# An evaluation of the use of the nitrification inhibitor dicyandiamide (DCD) to reduce nitrogen losses from intensive sheep winter grazing systems.

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of the requirements for the Degree of
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at
Lincoln University

By Matthew Alan Wild

Lincoln University
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# Abstract


The intensification of modern pastoral agriculture has led to an increase in nitrate ($NO_3^-$) leaching and nitrous oxide ($N_2O$) gas emissions from animal grazing systems, leading to environmental degradation. The use of nitrification inhibitor technology has recently been shown to reduce $NO_3^-$ leaching losses and $N_2O$ emissions from dairy pasture systems. However, there is no published data on the effect of using an inhibitor to reduce these losses in sheep winter grazing systems. This research project was therefore conducted to quantify the effect of using dicyandiamide (DCD) to reduce $NO_3^-$-N leaching and $N_2O$ emissions in an intensively winter grazed pastoral sheep system.

A trial was conducted at Lincoln University comprising of sixteen lysimeters of Templeton silt loam soil, sown in a ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture mixture. Four treatments (control, control + DCD, urine, urine + DCD) were applied in May 2009, with urine patches applied at an N loading rate equivalent to 300 kg N $ha^{-1}$. DCD treatments were applied at 10 kg DCD $ha^{-1}$. Simulated rainfall was applied in the spring to supplement natural rainfall in order to produce a similar water input to Southland averages conditions. Nitrous oxide gas collections and analyses were made twice a week over the first month, followed by once a week for the remainder of the experimental period. Drainage water was collected on a once or twice weekly basis depending on leachate volumes and was analysed to measure the $NO_3^-$-N concentration of the water.

Results showed that the application of DCD reduced the $N_2O$ emissions by 72% from sheep urine applied in the late autumn, from 4.55 kg $N_2O$-N $ha^{-1}$ without DCD to 1.31 kg $N_2O$-N $ha^{-1}$ over the four month experimental period. The application of DCD also reduced the amount of $NO_3^-$-N leached by 70% from sheep urine with a reduction from 147 kg $NO_3^-$-N ha$^{-1}$ to 44 kg $NO_3^-$-N ha$^{-1}$.

The data collected from the trial can be easily scaled up to a farm scale situation. Canterbury and Southland sheep farmers' 'break feed' approximately 1800 to 2000 s.u. ha$^{-1}$ over the winter period. Sheep urine patches (0.03 m$^2$ patch$^{-1}$) cover approximately 12% of the grazing area over 24 hours, therefore the N leaching and emission losses could be up to 18 kg N ha$^{-1}$ day$^{-1}$ and 0.54 kg N ha$^{-1}$ day$^{-1}$ respectively on grazed areas.

The use of a nitrification inhibitor has been shown to be beneficial environmentally in intensive sheep grazing situations, as it reduced N leaching and emission losses by up to 5.4 kg N ha$^{-1}$ and 0.15 kg N ha$^{-1}$ respectively in these conditions. In the future, intensive winter grazing systems may become a 'hot issue', where regional councils may target highly intensive farming systems. Results also indicate that the use of DCD may be an important tool to sustain intensive sheep grazing systems in the long term.

**Keywords:** nitrate leaching, nitrous oxide, nitrification inhibitor, dicyandiamide (DCD), *eco-n*, sheep, intensive winter grazing, urine patch, water quality.

# Acknowledgements

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# Chapter 1
# Introduction

Nitrate leaching from agricultural systems has been linked to increasing $NO_3^-$ concentrations in ground and surface waterways globally (Di & Cameron, 2002a). This has become a major environmental issue in the agricultural sector; due to the toxic effects it has in these waterways, having a negative effect on water life, humans and plants alike. These increasing concentrations have resulted from production intensification, through increasing stocking rates and fertiliser application rates and with changes in farm management practices.

Over several years of research, scientists have been studying the impacts of nitrate ($NO_3^-$) leaching from soils into ground water and mitigation of this problem e.g. Di and Cameron (2003) and Monaghan *et al*. (2007a). Cow urine is the main source of $NO_3^-$ leaching losses from grazed pasture systems and therefore the main focus of this previous research has been on reducing the amount of $NO_3^-$ that is leached from dairy cow urine deposited on to grazed pasture. Significant reductions in $NO_3^-$-N leaching losses of 60-70% have been observed with the use of the nitrification inhibitor dicyandiamide (DCD) under grazed dairy pasture systems (Di & Cameron, 2002a, 2004a, 2005).

Urine patches are also the main source of nitrous oxide ($N_2O$) greenhouse gas emissions from grazed pastures and research has also been conducted to find ways to reduce these emissions. It has recently been found that the application of DCD not only reduces $NO_3^-$ leaching losses but also reduces $N_2O$ emissions (Di & Cameron, 2002a). Reductions in $N_2O$ emissions of up to 80% have also been reported (Di & Cameron, 2002a, 2003, 2004b, 2006).

Livestock are typically stocked at 10-14 s.u. $ha^{-1}$ on sheep farms in Southland and Canterbury between late spring and early autumn. Leaching is not a major problem due to pasture being able to utilise deposited N from urine and soil moisture conditions generally not being overly saturated. Consequently, the use of DCD would probably be of minimal benefit. Nitrogen losses only become a problem over the winter and early spring periods when pasture growth is limited (resulting in limited N uptake) and therefore intensive grazing management is used, where ewes are fed rations in a big mob to slow intake, making the pasture last longer over winter. In addition, livestock are fed supplements, extending the time that animals spend grazing in one area, and are often wintered on more freely drained soils to minimise the effect of trampling, thus increasing the potential for N loss. Work in this area is urgently needed because when sheep are 'break fed' on pasture in Southland and Canterbury, the stocking rate is markedly increased in the relatively small area being grazed. This high intensity stocking rate increases the relative proportion of the area receiving animal urine and as a result potentially increases the amount of $NO_3^-$ leaching and $N_2O$ gas emissions.

It is not possible to make these measurements in an actual sheep grazing system due to too much variation occurring in the field. Therefore for the measurements to be precise and accurate the trial has to be carried out using lysimeter technology. These results can then be used to calculate the potential leaching losses and gas emissions that would occur under field conditions and to calculate the effectiveness of the inhibitor at the paddock scale.

The objective of this study is to assess the potential effect of using a nitrification inhibitor (DCD) to reduce $NO_3^-$ leaching losses and $N_2O$ emissions from intensively grazed pastoral sheep systems during winter and spring. The hypothesis being tested in this trial is that the application of the nitrification inhibitor DCD will reduce the amount of the $NO_3^-$ leaching loss and $N_2O$ gas emissions from a typical intensive sheep winter 'break feed' system used in Canterbury and Southland.

# Chapter 2
# Literature Review

## 2.1 Introduction

Nitrate leaching from agricultural production has been linked to increasing $NO_3^-$ concentrations in ground and surface waterways globally (Di & Cameron, 2002a). This has become a major environmental issue in the agricultural sector due to the negative effects it has on water quality and human health. The increasing $NO_3^-$ concentrations have occurred with the intensification of production, through increasing stocking and fertiliser application rates and changes in farm management practices.

In regions such as Southland, where a cool, moist environment predominates, leaching is particularly high. This is due to a high rainfall climate, and a four month winter period during which pasture growth and plant N uptake is limited due to cold conditions. The pastoral grazing systems are generally rotationally grazed over this period with the use of 'break feeding' on either pasture or forage crop. This reduces the amount of pugging on the property, while stock are grazing on the waterlogged soils. Many Canterbury and Southland farms are run intensively, with high stock numbers being grazed in confined areas over winter periods to increase feed consumption and reduce wastage when growth rates are limited.

Past research has focused on the issues of $NO_3^-$-N leaching and $N_2O$ emissions from dairy cows and the potential of nitrification inhibitor technology to reduce these N losses from grazed pasture systems in New Zealand (Di & Cameron, 2002a, 2002b, 2003, 2004a, 2005, 2008; Moir *et al.*, 2007). Although there are suggestions that nitrification inhibitors reduce $NO_3^-$-N leaching from low cow urine N concentrations (Di & Cameron, 2007), it is unclear whether $NO_3^-$ leaching from sheep urine can be influenced. The focus of this study is to measure nitrate reductions in intensive sheep winter grazing systems using nitrification inhibitor technology.

## 2.2 The nitrogen cycle

Nitrogen can be found in many different forms in the environment, with 18 x $10^{15}$ and 3.8 x $10^{15}$ tonnes being found in the earth's crust and atmosphere (mainly $N_2$ gas) respectively (McLaren & Cameron, 1996). Soil N is the main source of N for plant growth and eventually animal nutrition. Nitrogen is an essential element for plant functions, being used as a primary constituent of the basic amino acids (the building blocks of proteins such as enzymes) and nucleic acids (Kapal, 2008). Nitrogen is primarily obtained from the atmosphere, through N fixation by legumes and biological processes in the soil (Cameron, 1992).

Nitrogen is transferred from one form to another through a variety of processes within the soil/plant/atmosphere system. This transfer is generally referred as the "nitrogen cycle" (Figure 2.1), which forms an important integral part of the overall cycle of N in the earth system. The involvement of transformations and transfers of N within the cycle is important for maintaining N supply for plant growth.

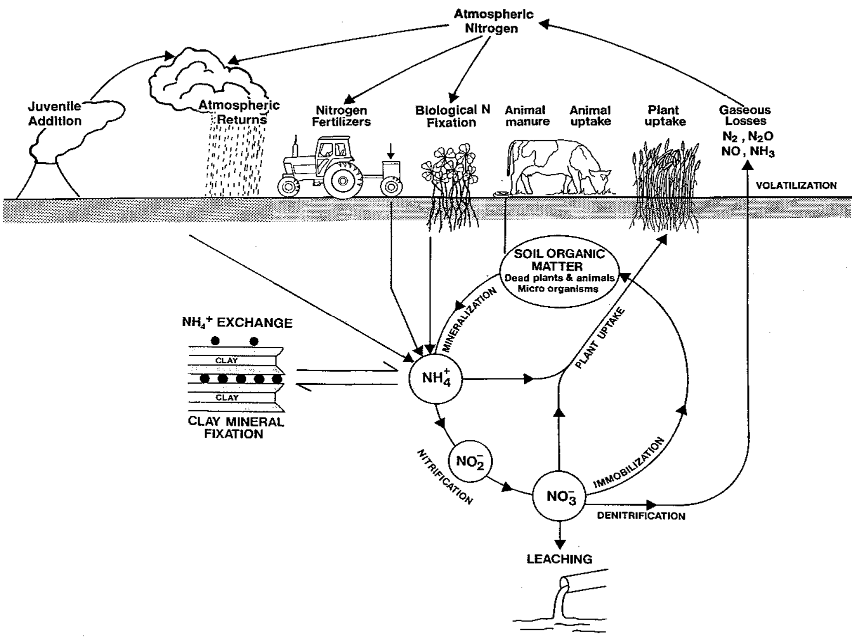


**Figure 2.1: The soil nitrogen cycle (Cameron, 1992).**

Nitrogen may be plentiful in the soil but is often present in forms that are unavailable to plants. Nitrogen is present as three main forms in the soil (Figure 2.2): (i) organic compounds in plant material, soil organisms and soil humus; (ii) ammonium ($NH_4^+$) ions held by clay minerals; and (iii) mineral N in soil solution ($NH_4^+$; $NO_3^-$; nitrite, ($NO_2^-$)) in soil solution (McLaren and Cameron, 1996). Ninety five percent of soil N is held in organic matter

(Cameron, 1992). Kapal (2008) stated that <2% of the N in soil organic matter is available for plant uptake as mineral N. Soils typically contain 0.1 to 0.3% N within the top 15 cm depth, equating to between 2,500 and 7,500 kg N $ha^{-1}$ (Cameron, 1992).

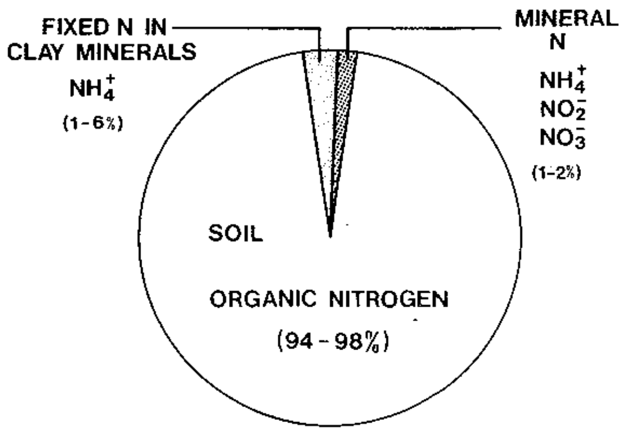


**Figure 2.2: Distribution of soil nitrogen (McLaren & Cameron, 1996).**

### 2.2.1 Plant uptake

A lack of N often limits pasture growth during autumn and spring months due to inadequate quantities of soil N being available, and from high N plant requirements, resulting from favourable climatic conditions (Moir *et al.*, 2007). Nitrogen can be taken up by plants either as $NH_4^+$ or $NO_3^-$. Nitrate may be absorbed and translocated unaltered within the plant, or converted to ammonia ($NH_3$) in the roots (McLaren & Cameron, 1996). This $NH_3$ is converted to amino acids, amides or amines, which are then translocated through the plant. Finally these compounds are converted into protein used for plant growth. This process can be reversed, with proteins being hydrolysed to form amino acids, a process called proteolysis. This allows the transferral of N from older tissue to younger growing ones during periods of N deficiency (Figure 2.3).

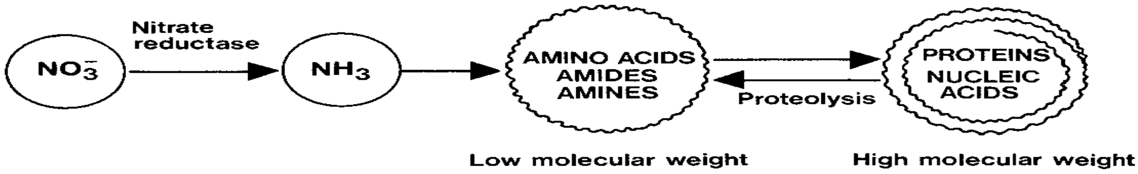


**Figure 2.3: Nitrogen metabolism in plants (McLaren & Cameron, 1996).**

The amount of N taken up by crops varies with crop type and the amount of protein required. Table 2.1 shows that grass has the highest N requirement of crops grown in Southland, with 300 kg N $ha^{-1}$ being required by grass pastures for a 10 t DM $ha^{-1}$ crop.

**Table 2.1: Amount of N taken up by various agricultural crops grown in Southland (Olsen & Kurtz, 1982).**

| Crop | Dry matter production (t DM $ha^{-1}$) | Nitrogen uptake (kg N $ha^{-1}$) |
|---|---|---|
| Wheat- grain | 5.5 | 110 |
| Wheat- straw | 6.0 | 45 |
| Grass | 10.0 | 300 |

### 2.2.2 Nitrification

The process of "nitrification" involves the conversion of soil $NH_4^+$ into $NO_3^-$ by oxidation reactions (Cameron, 1992). This reaction is brought about by the activity of two specific groups of autotrophic bacteria (i.e. they obtain carbon from carbon dioxide). Firstly, the reaction involves $NH_4^+$ being converted to $NO_2^-$ by *Nitrosomonas* and *Nitrosospira* (Figure 2.4):

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{energy}$$

**Figure 2.4: Reaction involved with first step of nitrification (Cameron, 1992).**

The second step of nitrification involves the oxidation of $NO_2^-$ to $NO_3^-$ by a single genus of bacteria called *Nitrobacter* (Figure 2.5). This conversion takes place rapidly and therefore nitrite rarely accumulates in the soil.

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{energy}$$

**Figure 2.5: Reaction involved with second step of nitrification (Cameron, 1992).**

Other nitrification processes are proposed, such as heterotrophic oxidation and photochemical methods, but these are of less significance compared with autotrophic bacterial activity. Heterotrophic nitrification is only likely to be of importance where autotrophs are inactive as these are sensitive to changes in soil conditions such as changes in soil pH, moisture content, temperature, nutrient status and vegetation (McLaren & Cameron, 1996). Ammonia-oxidising archaea (AOA) are present in large numbers in soils and until recently it was thought that they may also have a potential role in the N cycle. However, recent research has shown that ammonia-oxidising bacteria (AOB) are the most important type (Di *et al.*, 2009). AOA contain an *amoA* gene and this raised the prospect of it being important in nitrification. The *amoA* gene encodes a subunit of ammonia monooxygenase, which is involved in the first step of the nitrification process. However, Di *et al. (2009)* found that even though large numbers of AOA and AOB where present in various soils, it was only the AOB population abundance and activity that increased following addition of urine-N substrate (Figure 2.6a). The AOA population or activity showed no positive response (Figure 2.6b). Di *et al.* (2009) therefore concluded that these results demonstrate that AOB is the most important for nitrification in soils under animal urine patches.

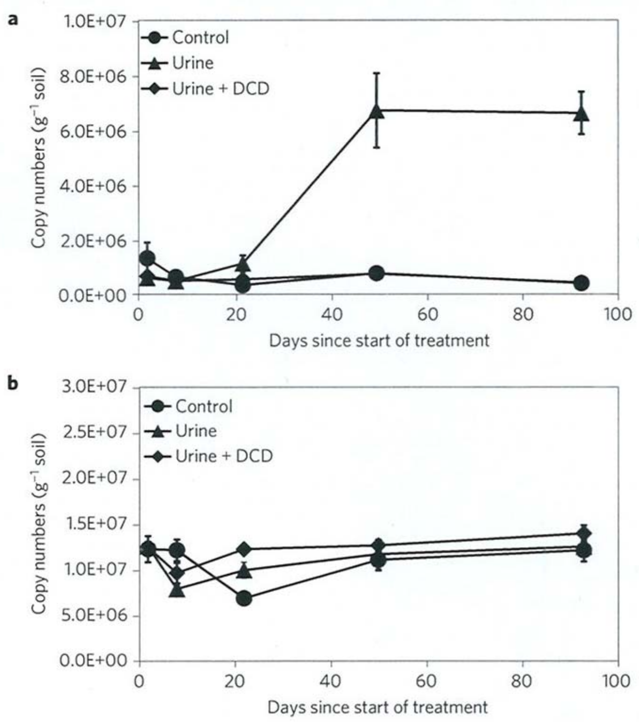


**Figure 2.6: Dynamics of AOB (a) and AOA (b) populations in a Waikato soil in an incubation study. The error bars indicate the S.E.M. (Di *et al.*, 2009).**

## 2.3 Environmental issues

### 2.3.1 Nitrate leaching

Nitrate leaching losses occur when an over supply of N in the soil solution from input processes such as mineralisation or urine excretion is not matched by subsequent uptake from pasture growth. Pasture plants are able to utilise approximately 300 kg N $ha^{-1}$ $year^{-1}$ (Haynes & Williams, 1993), leaving excess $NH_4^+$, which is converted to $NO_2^-$ and finally $NO_3^-$ by nitrifying bacteria. Nitrate is negatively charged, so is repelled by negatively charged soil clay particles (Cameron & Haynes, 1986). Leaching rates peak during winter and early spring, due to precipitation exceeding evapotranspiration during these months. Smith *et al.* (2008) found that leaching losses averaged 26 kg N $ha^{-1}$ over the winter and spring months in Southland.

Nitrate leaching from agricultural land through fertiliser application and livestock returns are of concern environmentally. Nitrate that is leached from the soil, inevitably ends up in waterways where it interferes with natural ecosystems causing eutrophication (Silva *et al.*, 2005). Nitrogen fertiliser has contributed to the intensification of New Zealand farming systems, which has led to increasing stocking rates and therefore increased $NO_3^-$ leaching. This is of concern as increasing $NO_3^-$-N concentrations can cause hazardous consequences in terrestrial, freshwater and marine ecosystems, as well as potentially affecting human health (WHO, 1984). Cameron and Haynes (1986) stated that non point sources contribute up to 90% of the $NO_3^-$-N leached into waterways, where 80% of this directly relates to agricultural input. Pollution from $NO_3^-$-N leaching can result in eutrophication of lakes and rivers, excessive algal bloom growth and eventually death of aquatic life.

A water body is classed as eutrophic when total N concentration reaches between 0.4 and 6 mg N $L^{-1}$ (Di & Cameron, 2002a). Inorganic N levels need to be kept below 0.3 $\mu g$ N ml $L^{-1}$ (Cameron & Haynes, 1986). Above this level, algal growth is rapidly stimulated and becomes a concern. High $NO_3^-$ levels may also affect human health, although the actual risk is disputed in the literature (WHO, 1984).

Elevated $NO_3^-$ intake may contribute to methemoglobinemia (blue baby syndrome) in formula-fed infants. Some researchers propose that other conditions such as gastric cancer, thyroid hypertrophy, reproductive toxicity and ulceration of the mouth and stomach lining could be affected by $NO_3^-$ (Silva *et al.*, 2005). However, such concerns are rejected by other researchers and the World Health Organisation (Addiscott & Benjamin, 2004; WHO, 2007).

The causes of methaemoglobinaemia are disputed in the literature. Cameron and Haynes (1986) propose that it is caused by consumed $NO_3^-$ being rapidly absorbed from the stomach into the blood supply. From here, the iron of haemoglobin is readily oxidised into a ferric state, forming methemoglobin. This product cannot function in the oxygen transport system and cellular anoxia can occur. If >50% of haemoglobin is oxidised, death generally occurs. However, Addiscott and Benjamin (2004) maintain that methaemoglobinaemia is caused by $N_2O$, not $NO_3^-$, and is caused purely by gastrointestinal infections with little or no relation to the quantity of $NO_3^-$ consumed.

The World Health Organisation recognises the strong link between gastrointestinal infections and methaemoglobinaemia, and accordingly has issued a maximum guideline value of 11.3 mg N $L^{-1}$ in water used for making infant formula. However, infants can continue to be given water up to 22.6 mg N $L^{-1}$, provided the water is boiled to reduce the risk of gastrointestinal infection (WHO, 2007). In New Zealand the WHO guideline value of 11.3 mg N $L^{-1}$ is stipulated by the New Zealand Ministry of Health as the maximum allowable concentration in drinking water to safeguard human health (Di & Cameron, 2004a).

### 2.3.2 Nitrous oxide gas emissions

Nitrous oxide gas is a by-product produced also from the application of N. Nitrous oxide is a greenhouse gas which is contributing to global warming and the depletion of the stratospheric ozone layer (Di & Cameron, 2002b; Clough *et al.*, 2004). Since 1950, the atmospheric concentration of $N_2O$ has increased by 17% (IPCC, 2001), with a predicted increasing rate of 0.25% $annum^{-1}$ (McLaren & Cameron, 1996). The long term global warming potential is approximately 310 times that of $CO_2$, with an atmospheric lifetime of an estimated 130 years (Di & Cameron, 2006).

Total $N_2O$ emissions comprise 33.4% of New Zealand's total agricultural emissions (Clough *et al.*, 2007). Nitrous oxide contributes approximately 20% of New Zealand's total greenhouse gas emission inventory, with animal excreta making up 87% of this total emission in 2007 (MFE, 2009). New Zealand is a signatory to the Kyoto Protocol and therefore needs to meet the target of reducing excess greenhouse gas emissions back to 1990 levels (Di & Cameron, 2008).

In 2004, total $N_2O$ gas emissions from New Zealand's agriculture was equal to 39.76 Gg $N_2O$-N, which was 24% higher than levels in 1990 (Clough & de Klein, 2006). These $N_2O$ emissions are dominated by N excreta deposited during grazing and from fertiliser. The IPPC guidelines categorises $N_2O$ emissions into 'direct emissions' from agricultural land, emissions from animal waste and 'indirect emissions' (Clough *et al.*, 2007).

Direct emissions from agricultural land caused by the N excreted from grazed animals are given as an emissions factor ($EF_{3PR\&P}$) and the New Zealand specific default value is 0.01 kg $N_2O$-N $kg^{-1}$ N excreted. In addition, leaching of N is the main source of indirect $N_2O$ emissions, which is a function of the total N inputs (fertiliser and excreta N) and the fraction of total N inputs that is leached ($Frac_{LEAC}$) (New Zealand specific default value 0.07 kg $N_2O$-N $kg^{-1}$ N leached). The mass of N leached is multiplied by a further emission factor $EF_5$ to calculate the amount of $N_2O$ produced from N leaching.

Nitrous oxide is formed through many processes associated with microbial nitrification and biological denitrification in the soil (McLaren & Cameron, 1996). This gas is produced under anaerobic conditions through the activity of bacteria causing denitrification. Under these conditions $NO_3^-$ is reduced to nitric oxide (NO), $N_2O$ and finally to dinitrogen gas ($N_2$) (Figure 2.7).

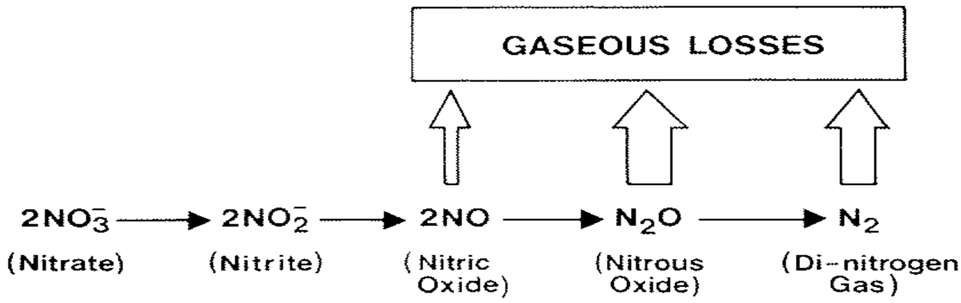


**Figure 2.7: General sequence of reduction steps in biological denitrification processes (McLaren & Cameron, 1996).**

All these gases can subsequently be lost from the soil into the atmosphere. The rate of denitrification is enhanced in wet, waterlogged soils due to a lack of oxygen, high concentration of aqueous $NO_3^-$, readily available sources of carbon to support micro-organism activity, and soil temperatures >5 ºC (Cameron, 1992).

Nitrous oxide can also be produced in aerobic soils during the nitrification process, where $NH_4^+$ is converted through to $NO_2^-$ and then $NO_3^-$, and intermediate products produced react to form the gas (Figure 2.1).

## 2.4 Environmental impacts of winter grazing

### 2.4.1 Dairy grazing systems

The New Zealand dairy industry plays a vital role in New Zealand's economy. The dairy sector is currently the biggest component of the agricultural industry, with over 4.35 million dairy cows being milked as of June 2009, (MAF, 2009). This industry is continuing to expand, replacing intensive sheep finishing systems on flat to rolling country. Approximately 1,440,000 hectares were used for dairy farming in the 2007/2008 season (MAF, 2009).

A typical New Zealand dairy system comprises dairy cows being rotationally grazed and fed high pasture allowances to maximise milk production. Under rotational feeding, pre-grazing targets are important for pasture quality and fed intake of cows. The ideal pre-grazing cover varies between 2200 kg DM $ha^{-1}$ with the use of no N and 2800 kg DM $ha^{-1}$ with a 50 kg N $ha^{-1}$ fertiliser application (Holmes & Roche, 2007). Cows are generally grazed to a post-grazing residual of 1500 kg DM $ha^{-1}$ (35-40 mm) during late autumn and winter. Autumn pastures are often hard grazed to a lower residual of 1200 kg DM $ha^{-1}$ to remove any accumulated old pasture from the sward, thus improving pasture quality (Holmes & Roche, 2007).

High pasture intakes (11-16 kg DM $head^{-1}$ $day^{-1}$) are required to meet the requirements for energy over the milking season. Over the winter months, intakes are reduced (6-7 kg DM $head^{-1}$ $day^{-1}$) while the cows are not being milked (Webby & Bywater, 2007). The main determinants of feed demand include the time of year, stocking rate (cows $ha^{-1}$ and feed required $cow^{-1}$) and dates at which cows calve and dry off. On average cows are grazed at a stocking density between 2.2 cows $ha^{-1}$ to 4.3 cows $ha^{-1}$ on pasture producing 17 to 20 t DM $ha^{-1}$ $annum^{-1}$ (Holmes & Roche, 2007). This is dependant on pasture growth rates and supply availability, the type of forage on hand and the type of cow breed being grazed and its feed requirements.

Higher feed intakes result in a large amount of the N ingested in the feed (70-90%) being excreted back onto the pasture as dung and urine (Di & Cameron, 2007). The proportion of N returned is dependant on pasture quality and content. In a good quality pasture with 4% N content, 70-80% of excreta N is excreted in the urine, with 70-90% of this excreted N being in the form of urea. It is estimated that a cow may urinate approximately 10-12 times per day, with each urine patch covering approximately 0.35 $m^2$ (Moir *et al.*, 2006). Poor utilisation of pasture N reflects a simple feature of the pasture-animal relationship where plants require higher N concentrations for optimum growth rates than required by grazing ruminants for protein synthesis (Haynes & Williams, 1993).

Furthermore, N losses have increased with the intensification of New Zealand dairy systems through increased stocking rates and the supplementation of pastures with N fertiliser, generally in the form of urea, to strategically boost pasture production during periods of shortage (de Klein & Ledgard, 2005).

Stocking rate is the key driver of N losses, with modern agricultural systems being more intensely operated than in the past as a result of improved soil fertility, pasture species and pasture management practices. For example, Monaghan *et al.* (2005) stated that dairy systems have been intensified with stocking densities varying between 1.9 and 2.6 cows ha$^{-1}$. These increases have resulted in greater pressure on the environment, because at higher stocking rates higher levels of urine excretion occur in one area. In Southland $NO_3^-$ leaching losses from low to moderately stocked (2 cows ha$^{-1}$) pastures were found to be within the acceptable limits for drinking water quality (Section 2.1.1). However, when grazed more intensively $NO_3^-$ leaching losses were found to be remarkably increased, with $NO_3^-$ being above acceptable levels with stocking rates above 2.5 cows ha$^{-1}$ and fertiliser application rates above 200 kg N ha$^{-1}$ (Figure 2.8).

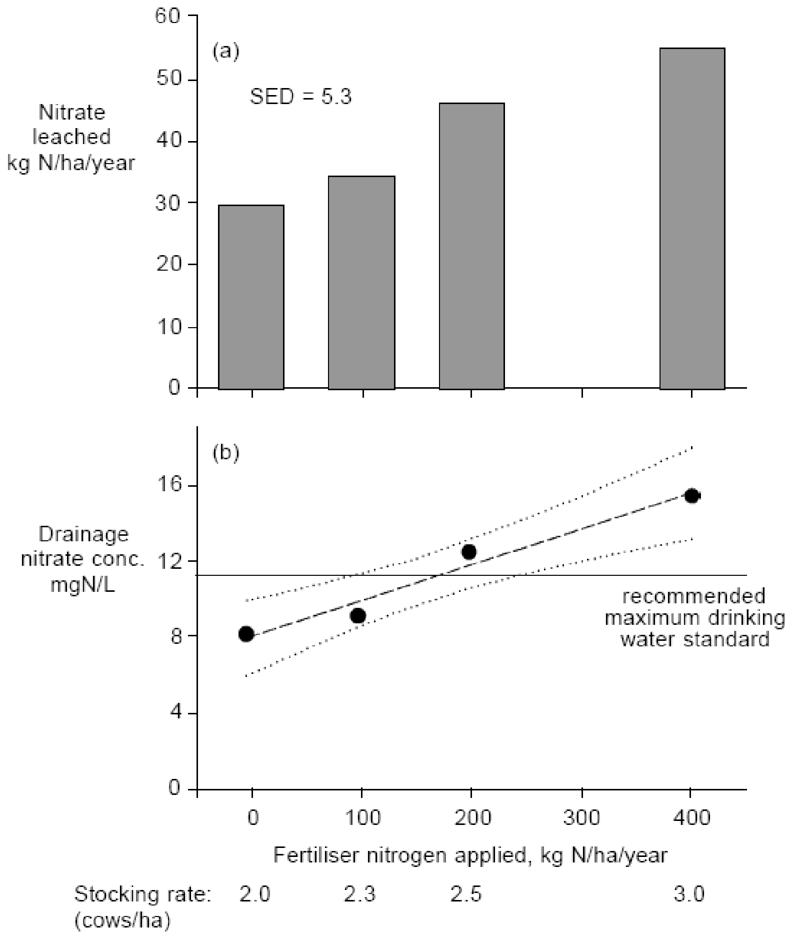


**Figure 2.8: Nitrate leaching losses under varying N inputs and stocking rates, represented as (a) mean annual amounts leached (kg N ha$^{-1}$ year$^{-1}$), and (b) mean nitrate-N concentrations in drainage water (mg l$^{-1}$, dotted lines represent upper and lower 95% Confidence Limits) (Monaghan *et al.*, 2000).**

Monaghan *et al.* (2005) found similar results, where an increase in stocking rate caused an increase in $NO_3^-$ leaching due to large quantities of N being excreted per hectare. When nil N fertiliser was applied an increase from 13 s.u. $ha^{-1}$ to 20 s.u. $ha^{-1}$, or 30.1 s.u. $ha^{-1}$ caused an increase in $NO_3^-$ leaching of 16% and 29%, respectively (Table 2.2).

**Table 2.2: Annual $NO_3^-$-N losses (kg $ha^{-1}$) in drainage water under various fertiliser and stocking rates (modified from Monaghan *et al.*, 2005).**

| | Fertiliser Treatment (kg N $ha^{-1}$) | | |
|---|---|---|---|
| **Stock units $ha^{-1}$ (cows $ha^{-1}$)** | 0 N | 100 N | 200N |
| 13 (1.6) | 25 | 29 | 33 |
| 20 (2.5) | 30 | 31 | 48 |
| 30.1 (3.8) | 35 | 42 | 57 |

It can be seen from this section and reading through the literature that stocking rate intensity influences the amount of N loss, and therefore research needs to be conducted to reduce $NO_3^-$ leaching losses under high stocking rates.

### 2.4.2 Sheep grazing systems

In New Zealand there are an estimated 38 million sheep being farmed, with most found in the Manawatu, Canterbury, Otago and Southland regions (MAF, 2009). In a typical Canterbury or Southland sheep winter grazing system, ewes are ‘break fed’ behind electric fences, with pastures being hard grazed to slow intake and spread pasture allowance further over the winter and to reduce feed shortages (Halford, 1974).

This method of grazing allows pasture to accumulate for use at lambing when it is needed most (Sheath *et al.*, 1987). The winter rotation length is dependant on seasonal growth, with cold areas such as Southland requiring a winter rotation length of 80-100 days due to low pasture growth rates (Sheath *et al.*, 1987). When under these intensive situations sheep generally grazed pastures down to approximately 500 kg DM $ha^{-1}$ (Sheath *et al.*, 1987). This is significantly lower than that which cows graze to as discussed in Section 2.2.1.

However, in a situation where pasture is going to be intensively grazed over winter, high soil fertility is required to provide adequate pasture supply coming into winter (Brown & Harris, 1972). With a high quality pasture mix such as ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), growth rates of 1400 kg DM $ha^{-1}$ may be achieved in the winter period of mid-May to mid-August (Figure 2.9).

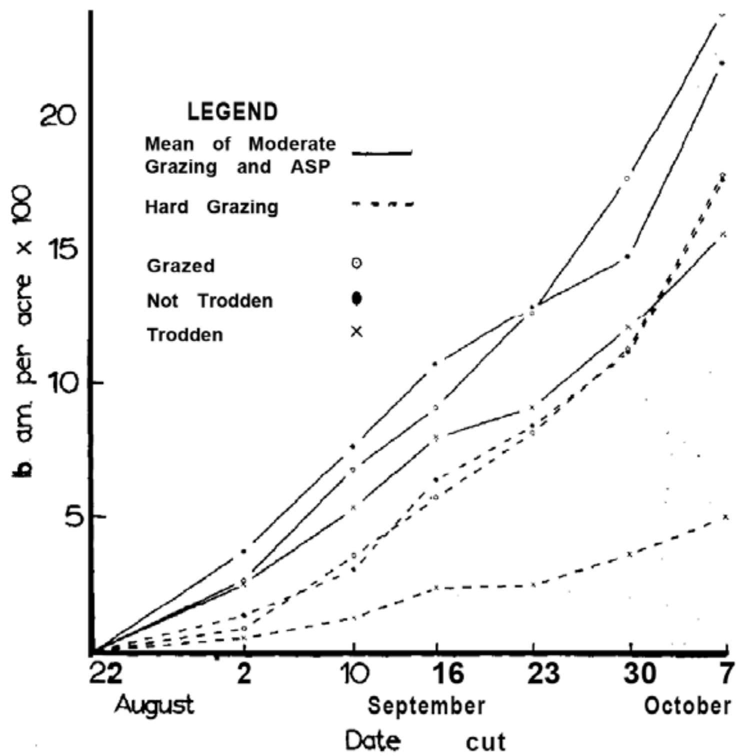


**Figure 2.9: Spring yields of total herbage following different winter grazing treatments (Brown & Harris, 1972).**

The use of precise grazing management practices is crucial during late spring, summer and autumn to produce feed of high quality (Lambert *et al.*, 2004). Farmers can generate high quality pasture by grazing to low pasture residuals (G. Edwards, personal communication, 2008). Korte (1981) reported that significantly less green herbage (249 kg DM ha$^{-1}$ vs. 783 kg DM ha$^{-1}$) and low quality dead herbage accumulated under hard grazing (Table 2.3).

**Table 2.3: Pasture composition at 95% light interception after hard and lax grazing treatments (Korte, 1981).**

| | Lax grazing | Hard grazing |
|---|---|---|
| **Date** | 7 February | 25 February |
| **Herbage Mass (kg DM ha$^{-1}$)** | | |
| Ryegrass stem | 783 | 249* |
| Dead herbage | 1527 | 300* |

** Hard significantly different from Lax ($P < 0.05$)*

Hard grazing also promotes increased tiller density, especially during spring (Brock, 2006). Korte (1981) reported that hard grazing in the spring produced leafy swards, which had a higher vegetative tiller density than stemmy swards in the subsequent summer. The difference was still apparent in June, despite both treatments having been grazed similarly for several months (hard 8660, lax 6720 tillers $m^{-2}$). Figure 2.10 illustrates the increased vegetative tiller density associated with hard grazing and a reduction in reproductive tillers, particularly during spring/early summer.

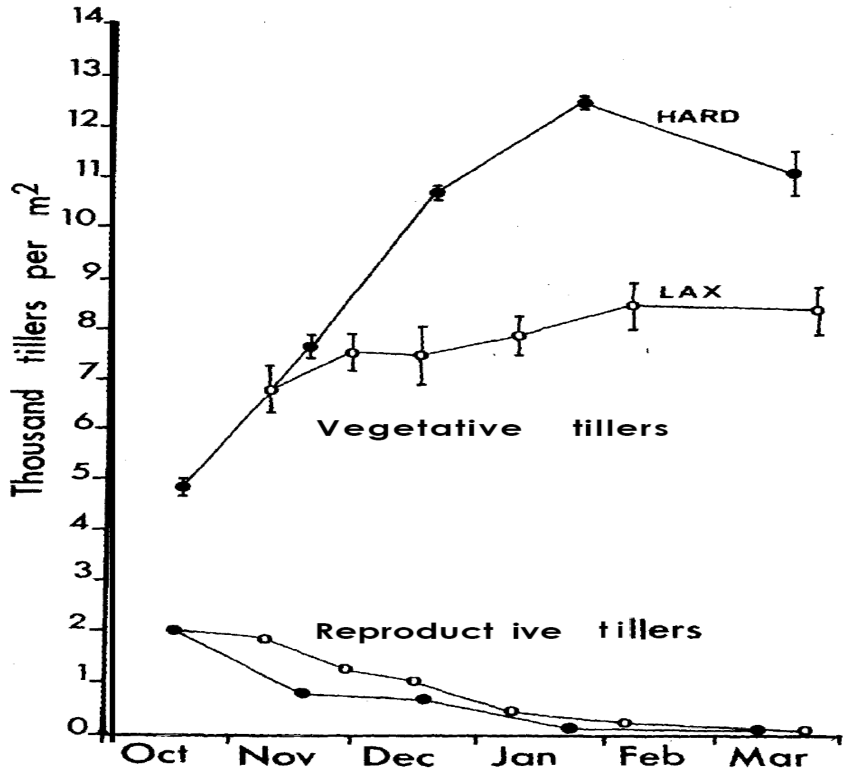


**Figure 2.10: Ryegrass tiller density at each grazing measured in fixed frames. Reproductive and vegetative tillers were respectively tillers with and without visible stem elongation (Korte, 1981).**

Conversely, lax and/or infrequent spring grazing produces swards with a higher proportion of reproductive stem and dead herbage (Butler & Chu, 1988). This is a result of higher post-grazing residuals with lax grazing. Summer swards that become overly long and reproductive lose quality due to lignification and increased cellulose. Furthermore, long rank pasture produces a higher proportion of dead matter in the bottom of the sward, which is then carried over into the winter, reducing feed quality.

Maintaining lower pasture covers also leads to increased clover content. This is due to hard grazing reducing the competition from the dominant ryegrass, providing the clover with the space and light it requires. The subsequent increase in clover content augments pasture quality, as legumes have a higher nutritive value and animal voluntary intake than grasses (Lambert *et al.*, 2004; Brock, 2006).

All-grass winter sheep feed systems are commonly used in Southland and Canterbury. This is because these systems provide a better control of feed intake to suit the availability of pasture production. These systems are also low cost, simple to plan, have a low labour requirement and high pasture utilisation which improves pasture quality. Finally, they allow the even distribution of dung and urine over the whole area, reducing fertility transfer (Watson, 1978).

Sheep are managed differently to dairy cattle due to their differing grazing patterns, nutrient and intake requirements. Under intensive sheep grazing systems, information on the average stocking rate used was limited. However, Morton and Baird (1990) provide information on the effect of stocking density on dung patch coverage (Table 2.4).

**Table 2.4: Mean no. of dung patches 4 $m^{-2}$, in each plot (Morton & Baird, 1990).**

| Stocking density (sheep $ha^{-1}$ $day^{-1}$) | Urine patches $m^{-2}$ in June |
|---|---|
| 900 | 6.66 |
| 1200 | 10.42 |
| 1500 | 11.08 |
| 1800 | 11.33 |
| LSD (5%) | 1.26 |

Over the winter, ewes have a low intake requirement 1-1.5 kg DM $ewe^{-1}$ $day^{-1}$ for true maintenance, (i.e. no change in liveweight) relative to cow intake requirements (Rattray *et al.*, 1987; Kenyon & Webby, 2007) and therefore sheep excrete a lower N concentration back onto the pasture. Sheep annually excrete 14.8 kg N $head^{-1}$ $annum^{-1}$, whereas cows can excrete up to 117 kg N $ha^{-1}$ $annum^{-1}$ (Clough *et al.*, 2007). Furthermore, sheep only excrete 300 kg N $ha^{-1}$ (9 g N $L^{-1}$) in a single urine patch, which is relatively small in comparison to dairy cows producing up to 1000 kg N $ha^{-1}$ in a single patch as stated in Section 2.1.1 (Williams & Haynes, 1990). Sheep urinate approximately 20 times a day with a single urine patching covering 0.03 $m^2$ (Morton & Baird, 1990; Haynes & Williams, 1993).

***Nitrate leaching losses and nitrous oxide emissions from sheep grazing systems***

Loss of N through leaching from grass-clover swards has been shown to be small when grazing animals are excluded from the system. It is thought that $NO_3^-$ leaching losses are lower under sheep grazing than under cattle grazing because sheep have a smaller bladder and urinate more often, spreading the distribution of N over a larger area (Di & Cameron, 2002a). However, high concentrations of sheep urine can nevertheless cause pulses of $NO_3^-$ to move below the root zone (Field *et al.*, 1984). A trial carried out by Di and Cameron (2007) measured the amount of leaching that occurred under a range of rates of urine deposition (300 kg N $ha^{-1}$, 700 kg N $ha^{-1}$ and 1000 kg N $ha^{-1}$) applied to pasture. The total leaching loss from 300 kg N $ha^{-1}$ (representing sheep urination) was found to reach 59.7 kg N $ha^{-1}$ compared with 22.8 kg N $ha^{-1}$ from the non-urine control ($P < 0.01$). The $NO_3^-$ concentration peaked significantly higher ($P < 0.01$) from 16.6 to 51.8 mg $NO_3^-$-N $L^{-1}$ when 300 kg N $ha^{-1}$ of cow urine was applied compared with the control (Figure 2.11).

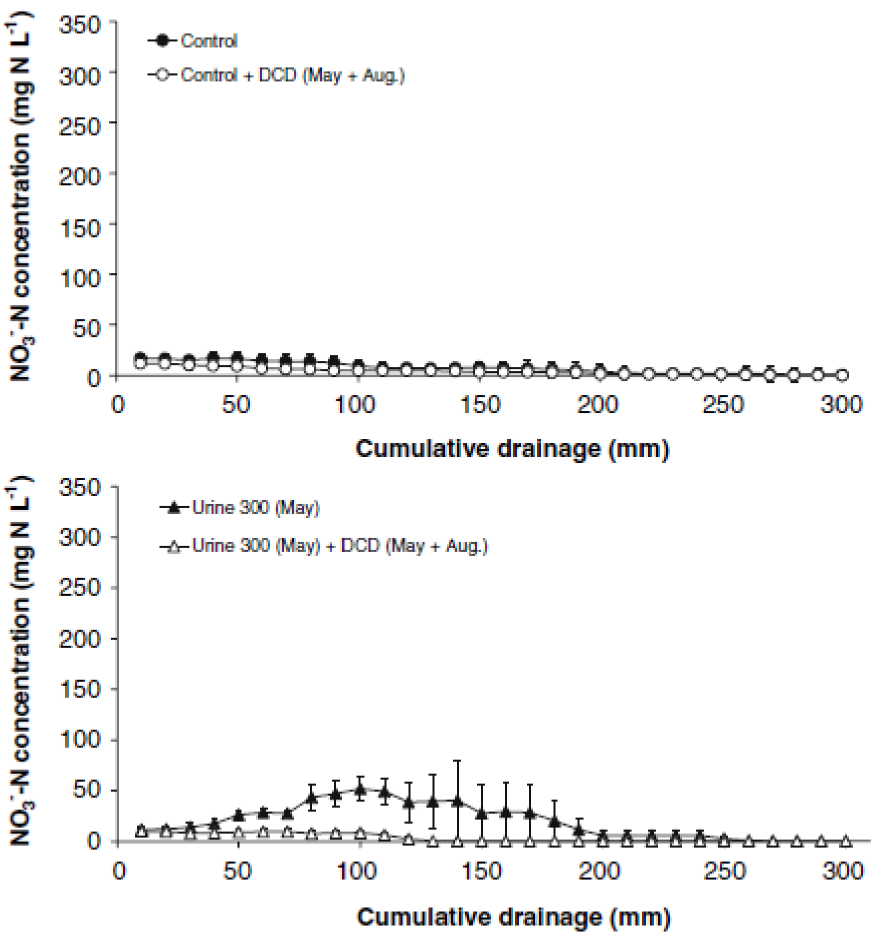


**Figure 2.11: Nitrate-N concentrations (± se) in the drainage water from lysimeters as affected by the urine application rate of 300 kg N $ha^{-1}$ and DCD application (Di & Cameron, 2007).**

However, when DCD was applied leaching losses were significantly ($P < 0.01$) reduced down to 12.4 kg N $ha^{-1}$ for the control and 9.9 kg N $ha^{-1}$ for the urine treatment. Ruz-Jerez *et al.* (1995) found that after 220-270 mm of drainage six to seven kg N ha $yr^{-1}$ was lost under sheep grazing on a sandy-loam soil. There is no published data on $N_2O$ emissions released from sheep urine.

## 2.5 Mitigation options

### 2.5.1 Nitrification inhibitor

The nitrification inhibitor DCD primarily slows the first stage of nitrification by deactivating the bacteria enzymes that convert $NH_4^+$ to $NO_3^-$ (Di & Cameron, 2004a) (Figure 2.12):

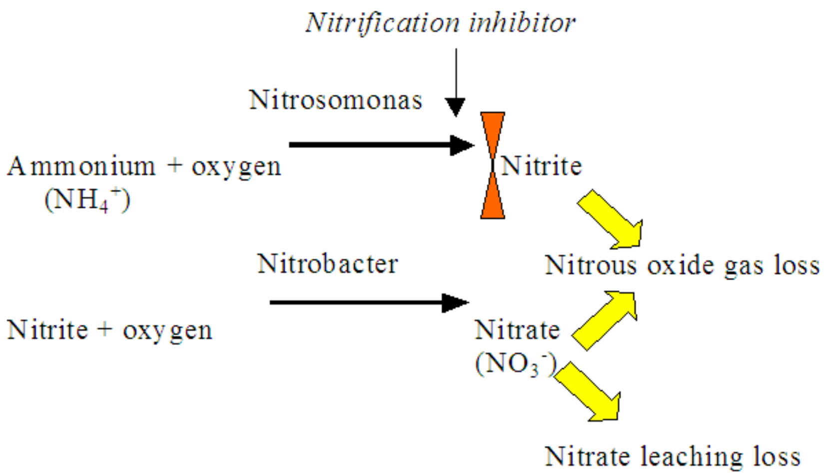


**Figure 2.12: The influence of DCD on nitrification, causing ammonium conversion to be slowed, reducing the loss of N (Cameron *et al.*, 2004).**

The nitrification inhibitor acts by 'binding' the site where $NH_4^+$ ions are converted to $NO_2^-$ (Figure 2.13) on an enzyme called *ammonia monooxygenase* produced by the *Nitrosomonas* and/or *Nitrosospira* bacteria (Di *et al.*, 2009).

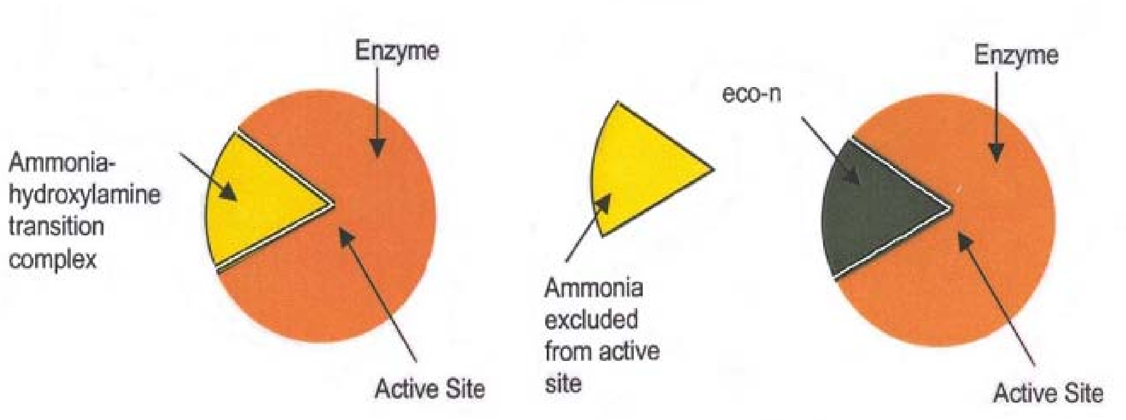


**Figure 2.13: The reaction of DCD on the ammonia monooxygenase enzyme (Christie & Roberts, 2004).**

On dairy farms, the DCD nitrification inhibitor technology *eco-n* (Ravensdown Fertiliser Co-operative Ltd) is applied in two 10 kg $ha^{-1}$ applications, one in May (autumn), and a second in August (spring), (Di & Cameron, 2007).

*Nitrification inhibitor effect on nitrate leaching*

Nitrate leaching has been found to be significantly reduced with DCD application. Di and Cameron (2004a) showed that a 74-76% reduction in $NO_3^-$ leaching occurred when DCD was applied to soil which had 1000 kg N ha$^{-1}$ of cow urine applied to it. When 200 kg N ha$^{-1}$ was applied with urine (1000 kg N ha$^{-1}$), 85 kg N ha$^{-1}$ was leached (Figure 2.10). When DCD was applied to this treatment in May the leaching loss was significantly reduced down to 20-22 kg N ha$^{-1}$. These results (Figure 2.14) show that the application of DCD is an effective way to reduce the amount of $NO_3^-$ leaching.

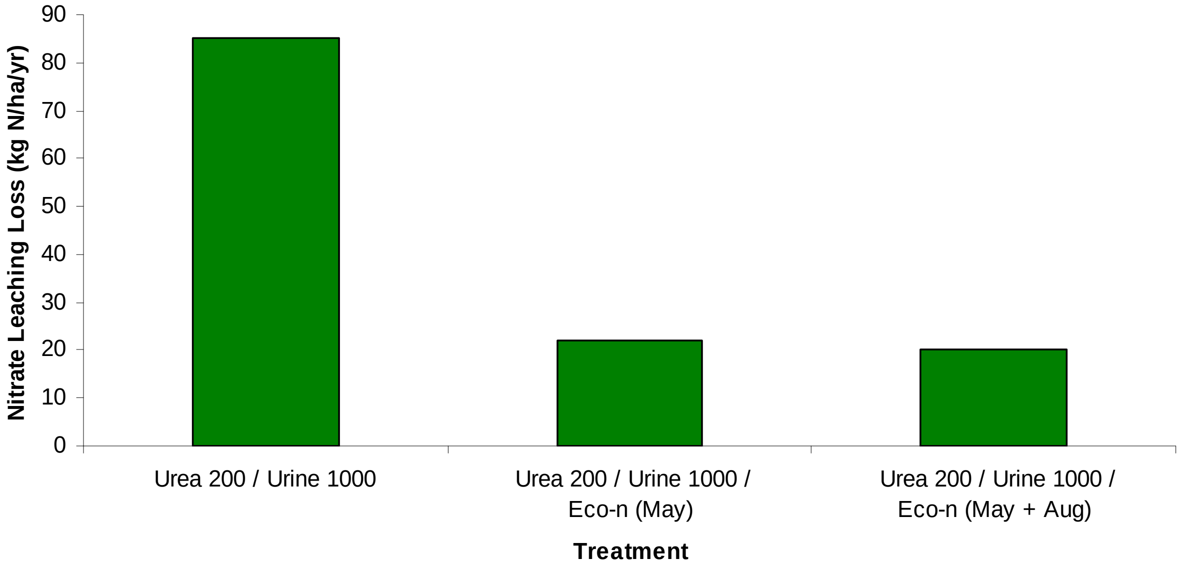


**Figure 2.14: Total $NO_3^-$-N leaching losses from urine patches (kg N ha year$^{-1}$) with combination of Urea (200 kg N ha$^{-1}$) and cow urine (1000 kg N ha$^{-1}$) and the affect of using DCD (Di & Cameron, 2004a).**

Similar results were reported by Di and Cameron (2005) when DCD was applied to 1000 kg N ha$^{-1}$ of cow urine and 200 kg N ha$^{-1}$ of urea. A 68% reduction was found where $NO_3^-$ leaching was reduced from 134 kg N ha$^{-1}$ to 43 kg N ha$^{-1}$. When reviewing a range of published data (Di & Cameron, 2002b, 2004a, 2005, 2007; Di *et al.*, 2007) on the effectiveness of DCD reducing $NO_3^-$ losses from urine patch areas in South Island soils, it was found from 14 data sets that leaching was reduced by an average 64% with the application of DCD in a dairy pasture system. Similarly, the use of DCD on simulated sheep urine patches (300 kg N ha$^{-1}$ of cow urine) was found to reduce $NO_3^-$ leaching by 83%, with the amount of $NO_3^-$ leached being reduced from 59.7 kg N ha$^{-1}$ to 9.9 kg N ha$^{-1}$ (Di & Cameron, 2007), however this was not significant ($P < 0.01$) (Table 2.5).

**Table 2.5: Total $NO_3^-$-N leaching losses as affected by cow urine application rate to simulate sheep urine (300 kg N $ha^{-1}$) and DCD (Di & Cameron, 2007).**

| Treatment | $NO_3^{-1}$-N leaching loss (kg N $ha^{-1}$) | % reduction by DCD |
|---|---|---|
| Control | 22.8 | n.a. |
| Control + DCD | 12.4 | 46 |
| Urine 300 | 59.7 | n.a. |
| Urine 300 + DCD | 9.9 | 83 |
| LSD (0.05) | 103.2 | n.a. |

***Nitrification inhibitor effect on nitrous oxide***

Nitrification inhibitors are not only beneficial for reducing $NO_3^-$ leaching, but also can be used as a mitigation strategy to reduce $N_2O$ emissions. Di and Cameron (2002b) measured an 82% reduction in $N_2O$ emissions with the application of DCD. Without DCD, the $N_2O$ emissions produced were 46 kg $N_2O$-N $ha^{-1}$, which was reduced to 8.5 kg $N_2O$-N $ha^{-1}$ with application of DCD. Di and Cameron (2003) also found that by treating grazed pasture soil with the DCD nitrification inhibitor, the $N_2O$ flux was reduced by 76% in the autumn and in spring (Table 2.6).

**Table 2.6: Total $N_2O$ emissions following urine treatments (Di & Cameron, 2003).**

| Treatments | Total $N_2O$ emissions (kg $N_2O$-N $ha^{-1}$) |
|---|---|
| Urea + Urine (autumn) | 26.7 (5.9) |
| Urea + Urine (autumn) + DCD (once after urine) | 7.0 (2.3) |
| Urea + Urine (autumn) + DCD (autumn & spring after urine) | 7.6 (1.1) |
| Urea + Urine (autumn) + DCD (once, mixed with urine) | 4.5 (1.3) |
| Urea + urine (spring) | 18.0 (2.9) |
| Urea + Urine (spring) + DCD (once after urine) | 4.5 (0.7) |
| Urea + Urine (spring) + DCD (quarterly) | 4.8 (0.9) |
| Urea + Urine (spring) + DCD (after urine & urea) | 2.5 (0.5) |
| LSD (P<0.05) | 7.6 |

A review of the published literature by Di and Cameron (2002b, 2003, 2006) and Di *et al*. (2007; 2009) showed an average reduction of 68% in $N_2O$ emissions was calculated from 23 datasets.

***Nitrification inhibitor effect on pasture production***

The use of a nitrification inhibitor can cause an increase in pasture production and N uptake by the plants. Pasture production in the dairy industry has been found to be increased by up to

20% with the application of DCD (Moir *et al.*, 2007). This is due to more N being held in the soil, facilitating plant growth. Di and Cameron (2004a) found that when Urea (200 kg N $ha^{-1}$) was applied with dairy urine (1000 kg N $ha^{-1}$), 15.9 t DM $ha^{-1}$ was produced. This was increased to 18.2 t DM $ha^{-1}$ and 21.1 t DM $ha^{-1}$ when DCD was applied in May, and May/August applications (Figure 2.15). The pasture increase is due to retained N in the root zone as a result of reduced N leaching in the urine patch.

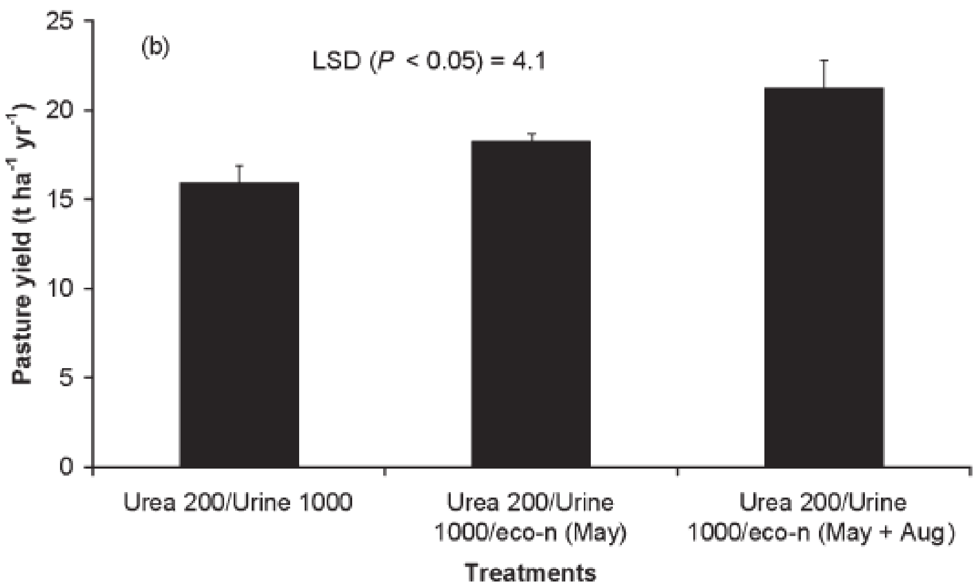


**Figure 2.15: Total annual herbage yield from lysimeters on an irrigated pasture in a sandy soil (Di & Cameron, 2004a).**

Pasture production was significantly ($P < 0.01$) increased from 4.42 t DM $ha^{-1}$ in the control to 10.82 t DM $ha^{-1}$ with the application of 300 kg N $ha^{-1}$ of cow urine, which represents the rate of N which sheep apply when they urinate. Pasture yields were significantly ($P < 0.01$) enhanced even further with the application of a nitrification inhibitor, with 5.41 t DM $ha^{-1}$ (control) and 13.31 t DM $ha^{-1}$ (300 kg N $ha^{-1}$ urine) being achieved (Di & Cameron, 2007).

***Other nitrification inhibitor benefits***

DCD is cheaper to produce than other inhibitors such as nitrapyrin, and it has a high water solubility, allowing it to be applied as a liquid or fine particle suspension. It is also less volatile than Nitrapyrin and decomposes into $NH_4^+$ and $CO_2$ in soil (Di & Cameron, 2002b). Furthermore, DCD is a suitable product due to being classified as a non-toxic substance (Amberger, 1989), with a low $LD_{50}$ value (10 g $kg^{-1}$ of bodyweight), which is approximately three times higher than common table salt.

The effectiveness of DCD is some what dependant on soil temperature. DCD has been shown to be most effective in cool (<10 ºC) soil temperatures, such as those found in Southland. Di and Cameron (2004b) analysed the impact of temperature on the effectiveness of DCD and reported that at lower temperatures the inhibitors half-life was extended. The authors showed that the half life is significantly increased from 25 days to 111 days when the temperature was decreased from 20 °C to 8 °C under 7.5 kg DCD $ha^{-1}$ (Table 2.7).

**Table 2.7: Breakdown rate constants and half-lives of DCD in the soil as affected by incubation temperature (Di & Cameron, 2004b).**

| Treatments | Breakdown rate constant per day | Half lives (days) | P |
|---|---|---|---|
| *Incubated at 8 °C* | | | |
| Urine + Urea + 7.5 kg DCD $ha^{-1}$ | 0.00567 | 111 | 0.04 |
| Urine + Urea + 15 kg DCD $ha^{-1}$ | 0.00542 | 116 | 0.04 |
| *Incubated at 20 °C* | | | |
| Urine + Urea + 7.5 kg DCD $ha^{-1}$ | 0.0266 | 25 | 0.001 |
| Urine + Urea + 15 kg DCD $ha^{-1}$ | 0.0373 | 18 | 0.002 |

### 2.5.2 Grazing management

***Standoff pads***

To reduce animal excretion impacts when soil is susceptible to leaching over winter months, the animals can be taken off the pasture in autumn (Di & Cameron, 2002a). In Southland the use of wintering pads could be suitable with sheep being housed and fed on a pad over the winter months. Sheath *et al.* (1987) stated that these concrete wintering pads reduce pugging on pastures, but suggested that there was no experimental evidence that justifies such options in the sheep industry.

However, Monaghan *et al.* (2007b) stated that wintering pads are a cost effective mitigation option for reducing $NO_3^-$ leaching in the dairy industry, where a 33% reduction in N losses occurred when cows were on the feed pad. Under the current system, 30 kg N $ha^{-1}$ $annum^{-1}$ can be lost into waterways from a dairy system, whereas when cattle are put onto a feed pad, Monaghan *et al.* (2007b) found that N losses were reduced down to 20 kg N $ha^{-1}$ $annum^{-1}$. Furthermore, due to nutrients being imported from off farm, the accumulation of 85% of these nutrients occurs in the pad area, which then can be applied out onto the farm in spring.

***Stocking Rate Reduction***

To reduce $NO_3^-$ leaching, the stocking rate could be reduced over the winter period, when leaching can peak. Piwowarczyk *et al.* (2007) showed that a higher stocking rate could cause greater leaching, due to higher urine and dung input and larger amounts of fertiliser being applied to encourage pasture growth. Peyraud and Delaby (2006) discovered that reducing the stocking rate (by grazing non-capital livestock off-farm) reduced the amount of urine input applied to the pasture. Under a stocking rate of 4.6 s.u. $ha^{-1}$, 220 kg N $ha^{-1}$ was excreted, which was reduced down to 178 kg N $ha^{-1}$ (19% reduction), when 3.7 s.u. $ha^{-1}$ were present. Alfaro *et al.* (2008) found that at a stocking rate of 5 steers $ha^{-1}$, $NO_3^-$ losses were 4.7 kg N $ha^{-1}$. When the stocking rate was decreased to 3.5 steers $ha^{-1}$, only a loss of 1.7 kg N $ha^{-1}$ occurred.

### 2.5.3 Riparian strips

The use of riparian strips is an appropriate mitigation method, which can be used to help reduce the risk of surface runoff of urine and fertiliser into waterways. A riparian strip is a buffer zone of vegetation which is planted at the water's edge. These intercept pollutants and nutrient loads through filtration, biological uptake and denitrification. The vegetation acts like a filtering system, reducing the amount of waste ending up in the waterway (Monaghan *et al.*, 2007b). The use of a sawdust layer is another type of buffering zone, which can be used to reduce waste movement into waterways. Schipper and Vojvodic-Vukocic (1998) found when a 1.5 m deep by 1.5 m wide sawdust wall was buried into the soil, losses to a waterway were reduced from 5-15 mg N $L^{-1}$ to <2 mg N $L^{-1}$. Fencing off waterways is another adequate solution, reducing stock access to these waterways to prevent stock defecation into the water (Monaghan *et al.*, 2007b).

## 2.6 Conclusions

- Nitrate leaching and $N_2O$ emissions have become an increasing environmental problem, and results from the intensification of agriculture. This intensification has had an impact on waterway pollution and causes risks to animal and human health.

- Use of a nitrification inhibitor technology (DCD) has been shown to reduce $NO_3^-$ leaching and $N_2O$ losses by up to 76% and 82% respectively in dairy pasture systems where urine-N loadings are high (1000 kg N $ha^{-1}$).

- Although there are suggestions that a nitrification inhibitor could be used to reduce $NO_3^-$ leaching from lower rate of urine N input, there is limited scientific information specifically examining the reduction of N losses from sheep urine patches under intensive winter ‘break fed’ situations. This study therefore aims to focus on this knowledge gap and establish whether N loss reductions in intensive sheep grazing systems are achieved with the use of nitrification inhibitor technology.

# Chapter 3
# Materials and Methods

## 3.1 Experimental design

The experimental design consisted of a $2^2$ complete randomised design. This design was made up of two treatments, (i) sheep urine and (ii) the nitrification inhibitor DCD, which were applied to lysimeters. Four different combinations of the two treatments were applied:

- Control (no urine)
- Control + DCD (10 kg $ha^{-1}$)
- Urine (300 kg N $ha^{-1}$) alone
- Urine (300 kg N $ha^{-1}$) + DCD (10 kg $ha^{-1}$)

Each treatment was replicated four times to give a total of 16 lysimeters.

## 3.2 Site description and preparation

The intact soil lysimeters were collected from a pasture soil (Templeton silt loam) on the Lincoln University Organic Farm. No grazing had occurred for at least four months at this site, so that the effects of previous urine deposition were negligible. Pasture composition was predominantly perennial ryegrass and white clover.

### 3.2.1 Soil Fertility

Soil samples (75 mm cores) were taken from the soil collection area on 23 March 2009, to examine soil nutrient status. Soil cores where analysed by Analytical Research Laboratories (ARL), Napier and the results are presented in Table 3.1. Soil pH was measured at a water: soil ratio of 2.5:1 (Blakemore *et al.*, 1987). The method of Olsen *et al.* (1954) was used to measure plant available soil P. Extractable soil sulphate was determined by method of Searle (1979). Soil extractable cations were measured by method of Schollenberger and Simon (1945). A modified method from Waring and Bremner (1964) and Keeney and Bremner (1966) was used to measure soil mineralisable N.

**Table 3.1: Average soil fertility results for the Templeton silt loam used in this study.**

| | | Exchangeable Cations | | | |
|---|---|---|---|---|---|
| pH | Olsen-soluble P $\mu g\ mL^{-1}$ | Calcium Quick Test Units | Magnesium Quick Test Units | Potassium Quick Test Units | Sodium Quick Test Units |
| 6 | 23 | 8 | 16 | 13 | 6 |

## 3.3 Lysimeter sampling

The trial was conducted using 16 plastic lysimeters, measuring 300 mm long and 200 mm in diameter. Once an appropriate site was found the lysimeter casings were placed on the ground and the pasture surface was broken to expose the soil. While holding the casing down with a foot, the soil was chipped away from around the outside of the lysimeter (Plate 3.1), forming a square like hole around the lysimeter.

**Plate 3.1: Lysimeter being collected by digging around the PVC casing.**

Once a 25 mm column was exposed beneath the lysimeter, the lysimeter casing was pushed downwards over the soil column (Plate 3.2). Once the pasture was level with the top of the lysimeter, hot liquid petroleum jelly was poured between the soil column and the lysimeter casing to stop any leachates from running down between the soil and the casing (Cameron *et al.*, 1990). Once the petroleum jelly was 'set' the cores were sliced off at the bottom with a spade and transferred to the lysimeter paddock facility at the Field Service Centre, Lincoln University.

**Plate 3.2: Digging and sealing of the lysimeter cores.**

In order to assist with drainage, 20 mm of soil was taken out of the base of each lysimeter and replaced with gravel to help aid leachate collection and to prevent soil blocking the drainage tubing. A base plate was sealed on to the bottom of each lysimeter with silicon. A small tube (5 mm diameter) was attached to the base plate in order for the leachates to be collected (Plate 3.4). Stainless steel collars were attached to the top of each lysimeter to provide an annular water trough to assist with gas collection (Plate 3.3).


**Plate 3.3: Lysimeters with fitted collars to assist with gas collection.**


**Plate 3.4: A) Removal of 20 mm of soil; B) drainage gravel; C) base plate.**

***Lysimeter installation***

The lysimeters were installed in a field trench facility. Firstly, an area was marked and dug out for the lysimeters to be put into. Eight 150 mm diameter pipes where laid at a 200 mm depth in the area adjacent to the lysimeter trench (Plate 3.5). Two 20 mm diameter holes where drilled into each pipe to allow the tubes from the lysimeters to run into the leachate containers in the main trench. The lysimeters where placed on top of the laid pipes, and once in place, soil was filled around each lysimeter until it was level with the existing soil level in the paddock.

**Plate 3.5: Lysimeters being put in place.**

In the trench, four metal boxes were put in place. Groups of four lysimeter tubes ran into each metal box containing a two-litre leachate collection container for each lysimeter (Plate 3.6).

**Plate 3.6: Leachate containers for $NO_3^-$ collection.**

### 3.3.1 Simulated grazing

Before urine and DCD application, the pasture was cut down to 500 kg DM $ha^{-1}$ residual (20 mm). This residual height was calculated from the survey of farms in Canterbury and Southland, as well as from literature (Sheath *et al.*, 1987), stating that sheep generally graze to this height when in intensive 'break feeding' situations. In order to simulate sheep grazing on a winter 'break' causing typical treading damage, the surface of each lysimeter was 'pugged up' by applying a continuous upward and downward pressure using a sheep hoof (Plate 3.7).

**Plate 3.7: Trampling of pasture with a sheep hoof.**

### 3.3.2 Treatment applications

Urine was collected from two sheep taken off pasture and put into 'sheep crates' on 18th of May 2009. Two litres of urine was collected into a container and then analysed to measure the N concentration. The N concentration was found to be 15 g N $L^{-1}$. Since the average N concentration of sheep urine is 9 g N $L^{-1}$ (Haynes & Williams, 1993) water was added to the urine solution to dilute the N concentration down to 9 g N $L^{-1}$. Urine was applied to eight lysimeters at the equivalent of 300 kg N $ha^{-1}$ (Di & Cameron, 2007) on the 19th of May 2009, which is the average concentration applied in a sheep urine patch (Haynes & Williams, 1993).

Calculation of applied urine volume is as follows:

$$300 \text{ kg N ha}^{-1} = 30 \text{ g N m}^{-2}$$

Surface area of each 200 mm diameter lysimeter $= \pi r^2$

$= 3.14 \times 0.1^2$

$= 0.0314 \text{ m}^{-2}$

Amount of urine required $= 30 \text{ g}^{-1} \text{ m}^{-2} \times 0.0314 \text{ m}^{-2}$

$= 0.942 \text{ g}$

Urine at 9 g $L^{-1}$ is equal to $9 \times 10^{-3}$ g $ml^{-1}$, therefore 0.942 g / $9 \times 10^{-3}$ g $ml^{-1}$ = 105 ml

Therefore urine application rate = 105 ml urine $lysimeter^{-1}$.

Flags were used to mark out which lysimeters were to receive urine. The 105 ml of urine was measured using a measuring cylinder and then tipped straight onto a lysimeter similar to how a sheep would urinate onto pasture (Plate 3.8). As the urine was applied, the flags were removed to prevent urine being applied twice to any lysimeter.

**Plate 3.8: Application of sheep urine with measuring cylinder.**

The nitrification inhibitor DCD application occurred an hour later using a fine suspension sprayer, simulating on-farm application by a commercial spray contractor (Clough *et al.*, 2007). Once again flags marked out were the DCD treatment was to be placed. In addition, lids were placed over the lysimeters which were not to receive any DCD in order to stop any contamination from wind blown DCD liquid.

The DCD was sprayed evenly over the whole surface area of each lysimeter, starting with a circular motion and then changing the motion frequently to make sure the whole area was covered (Plate 3.9). The applicator was an airbrush run by an air compressor, which sprayed a fine mist.

**Plate 3.9: DCD spray application.**

Once the treatments had been applied, 10 mm of water (315 ml lysimeter$^{-1}$) was applied to each lysimeter (Plate 3.10), to wash in the DCD as per recommendations for use of *eco-n* (Di & Cameron, 2007).

**Plate 3.10: Irrigation application.**

### 3.3.3 Irrigation

During periods of dry weather in the spring, natural rainfall was supplemented with simulated rainfall to make up to the average monthly rainfall for Southland. A measuring cylinder provided an accurate measurement of the amount of water applied and this was then transferred to a watering can for application (Plate 3.10). Towards the end of the trial, extremely dry conditions occurred and stimulated rainfall was needed to ensure that Southland rainfall conditions were simulated correctly.

## 3.4 Measurements

### 3.4.1 Gas collection and analysis

The gas chamber lid fitted into the specially constructed water trough collars around each lysimeter (Plate 3.11). Gas samples were taken between 1 pm and 2 pm twice per week for the first month and then once per week for the next three months (or until background emission levels were reached). Gas samples were collected at 0, 20 and 40 minute intervals using a syringe to transfer the gas into glass vials. The concentration of $N_2O$ in each gas sample was determined by gas chromatograph analysis (Clough *et al.*, 2007; Di *et al.*, 2007).

**Plate 3.11: Nitrous oxide gas collection.**

### 3.4.2 Leachate collection and analysis

Drainage water was collected from the base of each lysimeter on a one to two weekly basis, or whenever a high amount of rainfall caused a significant amount of drainage to occur (Plate 11). Flow injection analysis was used to determine the concentration of $NO_3^-$ and $NH_4^+$ in the leachate (Di & Cameron, 2002b, 2004c; Cameron *et al.*, 2007).

**Plate 3.12: Nitrate leachate collection.**

### 3.4.3 Pasture yield

Pasture yield and N uptake was not analysed in this trial, as the lysimeter size was not representative of a paddock scale. Furthermore, the yields produced are not representative of an annual pasture production as the trial was only carried out for four months during the winter period when growth rates are limited. The focus of this trial was to determine the effect of a nitrification inhibitor on reducing $NO_3^-$ leaching and $N_2O$ gas emissions under an intensive winter sheep grazing system.

### 3.4.4 Statistical Analysis

All total $NO_3^-$-N and $N_2O$-N data sets were statistically analysed to test for treatment effects by conducting an analysis of variance (ANOVA) and producing a 5% LSD using Genstat 11. The $NO_3^-$-N concentration interaction with cumulative drainage and $N_2O$-N flux data sets were statistically analysed using repeated measures REML, with 5% LSD.

# Chapter 4

# Results

## 4.1 Water inputs, drainage and temperature

The 2009 autumn in the Canterbury region was abnormally wet, with a severe rainfall event occurring in May (Figure 4.1). Over winter/spring, conditions were abnormally dry compared with previous years. Total water inputs over the trial period were equivalent to 471 mm over the four months (Figure 4.1). This comprised of 221 mm of natural rainfall and 250 mm of simulated rainfall. The amount of drainage was equivalent to 269 mm for the control (Figure 4.2). Drainage from urine and urine + DCD (10 kg ha$^{-1}$) treatments were found to be slightly lower (200 mm, Figure 4.6), probably partly due to a greater pasture dry matter yield, resulting in greater evapotranspiration losses. Most drainage occurred under saturated conditions and when soil temperatures and evapotranspiration losses were low. Daily 10 cm soil temperatures ranged from 2.4 ºC in late June to 17.7 ºC in early October (Figure 4.3).

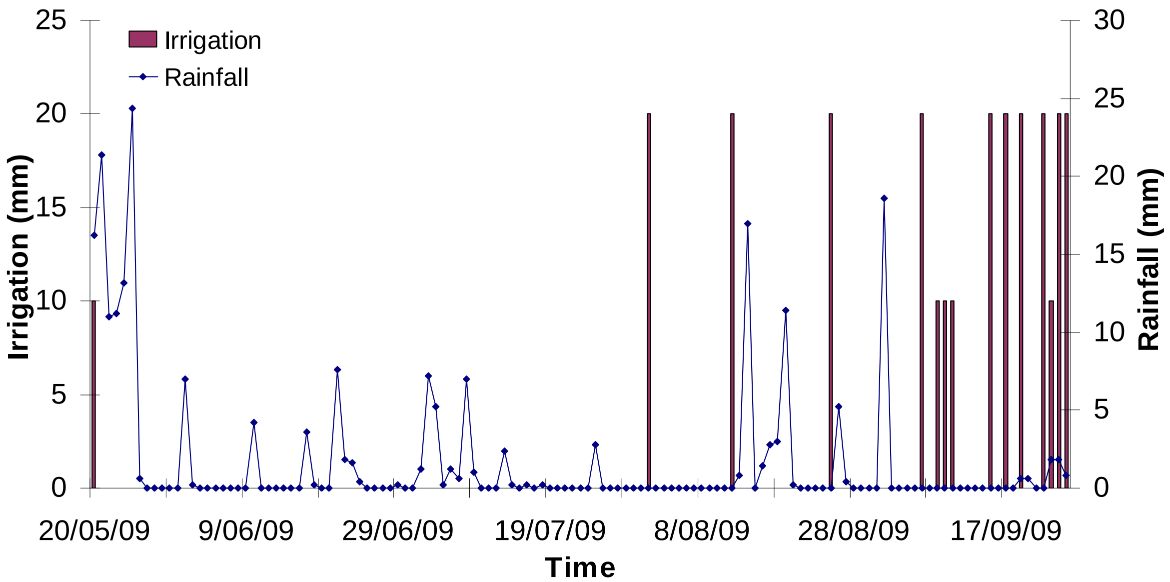


**Figure 4.1: Daily rainfall and simulated rainfall inputs (mm) to the lysimeters over the duration of the trial.**

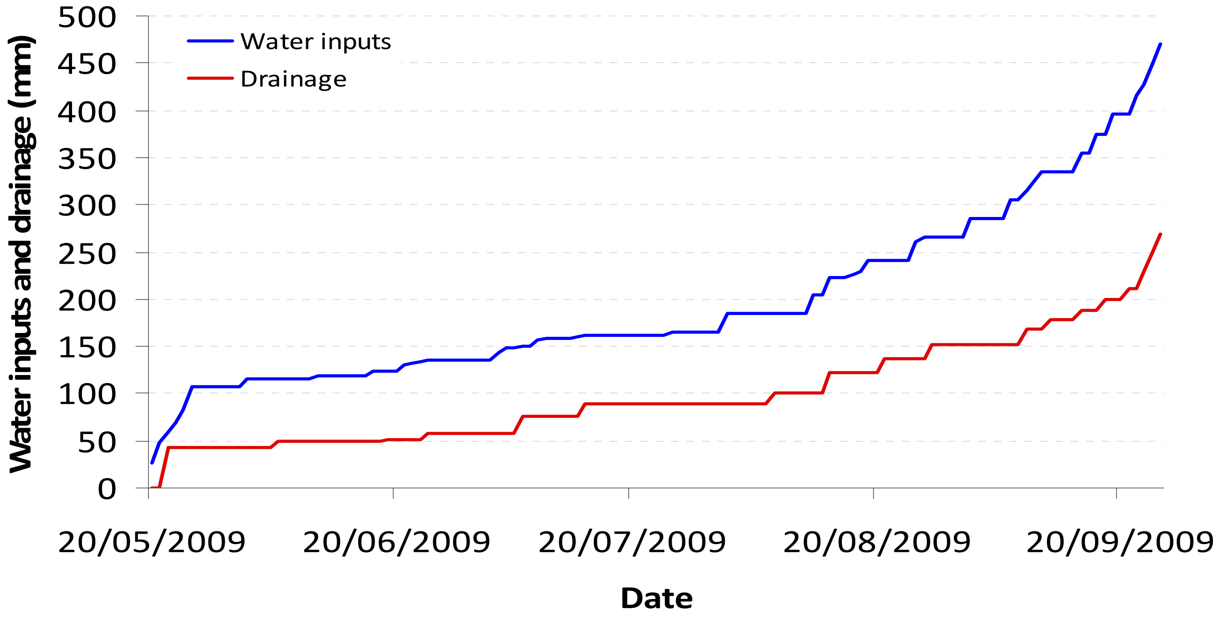


**Figure 4.2:** **Cumulative water inputs (rainfall + simulated rainfall) and drainage (mm) from the control lysimeters over the duration of the trial.**

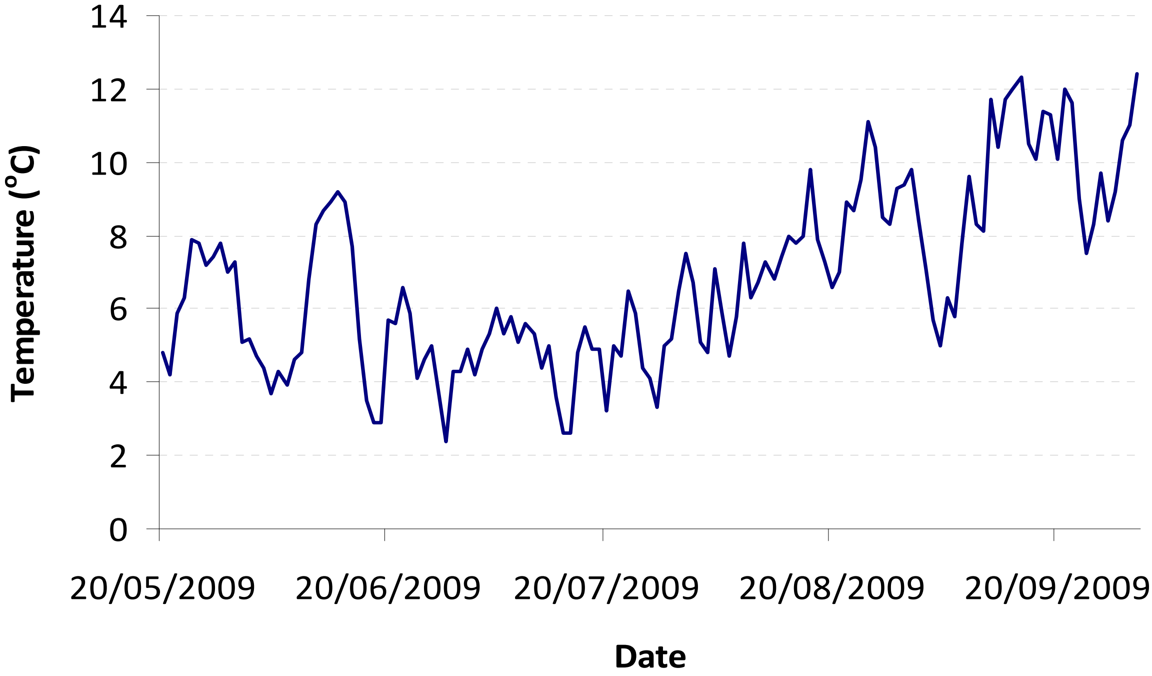


**Figure 4.3:** **Daily 10 cm soil temperature (°C) over the trial duration at Lincoln (NIWA, 2009).**

## 4.2 $N_2O$ gas emissions

When 300 kg N $ha^{-1}$ of sheep urine was applied without DCD to the soil in May, a steady increase in $N_2O$ flux occurred over the first seven days of the trial. A sharp peak emission of 167 g $N_2O$-N $ha^{-1}$ $day^{-1}$ occurred 23 days after urine application was made (Figure 4.4). Subsequent to peaking, the $N_2O$ flux dramatically declined within a few days before peaking for a second time (65.51 g $N_2O$-N $ha^{-1}$ $day^{-1}$). The flux from urine continued to fluctuate before a sudden decrease, 101 days after application, which resulted in the soil producing a zero flux for the remainder of the trial period.

When DCD was applied an hour after urine application, the daily $N_2O$ flux was significantly reduced ($P < 0.001$), peaking at 28.17 g $N_2O$-N $ha^{-1}$ $day^{-1}$. The incline to the peak was more gradual and took an extra week compared with the urine only application. The control or control + DCD treatments did not significantly ($P < 0.001$) differ from each other.

The total amount of $N_2O$ emitted during the experimental period (late May to early October) solely from the sheep urine treatment was 4.55 kg $N_2O$-N $ha^{-1}$ and was significantly reduced ($P < 0.01$) to 1.32 kg $N_2O$-N $ha^{-1}$ with the application of DCD. This was equivalent to a 72% reduction in $N_2O$ loss (Figure 4.5). The total $N_2O$ emissions from the control and control + DCD treatments over the trial duration were low, ranging from 0.24 to 0.28 kg $N_2O$-N $ha^{-1}$, which did not significantly differ from each other or the urine + DCD treatment (Figure 4.5). The total emission factor ($EF_3$) from the sheep urine application was reduced from 1.44% without DCD to 0.35% with the use of DCD (Table 4.1).

**Table 4.1: The influence of sheep urine (300 kg N $ha^{-1}$) and DCD on the emission factor (%) for animal urine ($EF_3$).**

| Treatment | EF3 (%) |
|---|---|
| Urine | 1.44 |
| Urine + DCD | 0.35 |

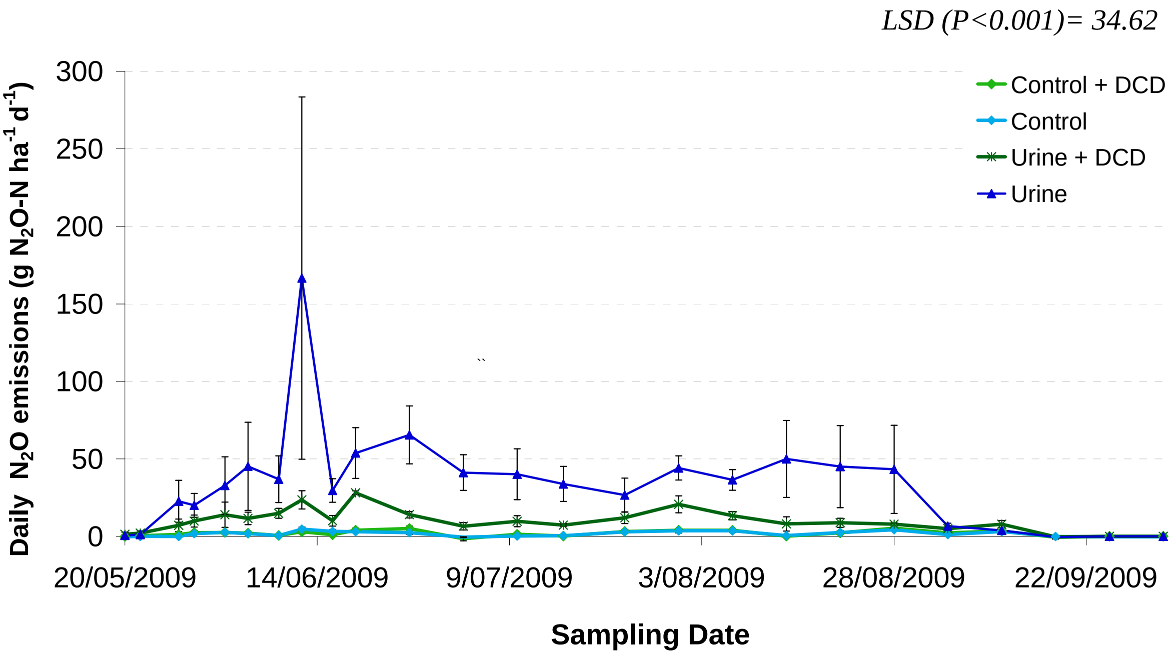


**Figure 4.4:** **Daily $N_2O$ emissions (g $N_2O$ $ha^{-1}$ $day^{-1}$) from the lysimeters (± SEM).**

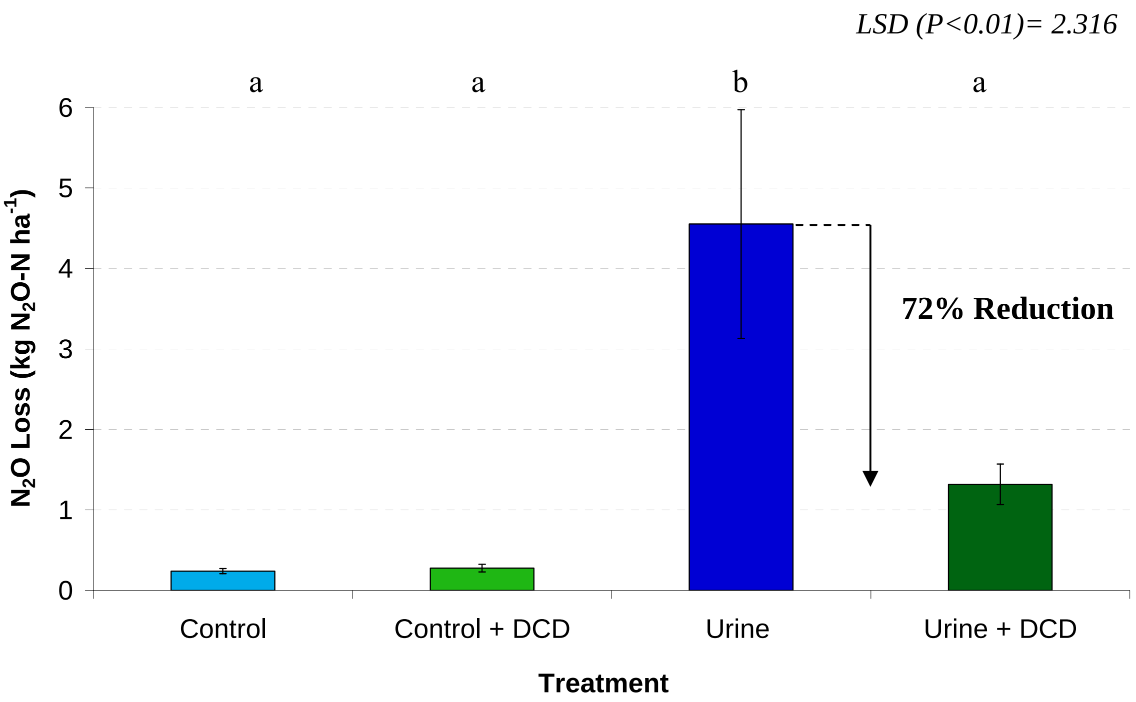


**Figure 4.5:** **Average total $N_2O$ gas losses (kg $N_2O$-N $ha^{-1}$) from all lysimeter treatments. Vertical bars indicate standard errors of the mean. Letters above bars indicate significant differences based on a 5% LSD test.**

## 4.3 Nitrate leaching losses

The N leached from the lysimeters was predominantly in the form of $NO_3^-$ with little or no $NO_2^-$ or $NH_4^+$ detected in the leachate. No $NO_3^-$-N concentration response was observed from any treatments until at least 30 mm of leachate had drained through the lysimeters. Nitrate concentrations from the control and control + DCD treatments were low (<1 mg N $L^{-1}$) throughout the four month trial period (Figure 4.6). The application of 300 kg N $ha^{-1}$ sheep urine produced the first $NO_3^-$-N response in drainage water, peaking at a significantly ($P < 0.001$) higher level than the other treatments at an average of 57.9 mg N $L^{-1}$ when 80 mm of cumulative drainage had drained through the urine lysimeters. A second $NO_3^-$-N concentration peak (33.5 mg N $L^{-1}$) occurred after 130 mm of drainage. When the DCD was applied at 10 kg $ha^{-1}$ to sheep urine, the concentrations followed a similar trend, with peaks occurring at 80 and 120 mm cumulative drainage. However, these $NO_3^-$-N concentration peaks in leachate were significantly ($P < 0.001$) lower at 20.7 mg N $L^{-1}$ and 10.28 mg N $L^{-1}$.

The total amount of $NO_3^-$-N leached from the soil over the five-month trial duration was found to be small in the control (1.06 kg N $ha^{-1}$) and control + DCD treatment (0.71 kg N $ha^{-1}$), although this 33% reduction was not statistically significant (Figure 4.7). The application of 300 kg N $ha^{-1}$ sheep urine to the lysimeter pasture caused a significant increase ($P < 0.01$) of 147.31 kg N $ha^{-1}$ in total $NO_3^-$-N leaching compared with the control. However, with the application of DCD to urine affected pasture, the total N leaching loss was significantly reduced ($P < 0.01$) to 44.82 kg N $ha^{-1}$. This represents a significant 70% reduction in N leaching loss. This result was not found to significantly differ ($P < 0.01$) from the control or control with DCD. The percentage of applied urine N (300 kg N $ha^{-1}$) lost in leaching without and with DCD averaged 49.10% and 14.94% respectively.

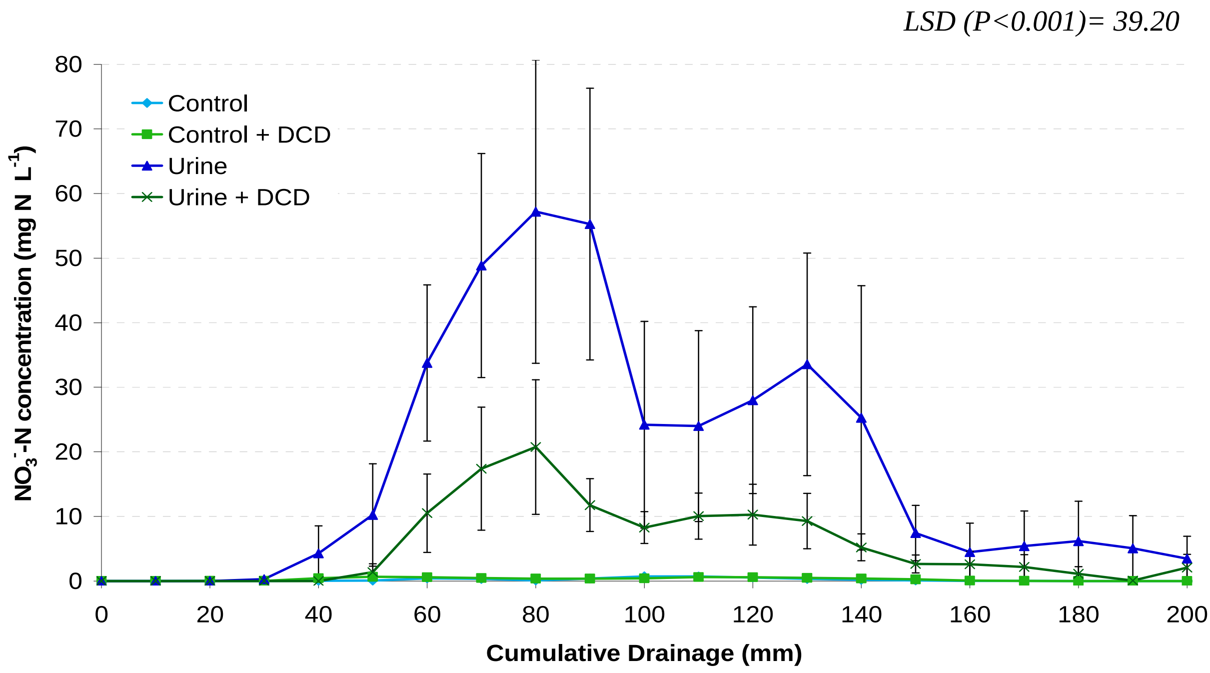


**Figure 4.6:** **$NO_3^-$-N concentrations (± SEM) in the drainage water from lysimeters as affected by urine and DCD treatments.**

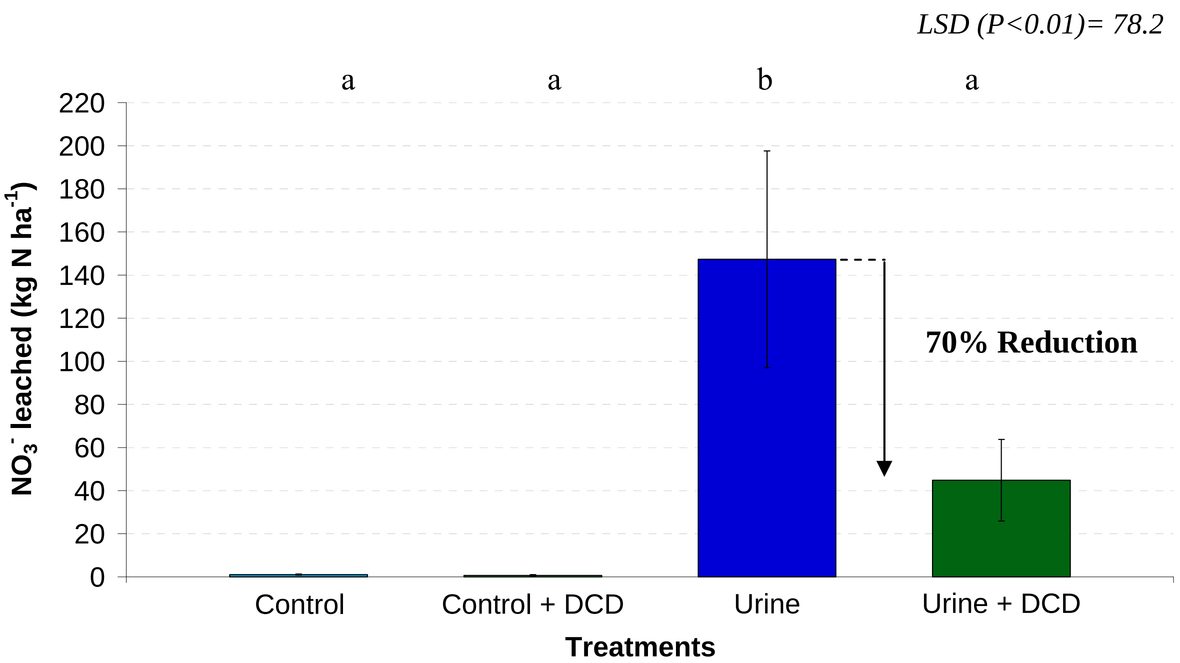


**Figure 4.7:** **Average total $NO_3^-$-N leaching losses (kg N $ha^{-1}$) from all lysimeter treatments. Vertical bars indicate standard errors of the mean. Letters above bars indicate significant differences based on a 5% LSD test.**

# Chapter 5
# Discussion

## 5.1 Nitrous oxide emissions

Sheep urine depositions have been shown to emit $N_2O$ gas in this trial even with the application rate (300 kg N $ha^{-1}$) being less than a third of that of cow urine (1000 kg N $ha^{-1}$) (Di & Cameron, 2007). However, a lower total amount was emitted from the sheep urine treatment (4.55 kg $N_2O$-N $ha^{-1}$) in this study compared with a cow urine application of a 1000 kg N $ha^{-1}$ (20.9 kg $N_2O$-N $ha^{-1}$) in a previous three month $N_2O$ emission study on a Templeton soil (Di *et al.*, 2007).

Despite the lower emissions, this is still an environmental problem in the sheep industry under intensive grazing situations, as the emission of $N_2O$ is harmful to the atmosphere because it has a global warming potential 310 times that of $CO_2$ (Di & Cameron, 2006). In New Zealand, $N_2O$ accounts for approximately one-third of total greenhouse gas emissions with 87% of this being contributed by animal excreta in 2007 (MFE, 2009). This study shows that treating soils with a nitrification inhibitor (DCD), is an effective way of mitigating $N_2O$ emissions from sheep urine patches. DCD applied in May significantly reduced $N_2O$ emissions from sheep urine patch areas by 72% reduction ($P < 0.01$), similar to previous results reported for dairy cow urine (Di & Cameron, 2005). In fact with DCD application, there was no difference between the $N_2O$ emissions from urine and the control treatments ($P > 0.05$). DCD had no effect on emissions from the control treatment ($P > 0.05$). Using DCD on sheep urine reduces the environmental impact as the urine is found to emit no more $N_2O$ than is released from the natural biological processes in the pasture soil when no urine is present (control).

The $N_2O$ flux from urine application increased at a steady rate over the first few weeks (Figure 4.1). This is due to saturated conditions causing anaerobic conditions, enhancing the anaerobic bacterial conversion of $NO_3^-$-N to $N_2O$-N through denitrification. The sudden occurrence of a high peak in June (three weeks after trial initiation) may have been a delayed effect from an extremely wet May and an increase in soil temperature over this period. Figure 4.3 shows that the average daily 10 cm soil temperature was over double the temperatures on either side of this period (mid June). An increase in soil temperature causes an increase in microbial activity and therefore increases the conversion rate of $NO_3^-$-N to $N_2O$ through denitrification (McLaren & Cameron, 1996). This large peak is associated with a wide range of variability in the data, shown by the large error bars when this peak occurred. This was due to one urine replicate peaking at approximately seven times greater concentration (515.88 g $N_2O$-N $ha^{-1}$) than the other three replicates, which ranged between 24 and 70 g $N_2O$-N $ha^{-1}$. In previous research, Di *et al.* (2007) reported that the $N_2O$-N flux from 1000 kg N $ha^{-1}$ cow urine peaked approximately seven weeks after urine application in a study on a Templeton silt loam. This peak was greater (522 g $N_2O$-N $ha^{-1}$ $day^{-1}$) than the average peak (167 g $N_2O$-N $ha^{-1}$ $day^{-1}$) due to a significantly higher N concentration being applied in the study by Di *et al.* (2007) compared to this study.

After seeing a decrease in the $N_2O$-N flux throughout July from the urine treatment, levels began to increase slightly (Figure 4.4), but this increase was not significant, due to wide variation between urine replicates. It took 122 days for the $N_2O$-N flux from urine application to reach zero, which is similar to the study by Di *et al.* (2007), where the flux decreased to zero after about 107 days.

Results from this study clearly demonstrate that even with a lower N application rate (300 kg N $ha^{-1}$) compared with dairy cow urine inputs, the application of DCD was just as effective at reducing $N_2O$-N from sheep urine inputs. Total emissions from urine was found to be 4.55 kg $N_2O$-N $ha^{-1}$ which is lower than the emissions found by Di and Cameron (2002b) from dairy cow urine at 1000 kg N $ha^{-1}$ (46 kg $N_2O$-N $ha^{-1}$). However, $N_2O$-N losses were still significantly reduced with DCD ($P < 0.01$) by 72% to 1.32 kg $N_2O$-N $ha^{-1}$.

This 72% reduction is similar to those found in previous studies, for example a 73% reduction on Lismore soils with the application of 1000 kg N $ha^{-1}$ (Di & Cameron, 2003). Similarly on a Templeton soil, Di *et al.* (2007) found that under a 1100mm annual rainfall, a 73% reduction in $N_2O$-N emissions occurred with the use of DCD on dairy cow urine (1000 kg N $ha^{-1}$).

The total $N_2O$-N emission factor ($EF_3$) from 300 kg N $ha^{-1}$ of sheep urine was 1.44%, which is above the New Zealand country specific emission factor of 1%. However, this specific factor is calculated for the whole year, where as the $EF_3$ calculated from this trial only takes the winter months into account and therefore would expect it to be lower on an annual basis. This value is slightly lower than those reported using cow urine in other studies. For example, the $EF_3$ was found to be 2% from Templeton soil by Di *et al.* (2007) and those reported by de Klein *et al.* (2003) for some New Zealand soils ranged between 0.3 to 2.5%. Di and Cameron (2003) reported a 2.2% emissions factor from a dairy cow urine patch of 1000 kg N $ha^{-1}$.

Ammonia volatilisation was not measured in this study, but because DCD inhibits the conversion of $NH_4^+$ to $NO_3^-$ thus maintaining higher $NH_4^+$ concentration in the soil for a longer period, this may increase the $NH_3$ loss through volatilisation (Di & Cameron, 2002b). However, volatilisation loss is dependant on factors such as temperature, pH and N source location and therefore an increase may not occur.

## 5.2 Nitrate leaching

Results from this study show that the treatment of the soil with 10 kg $ha^{-1}$ of DCD was effective in reducing $NO_3^-$-N leaching from sheep urine patches. The 70% reduction ($P < 0.01$) in the total annual $NO_3^-$-N leaching loss represents a substantial reduction in N losses. It is also similar to the 74% and 68% reductions in $NO_3^-$-N leaching from autumn applied 1000kg N $ha^{-1}$ urine achieved by Di and Cameron (2004a, 2005) respectively. This demonstrates that not only does DCD effectively reduce $NO_3^-$-N leaching from grazed dairy pastoral systems, but it can also be used effectively in sheep grazing systems where the urine-N loading rate is lower.

In this study, the total $NO_3^-$-N leaching losses from sheep urine without DCD (Figure 4.7) were 1.7 times greater than losses found in an earlier study conducted using 1000 kg N $ha^{-1}$ cow urine in the same soil type (Di & Cameron, 2002b). Even though a lower N loading rate was applied, a greater leaching result could have occurred due to double the water input being applied in a quarter of the time span and due to shallower lysimeters (300 mm deep) being used, compared with the 700 mm deep lysimeters in the trial by (Di & Cameron, 2002b).

When compared with a study involving an application of 300 kg N $ha^{-1}$ of cow urine (Di & Cameron, 2007), this sheep trial produced a greater leaching loss (by 87.61 kg N $ha^{-1}$) over a smaller time period (six months less). This was largely due to the greater water input occurring over the winter period (471 vs. 350 mm) and higher drainage volumes from this study (200 mm in four months as opposed to 300 mm drainage over 10 months - Di & Cameron, 2007). Even though a smaller amount of $NO_3^-$-N may be leached relative to 1000 kg N $ha^{-1}$ of cow urine, this is still a high loss from a small sheep urine patch. Because sheep urinate more often than cows (up to 20 vs. 12 times $head^{-1}$ $day^{-1}$) and are run at higher stocking rates when 'break fed' in a winter grazing system, the total leaching losses could actually be higher than dairy, hence the need to use DCD. For example, from dairy patches (1000 kg N $ha^{-1}$) it was found by Di and Cameron (2007) that 23% of N was leached, where as in this trial 49% of N was found to be leached from 300 kg N $ha^{-1}$.

The peak $NO_3^-$-N concentration (57.19 mg N $L^{-1}$ at 80 mm drainage) occurred earlier in this study than in other studies involving 1000 kg N $ha^{-1}$ dairy cow urine applied to deeper (700 mm vs. 300 mm) lysimeters. In previous studies, the concentration generally increased until at least 200mm of drainage before peaking occurred. Di and Cameron (2004a) found that the peak occurred at 210mm of cumulative drainage, while Di and Cameron (2005) found the $NO_3^-$-N concentration peaked at 250 mm of drainage from a Templeton silt loam.

Application of DCD to sheep urine treated lysimeters caused a similar pattern to previous studies on cow urine, with the peak concentration being 63% less with the use of DCD. The amount of N lost appears to be related to the quantity of water inputs applied, with the leaching rate potential increasing with water input. The $NO_3^-$-N concentration peak of 20.74 mg N $L^{-1}$ from the urine + DCD treatment is significantly lower ($P < 0.001$) than the urine only treatment. This peak was still found to be marginally above the recommended safe drinking water level of 11.3 mg N $L^{-1}$ set by the New Zealand Ministry of Health, but is a significant improvement compared with $NO_3^-$-N drainage concentration found from sheep urine alone (Di & Cameron, 2004a).

Even though there was a significant difference in peak concentrations ($P < 0.01$) between urine and urine + DCD treatments (Figure 4.6), the large error bars indicate large variability between replicate data sets for individual treatments. Large variability occurred due to the lysimeters being small and only four replicates representing each treatment. By being small, a minuscule incident can have a major impact on results. For example, a macropore may form in one lysimeter but not the rest in the same treatment and therefore that one lysimeter may drain faster, accelerating leaching. A greater number of replicates provide a more reliable average due to more figures being available to account for any misleading results.

## 5.3 Application into a field situation

The sheep urine patch has been identified as a source of N leaching loss, especially under intensive winter pastoral grazing systems. The trial was performed in a non-field situation to reduce any external variations such as sheep urinating on the control treatments and to be able to analyse exactly where urination has occurred. The data collected from the trial can then be integrated into a farm scale situation.

Even though this study has been conducted in Canterbury, the data can be transferred into a Southland high rainfall, high leaching environment, because the water inputs were similar to Southland rainfall. Therefore, farm scale calculations can be based on average farm management systems for Southland or wetter than average Canterbury conditions.

Furthermore, when air temperatures fall below 10 ºC, pasture growth begins to cease (McKenzie *et al.*, 1999). Therefore, plant activity is more limited over winter in Southland than Canterbury and consequently less N would be taken up by the roots, inducing a greater leaching potential. Applying DCD to a Southland sheep pastoral system would be appropriate as the effectiveness of the nitrification inhibitor is enhanced when temperatures are below 10 ºC, as often found over the winter-spring period in Southland (Di & Cameron, 2004b).

In order to calculate the amount of nitrogen loss at the field scale, a survey of typical winter 'break feeding' systems was conducted and the numbers of sheep per hectare counted in typical on-farm 'break feed' situations. The effectiveness of the nitrification inhibitor at the field scale can then be calculated and recommendations made on the best management practices for farmers to use in order to reduce $NO_3^-$ leaching losses and $N_2O$ emissions (Moir *et al.*, 2007).

From recent farm surveys, Canterbury and Southland winter 'break feeding' grazing systems were found to be running from 1800 to 2000 s.u. ha$^{-1}$ depending on pre-grazing pasture covers, the seasonal growth, the number of cattle and the amount of supplement on hand. Pre-grazing pasture covers averaged 1800-2000 kg DM ha$^{-1}$ in both Canterbury and Southland, where even though both are climatically different, the ewes still require the same feed amounts (1.2 kg DM s.u.$^{-1}$ day$^{-1}$) to meet maintenance and pregnancy requirements.

To put scientific trial data into context commercially, the presented data can be used to work out an approximate amount of leaching that may occur in an intensively grazed winter sheep system. However, sheep may urinate up to 20 times per day, with a single urine patch covering approximately 0.03 $m^2$ (Morton & Baird, 1990). At the average Canterbury and Southland intensive winter stocking rate of 1800-2000 s.u. $ha^{-1}$, this equates to approximately 12% of the area receiving sheep urine at a rate of 300 kg $ha^{-1}$ over a 24 hour period. The total N excreted by 2000 s.u. $ha^{-1}$ grazing behind an electric fence would therefore equal approximately 36 kg N $ha^{-1}$ $day^{-1}$.

Therefore, with a loss of 49% of N from 300 kg N $ha^{-1}$ of sheep urine in this trial, this means that potentially with 0.012 ha being covered in 24 hours by intensively grazed ewes, $NO_3^-$–N leaching and $N_2O$ emission losses could equate to approximately 18 kg N $ha^{-1}$ $day^{-1}$ and 0.54 kg N $ha^{-1}$ respectively, assuming there is no overlapping of sheep urine patches. However, when sheep are grazed intensively, there would be a greater chance of urine patch overlap which would further increase N loss above what has been calculated in this trial. With the use of a nitrification inhibitor (DCD), the total $NO_3^-$-N loss in a farm situation may be reduced by 70%, thus preventing 12.60 kg N $ha^{-1}$ day $^{-1}$ from being leached from the soil. Furthermore, the use of DCD may reduce $N_2O$ emissions by 72%, preventing 0.39 kg N $ha^{-1}$ day $^{-1}$ being emitted into the atmosphere.

These N losses are high, given that Ledgard *et al.* (2000) reported an average $NO_3^-$-N leaching loss from a dairy farm in New Zealand of 30 kg N $ha^{-1}$ $year^{-1}$ at a stocking rate of 16 s.u. $ha^{-1}$. Cameron *et al.* (2007) found that 39.9 kg N $ha^{-1}$ $year^{-1}$ was leached based on beef cattle being stocked at 13 s.u. $ha^{-1}$ and excreting 700 kg N $ha^{-1}$ on a farm in the Taupo district. This is higher than the figures quoted for dairy due to the trial being performed on a free draining pumice soil.

A paddock is only grazed once over the winter in a 120-150 day average rotation in Canterbury or Southland as animals often are fed on forage crops to supplement pasture growth. Paddocks may be re-grazed in late spring in Southland with ewes being set-stocked (12-14 s.u. $ha^{-1}$) for lambing. This increases the potential for further loss as more N is excreted onto the pasture, and some retained N from winter grazing may become susceptible to leaching and denitrification. However, the quantity of N lost may be lower due to the stocking management being less intensive.

Further grazing during the year and any cultivation (due to plants being unable to take any retained N in the soil) could further increase N loss, meaning that the total annual losses could easily equate to, or even exceed, the annual N losses in intensively grazed dairy systems.

Applying a DCD treatment via a conventional spray vehicle may be difficult while sheep are on subdivided 'breaks'. However, managing the grazing system so that the sheep are shifted every four days may improve the ease of application, as the 'break' sizes will be a lot larger, making it easier for an applicator truck to drive around the grazed area. Even though DCD was applied one hour after urine application, this is practically impossible in the field especially if running several mobs. In the dairy industry *eco-n* is recommended to be applied within seven days of grazing to maximise the effectiveness, and a similar strategy could be used for sheep. However, it may be more effective to apply the DCD before grazing winter pasture. DCD is still effective for several months after application (Cameron *et al.*, 2004), and this means it will start working on urinations as soon as they are deposited, rather than only several days after they are deposited.

## 5.4 Lucrative and future value of using DCD in the sheep industry

Firstly, reducing $NO_3^-$-N leaching from sheep urine with the use of a DCD product provides environmental benefits such as reducing $NO_3^-$ contamination of ground and surface water. This is due to DCD inhibiting the bacterial conversion of $NH_4^+$ to $NO_3^-$-N through nitrification, keeping N in the $NH_4^+$ form for longer (Amberger, 1989). This results in less $NO_3^-$-N diffusing into waterways, reducing eutrophication and thus improving water quality. Furthermore, the use of DCD in the sheep industry would reduce the amount of greenhouse gas emissions (specifically $N_2O$), reducing the rate of depletion of the stratospheric ozone layer (Clough *et al.*, 2004).

Applying a nitrification inhibitor to pasture would allow farmers to continue farming sustainably while farming at a higher stocking rate. This therefore improves the farm profitability, as a greater number of animals are able to be stocked for the same environmental impact, thus increasing production out the farm gate. At a standard stocking rate (10-14 s.u. $ha^{-1}$) leaching would most likely not be a problem in the sheep industry due to a smaller N output being excreted over a large area. However, when the stocking rate is intensified, the issue of leaching and gas emission becomes more important, due to a greater number of animals excreting N in a confined area. This is the key driver for using DCD as a mitigation option.

In the future, intensive winter grazing systems may become a ‘hot issue’, where regional councils may target the highly intensified farming systems rather than the farming classes as they do currently. Using a nitrification inhibitor could allow sheep farmers to be recognised for reducing N emissions by the councils and be allowed to continue farming at a higher stocking rate than those which aren’t reducing N emissions to the environment.

# Chapter 6
# Conclusions

This study has clearly identified that N losses are not only a concern in the dairy industry, but are also a concern in intensified sheep grazing systems such as winter ‘break feeding’ systems typically used in Canterbury and Southland. Over the late autumn-winter-spring period, $NO_3^-$-N leaching and $N_2O$ gas emissions are potentially at their greatest due to the presence of cool/moist weather producing drainage and anaerobic soil conditions. Results from this study clearly indicate great potential for the use of nitrification inhibitor technology in the sheep industry with significant ($P < 0.01$) 70% reductions in $NO_3^-$-N leaching losses and 72% reductions in $N_2O$ gas emitted with the application of DCD at 10 kg ha$^{-1}$ on sheep urine patches (300 kg N ha$^{-1}$) deposited in May. This trial has shown that the DCD is just as effective on sheep urine as cow urine, even with a third of the N loading rate in a single urine application.

The data collected from the trial can be easily integrated into a farm scale situation, with Canterbury and Southland sheep farmers ‘break feeding’ 1800 to 2000 s.u. ha$^{-1}$ over the winter period and sheep urine patches (0.03 m$^2$ patch$^{-1}$) covering approximately 12% of the grazing area over 24 hours. From these factors, it could be expected to see N leaching and emission losses of up to 18 kg N ha$^{-1}$ day$^{-1}$. This is considered relatively high in comparison with that from cow urine, with losses of 30 kg N ha$^{-1}$ year $^{-1}$ being reported from dairy pastures. Therefore the use of a nitrification inhibitor is beneficial environmentally in intensive sheep grazing situations, as it could reduce N losses by below 5.4 kg N ha$^{-1}$day$^{-1}$ in these conditions.

In the future, intensive winter grazing systems may become a ‘hot issue’ where regional councils may target the highly intensified farming systems rather than the stocking classes as so currently. Therefore, the use of DCD in regards to this could be beneficial to sheep farmers, as this may allow a higher stocking rate to be run.

## Suggestions for further sheep grazing research:

- Field trials need to be conducted the use of lysimeters incorporated into an actual full scale farming system, measuring the $NO_3^-$ and $N_2O$ losses from sheep urine with and without DCD, where sheep have intensively grazed paddocks behind electric fences.

- The effect of DCD on pasture yield and N uptake from sheep urine to work out whether this is a further benefit for sheep farmers.

- Quantifying the amount of DCD needed to be applied for sheep urine; through looking at varying application rates to improve the economic variability of applying DCD for sheep farmers. It is thought that, since a lower rate of N per ha is applied, less DCD may be needed per ha.

- Quantify the amount of DCD product in excess when applied to sheep urine patches of 300 kg N $ha^{-1}$ and the period of time DCD lasts in the soil in a intensively winter grazed system.

- The effect of pasture management on the amount of $NO_3^-$ and $N_2O$ losses when applying annual N and P fertiliser applications and involving hay or silage cut and carry systems.

- Furthermore, the application of DCD could be incorporated whilst sheep are intensively feed on brassica crops over winter, where potential leaching could be higher from greater soil exposure.

- DCD applications could be made in Southland looking at the effect of the cool and wet conditions experienced in this region where leaching is potentially at greater risk. These applications could also be incorporated on different soil types with a range of soil textures.

- Application of DCD could be examined to see the minimisation of N losses becoming effective as soon as urine is excreted onto the pasture as found in the dairy industry.

# References


Addiscott, T. M., & Benjamin, N. (2004). Nitrate and human health. *Soil Use and Management, 20* (2), 98-104.

Alfaro, M., Salazar, F., Iraira, S., Teuber, N., Villarroel, D., & Ramirez, L. (2008). Nitrogen, phosphorus and potassium losses in a grazing system with different stocking rates in a volcanic soil. *Chilean Journal of Agricultural Research, 68*, 146-155.

Amberger, A. (1989). Research on Dicyandiamide as a nitrification inhibitor and future outlook. *Communications in Soil Science and Plant Analysis, 20* (19 and 20), 1933-1955.

Blakemore, L. C., Searle, P. L., & Daly, B. K. (1987). Methods for chemical analysis of soils. In *New Zealand Soil Science Bureau* (pp. 11). Lower Hutt.

Brock, J. L. (2006). Grazing management of white clover in mixed pastures. *Proceedings of the New Zealand Grassland Association, 68*, 303-307.

Brown, K. R., & Harris, A. J. (1972). Wintering without crops - the problems involved. *Proceedings of the New Zealand Grassland Association, 34* (6), 49-56.

Butler, B. M., & Chu, A. C. P. (1988). The influence of spring grazing management on pasture quality. *Proceedings of the New Zealand Grassland Association, 49*, 131-134.

Cameron, K. C. (1992). Nitrogen in soil. In W. A. Nierenberg (Ed.), *Encyclopaedia of Earth System Science* (Vol. 3, pp. 307-317). San Diego Academic Press.

Cameron, K. C., & Haynes, R. J. (1986). Retention and movement of nitrogen in soils. In R. J. Haynes (Ed.), *Mineral Nitrogen in the Plant-Soil System* (pp. 166-219). Orlando: Academic Press.

Cameron, K. C., Harrison, D. F., Smith, N. P., & McLay, C. D. A. (1990). A method to prevent edge flow in undisturbed soil cores and lysimeters. *Australian Journal of Soil Research, 28* (6), 879-886.

Cameron, K. C., Di, H. J., Moir, J., & Roberts, A. (2007). Reducing nitrate leaching losses from a Taupo pumice soil using a nitrification inhibitor, *eco-n*. *Proceedings of the New Zealand Grassland Association, 69*, 131-135.

Cameron, K. C., Di, H. J., Moir, J., Christie, R., & van der Weerden, T. (2004). *Clean and green with 'eco-n'*. Paper presented at the Proceedings SIDE Conference, Invercargill, New Zealand.

Christie, R. G., & Roberts, A. (2004). *Using the nitrification inhibitor (eco-n) on New Zealand dairy farms*. Paper presented at the Large Herds Conference Proceedings, Napier, New Zealand.

Clough, T. J., & de Klein, C. A. M. (2006). *Review of New Zealand's indirect emission factor ($EF_5$)*: Ministry of Agriculture and Forestry.

Clough, T. J., Kelliher, F. M., Sherlock, R. R., & Ford, C. D. (2004). Lime and soil moisture effects on nitrous oxide emissions from a urine patch. *Soil Science Society of America, 68* (5), 1600-1609.

Clough, T. J., Di, H. J., Cameron, K. C., Sherlock, R. R., Metherell, A. K., Clark, H., & Rys, G. (2007). Accounting for the utilization of a $N_2O$ mitigation tool in the IPCC inventory methodology for agricultural soils. *Nutrient Cycling in Agroecosystems, 78*, 1-14.

de Klein, C. A. M., & Ledgard, S. F. (2005). Nitrous oxide emissions from New Zealand agriculture - key sources and mitigation strategies. *Nutrient Cycling in Agroecosystems, 72* (1), 77-85.

de Klein, C. A. M., Barton, L., Sherlock, R. R., Li, Z., & Littlejohn, R. P. (2003). Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. *Australian Journal of Soil Research, 41* (3), 381-399.

Di, H. J., & Cameron, K. C. (2002a). Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutrient Cycling in Agroecosystems, 64* (3), 237-256.

Di, H. J., & Cameron, K. C. (2002b). 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* (4), 395-403.

Di, H. J., & Cameron, K. C. (2003). Mitigation of nitrous oxide emissions in spray-irrigated grazed grassland by treating soil with dicyandiamide, a nitrification inhibitor. *Soil Use and Management, 19*(4), 284-290.

Di, H. J., & Cameron, K. C. (2004a). Treating grazed pasture soil with a nitrification inhibitor, *eco-n*™, to decrease nitrate leaching in a deep sandy soil under spray irrigation - a lysimeter study. *New Zealand Journal of Agricultural Research, 47* (3), 351-361.

Di, H. J., & Cameron, K. C. (2004b). Effects of temperature and application rate of a nitrification inhibitor, dicyandiamide (DCD), on nitrification rate and microbial biomass in a grazed pasture soil. *Australian Journal of Soil Research, 42* (8), 927-932.

Di, H. J., & Cameron, K. C. (2004c). Effects of the nitrification inhibitor dicyandiamide on potassium, magnesium and calcium leaching in grazed grassland. *Soil Use and Management, 20* (1), 2-7.

Di, H. J., & Cameron, K. C. (2005). Reducing environmental impacts of agriculture by using a fine particle suspension nitrification inhibitor to decrease nitrate leaching from grazed pasture. *Agriculture, Ecosystems and Environment, 109* (3/4), 202-212.

Di, H. J., & Cameron, K. C. (2006). Nitrous oxide emissions from two dairy pasture soils as affected by different rates of a fine particle suspension nitrification inhibitor, dicyandiamide. *Biology and fertility of soils, 42* (6), 472-480.

Di, H. J., & Cameron, K. C. (2007). Nitrate leaching losses and pasture yields as affected by different rates of animal urine nitrogen returns and application of a nitrification inhibitor - a lysimeter study. *Nutrient Cycling in Agroecosystems, 79* (3), 281-290.

Di, H. J., & Cameron, K. C. (2008). Sources of nitrous oxide from $^{15}$N-labelled animal urine and urea fertiliser with and without a nitrification inhibitor, dicyandiamide (DCD). *Australian Journal of Soil Research, 46*, 76-82.

Di, H. J., Cameron, K. C., & Sherlock, R. R. (2007). Comparison of the effectiveness of a nitrification inhibitor, dicyandiamide, in reducing nitrous oxide emissions in four different soils under different climatic and management conditions. *Soil Use and Management, 23* (1), 1-9.

Di, H. J., Cameron, K. C., Shen, J. P., Winefield, C. S., O'Callaghan, M., Bowatte, S., & He, J. Z. (2009). Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience, 2* (9), 621-624.

Field, T. R. O., Roger Ball, P., & Theobald, P. W. (1984). Leaching of nitrate from sheep-grazed pastures. *Proceedings of the New Zealand Grassland Association, 46* (34), 209-214.

Halford, R. E. (1974). Grass wintering and its implications in Southland. *Proceedings of the New Zealand Grassland Association, 34* (5 ), 42-48.

Haynes, R. J., & Williams, P. H. (1993). Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Advanced Agronomy, 49*, 119-199.

Holmes, C. W., & Roche, J. R. (2007). Pastures and supplements in dairy production systems. In P. V. Rattray, I. M. Brookes & A. M. Nicol (Eds.), *Pasture and supplements for grazing animals: Occasional publication No. 14* (pp. 221-242). Hamilton, New Zealand: New Zealand Society of Animal Production (Inc.).

IPCC. (2001). *Climate change 2001. The scientific basis. Contribution of Working Group I to the third assessment report of the Intergovernmental Panel on Climate Change (IPCC)*: Cambridge University Press, UK.

Kapal, D. B. (2008). Influence of a legume green manure crop on barley straw/stubble decomposition and soil nitrogen retention and availability. A thesis submitted in partial fulfilment of the requirements for the degree of Master of Applied Science at Lincoln University.

Keeney, D. R., & Bremner, J. M. (1966). Comparison and evaluation of laboratory methods obtaining an index of soil nitrogen availability. *Agronomy Journal, 58*, 498-503. .

Kenyon, P. R., & Webby, R. W. (2007). Pastures and supplements in sheep production systems. In P. V. Rattray, I. M. Brookes & A. M. Nicol (Eds.), *Pasture and supplements for grazing animals: Occasional publication No. 14* (pp. 255-273). Hamilton, New Zealand: New Zealand Society of Animal Production (Inc.)

Korte, C. J. (1981). Grazing management of perennial ryegrass/white clover pasture in late spring. *Proceedings of the New Zealand Grassland Association 43* (10), 80-84.

Lambert, M. G., Clarke, D. A., & Litherland, A. J. (2004). Advances in pasture management for animal productivity and health. *New Zealand Veterinary Journal 52* (6), 311-319.

Ledgard, S. F., de Klein, C. A. M., Crush, J. R., & Thorrold, B. S. (2000). Dairy farming, nitrogen losses and nitrate-sensitive areas. *Proceedings of the New Zealand Society of Animal Production, 60*, 256-260.

MAF. (2009). *Situation and outlook for New Zealand agriculture and forestry*. Retrieved August 24, 2009, from http://www.maf.govt.nz/mafnet/rural-nz/statistics-and-forecasts/sonzaf/2009/09-dairy.pdf

McKenzie, B. A., Kemp, P. D., Moot, D. J., Matthew, C., & Lucas, R. J. (1999). Environmental effects on plant growth and development In J. White & J. Hodgson (Eds.), *New Zealand Pasture and Crop Science* (pp. 29-44). New York: Oxford University Press.

McLaren, R. G., & Cameron, K. C. (1996). *Soil Science: Sustainable production and Environmental Protection* (2nd ed.). South Melbourne, Australia: Oxford University Press.

MFE. (2009). *New Zealand's Greenhouse Gas Inventory 1990-2007*. Wellington, New Zealand: Ministry for the Environment.

Moir, J. L., Cameron, K. C., & Di, H. J. (2007). Effects of the nitrification inhibitor dicyandiamide on soil mineral N, pasture yield, nutrient uptake and pasture quality on a grazed pasture system. *Soil Use and Management, 23* (2), 111-120.

Moir, J. L., Fertsak, U., Cameron, K. C., & Di, H. J. (2006). The spatial distribution and area coverage of urine depositions in grazed dairy or sheep and beef pastures in New Zealand. In *Proceedings of the 18th World Congress of Soil Science, Session 160, Commission 3.5*. Philadelphia, USA: International Union of Soil Sciences.

Monaghan, R. M., Paton, R. J., Smith, L. C., & Binet, C. (2000). Nutrient losses in drainage and surface runoff from a cattle-grazed pasture in Southland. *Proceedings of the New Zealand Grassland Association, 62*, 99-104.

Monaghan, R. M., Paton, R. J., Smith, L. C., Drewry, J. J., & Littlejohn, R. P. (2005). The impacts of nitrogen fertilisation and increased stocking rate on pasture yield, soil physical condition and nutrient losses in drainage from a cattle-grazed pasture. *New Zealand Journal of Agricultural Research, 48* (2), 227-240.

Monaghan, R. M., Hedley, M. J., Di, H. J., McDowell, R. W., Cameron, K. C., & Ledgard, S. F. (2007a). Nutrient management in New Zealand pastures – recent developments and future issues. *New Zealand Journal of Agricultural Research, 50*, 181-201.

Monaghan, R. M., Wilcock, R. J., Smith, L. C., Tikkisetty, B., Thorrold, B. S., & Costall, D. (2007b). Linkages between land management activities and water quality in an intensively farmed catchment in southern New Zealand. *Agriculture Ecosystems and Environment, 118*, 211–222.

Morton, J. D., & Baird, D. B. (1990). Spatial distribution of dung patches under sheep grazing. *New Zealand Journal of Agricultural Research 33* (2), 285-294.

NIWA. (2009). *Met Station Climate Data*: 'Broadfields', Lincoln, Canterbury.

Olsen, R. A., & Kurtz, L. T. (1982). Crop nitrogen requirements, utilisation and fertilisation. In F. J. Stevenson (Ed.), *Nitrogen in Agricultural Soils* (pp. 567-604). Madison, Wisconsin: American Society of Agronomy Inc.

Olsen, S. R., Cole, C. V., Watanabe, F. S., & Dean, L. A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *United States Department of Agriculture 939*, 19.

Peyraud, J. L., & Delaby, L. (2006). Grassland management with emphasis on nitrogen flows. In A. Elgersma & J. Dijkstra (Eds.), *Fresh herbage for dairy cattle: The key to sustainable food chain* (pp. 103-123). Dordrecht, The Netherlands: Tamminga. Springer.

Piwowarczyk, A., Hennessy, D., Richards, K., Murphy, J. J., & Holden, N. M. (2007). *Effect of stocking rate, N fertilisation level and grazing season length on nitrate leaching from a free draining soil type*. Retrieved April 20, 2009, from http://www.ucd.ie/biosys/2007%20research%20report/pdf%20files/Piwowarczyk%20A.pdf

Rattray, P. V., Thompson, K. F., Hawker, H., & Sumner, R. M. W. (1987). Pastures for sheep production. In A. M. Nicol (Ed.), *Livestock feeding on pasture: Occasional publication No. 10* (pp. 89-103). Ruakaura, New Zealand: New Zealand Society of Animal Production. .

Ruz-Jerez, B. E., White, R. E., & Ball, P. R. (1995). A comparison of nitrate leaching under clover based pastures and nitrogen fertilised grass grazed by sheep. *Journal of Agricultural Science of Cambridge, 125*, 361-369.

Schipper, L., & Vojvodic-Vukocic, M. (1998). Nitrate removal from groundwater denitrification wall amended with saw dust: field trial. *Journal of Environmental Quality, 27*, 664-668.

Schollenberger, C. J., & Simon, R. H. (1945). Determination of exchange capacity and exchange bases in soil-ammonium acetate method. *Soil Science Society of America, 59* (1), 13-24.

Searle, P. L. (1979). Measurement of adsorbed sulphate in soils – effect of varying soil extract ratios and methods of measurement. *New Zealand Journal of Agricultural Research, 22*, 287-290.

Sheath, G. W., Hay, R. J. M., & Giles, K. H. (1987). Managing pastures for grazing animals. In A. M. Nicol (Ed.), *Livestock feeding on pastures: Occasional publication No. 10* (pp. 65-74). Ruakaura, New Zealand: New Zealand Society of Animal Production.

Silva, R. G., Holub, S. M., Jorgensen, E. E., & Ashanuzzaman, A. N. M. (2005). Indicators of nitrate leaching loss under different land use of clayey and sandy soils in southeastern Oklahoma. *Agriculture, Ecosystems and Environment 109* (3-4), 346-359.

Smith, L. C., de Klein, C. A. M., Monaghan, R. M., & Catto, W. D. (2008). The effectiveness of dicyandiamide in reducing nitrous oxide emissions from a cattle-grazed, winter forage crop in Southland, New Zealand. *Australian Journal of Experimental Agriculture, 48* (1-2), 160-164.

Waring, S. A., & Bremner, J. M. (1964). Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. *Nature, 201*, 951-952.

Watson, D. G. (1978). Grass wintering of ewes in coastal Southland. *Proceedings of the New Zealand Grassland Association 40*(76-80)

Webby, R. W., & Bywater, A. C. (2007). Principles of feeding planning and management. In P. V. Rattray, I. M. Brookes & A. M. Nicol (Eds.), *Pasture and supplements for grazing animals: Occasional publication No. 14* (pp. 189-220). Hamilton, New Zealand: New Zealand Society of Animal Production.

WHO. (1984). Recommendations. In *Guildelines for Drinking Water 1*. Geneva: World Health Organisation.

WHO. (2007). Nitrate and nitrite in drinking water, back-ground document for development of WHO Guidelines for drinking water quality. In. Geneva: World Health Organisation.

Williams, P. H., & Haynes, R. J. (1990). Influence of improved pastures and grazing animals on nutrient cycling within New Zealand soils. *New Zealand Journal of Ecology, 14*, 49-54.

# Appendix A: Trial design - complete randomised design.