental land area. Gully and Medium slopes produced a combined total of 19% of total N_2O emissions, while representing a combined land area of 69%. Nitrous oxide emissions were negligible from Steep slopes, which represented 10% of the land area. These short term measurements during soil conditions for peak N_2O emissions suggest mitigation options should be targeted at reducing emissions from Campsites in hill country. In addition, the significant relationship between slope and N_2O emissions suggests that slope may be a suitable parameter for upscaling N_2O emissions from sheep grazed hill country.

Keywords: Nitrous oxide emissions; Topography; Sheep grazed: Hill country

6.1 Introduction

Nitrous oxide emissions from agricultural soils contribute approximately 16% of New Zealand's (NZ) greenhouse gas profile, and are of considerable environmental concern (Ministry for the Environment 2010b). The production of N_2O gas from agricultural soils is highly variable both in space and in time. This is due to the large number of factors that influence the evolution of N_2O gas during the soil processes of nitrification and denitrification (Firestone and Davidson 1989; Harrison and Webb 2001; McTaggart *et al.* 2002). It is generally agreed that the main influences on N_2O production are soil moisture and therefore rainfall and soil drainage type (Abbasi and Adams 2000; de Klein *et al.* 2003; Dobbie and Smith 2001), and soil nitrate (NO_3^-) supply and therefore grazing, fertiliser management and animal behaviour (Carran *et al.* 1995; Clayton *et al.* 1997; Hoogendoorn *et al.* 2008; Letica *et al.* 2010).

In hill country the distribution of soil moisture and N inputs to soils is influenced by topography. The challenge in estimating N_2O emissions from hill country pastures therefore is not only in estimating how much N_2O is emitted in total (kg N_2O-N/ha.y), but also estimating when and where in the landscape those emissions occur. Nitrogen cycling in hill country is heavily influenced by topography and aspect, because stock

transfer excretal N between slopes due to their preference to camp on flat and sheltered areas (Ball *et al.* 1982; Carran *et al.* 1995; Gillingham and During 1973; López *et al.* 2003b). Carran and Saggar (2004a) used a nutrient transfer model developed by Saggar *et al.* (1990) to explain the variations in excretal N distribution across slope and aspect classes in hill country. The model assumed that 60, 30 and 10% of dung and 55, 31 and 14% of urine was deposited by sheep on low, medium and steep slopes, respectively. In addition, Saggar *et al.* (1990) proposed that a significant proportion of excretal N was deposited on low slopes that were sheltered from prevailing north westerly winds, and that although steep exposed areas may be grazed, only a small proportion of N was returned to these areas via excreta. Therefore N_2O emissions are expected to be elevated in sheltered flat areas in hill country.

Natural ephemeral gully systems occur in many hill country landscapes, and soil drainage and moisture status within these areas may also influence the spatial and/or temporal distribution of N_2O emissions from these areas in hill country. Penncock *et al.* (1992) examined landscape patterns of denitrification in undulating terrain and found that denitrification rates were highly spatially correlated, being highest in the depression areas and lowest on the shoulders of slopes. Florinsky *et al.* (2004) also found that N_2O emissions were affected by both the soil moisture and organic matter levels which in turn were influenced by local topography.

Few field studies have measured the spatial distribution of gaseous N losses from hill country pastures in NZ (Carran *et al.* 1995; Hoogendoorn *et al.* 2008). Based on the known variability in soil moisture, fertility and physical properties between slope classes, N_2O emissions from hill country pastures are anticipated to be highly spatially variable. The objective of this short term study was to measure N_2O emissions from four distinct land classes (Campsites 0-12°, Medium slopes 13-25°, Steep slopes > 26°, and Gullies with drainage areas), and provide quantitative information on the spatial distribution and magnitude of N_2O emissions from sheep grazed hill country soils. As soil NO_3^- supply and moisture are crucial for the process of denitrification we hypothesised that N_2O losses from sheep grazed hill country areas would be in the order Gullies> Campsites> Medium slopes> Steep slopes. Using the data from this short term study we also made an assessment of the relative contribution of these four

distinct land classes to the total combined paddock N_2O emissions, based on the relative areas each of these slope classes occupied within the paddocks. We discuss the implications of the distribution of N_2O emissions measured in this study for estimating N_2O emissions from hill country and for targeting N_2O mitigation options.

6.2 Methods

6.21 Site description

The experiment was established on Ballantrae Research Farm in the southern Hawke's Bay, NZ. The predominant soil type was a Wilford Hill soil, a poorly drained Mottled Argillic Pallic soil (Hewitt 1998). The experimental area was situated *c.* 200 to 350 m a.s.l. on steep (*c.* 5 - 45º) heavily dissected hill country (Zhang *et al.* 2006), with an average annual rainfall of 1200 mm. The site consisted of 2 sheep grazed paddocks that for the previous 4 years had N supplied via excreta only. Single superphosphate had been applied to paddocks (250 to 300 kg SSP/ha.y) over the previous 4 years in autumn or winter as a single annual dressing. Nitrous oxide flux and soil physical and chemical parameters were measured in the winter of 2008.

Within each paddock, 12 plots (2 m^2) were established: 3 Low slope (0 to 12º, LS), 3 Medium slope (13 to 25º, MS), 3 Steep slope (26º +) and 3 Gully site (GS) plots, giving a total of 24 plots. Over the measurement period July 8 to July 24 stock was excluded from the paddocks. The last grazing episodes prior to sampling were 20 - 23 June in paddock 1 (61 mixed age ewes/0.33 ha) and 25 - 26 May in paddock 2 (50 MA ewes/0.28 ha).

6.22 Nitrous oxide flux

Nitrous oxide emissions were measured using a static chamber method as described by Saggar *et al.* (2004a) in this short term study. Nine gas measurements were made in the winter from 8 to 24 July, 2008 at intervals of 1 to 4 days depending on rainfall. Sampling occasions were chosen to occur 1 to 2 days before significant rainfall events (>10 mm), and within 1 to 2 days following the event, in an effort to

capture the entire N_2O production curve which is low in drier conditions prior to rainfall and then typically spikes in response to increased soil moisture during and after rainfall. The closed static chamber method, as well as all analytical methods and calculations to determine N_2O concentrations in samples are described in detail by Sagger *et al.* (2004a). Briefly, on the day prior to sampling 250 mm sections of PVC pipe (200 mm deep) were inserted 10 -15 mm into the ground and left to 'settle' for at least a day in the soil at each sampling site. Chamber heights were measured at four points around the chamber circumference so that chamber headspace volume could be calculated. On the sampling day modified PVC 'sewer hatches' were fastened to the top of each pipe. The hatch rims had an internal locking system with a greased rubber O-ring that formed an air tight seal. Two gas samples were taken with 60 mL polypropylene syringes fitted with 3 way stop cocks at 0 and 60 minutes (T_0 and T_{60}) after sealing. Ambient air samples were taken from outside the chamber at the start and conclusion of each gas sampling occasion. Soil (50 mm depth) and chamber headspace temperature (ºC) were recorded at the start and completion of each sampling occasion to give an average chamber and soil temperature over the measuring period.

Gas samples were transported back to the laboratory at Massey University and transferred to labelled 12 mL sealed glass vials. The samples were therefore over pressurised. Glass vials were stored at room temperature and analysed within 1 month. Gas samples were analysed at Landcare Research, Palmerston North, using a Shimadzu GC-17A gas chromatograph with a 63Ni-electron capture detector and oxygen-free N as carrier gas. The oven, valve and detector operating temperatures were 65, 100 and 280º C, respectively. The background samples were used as reference samples for calculating N_2O gas fluxes. According to the method of the US Environmental Protection Agency (EPA) and International Accreditation New Zealand (IANZ), the precision of the measurement technique (Gas Chromatograph) used to determine N_2O gas concentrations in samples was 0.74% (refer to Chapter 3, Hedley *et al.* 2006). Nitrous oxide fluxes were calculated (de Klein *et al.* 2003) by linear interpolation of the 2 samples and corrected for temperature and the ratio of cover volume to surface volume as follows:

$$N_2O \text{ flux} = \frac{\delta N_2O}{\delta t} * \frac{M}{Vm} * \frac{V}{A}$$

where N_2O flux is the hourly emission (mg N_2O-N/m^2.h), δN_2O is the increase in headspace N_2O during the enclosure period (ppm), δt is the enclosure period (h), M is the molar weight of N in N_2O (g/mol), Vm is the molar volume of gas at the sampling temperature (L/mol), V is the headspace volume (m^3), and A is the area covered (m^2). Hourly emissions were integrated over time for each individual plot to estimate the total emission over the measurement period.

6.23 Climatic factors

Air, soil and surface temperature (ºC), solar radiation (MJ/m^2) (mm/d) were recorded at a meteorological station approximately 50 m from the study site (Table 6.1). The historical average of measured climatic variables for the winter season in the area are also included in Table 6.1. Daily rainfall (mm/d) was also recorded at this site (120 mm over the measurement period).

Table 6.1 Climatic variables recorded on gas sampling days at Ballantrae meteorological station; approximately 50 m from experimental site, and winter averages (1980-2010) as calculated by Tait *et al.* (2006)

Sample date	Solar radiation (MJ/m^2)	Air temperature (°C)	Soil temperature at 10 cm depth (°C)	Surface temperature (°C)
8/07/08	1.1	9.7	6.5	5.9
9/07/08	0.7	7.5	6.7	5.5
10/07/08	0.8	8.9	6.9	6.3
14/07/08	1.4	13.2	9.6	9.7
15/07/08	0.9	11.3	9.1	8.8
17/07/08	1.0	11.9	8.3	7.4
18/07/08	0.5	9.5	8.0	7.4
21/07/08	0.8	12.6	9.9	9.5
24/07/08	0.5	10.4	9.2	8.4
Historical winter daily average	n/a	8.7	7.4	n/a

n/a: data not available

6.24 Soil factors

On each sampling occasion, 3 soil cores (75 mm depth x 25 mm width) were removed from the soil directly beneath each chamber once gas sampling was completed. Soil cores were bulked for each plot, sieved (2 mm) and analysed separately for soil NO_3^- and NH_4^+ by extracting 15 g field moist soil in 50 mL 0.5M

K_2SO_4 (Hatch *et al.* 2000), for pH in a suspension of 10 g of field moist soil in 30 mL RO water, and soil moisture was determined by weighing soil samples before and after drying at 105ºC for 24 h.

Several months after gas sampling had been completed when the gullies had dried sufficiently to be able to extract undisturbed soil cores, 4 aluminium rings (50 mm depth x 100 mm diameter) were inserted perpendicular to the soil surface contour into the soil in each plot to collect intact sub replicate soil cores. The intact core samples were used to determine bulk density and pore size distribution according to the method of Drewry *et al.* (1999). The mean bulk density of the four core sub replicates for each plot was calculated. The estimated WFPS at the time of sampling was calculated by dividing the volumetric soil moisture content (obtained by multiplying the gravimetric soil moisture by the mean bulk density value for each plot) by the mean total porosity for each plot.

6.25 Topography

A digital elevation map derived by photogrametry from digital orthographical aerial photos of the paddocks was provided by NZ aerial mapping Ltd (Figure 6.1). The total land areas of the experimental paddocks and the proportion of area covered by each slope class were estimated based on this information. Gully areas (which were identified as depression areas in the landscape) were estimated based on the combination of elevation and topographical contour information derived from the information supplied for this trial. The actual slope (º) where each chamber was placed at each sampling occasion was also recorded using a Pro SmartLevel (Series 200, Macklanburg-Duncan).

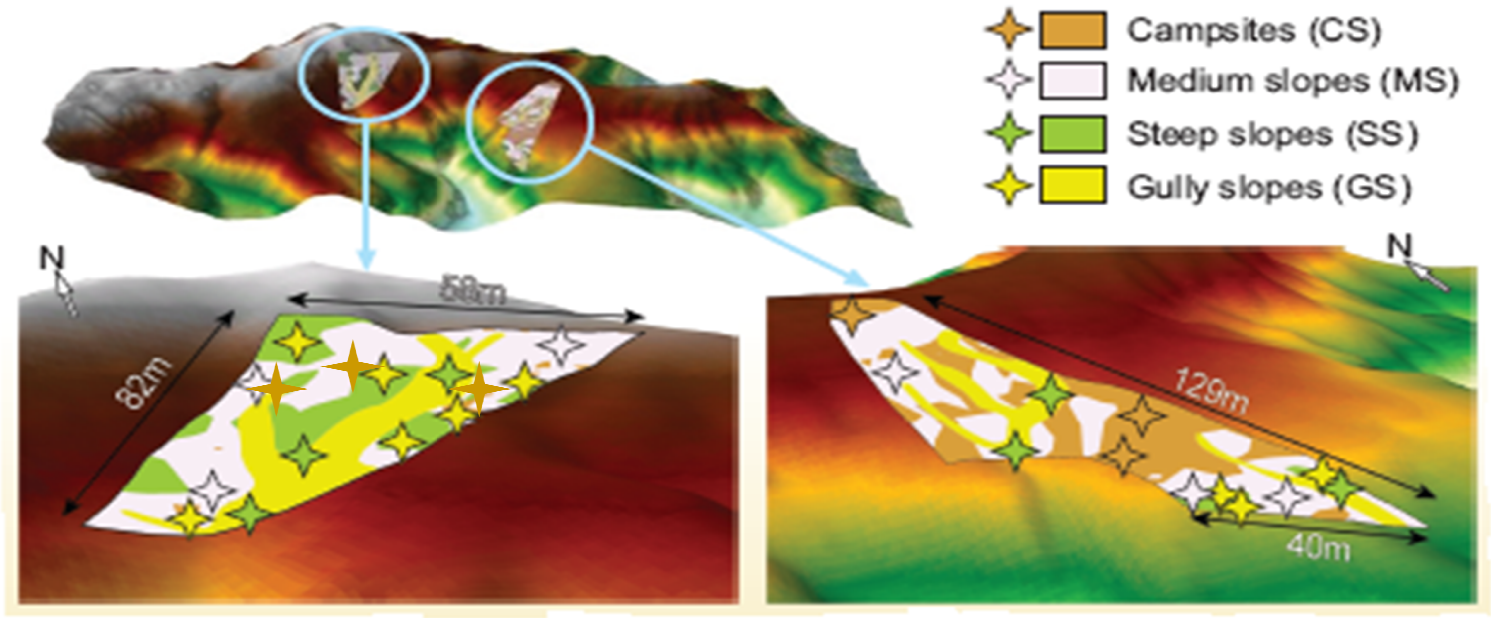


Figure 6.1 Digital elevation map (DEM) of land area in experimental paddocks 1 and 2: Campsites (CS), Medium slopes (MS), Steep slopes (SS) and Gully slopes (GS); depiction of the hillscape is a vertical exaggeration of the landscape and is not to scale. Stars indicate the location of sampling areas.

6.26 Statistical analyses

The distribution of all variables was assessed by inspection of residual plots and it was clear that a log-normal distribution was appropriate for daily N_2O emissions (g N_2O-N/ha.d) data. All other data was normally distributed. Due to the occurrence of negative gas flux values, a constant value was added to all data prior to log transformation: ln (N_2O flux+c). The value of c (1.965 and 0.571 for daily treatment mean and daily plot mean, respectively) was estimated to optimize the Anderson-Darling statistic for the normal distribution.

Bulk density, pore size distribution and field capacity were analysed by ANOVA with the block structure given by subreplicate within plot and the treatment structure by slope class, paddock and their interaction. Mean logged emissions (ln (g N_2O-N/ha.d+1.965)), soil mineral N levels (NO_3^- and NH_4^+), soil pH, headspace temperature were also analysed by ANOVA with block structure given by date (sampling occasion) within plot, and the treatment structure by slope, paddock variance and their interaction.

Relationships between mean plot N_2O emissions logged (ln (g N_2O-N/ha.d) + 0.571)) data and mean plot slope, soil NH_4^+, NO_3^- and WFPS were analysed by Stepwise Regression. The mean values for each of these parameters for each sample date were obtained from ANOVA's with block structure given by plot/date and treatment structure by paddock/treatment/date. The ANOVA also estimated missing values using an iterative algorithm (GenStat 2006). When soil NO_3^- concentrations were below the level of detection, calculations were carried out using the minimum detectable concentration.

All analyses were conducted using the statistical package GenStat version 9 (GenStat 2006).

6.3 Results

6.31 Nitrous oxide emissions

Arithmetic mean daily N_2O emissions (g N_2O-N/ha.d) over the 16 day measurement period were in the order Campsite> Gully> Medium> Steep plots (Table 6.2). The mean N_2O emissions measured from Campsite plots were almost 6 times those of the Gullies, and Gully mean daily N_2O emissions were almost twice those of Medium plots (Table 6.2). Mean N_2O losses from the Steep plots were close to zero (Figure 6.2 and Table 6.2). Nitrous oxide emissions varied considerably from day to day (Figure 6.2) and an emission spike occurred in most plots on July 14 or 15. The daily N_2O emissions within slope class plots were generally similar in both paddocks, although N_2O emissions from the Campsites in Paddock 1 were consistently higher than those in Paddock 2 (Figure 6.2 and Table 6.2). There was also one Gully plot in Paddock 2 that exhibited one large flux (Figure 6.2). The Steep plots in paddock 2 consistently recorded negative N_2O emissions, and the remainder of plots in this slope class were very low (<2 g N_2O-N/ha.d, Figure 6.2). In total 14% of N_2O emissions were negative fluxes, as determined by the Landcare Research (Palmerston North) GC.

Table 6.2 Summary of N_2O emissions (g N_2O-N/ha.d), mean losses and range for slope treatments; Campsites (CS), Medium slopes (MS), Steep slopes (SS) and Gullies (GS) and overall slope means with se. Mean WFPS (%), soil NO_3^- (mg NO_3-N/kg dry soil) and overall slope mean for plots are also included

	Paddock 1				Paddock 2				
	CS 1	**CS 2**	**CS 3**	**Paddock summary**	**CS 1**	**CS 2**	**CS 3**	**Paddock summary**	**Slope summary**
Mean N_2O (g/ha.d)	14.6	4.1	28.3	46.9	2.3	1.5	3.4	7.3	27.1
Min	0.5	0.1	-5.5	-1.6	1.9	0.2	1.3	1.1	0.3
Max	68.6	37.7	180.0	95.4	8.1	5.6	16.0	9.9	52.7
SE	7.8	5.6	22.7		0.8	0.7	2.2		
Mean WFPS (% v/v)	90.8	86.3	85.5	87.5	91.7	88.7	89.0	89.8	88.7
Mean NO_3-N (mg/kg soil)	261.3	281.5	420.3	321.0	433.5	491.6	343.1	422.7	392.8
	MS 1	**MS 2**	**MS 3**		**MS 1**	**MS 2**	**MS 3**		
Mean N_2O (g/ha.d)	0.8	0.4	0.2	1.4	0.6	0.9	1.7	3.2	3.2
Min	-0.8	-2.9	-0.4	-1.4	-0.1	0.3	0.9	0.4	-0.5
Max	6.5	3.1	1.8	3.8	3.9	3.7	5.5	4.4	4.1
SE	0.9	0.8	0.3		0.5	0.5	0.6		
Mean WFPS (% v/v)	51.4	58.1	58.8	56.1	73.5	69.4	66.2	69.7	62.9
Mean NO_3-N (mg/kg soil)	14.0	103.2	58.7	58.6	286.3	307.1	200.0	264.5	138.5
	SS 1	**SS 2**	**SS 3**		**SS 1**	**SS 2**	**SS 3**		
Mean N_2O (g/ha.d)	-0.1	0.4	0.1	0.4	0.2	-0.1	-0.1	0.1	0.3
Min	-1.9	-0.4	-0.5	-0.9	-1.1	-3.1	-2.9	-2.4	-1.7
Max	0.5	1.8	1.2	1.2	1.4	1.6	0.2	1.1	1.2
SE	0.3	0.3	0.2		0.3	0.6	0.4		
Mean WFPS (% v/v)	54.7	58.5	60.7	58.0	59.3	56.0	68.8	61.4	59.7
Mean NO_3-N	25.7	37.9	41.1	34.9	108.3	32.5	29.8	56.9	32.6

(mg/kg soil)									
	GS 1	**GS 2**	**GS 3**		**GS 1**	**GS 2**	**GS 3**		
Mean N_2O (g/ha.d)	0.8	1.5	1.6	4.1	1.0	0.1	4.7	5.8	5.0
Min	0.2	-2.6	0.8	-0.5	0.4	-2.1	-0.4	-0.7	-0.6
Max	4.3	20.3	6.2	10.3	4.6	1.8	34.8	13.7	12.0
SE	0.6	2.5	0.7		0.6	0.6	4.4		
Mean WFPS (% v/v)	54.8	36.4	85.9	59.0	83.2	82.1	82.3	82.5	70.9
Mean NO_3-N (mg/kg soil)	236.4	306.6	241.4	261.5	325.1	179.1	233.2	245.8	290.5

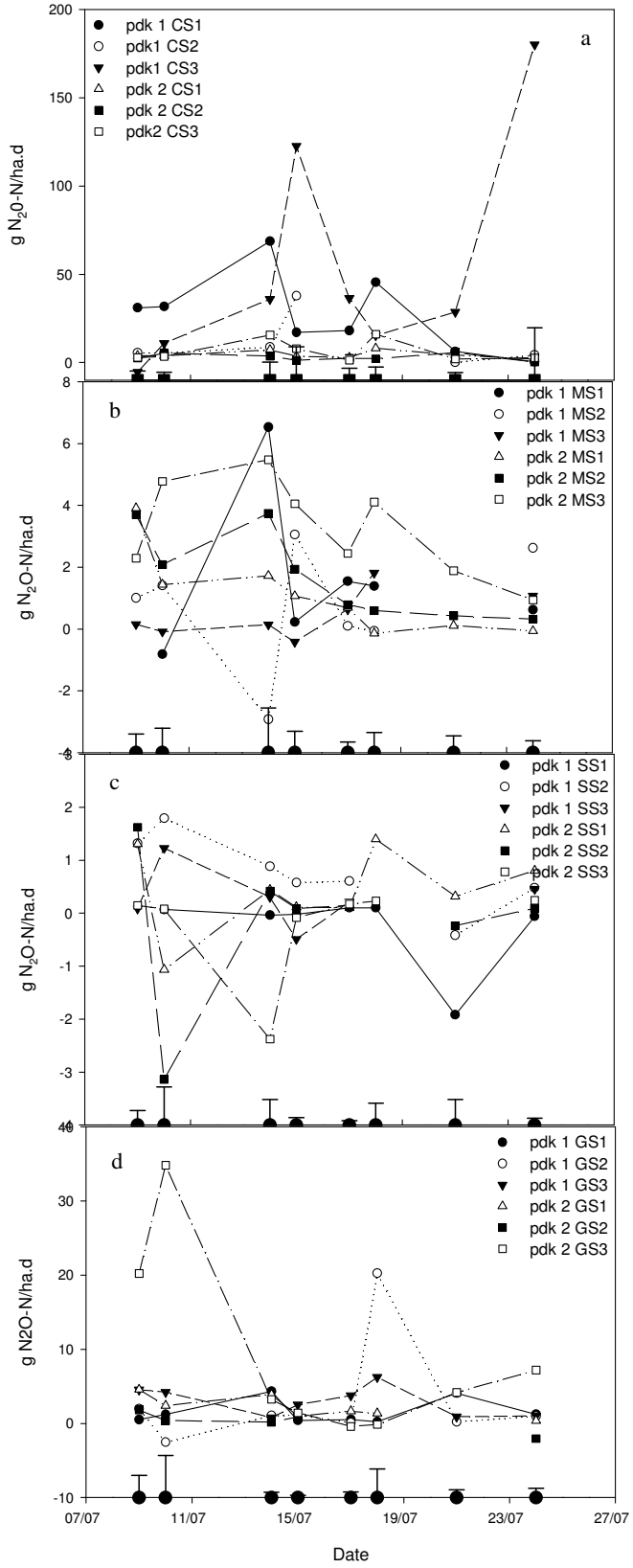


Figure 6.2 Daily N_2O emissions (g N_2O-N/ha.d) in paddocks 1 and 2 from all plots in slope classes; a) Campsites (CS), b) Medium slopes (MS), c) Steep slopes (SS) and d) Gullies (GS). Bars indicate sem.

Prior to statistical analysis of the N_2O emission (g N_2O-N/ha.d) data, a constant (1.965) was added to the slope treatment means for each measurement day before transforming the data (as described in the previous section). Mean N_2O emissions (ln (g N_2O-N/ha.d+1.965)) remained in the order Campsite> Gully> Medium> Steep (Figure 6.3) following this procedure. Logged N_2O emissions were significantly higher ($P< 0.001$) from Campsites, and significantly lower ($P< 0.001$) from the Steep slopes compared to Gully and Medium slopes.

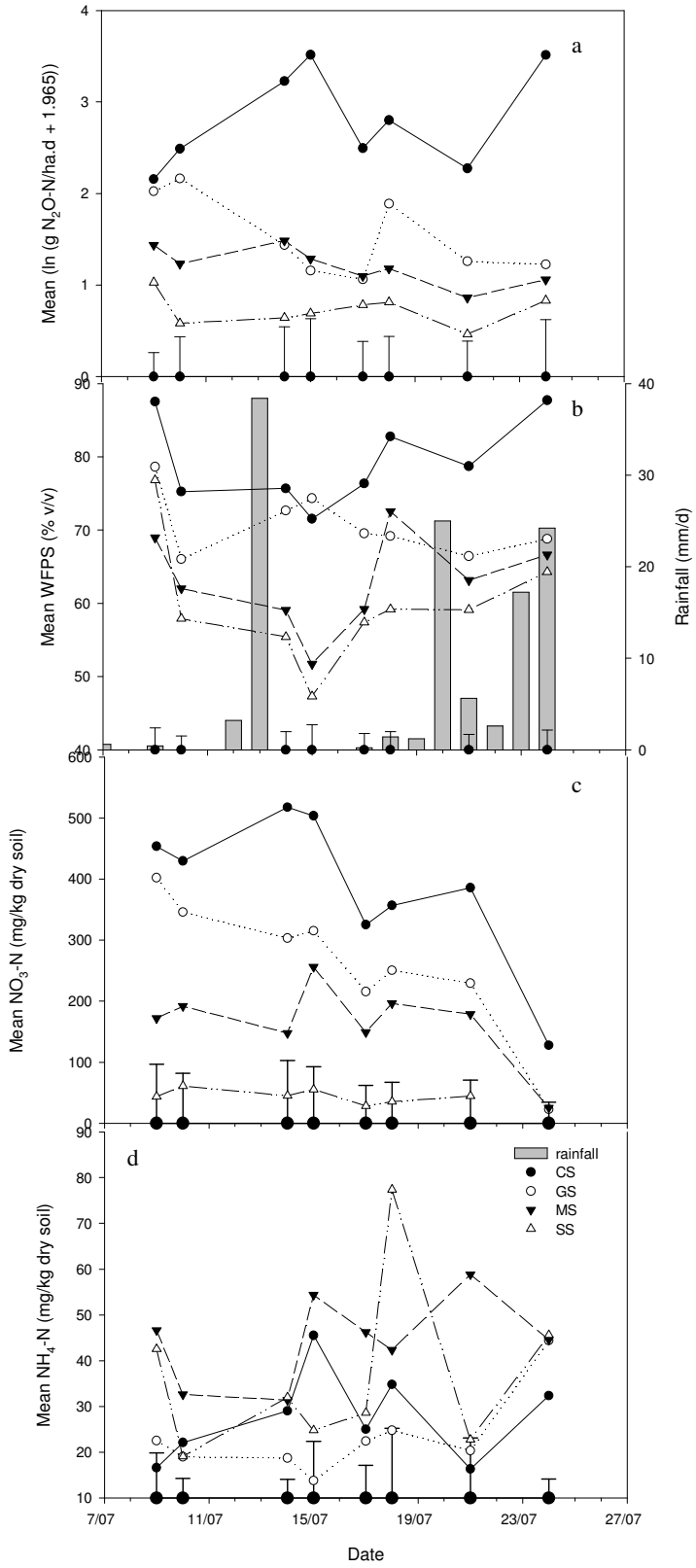


Figure 6.3 Time series of mean a) N_2O emissions (ln (g N_2O-N/ha.d+1.965)), b) WFPS (%) and rainfall distribution (mm/d), c) soil NO_3^- (mg NO_3-N/kg dry soil), d) soil NH_4^+ (mg NH_4-N/kg dry soil) for slope treatments; Campsites (CS), Medium slopes (MS), Steep slopes (SS) and Gullies (GS). Bars indicate treatment sem.

6.32 Topography

Estimated total land areas were 0.333 and 0.282 ha for paddock 1 and 2, respectively (Table 6.3). The proportions of land area estimated to represent each slope class were in the order Medium (41%)> Campsite (39%)> Gully (19%)> Steep (2%) for paddock 1 and Medium (53%)> Gully (25%)> Steep (19%)> Campsite (3%) for paddock 2 (Table 6.3). When the land areas of both paddocks were combined the land area represented by each of the slope classes was Medium (47%)> Campsite= Gully (22%)> Steep (10%, Table 6.3).

Table 6.3 Summary of estimated land area (ha) covered by slope classes in experimental paddocks: Campsites (CS), Medium slopes (MS), Steep slopes (SS) and Gullies (GS)

Paddock	Slope code	Slope class (º)	Estimated area (ha)	Mean measured slope (º)	Proportion of total (%)
1	**CS**	**0-12**	0.128	6.7 (2.9-11.1)	39
	MS	**13-25**	0.136	17.8 (10.7-21.7)	41
	SS	**>26**	0.007	30.9 (22.1-40.8)	2
	GS	**n/a**	0.062	10.4 (3.5-16)	19
Total			**0.333**		**101**
2	**CS**	**0-12**	0.008	7.8 (0.3-12.7)	3
	MS	**13-25**	0.151	20.5 (13.9-24.9)	53
	SS	**>26**	0.053	32.7 (25-42.7)	19
	GS	**n/a**	0.070	11.9 (1.8-20.5)	25
Total			**0.282**		**100**
Combined	**CS**	**0-12**	0.136	7.3	22
	MS	**13-25**	0.287	19.2	47
	SS	**>26**	0.060	31.8	10
	GS	**n/a**	0.132	11.2	22

6.33 Climate

In the week preceding the first day of measurements approximately 30 mm of rain fell at this site (*data not displayed*). During the measurement period a total of 120 mm rainfall was recorded. Rainfall distribution (mm/d) over the measurement period is displayed in Figure 6.3.

The range of temperatures recorded during the measurement period was widest on Steep and narrowest for Gully plots. No effect of slope was detected for chamber or soil temperatures (*data not displayed*).

6.34 Soil physical and chemical properties

The range of bulk density values measured was large (Table 6.4). There were a large number of low bulk density values in the Gully plots, particularly in paddock 1, and therefore a low mean bulk density value was determined for this treatment. Bulk density increased significantly as slope increased ($P < 0.01$, Table 6.4). Mean bulk density determined for each treatment was used to estimate daily WFPS for the soil samples taken during the gas sampling campaign and these values are displayed in Table 6.4.

The range in mean field capacity (FC) values was between 45 and 52% across all plots and significantly decreased as slope increased ($P < 0.01$, Table 6.4).

The ANOVAs for other soil physical parameters found that mean total porosity (%), pores 30 – 300 µm and WFPS (%) significantly decreased ($P < 0.01$, $P < 0.05$ and $P < 0.01$, respectively) as slope increased in the order of Gully< Campsite< Medium< Steep (Table 6.4). Macroporosity (> 300 µm %) was not significantly affected by slope class (Table 6.4).

Table 6.4 Mean pore size distribution (<30, 30-300, >300 µm), bulk density (g/cm^3) and WFPS (%) for slope treatments; Campsites (CS), Medium slopes (MS), Steep slopes (SS) and Gullies (GS), with ranges in parenthesis. Data are the means of 4 sub-replicate samples from plots for; pore size distribution, total porosity and bulk density (0-50 mm depth). Data for WFPS are the means and ranges estimated from all sampling occasions

	Pore distribution (v/v %)					
Slope	**<30 µm (field capacity)**	**30-300 µm**	**>300 µm**	**Bulk density (g/cc)**	**Total porosity (%)**	**WFPS (v/v %)**
CS	50.4 (35.9-59.4)	9.5 (6.9-10.0)	6.1 (4.0-8.7)			
MS	47.5 (38.8-54.8)	9.2 (6.0-13.2)	8.9 (3.9-13.0)	0.88 (0.78-1.02)	66.0 (60.7-70.2)	88.7 (32.9-100.0)
SS	45.4 (40.8-49.5)	8.5 (6.7-11.7)	9.3 (5.0-14.0)	0.89 (0.81-0.96)	65.7 (62.9-69.0)	62.9 (34.9-89.4)
GS	52.4 (41.3-61.4)	12.5 (6.4-12.8)	6.6 (7.1-11. 6)	0.96 (0.86-1.13)	63.1 (56.4-66.9)	59.7 (31.7-91.7)
Sig.	**	*	ns	0.74 (0.38-0.98)	71.7 (62.2-85.5)	70.8 (52.9-100.0)
sed	1.9	1.4	1.4	**	**	**

*: P< 0.05
**: P< 0.01
ns: not significant

All soil chemical variables were significantly affected by slope class (Table 6.5). Soil NH_4^+ (mg NH_4-N/kg dry soil) was significantly higher ($P< 0.01$) in Medium and Steep slopes compared to the Gully and Campsites. Mean soil NH_4^+ data for the Gully slopes however was influenced by one high value measured on July 18 (Figure 6.3). There were no other observable trends for soil NH_4^+ levels over the time series in Figure 6.3. Soil NO_3^- (mg NO_3-N/kg dry soil) and pH decreased significantly ($P< 0.001$) as slope increased in the order Steep< Medium< Gully< Campsites (Table 6.5). The range in soil NO_3^- levels for all treatments was large (Table 6.5). In the time series, mean soil NO_3^- levels increased and decreased at similar times across all slope treatments over the measurement period (Figure 6.3).

Table 6.5 Mean soil NH_4^+, NO_3^- (mg N/kg dry soil) and pH (25 x 75 mm depth) for slope treatments; Campsites (CS), Medium slopes (MS), Steep slopes (SS) and Gullies (GS), with ranges in parenthesis

	mg NO_3-N/kg dry soil	**mg NH_4-N/kg dry soil**	**pH**
CS	392.8 (50.4-1007.5)	25.8 (7.7-104.8)	5.9 (5.6-6.3)
MS	138.5 (4.7-382.5)	43.3 (12.6-123.5)	6.0 (5.5-6.5)
SS	32.6 (4.6-133.6)	33.1 (8.3-138.5)	5.7 (5.4-6.0)
GS	290.5 (5.5-519.8)	26.1 (6.0-75.5)	5.6 (5.2-5.9)
Sig.	***	*	***
s.e.d	39. 6	7.6	0.1

*: P< 0.05
***: P< 0.001

6.35 Nitrous oxide, topographical, soil physical and chemical interactions

Nitrous oxide emissions, soil NO_3^-, soil NH_4^+ and WFPS were highly spatially and temporally variable, even within slope treatments (Table 6.2 and Figure 6.2). Despite this variation, all these variables were significantly higher in the Campsite plots and significantly lower in the Steep slopes ($P< 0.001$ and $P< 0.01$ for soil NO_3^- and WFPS, respectively, Tables 6.5 and 6.4). There were no linear relationships between soil variables or slope and N_2O emissions, when the individual data points were plotted (Figure 6.4). All high N_2O emissions however occurred on slopes < 20° (Figure 6.4).

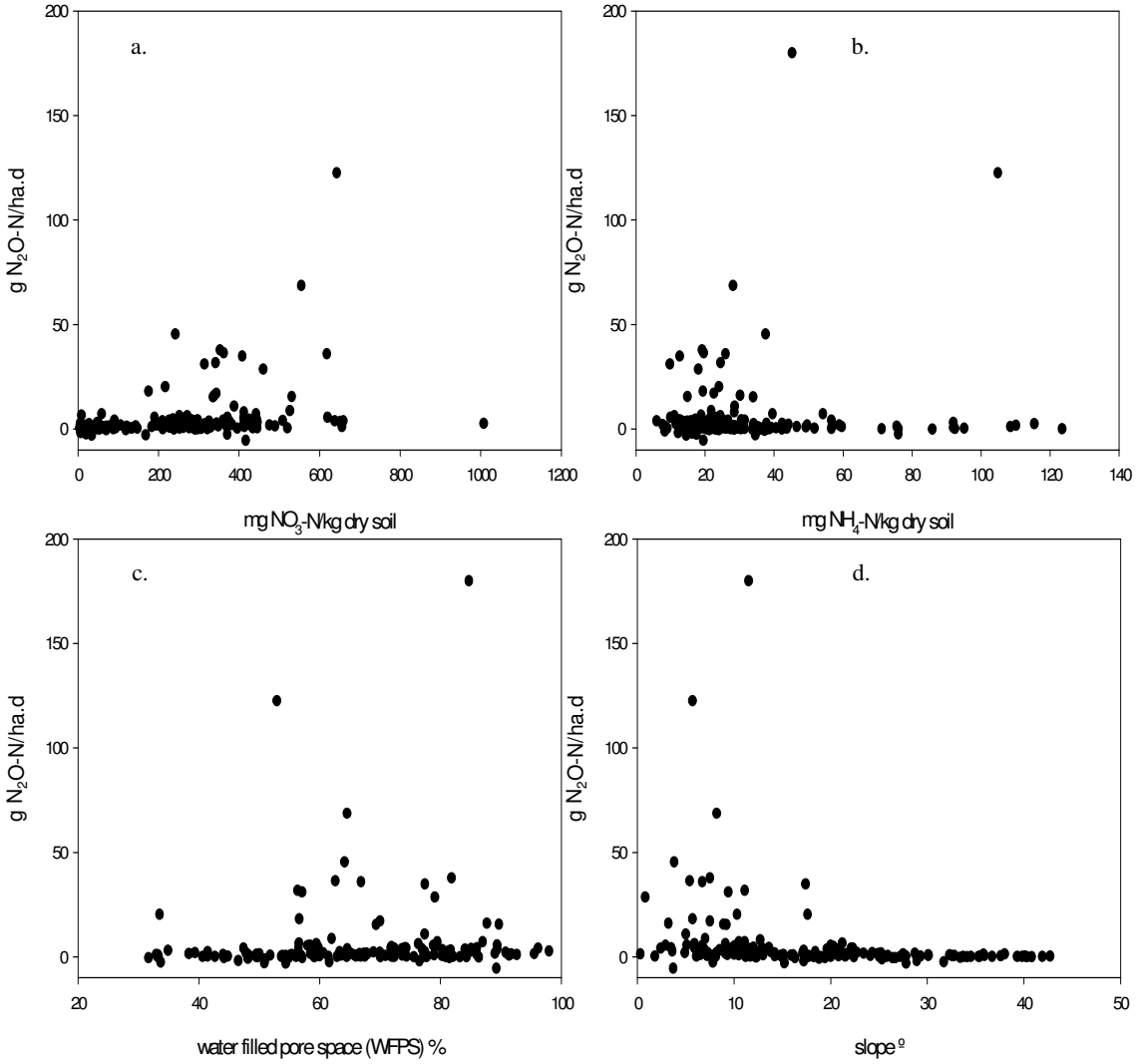


Figure 6.4 Relationship between individual data points of g N_2O-N/ha.d and a) soil NO_3^- (mg NO_3-N/kg dry soil), b) soil NH_4^+ (mg NH_4-N/kg dry soil), c) WFPS (%), d) slope º

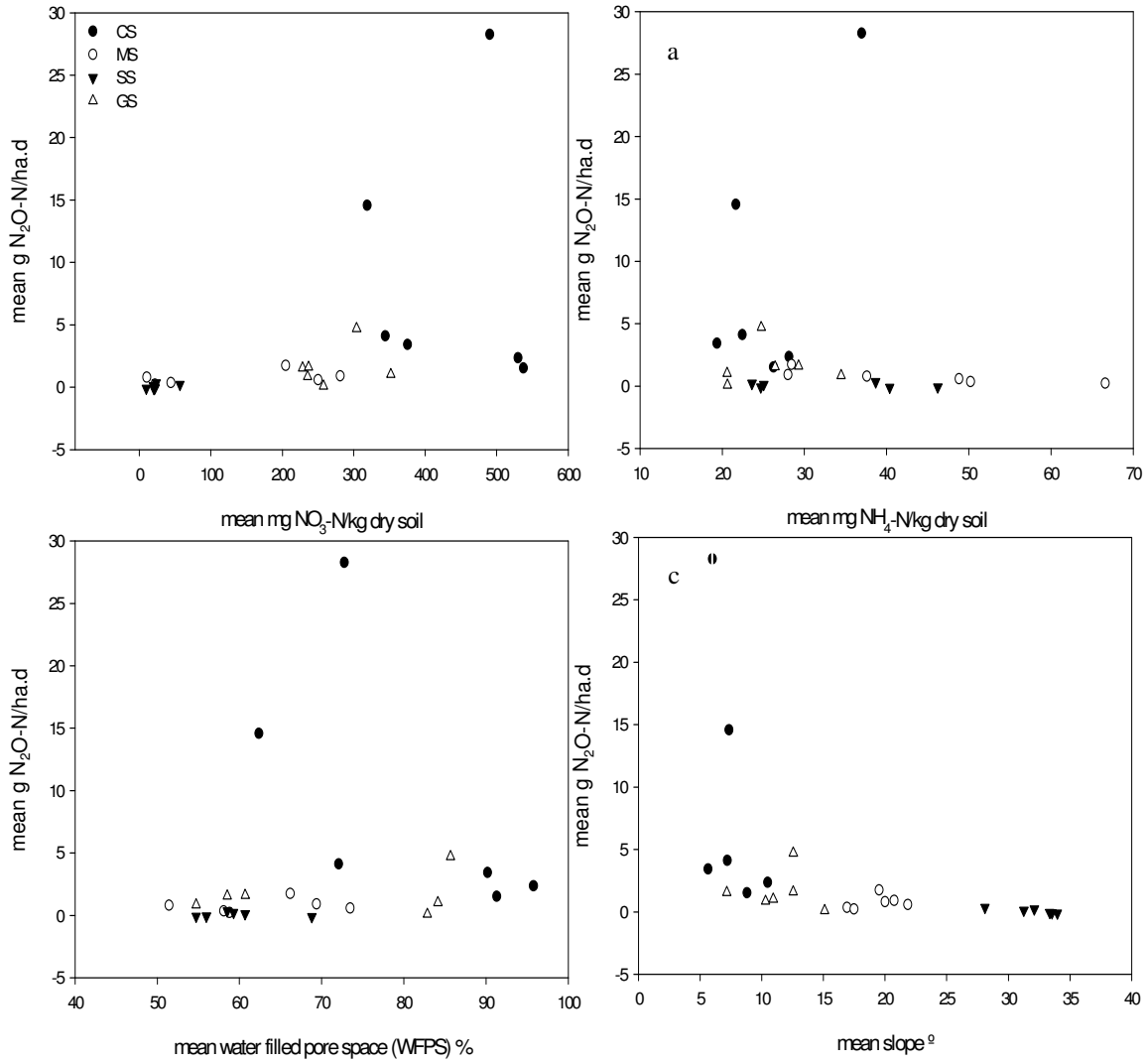


Figure 6.5 Relationship between plot mean g N_2O-N/ha.d and a) soil NO_3^- (mg N/kg dry soil), b) soil NH_4^+ (mg N/kg dry soil), c) WFPS %, d) slope (º)

When some of the temporal and spatial variability in the N_2O emission data was removed by calculating plot means, relationships between soil variables soil NO_3^-, NH_4^+ and WFPS remained weak (Figure 6.5). Mean N_2O emissions increased on mean slopes < 10° (Figure 6.5).

The N_2O flux data were log transformed to perform regression analyses. Due to the occurrence of negative gas fluxes, a constant (0.571) was added to daily plot mean N_2O data for each measurement day before transforming the data (as described in the methods section). Plot means for soil NO_3^-, NH_4^+ and WFPS were used to perform the regression analysis against plot mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)). The linear regression analyses in Figure 6.6 found that mean slope was significantly negatively related ($P< 0.01$), and mean soil NO_3^- was significantly positively related ($P< 0.01$) to mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)). The relationship between mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) and slope however was better described by a logarithmic curve (Figure 6.7).

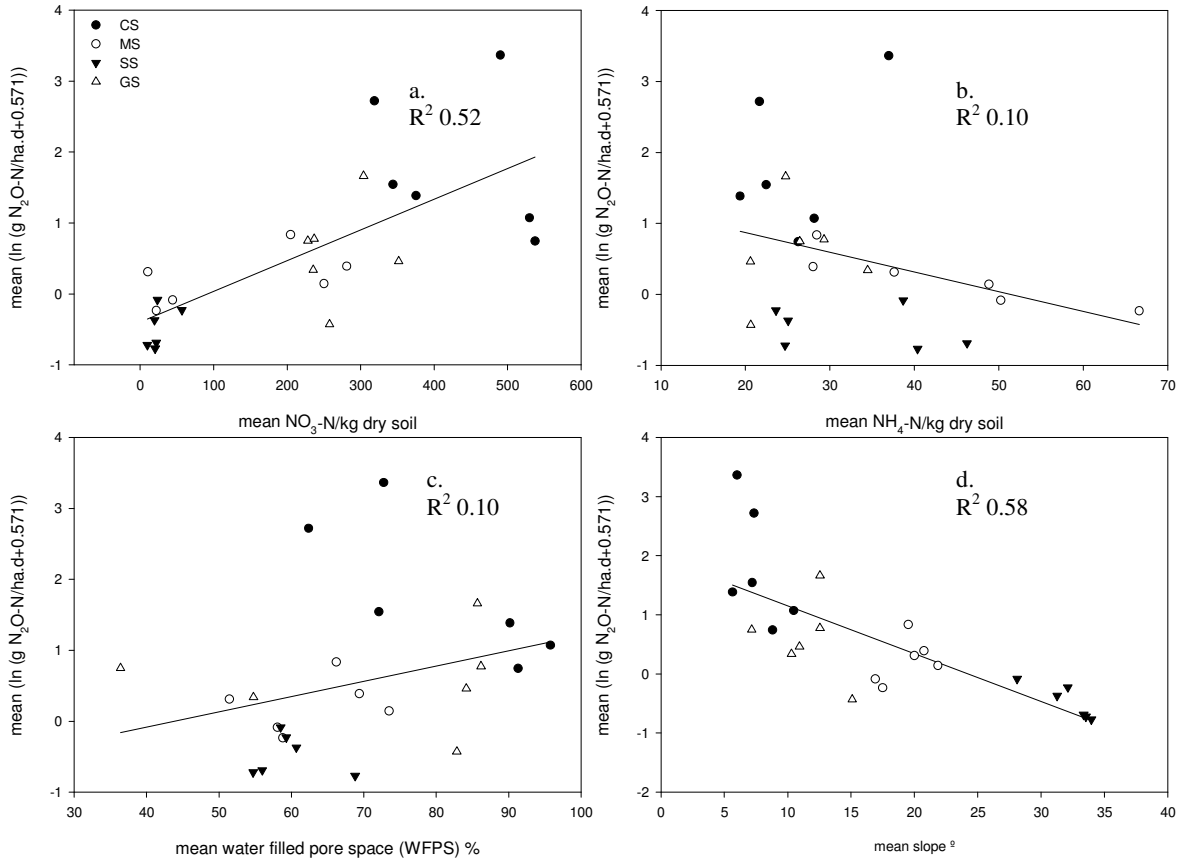
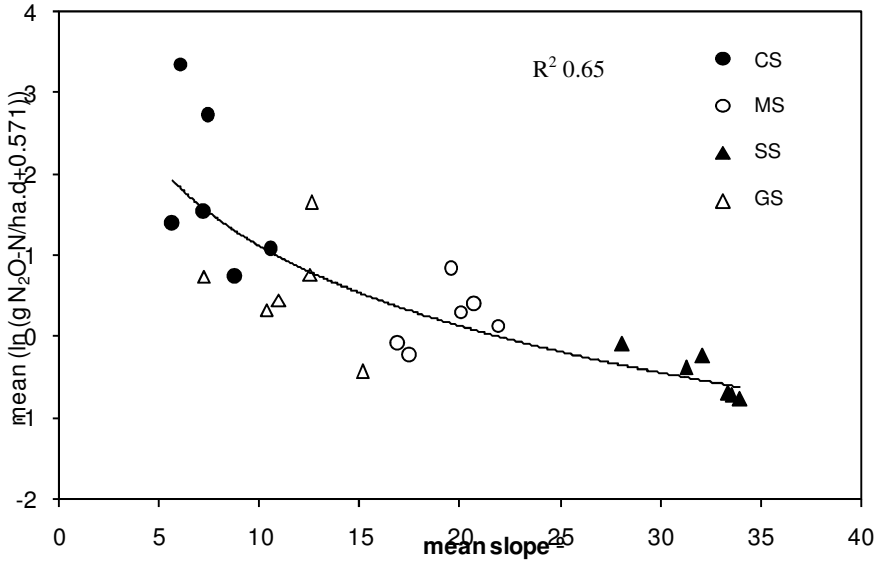


Figure 6.6 Linear relationship between mean plot N_2O emissions (ln (g N_2O-N/ha.d+0.571)) and a) soil NO_3^- (mg NO_3-N/kg dry soil), b) soil NH_4^+ (mg NH_4-N/kg dry soil), c) WFPS (%), d) slope (º)



6.7 Logarithmic relationship between mean plot N_2O emissions (ln (g N_2O-N/ha.d+0.571)) and slope (º)

In contrast to the previous chapter, a 3-dimensional graph of mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) against soil NO_3^- and WFPS (Figure 6.8a) did not show any clear relationship. Although, N_2O emissions generally increased as soil NO_3^- increased. However, a similar 3-dimensional graph of mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) against soil NO_3^- and land slope (Figure 6.8b) did demonstrate that high N_2O emissions only tended to occur on low slopes and when soil NO_3^- were high.

Table 6.6 Stepwise regression analysis of the linear relationship between mean plot N_2O emissions (ln (g N_2O-N/ha.d+0.571)), slope (°), soil NO_3^- (mg NO_3-N/kg dry soil) and WFPS (%)

Constant	Fitted terms						
Mean (ln (g N_2O-N/ha.d+0.571))	**Mean slope °**	**Mean mg NO_3-N/kg dry soil**	**Mean WFPS %**	**Residual mean squares**	**Variation %**	**s.e.**	**P**
*	*			0.48	55.9	0.7	0.001
*	*	*		0.46	57.8	0.7	0.172
*	*	*	*	0.45	58.8	0.7	0.231

The 3-dimensional relationship in Figure 6.8a shows that mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) generally increased as soil NO_3^- increased. There was no consistent relationship between mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) and WFPS. This was the likely reason that no significant relationship was detected between N_2O mean emissions (ln (g N_2O-N/ha.d+0.571)) and WFPS in either of the regression analyses (Figure 6.6 and Table 6.6). The 3-dimensional relationship in Figure 6.8b demonstrates that both mean soil NO_3^- levels and N_2O emissions (ln (g N_2O-N/ha.d+0.571)) generally increased as slope decreased.

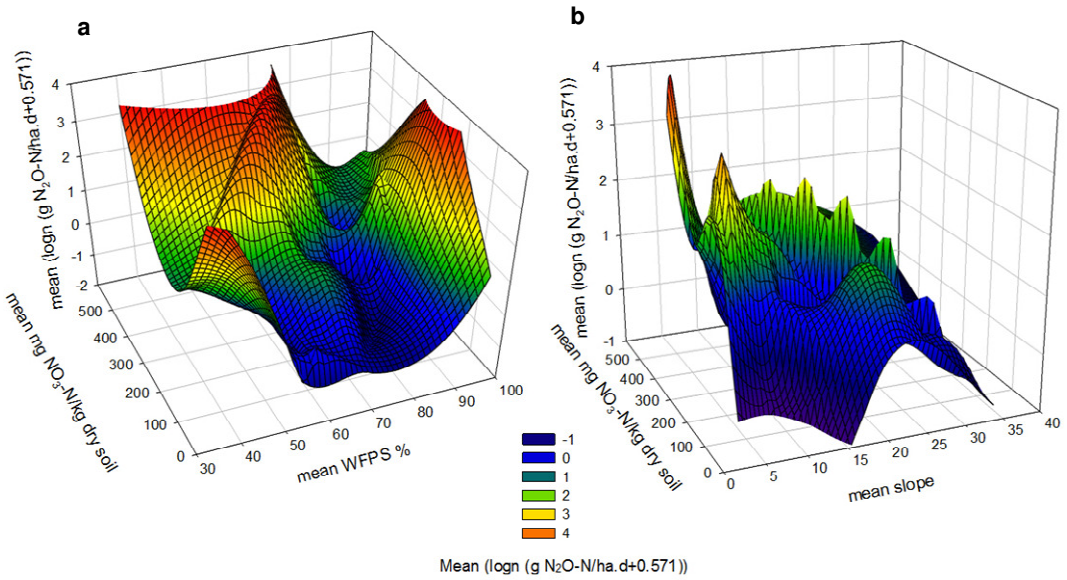


Figure 6.8 Three dimensional relationship between mean a) plot N_2O emissions (ln (g N_2O-N/ha.d+0.571)), soil NO_3^- (mg NO_3-N/kg dry soil) and WFPS, b) plot N_2O emissions (ln (g N_2O-N/ha.d+0.571)), soil NO_3^- (mg NO_3-N/kg dry soil) and slope (º) in sheep-grazed hill country

Nitrous oxide emissions from plots were integrated to estimate the total loss from the paddock areas over the measurement period for each slope class. The combined total N_2O emissions were in the order CS > MS = GS > SS (0.05, 0.01, 0.01 and 0.00 kg N_2O-N/ slope class over the measurement period, respectively), which represented 81%, 10%, 9% and 0% of the total emissions, based on the proportion of paddock area estimated for each slope class (Table 6.7). The results in Table 6.7 demonstrate that the high proportion of N_2O emissions estimated to come from Campsites (on a combined area basis), was heavily influenced by N_2O emissions from paddock 1 Campsite plots. The Medium and Gully slope results were similarly influenced by N_2O emissions in paddock 2.

Table 6.7 Estimated proportions of N_2O emissions produced from slope classes; CS (Campsites), MS (Medium slopes), SS (Steep slopes), GS (Gully slopes)

Slope code	Slope class (º)	Area covered by slope class (ha)	kg N_2O-N/ha slope class	Total N_2O-N/measured slope class (kg N)	N_2O-N released from slope classes (%)
Paddock 1					
CS	0-12	0.128	0.41	0.158	93
MS	13-25	0.136	0.01	0.006	2
SS	>26	0.007	<0	0.000	0
GS	n/a	0.062	0.04	0.007	4
		0.333		**0.171**	**100**
Paddock 2					
CS	0-12	0.008	0.07	0.001	6
MS	13-25	0.151	0.03	0.005	53
SS	>26	0.053	0.00	0.000	0
GS	n/a	0.070	0.05	0.004	41
		0.282		**0.009**	**100**
Combined					
CS	0-12	0.136	0.392	0.053	81
MS	13-25	0.287	0.022	0.006	10
SS	>26	0.060	0.000	0.000	0
GS	n/a	0.132	0.045	0.006	9
Total		**0.615**		**0.065**	**100**

6.4 Discussion

The results of this short term study demonstrate that mean N_2O emissions from Campsite plots were significantly and consistently higher than those from the plots of other slope classes (Figure 6.2 and Table 6.2). The results also indicate that average N_2O emissions from the Steep slope class were negligible. The mean N_2O emissions estimated for Gullies (5.0 g N_2O-N/ha.d, Table 6.2) were lower than expected, and not significantly different from the mean N_2O emissions from Medium plots. The hypothesis that N_2O emissions from Gullies would be higher than other slope classes was based on the knowledge that gully areas tend to be wet for extended periods, and that soluble NO_3^- is likely to be transported to depression areas in soil solution. The lower than expected results from Gullies may indicate that complete denitrification to di-nitrogen (N_2) is taking place due to the saturated soil conditions, or that N_2O emissions are restricted through standing water in these areas. It was observed in the field that immediately following rainfall, gas chambers in Gullies were often situated in standing water. It could also be possible that NO_3^- supply for denitrification in these areas may be reliant on *in situ* nitrification which may be inhibited due to the anoxic state of saturated soils in gully areas, which is common in winter at this site. Nitrous oxide emissions from the Gully plots were also relatively stable compared to the 'spiky' distribution observed for Camp and Medium slopes (Figure 6.2), which is common when measuring N_2O emissions from agricultural soils (Yates *et al.* 2006).

The results in this Chapter were generally similar to those reported by others (Carran *et al.* 1995; Hoogendoorn *et al.* 2008; Letica *et al.* 2010; Parker 2008; Saggar *et al.* 2007b), except in the Campsite plots used in this study. Mean N_2O emissions in Campsite plots in our study were much higher (27.1, range -5.5–180.0 g N_2O-N/ha.d, Table 6.2), than results from previous workers in hill country. For example Parker (2008) also found that N_2O emissions were significantly higher from flat wet areas compared to steep slopes, although lower N_2O emissions from low slopes (0 to 6 g N_2O-N/ha.d) were measured by this worker. Similarly, Hoogendoorn *et al.* (2008) found that mean daily N_2O emissions from flat to medium slopes in 0 N paddocks were only 2 to 5 g N_2O-N/ha.d at Ballantrae (North Island), and 3 to 11 g N/ha.d in rolling hill country at Invermay (South Island). Carran *et al.* (1995) showed that N_2O

emissions from a steep sheep grazed hill country site was generally > 5 g N_2O-N/ha.d during the 9 month measurement period, and the stock camping area rarely exceeded 10 g N_2O-N/ha.d during the same sampling campaign also. Mean N_2O emissions (9 g N_2O-N/ha.d) from rolling hill country 0 N paddocks in Letica *et al.* (2010) were similar to all slopes except Campsites used in the current study. The mean N_2O emissions from grazed and un-grazed sheep pastures receiving 80 kg N/ha.y on flat land in the Manawatu region in NZ (7.4 and 3.4 g N_2O-N/ha.d in Saggar *et al.* (2007b)) were also lower than results from Campsites in the current study.

There were also a number of negative fluxes calculated in the data set (14% of flux values, Table 6.2 and Figure 6.2), which was similar to the results in Chapter 5 (fertiliser N rate trial). As in Chapter 5, attempts were made to determine whether the negative fluxes measured could be explained by variability in sampling and analytical methods, or were likely to be "real". Two approaches were used. As noted in Section 3.37, Hedley *et al.* (2006) had assessed the precision of the analytical equipment used in this study to determine concentrations of N_2O. These workers reported a minimum detection limit (MDL) for N_2O of 7.4 ppb and from this calculated a flux-detection limit of (FDL) of 0.0019 mg N_2O-N/m^2.h. The equivalent FDL for this study would be 0.003 mg N_2O-N/m^2.h , after chamber dimension corrections have been made to the calculation. Therefore approximately 39% of the negative fluxes calculated in this study were within this FDL. However errors that may arise during the collection of gas from the chambers in the field were not considered by Hedley *et al.* (2006). The likely importance of such field-based errors was highlighted by the observation that the three largest negative fluxes in the current study resulted from t0 measurements that were abnormally high. The variations in t0 measurements were therefore used to estimate the errors in the sampling and analysis of N_2O in the current study, as in Chapter 5. Based on the measured variability in the t0 N_2O concentrations and using the same assumptions outlined in Chapter 5, the calculated FDL was approximately 0.017 mg N_2O-N/m^2.h. This is much greater than the FDL (0.003 mg N_2O-N/m^2.h) calculated from analytical errors alone by Hedley *et al.* (2006). If a FDL of 0.017 mg N_2O-N/m^2.h is assumed, then the vast majority of apparent negative fluxes are within this FDL and should therefore be interpreted with caution as conclusive evidence of negative N_2O fluxes in hill country. All the calculated N_2O fluxes (both positive and

negative) were however included in calculations of average fluxes from different slope categories within the paddocks

The recent grazing in paddock 1 (two to three weeks prior to the start of the measurement period), and therefore the recent addition of substrate N for nitrification and subsequent denitrification may have influenced the results. It is also important to note that the higher N_2O emissions measured in this study may be due to the fact that measurements were taken during optimum conditions for N_2O emissions (i.e. high rainfall and soil moisture conditions). Measurements were also taken over a short period, and therefore may not reflect the longer term trend for N_2O emissions from each slope class. For example, the depression gully areas typically remain wet for longer over the summer period and may therefore produce more N_2O emissions compared to campsites at that time of the year. The possibility of seasonal variation in soil conditions as influenced by slope, highlights an important area of future N_2O research in hill country for accurate spatial and temporal integration of annual N_2O losses in sheep grazed hill country pastures.

Nitrous oxide gas measurements did not start until a significant amount of rainfall had occurred to ensure soil moisture levels at the site were consistently high over a two to three week period, and sufficiently elevated (i.e. at or above field capacity) to provide optimum conditions for N_2O gas production (Saggar *et al.* 2007b). Measurements were therefore made in the winter, as this is historically a high rainfall season in North Island hill country in New Zealand. There were several significant rainfall events (>10 mm/d) over the measurement period (Figure 6.3). Rainfall, mean WFPS and N_2O emissions (ln (g N_2O-N/ha.d+1.965)) were not consistently related in the time series graph over the measurement period (Figure 6.3). This is different to the observations made by de Klein *et al.* (2003) and Saggar *et al.* (2007b), where N_2O emissions and WFPS typically spike at similar times in response to rainfall events. This implies that soil processes other than denitrification may have been a source of N_2O at certain times during the measurement period. Chamber temperatures ranged between 7.1 and 20.2 ºC over the measurement period; however no effect of slope on temperature was detected (*data not shown*). Gillingham (1973) measured significant differences in grass temperatures on north and south aspects in North Island hill country and daily mean grass temperatures remained below 5 ºC for most of the

winter season on southerly slopes. Grass temperatures however never fell below this threshold at any stage of the year on northerly slopes. Aspect was not recorded for the position of plots, but this may have influenced the results and help to explain the higher mean N_2O emissions from campsites in paddock 1 compared to paddock 2 in the current study.

The consistently higher and wider ranging N_2O emissions measured in paddock 1 compared to paddock 2 demonstrates the range of N_2O emissions that may occur even within slope classes, particularly in Campsite plots (Table 6.2 and Figure 6.2). It also suggests that parameters other than those measured in this study may influence spatial variation in N_2O emissions in this dissected pastoral environment. Complete denitrification and the reduction of NO_3^- to N_2 in wet conditions may also explain the lower N_2O emissions from paddock 2, as it was observed that paddock 2 was wetter than paddock 1. Mean WFPS however was similar between the two paddocks (Table 6.2). Climatic variables such as elevation and aspect have the potential to further influence N dynamics in hill country environments by creating temperature, solar radiation and soil moisture differences (Gillingham, 1973). Many of these variables can also modify animal behaviour and therefore excreta N transfer, as in Sakadevan *et al.* (1993b). Changes in soil texture or drainage class over short distances in hill country may also impact on soil moisture levels and subsequent N_2O emissions, as in Ball *et al.* and McTaggart *et al.* (1997; 2002). The fact that paddock 1 was grazed closer to the time of experimentation could have influenced results by leading to elevated soil mineral N levels in paddock 1, due to the recent addition of substrate N via excretal N deposition. The results however show that mean NO_3^- levels were higher in paddock 2 over the measurement period (Table 6.2), and NH_4^+ levels were similar between paddocks (*data not shown*).

The soil cores taken for the determination of bulk density and pore distribution were unable to be removed from the chamber areas immediately after gas sampling. This was due to the destructive nature of the sampling technique as well as the difficulty of removing "undisturbed" samples from extremely wet or saturated soils in many of the plots. As a result, the WFPS values estimated for each sampling occasion were based on bulk density measurements taken from 4 samples within each plot area on one occasion. Bulk density measurements for Gully slopes (Table 6.4) were likely

affected by the large organic layer observed in many of the samples taken from these areas. The often thick dung layer on Campsites (Letica e*t al.* 2006) may also have affected bulk density estimates in soil cores taken from these sites. There was however a good level of consistency in the values determined for bulk density within slope classes in this study (Table 6.4). Paddocks also remained under a similar grazing rotation during the time it took for soils to become suitable for sampling. Therefore bulk densities were not expected to change markedly within this time frame (Lambert *et al.* 2000).

The ANOVA detected a significant difference in bulk density between slopes in our study (Steep slopes< Medium slopes< Campsites< Gullies, P< 0.01), which was also found in Lambert *et al.* (2000), (steep slopes< medium slopes< flat slopes). The significant effect of slope was not detected in the work by Parker (2008). Most notable in our study was the low mean bulk density (0.74 g/cc) estimated for paddock 1 Gully slopes compared to other slope classes (Table 6.4). Most bulk density measurements in this study were within the range measured in earlier work on Ballantrae (mean 0.97 and range 0.78 to 1.11 g/cc, *data unpublished*), and were lower than values obtained by Parker (2008, 0.99 to 1.04 g/cc).

There are no porosity or pore size distribution data available for Ballantrae hill slopes to compare our results to. Mean FC for each slope class decreased significantly (P< 0.001, Table 6.4) as slope increased. Total porosity was similarly affected by slope (P< 0.001, Table 6.4), as expected based on bulk density results. These results are not dissimilar to Velthof *et al.* (2000) and Pierson and Mulla (1990).

Mean WFPS decreased significantly (P< 0.001) as slope increased in the current study (Table 6.4). There was a clear separation between mean WFPS levels in Campsites and Gullies compared to Medium and Steep slopes throughout the measurement period (Figure 6.3). Similar observations of the effect of slope on WFPS were made by Parker (2008). Velthof *et al.* (2000) and Pierson and Mulla (1990) also found that soil moisture content was greatest in the foot and toe slopes due to run off from the upper slopes. Velthof *et al.* (2000) observed that higher soil moisture contents in foot slope areas were due to the occurrence of slower draining clay soil.

Mean soil mineral N levels in the current study (32-393 mg NO_3-N and 26-43 mg NH_4-N/kg soil) were much higher than those found by previous workers and the reason for this is not clear. For example, mean mineral N levels in Parker (2008) were 5 mg NO_3-N and 6 mg NH_4-N/kg soil, however the significant slope effect on soil NO_3^- levels (flat slopes> steep slopes) was also detected by this worker. Mean soil NO_3^- levels in Letica *et al.* (2006) were 30 to 96 mg NO_3-N/ kg soil, however the effect of slope was detected again (flat slopes > steep slopes). The effect of slope was very pronounced for soil NO_3^- levels and highly significant in the current study (Campsites> Gullies> Medium slopes> Steep slopes, P< 0.001, Table 6.5). Soil NH_4^+ levels (17 to 38 mg NH_4-N/kg soil) were similar in Letica *et al.* (2006) however no significant slope effect was detected. Mineral N was not specifically measured in Lambert *et al.* (2000) however the soil fertility parameters that were measured (i.e. Olsen P, Total N, exchangeable K) generally declined with increasing slope.

Soil pH also decreased significantly as slope increased in this study (Campsites> Gullies> Medium slopes> Steep slopes, P< 0.001). Parker (2008) detected the same effect of slope on soil pH levels (flat slopes> steep slopes). Slope had no effect on pH levels in Lambert *et al.* (2000) or in Letica *et al.* (2006). Mean pH levels were 5.4 and 5.3 in these studies, respectively. Slope effects on soil properties at Ballantrae were not measured by Stevens *et al.* (2008) however mean soil pH was 5.2 in 2004 and was 5.4 when last measured in 2008 in the same 0 N treated paddocks. This change over the 4 years of the trial was not significant.

The individual data points in this short term study demonstrate that there was a high degree of spatial and temporal variation in the magnitude of N_2O emissions (g N_2O-N/ha.d), as well as other soil variables (NO_3^-, NH_4^+ and WFPS), even within slope classes (Figures 6.2 and 6.4 and Tables 6.2, 6.4 and 6.5). The relationships between individual N_2O emission measurements and the corresponding soil variables were generally weak, although high N_2O emissions only occurred on slopes < 20° (Figure 6.4). The results in this Chapter are in contrast to the positive (but not significant) relationship between N_2O emissions (g N_2O-N/ha.d), soil NO_3^- (mg NO_3-N/kg dry soil) and WFPS (%) in Chapter 5. Numerous workers have also described a similar relationship between these variables previously (Clayton *et al.* 1997; de Klein *et al.* 2003; Dobbie and Smith 2001; Letica *et al.* 2010).

In this study soil variables (NO_3^-, NH_4^+ and WFPS), slope and N_2O emissions were then averaged to reduce variation in the data, but the relationships between mean N_2O emissions and mean soil variables (NO_3^-, NH_4^+ and WFPS) remained weak (Figure 6.5). The relationship between mean N_2O emissions and slope however was improved, showing that high N_2O emissions (mean g N_2O-N/ha.d) occurred on a mean slope of < 10° (Figure 6.5). Mean N_2O emissions were then log transformed (ln (g N_2O-N/ha.d+0.571)) for regression analysis against mean soil variables (NO_3^-, NH_4^+ and WFPS) and slope. Mean soil NO_3^- and slope were significantly related to mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) in the linear regression analysis (P> 0.01 Figure 6.6). The relationship between mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) and slope however was best described by a logarithmic curve (Figure 6.7). The step wise regression found that the relationship between mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) and slope was significant (P< 0.001), explaining 55.9% of the variance. Adding soil NO_3^- and WFPS improved the relationship, but not enough to be significant (Table 6.6).

The value of investigating the relationship of raw, mean or log transformed N_2O emissions data to soil variables (soil NO_3^-, NH_4^+ and WFPS) and slope is highlighted in this study. Raw (actual) N_2O fluxes were poorly related to soil variables. This may suggest that even removing soil samples from directly beneath gas chambers themselves was not sufficient to obtain a true representation of the soil conditions in the "hot spots" where denitrification was occurring. The method of measuring the actual slope at each gas sampling site did however prove valuable and demonstrated that N_2O emissions only increased on slopes < 20° and were significantly higher on slopes < 10°. By meaning the data the variation was reduced and did allow some trends in data to be observed (Figure 6.5), although it was still only land slope that appeared to have a consistent relationship with N_2O emissions. However, fitting the N_2O data to a log-normal distribution allowed us to determine by simple linear regression that mean slope and soil NO_3^- were significantly related to mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) (P< 0.01, Figure 6.6). The relationship between mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) and slope however was best described by a logarithmic relationship (Figure 6.7). In the step wise regression mean slope was significantly (P< 0.001) negatively related to N_2O emissions, and had the

strongest relationship with mean N_2O emissions (ln (g N_2O-N/ha.d+0.571)) of all variables measured (Table 6.6). The fact that other soil variables did not significantly improve the step wise regression analysis may suggest that slope was acting as an easily measureable variable that encompassed the general trend that soil NO_3^-, WFPS and N_2O emissions were all higher in Campsites, and much lower on Steep slopes. Medium and Gully plots had generally low to intermediate levels for most soil variables and N_2O emissions by comparison.

The effect of slope on increasing soil nutrient levels (e.g. soil N, P and C), as well as N cycling, subsequent pasture growth rates and annual net herbage yields, has been well documented by numerous workers (Ball *et al.* 1982; Bowatte 2003; Carran and Saggar ; Florinsky *et al.* 2004; Gillingham 1982; Lambert *et al.* 2000; Letica *et al.* 2006; Parker 2008; Sakadevan *et al.* 1993a). Integration of measured N_2O emissions estimated that 81% of N_2O emissions were produced on Campsite slopes (0-12°), which represented only 22% of the total experimental land area in this study. The significant relationship between N_2O emissions and slope class therefore appears to be a succinct method to summarise the combined effect and relationships of many soil and environmental parameters on N_2O emissions in sheep grazed hill country. As measuring all of these soil and environmental parameters to predict or estimate N_2O emissions is often complex and difficult, 'slope class' appears to be a convenient and adequate way of spatially integrating N_2O emissions from sheep grazed hill country. The limitation of our study was its short duration and the low replication of very discrete slope class areas. However the literature suggests that the spatial patterns of N_2O emissions we observed in the current study often persist for extended periods of time (Velthof *et al.* 2000). In addition, some low lying Campsite areas may also have been included as Gullies in the reproduced DEM model due to the inability of the model to separate low slope areas and depression or gully areas in some cases. Visual inspection of the site suggested that this was an insignificant variation in the estimation of slope class coverage. The results of this study suggest that determining the long term trends for N_2O emissions from different slope classes may have important implications for estimating N_2O emissions from sheep grazed hill country.

6.5 Conclusions

The mean N_2O emissions estimated for the Gully slope treatment were lower than anticipated and therefore the null hypothesis, that emissions would be in the order Gullies> Campsites> Medium slopes> Steep slopes, was rejected. Based on these short term measurements, it was extrapolated that 81% of total N_2O emissions measured in this study were produced from Campsites, which represented only 22% of the total experimental land area. The Medium and Gully slopes produced a combined total of 19% of N_2O emissions, yet represented 69% of the experimental land area. The Steep slopes represented 10% of land area and produced negligible amounts of N_2O emissions in this study. Over the short term, high rainfall measurement period in this study Campsites produced the vast majority of N_2O emissions in sheep grazed hill country receiving no fertiliser N. This study suggested that 'slope class' was highly correlated with N_2O emission and this provides a convenient and easily measureable parameter for spatially integrating emissions from hill country pastures. The results also suggest N_2O mitigation options in hill country could be targeted at reducing emissions from Campsites. The cost of mitigation technologies such as the application of nitrification inhibitors could be substantially reduced by targeting application to hot spot N_2O emission areas, such as campsites in sheep grazed hill country. In addition, the highly significant relationship between slope class and N_2O emissions suggests that slope may be a suitable parameter for upscaling N_2O emissions from sheep grazed hill country.

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CHAPTER 7 GENERAL DISCUSSION

7.1 Nitrous oxide emissions from New Zealand agriculture

In the most recent National Inventory Report (NIR) of total greenhouse gas (GHG) emission, the agriculture sector was NZ's largest source of total emissions (47% or 34,826 Gg CO_2-e) and the second largest contributor was the energy sector (45% or 22,839 Gg CO_2-e, Ministry for the Environment 2010b). New Zealand's GHG profile is therefore unique amongst developed countries where GHGs from agriculture typically contribute less than 10% of the total GHG profile (Ministry for the Environment 2010b). New Zealand's agricultural GHG emissions are dominated by methane (CH_4) from enteric fermentation and nitrous oxide (N_2O) from excreta deposition on pasture, as well as from increasing amounts of nitrogenous fertiliser applications. Agriculture alone was responsible for 96% (11, 434 Gg CO_2-e) of total N_2O emissions in the most recent Inventory (Ministry for the Environment 2010b).

In hill country, nitrogen (N) cycling is spatially variable due to topographically driven differences in N inputs and soil moisture conditions. There is a body of literature to demonstrate that in hill country, soil N levels are generally higher in low sloped and sheltered areas where stock prefer to camp and therefore deposit most excreta, compared to steep and exposed faces where stock graze but do not camp (refer to Chapter 2). Greater rates of N cycling (in particular nitrification) in campsite areas, where the majority of dung and urine is deposited, are of considerable importance because of the potential to increase N losses through leaching and denitrification (de Klein and van Logtestijn 1994; Field *et al.* 1985; Letica *et al.* 2006; Parker 2008). Increases in N losses via N_2O emissions as a result of increased fertiliser N inputs and subsequent excretal N inputs have received very little attention in hill country. There is limited evidence to suggest that N_2O emissions may be lower and more spatially variable in hill country than on flat land, due to slope-driven differences in animal behaviour and the soil and climatic factors that affect N_2O emissions (Carran and Saggar 2004a; Hoogendoorn *et al.* 2008; Parker 2008). Spatial variation in N_2O emissions in hill country may reflect spatial variation in excretal N distribution or the

distribution of soil moisture and subsequent mineral N levels, as all of these factors are known to influence N_2O emissions. The cause for this spatial variation however is not yet clear in the literature. Currently in NZ's GHG inventory N_2O emission factors for fertiliser N (EF_1) and excreta N (EF_{3PRP}) are identical for all landscape units. That is to say, that all landscape units are currently assumed to produce the same amount of N_2O per kg N applied or excreted onto the soil surface.

7.2 Thesis objectives

The current thesis therefore aimed to investigate the influence of slope on nitrification rates (NP) and on N_2O emissions. The sites chosen for study were part of the wider 'Wise N Use' project to determine best management practices for fertiliser N use in hill country in NZ (refer to Chapter 3). For the purposes of the current Thesis, investigations into N_2O emission losses consisted of a series of short term paddock scale trials to determine the effect of increasing application rates of fertiliser N and the influence of topography on NP and N_2O emissions. The fertiliser N treatment rates selected for the investigations in this Thesis were chosen to determine the range of NP and N_2O emission rates that may occur under a range of management scenarios. The relationship between NP and N_2O emissions, and soil chemical (mineral N) and physical (pore size distribution, PSD and water filled pore space, WFPS) factors were also investigated. The study sites were typical of the topography encountered in sheep grazed hill country in the North (Ballantrae Farm) and South (Invermay Farm) Islands of NZ. Stock management however was generally more intensive than is typical for hill country.

A pilot investigation (Chapter 4) into the effect of a high fertiliser N rate and attendant increased excretal N deposition on the spatial distribution of nitrification potential (NP), and therefore potential soil nitrate (NO_3^-) supply, was conducted. Soil NP rates were measured in plots on Campsites and Medium slopes in hill country paddocks in the North and South Island, receiving 0 and 500 kg fertiliser N/ha.y. The pilot study found that NP increased in response to a high fertiliser N application rate in the rolling hill country of Invermay Farm in Otago, South Island only. Nitrification potential was not affected by the high fertiliser N application rate in the steep dissected country at Ballantrae Farm in Manawatu, North Island. Nitrification potential rates across

both farms were significantly higher in Campsites compared to Medium slopes. The conclusion therefore was that the Campsites used in this study demonstrated a higher risk of N losses due to the increased NO_3^- availability compared to Medium slopes. The results also showed that the high fertiliser N application rate in rolling hill country in the South Island resulted in increased NP activity and subsequent risk of N losses compared to areas that had not received fertiliser N. The recommendation was therefore made to consider avoiding fertiliser N application to Campsites in an effort to minimise N losses via leaching and denitrification from these areas in hill country.

Based on the fact that a high fertiliser N application rate significantly increased NO_3^- availability in rolling hill country in Otago, a short term study to investigate the effect of increasing fertiliser N application rates on N_2O emissions (Chapter 5) followed. The hypothesis was that N_2O emissions would be higher from rolling hill country paddocks receiving a high fertiliser N rate (500 kg N/ha.y), compared to those receiving low (i.e. 100 kg N/ha.y) or no fertiliser N. A range of soil physical (WFPS and PSD) and chemical (mineral N and pH) measurements were made at each gas sampling occasion to determine their relationship with N_2O emissions. A REML analysis detected a positive relationship between N_2O emissions, soil NO_3^- and WFPS levels, however this relationship was not consistent over the duration of the measurement period. The investigation found that N_2O emissions were highly spatially variable and that high fertiliser N rates (i.e. 500 kg N/ha.y) and the attendant increased stocking rate significantly increased N_2O emissions, even in the unseasonably dry conditions that were encountered. Mean daily N_2O emissions were 10 fold greater in the 500 N compared to the 100 N paddocks. The results in Chapter 5 were based on short term measurements and are therefore limited in their application. It is not appropriate therefore to extrapolate the data out to annual losses (kg N_2O-N/y), because the long term N_2O emission response is unknown based on the current data set and the excreta N inputs were not controlled. It is possible however to make a brief comparison between the results in Chapter 5 and the current inventory default factor for direct N_2O emissions from fertiliser (EF_1= 1%). The EF_1 is derived from the hourly emission measurements that are integrated over time to estimate total emissions for a known treatment (e.g. N fertiliser rate) over the measurement period (de Klein *et al.* 2003). The emission factor is the calculated as:

$$EF_1 = \frac{(N_2O_f - N_2O_c)*100}{N_r}$$

where EF_1 is the direct N_2O emission factor from fertiliser N application, N_2O_f is the total N_2O emissions (kg N_2O-N/ha) from the fertiliser treatment, N_2O_c is the total N_2O emissions (kg N_2O-N/ha) from the control treatment, and N_r is the fertiliser nitrogen application rate (50 and 125 kg N/ha applied during the measurement period in the 100 and 500 N treatments, respectively). Using this method the EF_1= 1.7% in Chapter 5 (Fertiliser N rate study) for the 500N treatment and is higher than the current EF_1. Stock were not excluded from measurement plots in this study and therefore the additional (unaccounted) source of N may have influenced results in the 500 N treatment. It should also be noted that fertiliser was applied to the paddocks at given rates (100 and 500 kg N/ha.y), as opposed to within chambers and the spatial variation from the spreader may also be a factor that contributed to these results. For the 100N treatment EF1= 0.01%. While stock also had access to these sites, the un-seasonably dry conditions and the variability of fertiliser application may have influenced the low results for the 100N treatment, which were lower than the current EF_1 would have predicted. Although there are obvious limitations with short term data sets the exercise of evaluating the data by comparison to national standards is nonetheless valuable because it generates discussion. This small data set demonstrates the importance of being able to control and/or account for contributing factors (such as stock management), as well as measuring over a range of seasonal and soil conditions (dry and wet years). Based on the results in Chapter 5 it was recommended that the possibility of significant N_2O emissions outside of what are considered to be optimal conditions for denitrification should be considered under intensive conditions when making recommendations for fertiliser N management for rolling sheep grazed hill country pastures.

Because the pilot study (Chapter 4) showed that NP was heavily influenced by topographically-driven differences (i.e. slope), the spatial distribution of N_2O emissions at Ballantrae Farm was investigated in 2 sheep grazed paddocks receiving N via excretal N returns only for 4 years prior to measurements. Nitrous oxide emissions were measured from 4 distinct slope classes; Campsites (0-12º), Medium slopes (13-25º), Steep slopes (>26º), and Gully areas. A range of soil physical (WFPS

and PSD), soil chemical (mineral N and pH levels), and slope measurements were also made on each gas sampling occasion to determine their effect on N_2O emissions. Given that NO_3^- and soil moisture are both crucial for the process of denitrification the hypothesis was that N_2O losses from sheep grazed hill country areas would be in the order Gullies> Campsites> Medium slopes> Steep slopes. The relationship between N_2O emissions and slope was best described as log-normal. Nitrous oxide emissions increased significantly in low slopes plots (campsites) and decreased significantly in steep slopes plots. Medium and gully slope plots had intermediate N_2O fluxes. A stepwise linear regression detected a significant negative relationship between slope and N_2O emissions, explaining 58% of the observed variability in emissions. Adding a range of soil variables subsequent to the slope variable did not improve the relationship. The N_2O emissions from the slope classes were spatially integrated to estimate paddock scale emissions and to determine the relative contribution of each slope class to total N_2O emissions at a paddock scale. Eighty one percent of total paddock N_2O emissions measured in this study were produced from Campsites, which represented only 22% of the paddock area. Gully and Medium slopes produced a combined total of 19% of total paddock N_2O emissions, yet represented 69% of the paddock area. Steep slopes represented 10% of the paddock area and produced a negligible amount of N_2O emissions. Again, this was a short term trial and therefore the data are limited in their application. A new frame work to upscale N_2O emissions in hill country was proposed by de Klein *et al.* (2009) to account for the influence of topographical variation on excreta return to different slope classes and subsequent N_2O emissions. There is sufficient information in Chapter 6 to make a comparison to the proposed frame work methodology:

$$N_2O_{hillcountry} = \Sigma_i(HLU_i * Nreturn_i * EF_{3i})$$

where $N_2O_{hillcountry}$ is the total N_2O emissions from hill country, HLU_i is the hill country land unit i, as defined by slope, aspect, soil type, fertility status and/or any other relevant factors; i= 1, ….n, $Nreturn_i$ is the amount of excreta N deposited in HLU_i, EF_{3i} is the N_2O emission factor for N deposited in HLUi.

These workers developed six possible EF_3 scenarios, involving a range of EF_3 factors (EF_{3i}) for hill country. Based on current results and measurements from previous chapters Scenario VI is the most appropriate for comparison (Table 7.1):

- Scenario I: EF_3 set at 1% for all HLUs as in the current NIR
- Scenario II: a relatively high EF_3 for all HLUs
- Scenario III: a moderate/reasonable EF_3 for all HLUs
- Scenario IV: a slightly less than moderate/reasonable EF_3 for all HLUs
- Scenario V: fairly low EF_3 for all HLUs
- Scenario VI: a very high EF_3 for areas that are expected to have high emissions, and a very low EF_3 for areas that are expected to have low emissions

Table 7.1 The range of emission factors used in different EF_3 scenarios in de Klein *et al.* (2009)

	EF_3 based on slope (% of N input emitted as N_2O-N) Farmlet slope class				
	1-10°	**11-20°**	**21-30°**	**31-40°**	**40°+**
Scenario I	1.00	1.00	1.00	1.00	1.00
Scenario II	2.00	1.50	1.00	0.50	0.05
Scenario III	1.50	1.00	0.60	0.30	0.05
Scenario IV	1.00	0.75	0.50	0.25	0.05
Scenario V	0.50	0.25	0.10	0.05	0.01
***Scenario VI**	2.50	1.00	0.20	0.050	0.001

*for current comparison to results from Chapter 6

Based on a calculated average annual stock rate of 23 SU/ha at Ballantrae (Woodford and Nicol, 2005) excreting 78 g N/SU.d (Whitehead, 1995) in the 0 N treatment for 3 days at each grazing period, for a notional 1 ha paddock the total N input was 53.8 kg N/ha in the grazing period prior to N_2O measurements. Excreta distribution (average of urine and dung) was therefore approximately 31, 17, and 6 kg N/ha, or 51, 31, and 12% on low, medium and steep slopes, respectively (Saggar *et al.* 1990; Rowarth 1987). Data from de Klein *et al.* (2009), suggests that the excretal N deposition to low slopes is 53-62% and is not significantly affected by the relative area of land in this slope category (CV% = 6.2).

Table 7.2 Calculated and measured N_2O emissions (kg N_2O-N) for low, medium and steep slopes based on the current default emission factor (EF_3), Scenario VI in the proposed hill country frame work, and measured N_2O emissions in Chapter 6

	kg N_2O-N Default EF_3 (1%)	kg N_2O-N Scenario VI EF_{3i}	kg N_2O-N Measured
Low 0-12°	0.312	0.780	0.053
Medium 13-25°	0.167	0.002	0.006
Steep 26°+	0.065	0.000	0.000
Gully n/a	n/a	n/a	0.006
Total	**0.544**	**0.782**	**0.065**

n/a: not applicable

Total N_2O emissions measured in Chapter 6 were much lower than estimates made using either EF_3 methodology (Table 7.2). The measurements may have been influenced by numerous uncontrolled factors, most notably the amount of time since the last grazing episodes within the trial paddocks (> 2 weeks in paddock 1 and >1 month in paddock 2). Therefore an accurate estimation and comparison to current and proposed methods for estimating N_2O emissions in hill country is difficult. The distribution of measured N_2O emissions certainly followed a similar pattern to the proposed Scenario VI which is encouraging. A re-assessment of the frame work was completed by de Klein *et al* (2010) and a seventh scenario (scenario VII) was subsequently added, with EF_3 values of 1, 0.1 and 0.001% for Low, Medium and Steep slopes, respectively. The new scenario was introduced to better reflect the differences in emission factors observed between slopes that were measured in the re-assessment. The inclusion of scenario VII in the re-assessment is also in closer agreement with the results in Chapter 6 in this Thesis. The average daily N_2O emission rate in sheep grazed country, receiving 0 N is ~3.4 g N_2O-N/ha.d (Saggar *et al.* 2007b), which is identical to a weighted average of 3.4 g N_2O-N/ha.d in the current study. However the limitations of the data in Chapter 6 make it difficult to have confidence in this estimate. It was concluded that 81% of N_2O emissions measured in Chapter 6 came from ~22% of the experimental area (campsites, 0-12°). The medium slope class (13-25°) covered ~46% of the land area, yet contributed only 10% to the N_2O emissions total, and emissions from steep slopes (~10% of land area) were negligible. Gully areas covered ~21% of the land area and contributed 9% of total N_2O emissions. Based on the results in Chapter 6 it was recommended that N_2O mitigation in hill country would be most effective if targeted at reducing emissions from Campsites, and also suggests that gully areas require closer research attention. In addition, the highly significant relationship between slope class and N_2O emissions

suggested that slope may indeed be a suitable parameter for upscaling N_2O emissions from sheep grazed hill country.

7.3 General conclusions

Some of the N cycling and N_2O emission rates reported in this Thesis were higher than in comparable studies, which was likely due to the high fertiliser N application rates and intensive stock management at these study sites. The results presented in this Thesis however contribute to the growing literature on N_2O emissions in NZ hill country. The data have limitations in their application, due to the short term nature of the trials. Longer term studies as well as greater plot replication and a wider range of farm situations would further broaden our understanding of N_2O emissions in hill country. The value of this data in particular will be to guide the experimental design of future work in hill country. For example, the negligible amounts of N_2O emissions produced on steep slopes may justify omitting these slopes from further studies on N_2O emissions. The time, money and resources could then be spent on improving the examination of those slope classes that make significant contributions to the total N_2O emissions from pastoral hill country.
This data may also be used to make better informed fertiliser N and grazing management decisions for sheep grazed hill country. For example based on the results of this thesis, it is recommended that fertiliser N application to stock campsites should be avoided given the significant and consistent elevation of nutrient N in these areas. In addition this study suggests that mitigation strategies in hill country could focus on reducing N_2O emissions from campsites. For example, targeted application of nitrification inhibitors to campsite and flat areas may significantly reduce N leaching and N_2O emissions at a lower cost than would be required to aerially apply the inhibitor over the whole hill country block.

Nitrogen cycling and N losses via N_2O emissions in hill country were highly variable both spatially and over time in this study. Measured soil variables NO_3^- and WFPS often had positive but not significant relationships with N_2O emissions, due largely to the spatial variation in soil conditions and N_2O emissions in hill country. The complete reduction of soil NO_3^- to di-nitrogen (N_2) may be part of the reason why the relationship between soil variables and N_2O emissions lacked consistency in this

study. Other processes such as nitrifier denitrification may also have affected the relationships between soil variables and N_2O emissions and these could be pathways for N loss that are investigated into the future. For example there is limited evidence to suggest that soil pH can have an indirect effect on the abundance or composition of the denitrifying community (distal control by pH, as measured by DEA) as well as directly through the kinetics of the denitrification reactions (proximal control by pH, as measured by N_2O/N_2 ratio), (Čuhel and Šimek 2011). However the relative importance of either process on final N_2O production from soils is not yet well understood. Also a distinct seasonal shift has been found to occur in denitrifying active communities and denitrifying gene expression, that also showed a significant variation depending on soil variables such as texture and organic carbon (Pastorelli et al. 2011). Clearly a more detailed knowledge of soil processes would increase our understanding of N dynamics in hill country considerably. The results throughout this Thesis however clearly show that nitrification potential (NP), soil moisture, soil mineral N levels and N_2O emissions were consistently higher in campsites on slopes 0 to 12°. The significant negative relationship between slope class and N_2O emissions suggests that slope class is more consistently related to N_2O emissions than the other soil variables measured in this work, and therefore broadly supports the use of slope class to upscale N_2O emission estimates from sheep grazed hill country.

CHAPTER 8 APPENDICES

Appendix 1 Blocking system and area (ha) of experimental paddocks on Ballantrae and Invermay Research Farms used in the 'Wise N-use' Trial. Treatments; 0, 100, 200, 300, 400, 500 & 750 kg N/ha.y

Farm	Block	Paddock/plot	N Treatment	Area (ha)
Ballantrae	1	E	0 N	0.33
		D	100 N	0.34
		A	200 N	0.24
		F	400 N	0.35
		C	500 N	0.37
		B	750 N	0.39
	2	I	0 N	0.33
		H	200 N	0.44
		J	300 N	0.29
		P	300 N	0.22
		L	400 N	0.40
		G	500 N	0.33
		K	750 N	0.25
	3	M	0 N	0.28
		Q	100 N	0.31
		O	200 N	0.20
		P	300 N	0.22
		N	400 N	0.27
Invermay	1	88	0 N	0.85
		87	100 N	0.90
		89	200 N	0.85
		86	300 N	0.95
		85	400 N	0.90
		84	500 N	0.95
	2	94	0 N	0.80
		93	100 N	0.80
		124	200 N	0.80
		125	300 N	0.90
		98	400 N	1.05
		79	500 N	0.96
	3	100	0 N	0.8
		101	100 N	0.90
		99	200 N	0.88
		95	300 N	0.70
		111	400 N	0.90
		126	500 N	0.85

CHAPTER 9 BIBLIOGRAPHY

Abbasi MK, Adams WA (2000) Gaseous N emission during simultaneous nitrification-denitrification associated with mineral N fertilization to a grassland soil under field conditions. *Soil Biology and Biochemistry* **32**, 1251-1259.

Alexander M (1965) Nitrification. In 'Soil Nitrogen'. (Ed. WC Bartholomew, FE) p. 615. (American Society of Agronomy.

Allen AG, Jarvis SC, Headon DM (1996) Nitrous oxide emissions from soils due to inputs of nitrogen from excreta return by livestock on grazed grassland in the U.K. *Soil Biology and Biochemistry* **28**, 597.

Ball BC, Horgan GW, Clayton H, Parker JP (1997) Spatial variability of nitrous oxide fluxes and controlling soil and topographic properties. *Journal of Environmental Quality* **26**, 1399-1409.

Ball RP, Luscombe PC, Grant DA (1982) Nitrogen in hill country. In 'Nitrogen Fertilisers in New Zealand Agriculture'. (Ed. PB Lynch) pp. 133-148. (Ray Richards Publisher: Auckland).

Bertora C, van Vliet PCJ, Hummelink EWJ, van Groenigen JW (2007) Do earthworms increase N_2O emissions in ploughed grassland? *Soil Biology and Biochemistry* **39**, 632-640.

Bhandral R, Saggar S, Bolan N, Hedley M (2003) Nitrous oxide fluxes in soil as influenced by compaction. *Proceedings of the New Zealand Grasslands Association* **65**, 259-264.

Bhandral R, Saggar S, Bolan NS, Hedley MJ (2007) Transformation of nitrogen and nitrous oxide emission from grassland soils as affected by compaction. *Soil and Tillage Research* **94**, 482-492.

Black C (1968) 'Soil-plant Relationships.' (John Wiley & Sons, Inc).

Blakemore L (1987) 'Methods for chemical analysis of soils.' (NZ Soil Bureau, Wellington: Lower Hutt, Wellington).

Bothe H, Ferguson S, Newton W (2007) 'Biology of the Nitrogen Cycle.' (Elsevier).

Bowatte S (2003) Urine nitrogen in hill country pasture soils. PhD Thesis, Massey University.

Burford JR, Bremner JM (1975) Relationships between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. *Soil Biology and Biochemistry* **7**, 389-394.

Campbell I (1977) 'Soils of Waikouaiti county, Otago, New Zealand.' (New Zealand Department of Scientific and Industrial Research: Wellington).

Carran A, Saggar S (2004a) Client Report. Ministry of Agriculture and Forestry. Nitrous oxide emission from hill country soil. AgResearch & Landcare Research, Palmerston North.

Carran RA, Saggar S (2004b) Client Report. Nitrous oxide emission from hill country soil. Draft p. 17. (Ministry of Agriculture and Fisheries.

Carran RA, Theobald PW, Evans JP (1995) Emission of nitrous oxide from some grazed pasture soils in New Zealand. *Australian Journal of Soil Research* **33**, 341-352.

Chapuis-Lardy L, Wrage N, Metay A, Chotte JL, Bernoux M (2007) Soils, a sink for N_2O? A review. *Global Change Biology* **13**, 1-17.

Clark DA, Lambert MG (1989) The use of fertiliser nitrogen for increased animal production from New Zealand hill pastures. Versailles, France p. 1269. (Association Française pour la Production Fourragère).

Clayton H, McTaggart IP, Parker J, Swan L, Smith KA (1997) Nitrous oxide emissions from fertilised grassland: a 2-year study of the effects of N fertiliser form and environmental conditions. *Biology and Fertility of Soils* **25**, 252-352.

Clough TJ, Sherlock RR, Mautner MN, Milligan DB, Wilson PF, Freeman CG, McEwan MJ (2003) Emission of nitrogen oxides and ammonia from varying rates of applied synthetic urine and correlations with soil chemistry. *Australian Journal of Soil Research* **41**, 421-438.

Conen F, Smith KA (1998) A re-examination of closed flux chamber methods for the measurement of trace gas emissions from soils to the atmosphere. *European Journal of Soil Science* **49**, 701-707.

Cowie J (1983) 'The soils of Ballantrae Farm, Woodville. N.Z. Soil Survey Report.'

Čuhel J, Šimek M (2011) Proximal and distal control by pH of denitrification rate in a pasture soil. *Agriculture, Ecosystems & Environment* **141**, 230-233.

de Klein C, Hoogendoorn C, Luo J, van der Weerden T (2010) Re-assessment of N_2O emissions from sheep grazed hill country using refined emission factors. . Ministry of Agriculture and FisheriesMAF POL 0910-11526 (09-10 EF3 in Hill Country), Mosgiel.

de Klein C, Hoogendoorn C, Manderson A, Saggar S, Giltrap D, Briggs C, Rowarth J (2009) Refinement of the framework for upscaling nitrous oxide emissions from hill country. MAF-POL 0809-11061 Hill Country EF3. AgResearch Ltd, Mogiel.

de Klein C, Ledgard S (2005) Nitrous Oxide Emissions from New Zealand Agriculture – key Sources and Mitigation Strategies. *Nutrient Cycling in Agroecosystems* **72**, 77-85.

de Klein CAM (1994) Denitrification in grazed grasslands in The Netherlands. PhD Thesis, University Utrecht.

de Klein CAM, Barton L, Sherlock RR, Li Z, Littlejohn RP (2003) Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. *Australian Journal of Soil Research* **41**, 381-399.

de Klein CAM, van Logtestijn RSP (1994) Denitrification and N_2O emission from urine-affected grassland soil. *Plant and Soil* **163**, 235-242.

Dendooven L, Anderson JM (1994) Dynamics of reduction enzymes involved in the denitrification process in pasture soil. *Soil Biology and Biochemistry* **26**, 1501-1506.

Di HJ, Cameron KC (2002) The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. *Soil Use and Management* **18**, 395-403.

Doak BW (1952) Some chemical changes in the nitrogenous constituents of urine when voided on pasture. *Journal of Agricultural Science* **42**, 162-171.

Dobbie KE, Smith KA (2001) The effects of temperature, water-filled pore space and land use on N_2O emissions from an imperfectly drained gleysol. *European Journal of Soil Science* **52**, 667-673.

Drewry JJ, Lowe JAH, Paton RJ (1999) Effect of sheep stocking intensity on soil physical properties and dry matter production on a Pallic Soil in Southland. *New Zealand Journal of Agricultural Research* **42**, 493-499.

Fair RJ, Jamieson HM, Hopkins DW (1994) Spatial distribution of nitrifying (ammonium-oxidizing) bacteria in soil. *Letters in Applied Microbiology* **18**, 162-164.

Fenchel T, Blackburn TH (1979) 'Bacteria and Mineral Cycling.' (Academic Press: London).

Ferguson S, Richardson D, van Spanning R (2007) Biochemistry and molecular biology of nitrification. In 'Biology of the Nitrogen Cycle'. (Ed. H Bothe, Ferguson, SJ & Newton, WE) p. 427. (Elsevier.

Field TRO, Ball RP, Theobald PW (1985) Leaching of nitrate from sheep-grazed pastures. *Proceedings of the New Zealand Grassland Association* **46**, 209-214.

Firestone M, Davidson E (1989) Microbial basis of NO and N_2O production and consumption in soil. In 'Exchange of trace gases between terrestrial ecosystems and the atmosphere'. (Ed. O M, Andreae, and E, A, Davidson) pp. 7-21. (John Wiley & Sons Ltd: Berlin).

Floate M (1970) Mineralisation of nitrogen and phosphorus from organic materials of plant and animal origin and its significance in the nutrient cycle in grazed upland and hill soils. *Journal of the British Grassland Society* **25**, 295-302.

Florinsky IV, McMahon S, Burton DL (2004) Topographic control of soil microbial activity: a case study of denitrifiers. *Geoderma* **119**, 33-53.

GenStat (2006) 'GenStat (20065) GenStat for Windows. Ninth Edition. VSN International Ltd., Oxford.' (VSN International Ltd: Oxford).

Gillingham AG (1973) Influence of physical factors on pasture growth on hill country. *Proceedings of the New Zealand Grasslands Association* **35**, 77-85.

Gillingham AG (1980) Phosphorus uptake and return in grazed, steep hill pastures. II. Above-ground components of the phosphorus cycle. *New Zealand Journal of Agricultural Research* **23**, 323-330.

Gillingham AG (1982) Topographic and management effects on dung distribution by grazing sheep. *Proceedings of the New Zealand Grassland Association* **43**, 161-170.

Gillingham AG, During C (1973) Pasture production and transfer of fertility within a long-established hill pasture. *New Zealand Journal of Experimental Agriculture* **1**, 227-232.

Grant DA, Lambert MG (1979) Nitrogen fixation in pasture V. Unimproved North Island hill country, "Ballantrae". *New Zealand Journal of Experimental Agriculture* **7**, 19-22.

Grant DA, Rumball PJ, Suckling FET (1973) Pasture improvement and potential productivity in southern North Island hil country. *Proceedings of the New Zealand Grasslands Association* **34**.

Harrison R, Webb J (2001) A review of the effect of N fertilizer type on gaseous emissions. *Advances in Agronomy* **73**, 65-108.

Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralisation, immobilisation, and nitrification. In 'Methods of Soil Analysis Part 2'. (Eds JM Bigham, SH Mickelson) pp. 985-1018. (Soil Science Society of America Book Series: Wisconsin).

Hatch DJ, Bhogal A, Lovell RD, Shepherd MA, Jarvis SC (2000) Comparison of different methodologies for field measurement of net nitrogen mineralization in pasture soils under different soil conditions. *Biology and Fertility of Soils* **32**, 287-293.

Hawke DJ (2001) Variability of N^{15} in soil and plants at a New Zealand hill country site: correlations with soil chemistry and nutrient inputs. *Australian Journal of Soil Research* **39**, 373-383.

Haynes RJ (1986) 'Mineral Nitrogen in the Plant-Soil System.' (Academic Press, Inc: Sydney).

Haynes RJ, Williams PH (1992) Changes in soil solution composition and pH in urine-affected areas of pasture. *Journal of Soil Science* **43**, 323-334.

Haynes RJ, Williams PH (1993) Nutrient Cycling and Soil Fertility in the Grazed Pasture Ecosystem. *Advances in Agronomy* **49**, 119-199.

Hedley C, Saggar S, Tate K (2006) Procedure for fast simultaneous analysis of the greenhouse gases: Methane, carbon dioxide, and nitrous oxide in air samples. *Communications in Soil Science and Plant Analysis* **37**, 1501-1510.

Hewitt AE (1998) 'New Zealand Soil Classification.' (Landcare Research Ltd, New Zealand: Lincoln).

Hoogendoorn C (2006) Wise use of fertiliser nitrogen on hill country pastures: Ballantrae trial.Palmerston North).

Hoogendoorn C, de Klein C, Rutherford A, Letica S, Devantier B (2008) The effect of increasing rates of nitrogen fertiliser and a nitrification inhibitor on the nitrous oxide emissions from urine patches on sheep grazed hill country pasture. *Australian Journal of Experimental Agriculture* **48**, 147-151.

Hutchinson G, Livingston G (2001) Vents and seals in non-steady-state chambers used for measuring gas exchange between soil and the atmosphere. *European Journal of Soil Science* **52**, 675-682.

IPCC (2006) 2006 International Panel on Climate Change Guidelines for National Greenhouse Gas Inventories. (IGES.

Jarvis SC, Hatch DJ, Roberts DH (1989) The effects of grassland management on nitrogen losses from grazed swards through ammonia volatilisation; the relationship to excretal N returns from cattle. *Journal of Agricultural Science* **112**, 205-216.

Kemmit SJ, Wright D, Goulding KWT, Jones DL (2006) pH regulation of carbon and nitrogen dynamics in two agricultural soils. *Soil Biology & Biochemistry* **38**, 898-991.

Ladd JN, Amato M, Oades JM (1985) Decomposition of plant material in Australian soils. III. Residual organic and microbial biomass C and N from isotope-labelled legume material and soil organic matter, decomposing under field conditions. *Australian Journal of Soil Research* **23**, 603-611.

Lambert G, Luccombe PC, Clark D (1982a) Soil fertility and hill country production. *Proceedings of the New Zealand Grassland Association Conference* **43**, 153-160.

Lambert GM, Clark D (1985) Effects of late-autumn nitrogen application on hill country pastures and sheep production. *Proceedings of the New Zealand Grassland Association* **47**, 211-216.

Lambert M, Renton S, Grant D (1982b) Nitrogen balance studies in some North Island hill pastures. In 'Nitrogen Balances in New Zealand Ecosystems'. (Ed. P Gandar) p. 263. (DSIR, New Zealand: Palmerston North).

Lambert MG, Clark DA, Grant DA, Costall DA (1986) Influence of fertiliser and grazing management on North Island moist hill country. 2. Pasture botanical composition. *New Zealand Journal of Agricultural Research* **29**, 1.

Lambert MG, Clark DA, Grant DA, Costall DA, Fletcher RH (1983) Influence of fertiliser and grazing management on North Island moist hill country. 1. Herbage accumulation. *New Zealand Journal of Agricultural Research* **26**, 95.

Lambert MG, Clark DA, Mackay AD, Costall DA (2000) Effects of fertiliser application on nutrient status and organic matter content of hill soils. *New Zealand Journal of Agricultural Research* **43**, 127-138.

Lambert MG, Luscombe PC, Clark DA (1982c) Soil fertility and hill country production. *Proceedings of the New Zealand Grasslands Association* **43**, 153-160.

Lambert MG, Mackay AD, DeVantier BP, McDougall DB, Barker DJ, Park-NG ZA (2003) Redefining the production potential of hill pastures using fertiliser nitrogen. *New Zealand Grasslands Association* **65**, 35-40.

Ledgard S, Brier G (1993) Response to re-application of phosphate fertilisers on hill pasture where fertiliser had been withheld for seven years. *Proceedings of the New Zealand Grasslands Association* **55**, 67-71.

Ledgard SF (2001) Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. *Plant and Soil* **228**, 43-59.

Ledgard SF, Brier, G.J., & Littler, R.A (1987) Legume production and nitrogen fixation in hill pasture communities. *New Zealand Journal of Agricultural Research* **30**, 413-421.

Ledgard SF, Steele KW (1992) Biological nitrogen fixation in mixed legume/grass pastures. *Plant and Soil* **141**, 137-153.

Leslie M, Aspin M, Clark H (2008) Greenhouse gas emissions from New Zealand agriculture: issues, perspectives and industry response. *Australian Journal of Experimental Agriculture* **48**, 1-5.

Letica S, Tillman R, Littlejohn R, Hoogendoorn C, Kemp P (2006) Spatial distribution of potential nitrification activity in two hill country pastures. *Proceedings of the New Zealand Grasslands Association* **68**, 369-373.

Letica SA, de Klein CAM, Hoogendoorn CJ, Tillman RW, Littlejohn RP, Rutherford AJ (2010) Short-term measurement of N_2O emissions from sheep-grazed pasture receiving increasing rates of fertiliser nitrogen in Otago, New Zealand. *Animal Production Science* **50**, 17-24.

López IF, Hodgson J, Hedderley DI, Valentine I, Lambert MG (2003a) Selective defoliation by sheep according to slope and plant species in the hill country of New Zealand. *Grass And Forage Science* **58**, 339.

López IF, Lambert MG, Mackay AD, Valentine I (2003b) The influence of topography and pasture management on soil characteristics and herbage accumulation in hill pasture in the North Island of New Zealand. *Plant and Soil* **255**, 421-434.

Lovell RD, Jarvis SC (1996) Effects of urine on soil microbial biomass, methanogenesis, nitrification and denitrification in grassland soils. *Plant and Soil* **186**, 265.

Luo J, White RE, Roger Ball P, Tillman RW (1996) Measuring denitrification activity in soils under pasture: Optimizing conditions for the short-term denitrification enzyme assay and effects of soil storage on denitrification activity. *Soil Biology and Biochemistry* **28**, 409-417.

Luscombe P, & Fletcher, RH (1981) Nitrogen fertiliser on grazed hill pastures. *Proceedings of the New Zealand Grassland Association* **43**, 171-181.

Luscombe PC (1979) Nitrogen fertiliser responses on hill country pastures. *Proceedings of the New Zealand Grassland Association* **41**, 155-162.

Marhan S, Scheu S (2005) Effects of sand and litter availability on organic matter decomposition in soil and in casts of Lumbricus terrestris L. *Geoderma* **128**, 155-166.

Massey University (1998) Schematic diagram of Auto-Analyser. In 'Institute of Technology and Engineering: Water Systems Engineering'. (Massey University: Palmerston North).

Matthias AD, Blackmer AM, Bremner JM (1980) A Simple Chamber Technique for Field Measurement of Emissions of Nitrous Oxide from Soils. *Journal of Environmental Quality* **9**, 251-256.

McDowell R, Drewry JJ, Paton RJ (2004) Effects of deer grazing and fence-line pacing on water and soil quality. *Soil Use and Management* **20**, 302-307.

McIntosh P (1985) 'Soils of Waiora Farm, Invermay Agricultural Centre, Otago, New Zealand.' (V.R. Ward, Government Printer: Wellington).

McLaren R, Cameron KC (1996) 'Soil Science. Sustainable production and environmental protection.' (Oxford University Press, Auckland).

McTaggart IP, Akiyama H, Tsuruta H, Ball BC (2002) Influence of soil physical properties, fertiliser type and moisture tension on N_2O and NO emissions from nearly saturated Japanese upland soils. *Nutrient Cycling in Agroecosystems* **63**, 207-217.

Ministry for the Environment (2006) New Zealand's Greenhouse Gas Inventory 1990-2004; The National Inventory Report and Common Reporting Format. Climate Change Office, Wellington.

Ministry for the Environment (2007) New Zealand's Greenhouse Gas Inventory 1990-2005. The national inventory report and common reporting format. Wellington.

Ministry for the Environment (2010a) Climate change (http://www.mfe.govt.nz/issues/climate/index.html

Ministry for the Environment (2010b) New Zealand's Greenhouse Gas Inventory 1990-2008. Ministry for the Environment, Wellington.

Monaghan RM, Barraclough D (1993) Nitrous oxide and dinitrogen emissions from urine-affected soil under controlled conditions. *Plant and Soil* **151**, 127.

Morton JD, Korte CJ, Smith DR, Watt BD, Smith RG (1993) Nitrogen use and farm performance on Wairarapa sheep and beef farms. *New Zealand Grasslands Association* **55**, 54-56.

Nunan N, Singh B, *et al.* (2006) Sheep-urine-induced changes in soil microbial community structure. *FEMS Microbiology Ecology* **56**, 310-320.

O'Connor KF (1961a) Nitrogen and grassland production in the mid-altitude zone of Canterbury, New Zealand. I. The effects of different levels of nitrogen fertiliser on herbage and nitrogen yields of cultivated pastures. *New Zealand Journal of Agricultural Research* **4**, 686-697.

O'Connor KF (1961b) Nitrogen and grassland production in the mid-altitude zone of Canterbury, New Zealand. II. The effects of different levels of calcium-ammonium nitrate on yields of cultivated pastures under different mowing frequencies. *New Zealand Journal of Agricultural Research* **4**, 698-708.

O'Connor KF (1961c) Nitrogen and grassland production in the mid-altitude zone of Canterbury, New Zealand. III. The effects of nitrogenous and other fertiliser materials on uncultivated pastures. *New Zealand Journal of Agricultural Research* **4**, 709-721.

Otago Regional Council (2006) GrowOTAGO climate and soil data p. http://www.growotago.orc.govt.nz/ (12 Apr 2006). (Otago Regional Council: Dunedin).

Parker L (2008) Study of denitrification in hill country: The effect of nitrogen availability and topography on gaseous emissions. Dissertation, Massey University.

Parkin TB, Venterea RT (2010) Sampling protocols. Chapter 3. Chamber-based trace gas flux measurements. (Ed. RF Follett).

Parsons LL, Smith MS, Murray RE (1991) Soil Denitrification Dynamics: Spatial and Temporal Variations of Enzyme Activity, Populations, and Nitrogen Gas Loss. *Soil Science Society of America Journal* **55**, 90-95.

Pastorelli R, Landi S, Trabelsi D, Piccolo R, Mengoni A, Bazzicalupo M, Pagliai M (2011) Effects of soil management on structure and activity of denitrifying bacterial communities. *Applied Soil Ecology* **49**, 46-58.

Pennock DJ, Van Kessel C, Farrell RW, Sutherland RA (1992) Landscape-scale variations in denitrification. *Soil Science Society of America Journal* **56**, 770-776.

Postma-Blaauw MB, Bloem J, Faber JH, van Groenigen JW, de Goede RGM, Brussaard L (2006) Earthworm species composition affects the soil bacterial community and net nitrogen mineralization. *Pedobiologia* **50**, 243-256.

Prosser J (2007) The ecology of nitrifying bacteria. In 'Biology of the Nitrogen Cycle'. (Ed. H Bothe, Ferguson SJ. & Newton, WE) p. 427. (Elsevier.

Radcliffe JE (1982) Effects of aspect and topography on pasture production in hill country. *New Zealand Journal of Agricultural Research* **25**, 485.

Roach C, Nemaia E, Ledgard SF, Brier G, McLay C (1996) Effects of long term differences in fertiliser history on hill country: seasonal pasture production, legume growth and soil physical phosphorus status. *Proceedings of the New Zealand Grasslands Association* **57**, 105-109.

Rochette P, Bertrand N (2003) Soil air sample storage and handling using polypropylene syringes and glass vials. *Canadian Journal of Soil Science* **83**, 631-637.

Rochette P, Eriksen-Hamel NS (2008) Chamber measurements of soil nitrous oxide flux: are absolute values reliable? *Soil Science Society of America Journal* **72**, 331-342.

Rooney D, Clipson N (2008) Impact of sheep urine deposition and plant species on ammonia-oxidising bacteria in upland grassland soil. *Candian Journal of Microbiology* **54**, 791-796.

Rossignol N, Bonis A, Bouzillé J-B (2006) Consequence of grazing pattern and vegetation structure on the spatial variations of net N mineralisation in a wet grassland. *Applied Soil Ecology* **31**, 62-72.

Rowarth JS (1987) Phosphate cycling in grazed hill country pasture: A Thesis presented in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Soil Science at Massey University. PhD, Massey University.

Rowarth JS, Gillingham AG, Tillman RW, Syers JK (1985) Release of phosphorus from sheep faeces on grazed hill country pastures *New Zealand Journal of Agricultural Research* **28**, 497-504.

Rus Jerez BE, Ball PR, Tillman RW (1988) The role of earthworms in nitrogen release from herbage residues. In 'Efficiency in Agricultural Soils'. (Eds DS Jenkinson, KA Smith) pp. 355-370. (Elsevier: LONDON).

Ruz-Jerez BE, White RE, Ball PR (1994) Long-term measurement of denitrification in three contrasting pastures grazed by sheep. *Soil Biology and Biochemistry* **26**, 29-39.

Saggar S, Andrew RM, Tate KR, Hedley CB, Rodda NJ, Townsend JA (2004a) Modelling nitrous oxide emissions from dairy-grazed pastures. *Nutrient Cycling in Agroecosystems* **68**, 243-255.

Saggar S, Bolan NS, Bhandral R, Hedley CB, Luo J (2004b) A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *New Zealand Journal of Agricultural Research* **47**, 513-544.

Saggar S, Giltrap DL, Li C, Tate KR (2007a) Modelling nitrous oxide emissions from grazed grasslands in New Zealand. *Agriculture Ecosystems & Environment* **119**, 205-216.

Saggar S, Hedley CB, Giltrap DL, Lambie SM (2007b) Measured and modelled estimates of nitrous oxide emission and methane consumption from a sheep-grazed pasture. *Agriculture, Ecosystems & Environment* **122**, 357-365.

Saggar S, Mackay AD, Hedley MJ, Lambert MG, Clark DA (1990) A Nutrient-Transfer Model to Explain the Fate of Phosphorus and Sulfur in Grazed Hill-Country Pasture. *Agriculture Ecosystems & Environment* **30**, 295-316.

Sakadevan K, Hedley MJ, Mackay AD (1993a) MIneralisation and fate of soil sulphur and nitrogen in hill pastures. *New Zealand Journal of Agricultural Research* **36**, 271-281.

Sakadevan K, Mackay AD, Hedley MJ (1993b) Influence of sheep excreta on pasture uptake and leaching losses of sulfur, nitrogen and potassium from grazed pastures. *Australian Journal of Soil Research* **31**, 151-162.

Sarathchandra SU (1978) Nitrification activities of some New Zealand soils and the effect of some clay types on nitrification. *New Zealand Journal of Agricultural Research* **21**, 615-621.

Sarathchandra SU, Perrott KW, Upsdell MP (1984) Microbiological and biochemical characteristics of a range of New Zealand soils under established pasture. *Soil Biology & Biochemistry* **16**, 177-183.

Schall R (1991) Estimation in generalized linear models with random effects. *Biometrika* **78**, 719-727.

Sherlock R, Goh K (1984) Dynamics of ammonia volatilization from simulated urine patches and aqueous urea applied to pasture. *Fertilizer Research* **5**, 181-195.

Sherlock RR, O'Connor MB (1973) The use of nitrogen on hill country. *Proceedings of the New Zealand Grasslands Association* **35 (1)**, 52-62.

Skiba U, Smith KA (2000) The control of nitrous oxide emissions from agricultural and natural soils. *Chemosphere - Global Change Science* **2**, 379-386.

Skiba U, Smith KA, fowler D (1993) Nitrification and denitrification as sources of nitric oxide and nitrous oxide in a sandy loam soil. *Soil Biology and Biochemistry* **25**, 1527-1536.

Smith MS, Tiedje JM (1979) Phases of denitrification following oxygen depletion in soil. *Soil Biology and Biochemistry* **11**, 261-267.

Stark S, Grellmann D (2002) Soil microbial responses to herbivory in an Arctic tundra heath at two levels of nutrient availability. *Ecology* **83**, 2736-2744.

Steele KW, Wilson AT, Saunders WMH (1980) Nitrification activity in New Zealand grassland soils. *New Zealand Journal of Agricultural Research* **23**, 249-256.

Steenvoorden JHAM, Fonck H, Oosterom HP (1986) Losses of nitrogen from intensive grassland systems by leaching and surface runoff. In 'Nitrogen Fluxes in Intensive Grassland Systems'. (Eds HG Van der Meer, JC Ryden, GC Ennik) pp. 85-98. (Martinus Nijhoff Publishers: Dordrecht).

Stevens D (2006) Wise use of N-fertiliser on hill country pastures: Invermay site.

Stevens D, Hoogendoorn C, Devantier B, Lambert M (2008) Final Report to FertResearch. Wise and sustainable rates of N fertiliser on hill country. AgResearch Limited.

Subarao G, Ito O*, et al.* (2006) Scope and strategies for regulation of nitrification in agricultural systems - challenges and opportunities. *Critical Reviews in Plant Sciences* **25**, 303-335.

Szili-Kovacs T, Szabo R, Halassy MT (2002) Restoration of sandy grasslands by immobilisation of soil nitrogen 3. Changes in soil microbial biomass C and N, and in mineral N between 2000 and 2002. *Agrokemia es Talajtan* **57** 133-146.

Tait A, Henderson R, Turner R, Zheng X (2006) Thin plate smoothing spline interpolation of daily rainfall for New Zealand using a climatological rainfall surface. *International Journal of Climatology* **26**, 2097-2115.

Tait A, Woods R (2007) Spatial interpolation of daily potential evapotranspiration for New Zealand using a spline model. *Journal of Hydrometeorology* **8**, 430-438.

Taylor J (1973) The analysis of designed experiments with censored observations. . *Biometrics* **29**, 35-43.

Tenuta M, Beauchamp EG (2003) Nitrous oxide production from granular nitrogen fertilizers applied to a silt loam soil. *Canadian Journal of Soil Science* **83**, 521-532.

Tiedje JM (1982) Denitrification. In 'Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties'. (ASA-SSSA: Madison).

Tiedje JM, Simkins S, Groffman PM (1989) Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. *Plant and Soil* **115**, 261-284.

Vallis I, Harper L, Catchpoole V, Weier K (1982) Volatilisation of ammonia from urine patches in a subtropical pasture. *Australian Journal of Agricultural Research* **33**, 97-107.

van der Weerden T (1999) Nitrous oxide emission and methane production and consumption by arable agriculture. PhD Thesis, Lincoln University.

Velthof G, van Groenigen J, Gebauer G, Pietrzak S, Jarvis S, Pinto M, Corre W, Oenema O (2000) Temporal stability of spatial patterns of nitrous oxide fluxes from sloping grassland. *Journal of Environmental Quality* **29**, 1397-1407.

Wakelin S, Gregg A, Simpson R, Li G, Riley I, McKay A (2009) Pasture management clearly affects soil microbial community structure and N-cycling bacteria. *Pedo biologia* **52**, 237-251.

Walker T, Thapa, BK., & Adams, AFR (1959) Studies on soil organic matter: 3. Accumulation of carbon, nitrogen, sulfur, organic and total phosphorus in improved grassland soils. *Soil Science* **87**, 135-140.

Walker T, W, Thapa B, K, Adams A, F,R (1959) Studies on soil organic matter: 3. Accumulation of carbon, nitrogen, sulfur, organic and total phosphorus in improved grassland soils. *Soil Science* **87**, 135-140.

Wallenstein MD, Myrold DD, Firestone M, Voytek M (2006) Environmetnal controls on denitrifying communities and denitrification rates: insights from molecular methods. *Ecological Applications* **16**, 2143-2152.

White J (1990) Hill and high country pasture. In 'Pastures. Their ecology and management'. (Ed. R Langer) p. 499. (Oxford University Press: Auckland).

Whitehead DC (1986) Sources and transformations of organic nitrogen in intensively managed grassland soils. In 'Nitrogen Fluxes in Intensive Grassland Systems'. (Ed. HG van der Meer, Ryden, J.C., Ennik, G.C) p. 113. (Martinus Nijhoff Publishers: Dordrecht).

Whitehead DC (1995) 'Grassland Nitrogen.' (CAB International: Wallingford).

Williams PH, Haynes RJ (1990) Influence of improved pastures and grazing animals on nutrient cycling within New Zealand soils. *New Zealand Journal of Ecology* **14**, 49-57.

Williams PH, Haynes RJ (2000) Transformations and plant uptake of urine N and S in long and short-term pastures. *Nutrient Cycling in Agroecosystems* **56**, 109-116.

Wise N use (2007) http://www.wisenuse.co.nz/.

Woodford K, Nicol A (2004) A reassessment of the stock unit system. Ministry of Agriculture and Fisheries, Wellington.

Yates T, Si B, Farrell R, Pennock DJ (2006) Probability distribution and spatial dependence of nitrous oxide emission: temporal change in hummocky terrain *Soil Science Society of America Journal* **70**, 753-762.

Yoshinari T, Hynes R, Knowles R (1977) Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. *Soil Biology and Biochemistry* **9**, 177-183.

Young SR, Black AS, Conyers MK (2002) Distribution of nitrification within surface soils under pasture. *Communications in Soil Science and Plant Analysis* **33**, 1507-1518.

Zhang BS, Valentine I, Kemp P, Lambert G (2006) Predictive modelling of hill-pasture productivity: integration of a decision tree and a geographical information system. *Agricultural Systems* **87**, 1-17.