THE ROLE OF INHIBITORS IN MITIGATING NITROGEN LOSSES FROM CATTLE URINE AND NITROGEN FERTILISER INPUTS IN PASTURES

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy (PhD)

in

Soil Science

at Massey University, Palmerston North, New Zealand.

Massey University

Jagrati Singh

2007

Abstract

The major land use in New Zealand is pastoral farming of sheep and cattle. In intensively grazed dairy-pasture systems, animals graze on nitrogen (N)-rich legume-based pastures, but do not efficiently utilize the N they ingest. On average only 10.5% of the N in forage-based animal feed is converted into milk and the remainder is excreted in dung and urine. In the pastures, a cow urine patch can typically contain up to 1000 kg N ha^{-1}. Nitrogen input, either in the form of cow urine or fertilizer, often exceeds immediate plant requirements and hence is susceptible to losses as ammonia (NH_3) volatilisation and nitrous oxide (N_2O) emissions and removal in drainage water through nitrate (NO_3^-) leaching. This loss of N from grazed pastures causes detrimental environmental impacts in the form of acidification and eutrophication of the soil and water bodies, global warming, destruction of stratospheric ozone, and NO_3^- toxicity.

Various approaches have been attempted to mitigate the economic and environmental impacts of N losses. One such approach is the use of Urease (UIs) and Nitrification (NIs) inhibitors. There have been extensive studies on the value of UIs in arable farming and NIs in grazed pastures. However, only limited work on the impact of UI and NI alone and in combination in influencing the N dynamics, and thus mitigating N gaseous losses from pastures, has been conducted.

This thesis examines the impact of UI (Agrotain; N-(n-butyl) thiophosphoric triamide) and NI (Dicyandiamide, commonly known as DCD), when applied alone or in combination to cow urine and urea fertiliser, on N losses through NH_3 and N_2O emissions and NO_3^- leaching, and on herbage production under glasshouse conditions and a field-plot study. The degradation rate of DCD, and its effect on nitrification and on N_2O emissions from four soils varying in their physical and chemical properties was also examined under laboratory incubations. The results from the field-plot study were then used to predict the effect of DCD on N_2O emissions reductions from urine by adapting the process-based NZ-DNDC model.

Both NH_3 and N_2O emissions have common sources in agriculture. Therefore, chambers were adapted to measure their emissions simultaneously using active and passive gas sampling. Active sampling involved continuous air flow and the use of acid

(0.05 M H_2SO_4 and 2% H_3BO_3) traps for NH_3 measurements and passive sampling involved collecting three gas samples over a one-hour period from a static chamber used for N_2O emissions.

The first glasshouse experiment used UI with urine or urea to assess its effect on NH_3 and N_2O emissions, changes in soil mineral-N and N uptake by pasture plants. The UI treatments also involved two commercial products, Sustain Yellow (urea coated with Agrotain and elemental S) and Sustain Green (urea coated with Agrotain). The use of UI effectively decreased total NH_3 emissions, as well as delaying the time of maximum NH_3 emissions from both urea (600 kg N ha^{-1}) and urine (476 kg N ha^{-1}) by 27% and 22%, respectively. The UI-induced decrease in NH_3 volatilization ranged from 42-48% when urea was applied @ 100 kg N ha^{-1}. Urease inhibitor was also effective in decreasing N_2O emissions significantly from urine and urea applied @ 100 kg N ha^{-1}. The addition of UI increased dry matter yield by 13-19% as compared to the urea-alone treatment.

In the second glasshouse study, NI (DCD) was added @ 25 kg ha^{-1} to urea (@ 25, 50 and 75 kg N ha^{-1}) and urine (@ 144, 290 and 570 kg N ha^{-1}) applied at different rates. Addition of DCD reduced N_2O emissions from both urea and urine and NO_3^- leaching from urine. Dicyandiamide reduced N_2O emissions by 34-93% from the added urea and 33-80% from the added urine. However, its use increased the amount of ammonium (NH_4^+) present in the soil by 3 to 13% both in the urea and urine treatments, and this NH_4^+ was susceptible to leaching and volatilisation losses. The addition of DCD, however, resulted in a 60-65% reduction in NO_3^- leaching from urine applied to pasture soil cores. It also caused a significant reduction in NO_3^--induced cation leaching. Leaching of K^+, Mg^{+2} and Ca^{+2} ions was reduced by 36-42%, 33-50% and 72%, respectively, with DCD applied to cattle urine (290 and 570 kg N ha^{-1}).

The combined use of UI and NI was more effective in controlling N gaseous losses than using them individually. The combination of UI and NI retarded NH_3 emissions by 70% in the urea treatment and by 4% in the urine treatment (field-plot study). It also considerably reduced N_2O emissions (50-51%) following the application of urea and urine (field-plot study) to pasture soil. With the combined inhibitors, there was a 14 and 38% increase in herbage yield from added urea and urine (field-plot study), respectively.

A laboratory incubation experiment was undertaken to study the effect of soil types and the rate of DCD application on the degradation kinetics of DCD. The rate of degradation of DCD varied among the four soils studied. The degradation was slowest (half-life period of 6 to 11 days) in an allophanic soil with a high concentration of organic matter. The effectiveness of DCD in inhibiting nitrification also varied depending on the nature and amount of soil organic matter and clay content. The maximum inhibition was observed in a soil with low organic matter and high clay content.

Finally, 'NZ-DNDC', a process-based model, was adapted and used to simulate the effect of DCD on emissions reduction using DCD inhibition values that vary according to different soil types. This model effectively simulated the effect of DCD on N_2O emissions reductions in Tokomaru silt loam following urine application. However, more field data are required from a range of pasture soils with contrasting amount of soil organic matter and clay content under differing climatic conditions to further test this model modification to predict emission-reductions with DCD application in different soil types

Acknowledgements

I would like to thank the Department of Soil Science, Massey University which made this study possible by providing me with the support and research facilities.

This thesis would not have been possible without the guidance, encouragement, support and patience of Dr. Nanthi Bolan, chief supervisor, and Dr. Surinder Saggar, associate supervisor. I would like to thank Dr.Bolan for providing great mentoring and teaching during the period of this study without which this thesis would not have been possible.

With the volume of work involved in this project, the technical assistance provided by Bob Toes, Leighton Parker, Ian Furkert and Ross Wallace made life a lot easier. Moira, thanks for your ever helping attitude, not only during preparation of this manuscript but throughout my studies. I would like to thank Mike for all his computer assistance and wonderful talks/dinners.

I would like to acknowledge Landcare Research, Palmerston North for providing me scholarship, allowing me to use their laboratories and research equipment, Carolyn Hedley for technical assistance, suggestions and patience while teaching me the operation of the GC. Thanks are also due to Dr. Des Ross for accommodating the proof-reading of my thesis during his busy schedule. Help by Donna Giltrap for her ever helping attitude in modelling work is greatly acknowledged.

I thank Summit Quinphos Ltd. for providing me with a doctoral scholarship for the duration of my study in Massey University.

My fellow post graduate students thank you very much. Looking at all of you working so hard constantly gave me my inspiration to go on. I am extremely thankful to my friend Rekha, for taking care of me and being there for me always during my thesis writing. I am highly indebted to all my friends Priyanca, Kshama, Parul, Rita, Roshan, Sudarshan, Sujit, Hari and Rohan for their support and for the get-togethers which were always a welcome break from the tedious PhD.

To my parents and sister, I have no words to thank you for your unconditional love, support and encouragement at every step in my life. I would like to thank my parents for always believing in me and for inspiring me during my ups and downs.

Sirjana, I kept taking out my frustration on you, thank you so much for always understanding me in such times.

I thank you all for helping me fulfil my ambition to do a PhD in Soil Science. Without your support and encouragement, I would not have the confidence of achieving my dream.

Table of Contents

List of Tables

List of Figures

List of Plates

Chapter 1

Introduction

1.1 The issue

The major land use in New Zealand is pastoral farming of sheep and cattle. These managed pastures are highly productive, with increased pasture production being the major goal for the pastoral farmers in order to achieve the highest per hectare animal productivity. The fertility of pasture soils can be substantially altered by grazing animals mainly through the deposition of dung and urine and their subsequent transformation and transport in soil (Saggar *et al.* 1990b; Haynes & Williams 1993). It is estimated that New Zealand agricultural soils annually receive about 3 million tonnes of N, with 1.58 million tonnes recycled from animal excreta, 0.9–1.1 million tonnes from biological N fixation (BNF), 0.33 million tonnes from fertilisers, and about 0.01–0.015 million tonnes from atmospheric deposition (Saggar 2004). In intensively grazed pasture systems (e.g., dairy cattle and sheep pastures), animals graze on N-rich legume-based pastures, but do not efficiently utilize the N they ingest. On average, only 10.5 % of the N in forage-based animal feed is converted into milk, meat or wool and the remainder is excreted in dung and urine. In grazed pastures, animal urine patches can typically add 500 kg N ha^{-1} for sheep and 1000 kg N ha^{-1} for cattle (Haynes & Williams 1993; Silva *et al.* 1999; Di & Cameron 2002a). Recently, fertiliser N inputs to grazed pastures have also increased sharply, and this increase is expected to continue in the foreseeable future. Nitrogen fertiliser use in 2003-04 has increased six times over that in 1990 (MfE, 2005). The in-situ N either from cattle urine or fertiliser is far in excess of immediate plant requirements and hence is susceptible to losses through ammonia (NH_3) volatilisation, nitrous oxide (N_2O) emission and nitrate (NO_3^-) leaching, causing a significant cost to society and the environment.

Nitrogen is extremely dynamic in grazed pastoral soils, undergoing continuous microbial transformations, leading to gaseous and leaching losses. Nitrogen is an important plant nutrient and its loss can affect both the quality and quantity of feed,

thereby leading to poor animal production. In New Zealand, increased interest in the loss of N through gaseous emissions and leaching from grazed pastures has occurred during the last decade mainly because of the environmental impacts of these losses. Moreover, as a signatory to the United Nations Framework Convention on Climate Change (UNFCC), New Zealand is required to maintain and report on its inventory of greenhouse gas emissions, and as New Zealand has also recently ratified the Kyoto Protocol, the pressure to reduce these losses is even greater.

In New Zealand, grazed pastures are identified as an important source of NH_3 and N_2O emissions. Ammonia emission contributes to the formation of ammonium nitrate and ammonium sulphate particulates and leads to various adverse human health effects (McCubbin *et al.* 2002). In addition, the deposition of atmospheric NH_3 may contribute to undesirable changes in our environment such as acidification of poorly buffered soils, eutrophication in lakes and streams (Schulze *et al.* 1989) and direct plant toxicity (Van derEerden 1982). Ammonia has a short life time in the atmosphere, but it can also act as a secondary source of nitric oxide (NO) and N_2O (Mosier *et al.* 1998). Nitrous oxide is a potent greenhouse gas which contributes to global warming (Bouwman 1990). It accounts for about 2-4 % of the total anticipated Global Warming Potential (Watson *et al.* 1992) and is also involved in catalytic destruction of stratosphere ozone.

Both gaseous N emissions and NO_3^- leaching are considered as a greater issue for dairying compared to sheep and beef farms. A number of studies have shown higher NH_3 and N_2O emissions (de Klein & Ledgard 2005; Saggar *et al.* 2005a, b) and NO_3^- leaching (Ryden 1984; Silva *et al.* 1999; Di & Cameron 2000; Ledgard 2001) from grazed pastures when compared to ungrazed pastures, with N losses also much higher from dairy-grazed than sheep-grazed pastures.

Various approaches have been attempted to mitigate the economic and environmental impacts of N losses. One such approach is the use of urease (UIs) and nitrification inhibitors (NIs). Traditionally, the research examining the value of inhibitors in mitigating N losses and thereby enhancing the efficiency of N fertilisers has been focussed mainly on arable soils (Stelly 1980; Prasad & Power 1995; Xu *et al.* 2000). Recently, there has been increasing interest in the use of commercially

formulated NIs and UIs (e.g., EcoN, N-Care and SustaiN[1]) in mitigating environmental impacts of N losses through leaching and gaseous emissions from animal excreta, fertiliser and effluent application in grazed pastures. Agrotain (NBPT) and Dicyandiamide (DCD) are used as UI and NI, respectively in these commercial products. However, there has been only a limited number of studies conducted in New Zealand to explore the use of UIs and NIs in mitigating N losses from cattle urine and urea in grazed pastures (Di & Cameron 2002b, 2003, 2004c). The study reported in this thesis was aimed at understanding the value of UI and NI alone and in combination with each other in influencing N dynamics of urine and urea fertiliser, and thus mitigating N gaseous losses from grazed pastures.

Both NH_3 and N_2O have common sources in agriculture, and to understand the processes involved in the production, emission and interaction of these gases simultaneous measurements are therefore required. However, methods adopted for measuring these gases are quite different from each other. Nitrous oxide fluxes are determined from the headspace samples periodically collected from closed chambers, whereas NH_3 flux measurements require an active flow of air. There is thus no technique currently available for measuring both the gases simultaneously. To help overcome this problem, the first priority of the research project was to design and test a novel technique that could be used to measure NH_3 and N_2O emissions simultaneously.

1.2 Research Objectives

This study was conducted with following objectives in mind:

- To design and test a novel technique to measure NH_3 volatilisation and N_2O emissions simultaneously.
- To quantify the effect of a urease inhibitor (Agrotain) on N transformation, and NH_3 and N_2O emissions from cattle urine and urea in pasture soil.

[1] EcoN: Ravensdown Fertiliser Co-operative Ltd.; N-Care: Ballance AgriNutrients Ltd.; SustaiN: Summit-Quinphos Ltd.

- To investigate the influence of a nitrification inhibitor (DCD) on N transformation, NH_3 and N_2O emissions, and leaching losses of NO_3^- and basic cations from cattle urine and urea fertiliser applications in pasture soil.
- To examine the combined effect of urease (Agrotain) and nitrification (DCD) inhibitors on N gaseous losses (NH_3 and N_2O) and N transformation from cattle urine and urea fertiliser applied to grazed pastures.
- To study the influence of soil type on DCD degradation and the effectiveness of DCD in inhibiting nitrification, and thus influencing N_2O emissions, in different soils.
- To assess the ability of NZ-DNDC (NZ-denitrification decomposition model) to simulate the effect of DCD on N_2O emissions from urine application.

The specific objectives of various laboratory, glasshouse and field experiments are outlined in the given chapters.

1.3 Thesis Structure

This thesis is divided into 9 chapters, including this Chapter 1 which gives an introduction of the environmental effects of intensification of dairying in New Zealand and the need to use mitigation options to inhibit N losses. This Chapter also highlights the main objectives of the various laboratory, glasshouse and field experiments covered in this thesis. Chapter 2 provides a review of the literature and is mainly divided into two sections. Sections 2.1 to 2.5 review the literature on N input and N gaseous losses. This section forms a part of the invited review chapter co-authored by me and published in Advances in Agronomy (Bolan *et al.* 2004). Sections 2.6 to 2.8 cover urease and nitrification inhibitors and their mode of action in the inhibition of N transformations in soil. It also details the effect of inhibitors on N losses. This section has been accepted for publication as a chapter in the book 'Chemical Bioavailability in Terrestrial Environment' (Ed. R. Naidu).

Chapter 3 describes the development of a chamber-based methodology for simultaneous measurement of N gaseous losses as NH_3 and N_2O. This method was used subsequently in all glasshouse and field experiments to examine the effect of inhibitors on the loss of N through gaseous emissions.

Chapter 4 covers glasshouse experiments which quantitatively determined the effect of UI (Agrotain) on N losses through NH_3 and N_2O emissions from N sources (i.e., cattle urine and urea) common to grazed pastures. This chapter also gives the effect of UI on pasture yield and N uptake from urea. In this study, commercial urea products containing UI (Sustain Yellow and Sustain Green) were used to examine the effect of UI (Agrotain) on N dynamics in pasture soils. Since Sustain Yellow also contains sulphur (S) coating, a separate glasshouse experiment was conducted to separate the effects of UI and S coating on N dynamics.

Chapter 5 quantifies the effect of NI (DCD) on NH_3 and N_2O emissions and also on N leaching as NH_4^+ and NO_3^- from cattle urine and urea applications to pasture soil. The effect of DCD on cation (K^+, Mg^{+2}, Ca^{+2}) leaching was also explored.

Chapter 6 examines the combined effect of UIs and NIs on N gaseous losses, and also on pasture production from urea and cattle urine application under both glasshouse and field conditions.

Chapter 7 describes an incubation study which was carried out to investigate the effect of different soil types on DCD degradation and its effectiveness in reducing N_2O emissions from urine. The effect of DCD on microbial biomass and microbial respiration as measured by CO_2 release is also reported in this chapter.

In Chapter 8 the NZ-DNDC model was used to estimate the reduction in N_2O emissions with the application of DCD to cattle urine, and the model output was compared with the measured data.

The findings of all these experiments are then summarized in Chapter 9 along with the main conclusions drawn from this PhD research, and the direction of future research is indicated.

The articles in scientific journals and conference proceedings that are based on the results presented in this thesis are listed below. Except for minor formatting changes so to be consistent with thesis presentation requirements, each chapter is written as a manuscript ready for publication. Due to the presentation of chapters as 'stand alone' manuscripts, some repetition of methodology and discussion of results may occur in the different chapters.

List of Publications:

Review Papers

Singh, J., Bolan, N. S., Saggar, S and Zaman, M (2007). The role of inhibitors in the mobilization and bioavailability of nitrogen in grazed pasture. In: Chemical Bioavailability in Terrestrial Environment. (Ed. R. Naidu). (in press)

Saggar, S., Bolan, N.S., **Singh, J.** and Blard, A. (2005). Economic and environmental impacts of increased nitrogen use in grazed pastures and role of inhibitors in mitigating nitrogen losses. New Zealand Science Review, 62, 69-74.

Bolan, N. S., Saggar, S., Luo, J., Bhandral, R. and **Singh, J**. (2004) Gaseous emissions of nitrogen from grazed pastures: processes, measurements and modelling, environmental implications, and mitigation. *Advances in Agronomy* **84**: 37-120.

Conference Proceedings

Singh, J., Saggar, S., Bolan, N.S. and Zaman, M. (2006). Influence of urease and nitrification inhibitors on ammonia and nitrous oxide emission under field conditions. Proceedings of workshop on 'Implementing Sustainable Nutrient Management Strategies in Agriculture'. (Ed L.D. Currie and J.A. Hanley), Massey University. Occasional Report No. 19. ISSN 0112-9902. pp 162-170.

Singh, J., Bolan, N. and Saggar, S (2005) Inhibitors and Urine-N dynamics in dairy pastures. Proceedings of the 3rd Dairy^3 Conference, Palmerston North, New Zealand, 11-13 April 2005.

Singh, J., Bolan, N., and Saggar, S. (2005). Influence of nitrification inhibitor on urine-N dynamics in pasture soils. In *Developments in fertiliser application technologies and nutrient management*. (Eds L.D. Currie and J.A. Hanly). Occassional Report No. 18. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, 9 – 10 February 2005. 193-199.

Singh, J., Saggar, S., and Bhandral, R. (2004). Mitigating gaseous losses of nitrogen from pasture soil with urease and nitrification inhibitors. *Supersoil 2004*: Program and Abstracts for the 3rd Australian New Zealand Soils Conference, University of Sydney, Australia, 5–9 December 2004. www.regional.org.au/au/asssi/supersoil2004

Singh, J., Bolan, N., and Saggar, S. (2003). A method for simultaneous measurement of ammonia volatilization and nitrous oxide emissions from intact soil cores. In *Tools for nutrient and pollutant management: Applications to agriculture and environmental quality.* (Eds L.D. Currie and J.A. Hanly). Occassional Report No. 17. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand. 224-231.

Bolan, N.S., S. Saggar, R. Bhandral and **J. Singh** (2003). Gaseous emission of nitrogen from farm effluents: economic and environmental implications. In: Balancing social, cultural, and economic and technical issues in land treatment policy (Technical Session 24), Proceedings of Annual Land Treatment Collective Conference, 92-102.

Conference Abstracts

Singh, J., Saggar, S., Bolan, N. S. and Giltrap, D. (2006). Degradation of dicyandiamide (DCD) and nitrous oxide emissions reduction in four soils – an incubation study. Soils & Society, New Zealand Soil Science Society Conference, Rotorua, New Zealand, 27-30 November 2006.

Giltrap, D., Saggar, S., **Singh, J** and Li, C. (2006). Modelling the effects of nitrification inhibitors on nitrous oxide emissions from grazed pastures. Soils & Society, New Zealand Soil Science Society Conference, Rotorua, New Zealand, 27-30 November 2006.

Bolan, N.S., **Singh, J.**, and Saggar, S. (2004). The role of inhibitors in mitigating nitrogen losses in grazed pasture. NZ Soil News 52, 52-55.

Singh, J., S. Saggar and N.S. Bolan (2003). Mitigating gaseous emissions of N from excretal and fertiliser N input in grazed pastures. In *Proceedings of the 2nd Joint Australia/New Zealand Forum on Non-CO_2 Emissions,* 20-22 October 2003 Melbourne, Australia. Abstract E12.

Chapter 2

Review of Literature

2.1 Introduction

Managed grasslands have a high demand for nitrogen (N) for plant growth. These grassland soils need a continuous supply of N inputs from various sources to meet animal feed demand and sustain productivity. Addition of N to soils not only increases plant productivity but also results in increased nitrate (NO_3^-) leaching and release of gaseous N such as ammonia (NH_3) and nitrous oxide (N_2O). Recent sharp increase in fertiliser N inputs to intensively managed and grazed grasslands has rekindled the debate on its impact on atmospheric, terrestrial and aquatic environments. There has been increasing interest in the use of nitrogen inhibitors in mitigating environmental impacts of N losses through leaching and gaseous emissions from excretal, fertiliser and effluent N inputs. This chapter gives an overview of the sources of N input to grazed grasslands, the dynamics of N in grassland soils, environmental impacts of N losses; discusses the role of inhibitors in improving N bioavailability and mitigating N losses; identifies the gaps and the limitations from the existing information; and concludes by presenting the main research needs to devise mitigation strategies with inhibitors.

2.2 Issues

Grasslands worldwide occupy 117 million km^2 of vegetated lands and provide forage for over 1800 million livestock units and wildlife (World Resources Institute 2000). These managed grasslands are highly productive, with increased pasture production being the major goal for the pastoral farmers for higher per hectare animal productivity. The fertility of grassland (pasture) soils can be substantially altered by grazing animals mainly through the deposition of dung and urine and their subsequent transformation and transport

in soils (Saggar *et al.* 1990a; Saggar *et al.* 1990b; Haynes & Williams 1993). In legume-based pastures, N is derived from biological fixation of atmospheric N (BNF), through the addition of manures and fertilisers, and the uneven deposition of animal excreta. In non-legume-based pastures, such as grass pastures in Europe, most N is derived from fertiliser and manure application. Although in legume-based pastures, most of the N is derived from BNF, a small amount of fertiliser N has been applied since mid nineties, during the early spring season mainly to overcome the deficiency caused by the slow rates of BNF and mineralisation of soil organic matter. Nitrogen is extremely dynamic in grazed pastoral soils, always changing or moving. Nitrogen is the major nutrient element that most strongly regulates pasture production but N is also a major contributor to environmental degradation.

The global N fertiliser demand is expected to grow at an average annual rate of 1.7% per annum, reaching 94.6 M tonnes N in 2008. It is estimated that New Zealand agricultural soils annually receive about 3 million tonnes of N, with 1.58 million tonnes from recycled animal excreta, 0.9–1.1 million tonnes from BNF, 0.33 million tonnes from fertilisers, and about 0.01–0.015 million tonnes from atmospheric deposition (Saggar 2004). Recently, fertiliser N inputs to New Zealand grazed pastures have increased sharply, and this increase is expected to continue in the foreseeable future. This increased use of reactive-N benefits society, but it also represents a significant cost to society through increased NO_3^- leaching and enhanced NH_3 and N_2O emissions. The increasing fertiliser N input to grazed pastures has rekindled the debate on its impact on atmospheric, terrestrial and aquatic environments (Parliamentary Commissioner for the Environment Report 2005).

Nitrogen is an important plant nutrient and its loss affects both the quality and quantity of feed and animal production. Nitrate leaching is one of the biggest environmental issues facing the New Zealand agriculture sector at present. Similarly, grazed pastures are identified as an important source of NH_3 and N_2O emissions. While NH_3 is implicated in acid rain, N_2O is involved in ozone depletion and global warming (i.e. greenhouse gas). Both NO_3^- leaching and gaseous emissions are considered a greater issue for dairy farms compared to sheep and beef farms. Results of studies conducted by Saggar and associates (Saggar 2004; Saggar *et al.* 2004b; Saggar *et al.* 2005a, b) show a 5- to 10-fold increase in N_2O emissions in grazed pasture compared with ungrazed pasture, and also a much higher N_2O emission factor for dairy-grazed than sheep-grazed soils.

Therefore, the increasing amounts of N going on to hill country could increase both leaching and gaseous emissions of N and create further problems in the future.

Many approaches have been used to improve the bioavailability of N, and mitigate the economic and environmental impacts of N losses. One such approach is the use of nitrification and urease inhibitors (NIs and UIs). Recently in New Zealand there has been an increasing interest in the use of commercially formulated NIs and UIs (e.g., EcoN, N-Care and SustaiN[2]) to reduce the loss of N through leaching and gaseous emissions, and enhance plant productivity.

The aim of this chapter is to discuss the role of inhibitors in the bioavailability of N. As N exists in many different inorganic and organic forms in soils and these N forms undergo several transformations, an understanding of N dynamics can help to illustrate the importance of N bioavailability and its fate in the environment. This chapter therefore summarises the various areas of this topic including: (i) a brief summary of N inputs and dynamics in grazed pastures; (ii) an outline of the environmental impacts of N losses; (iii) an illustration of the role of inhibitors in improving the N bioavailability and mitigating N losses; (iv) a brief description of research on the use of inhibitors in New Zealand; and (v) conclusions presenting the main research needs.

2.3 Sources of nitrogen input in grazed pastures

In grazed pastures, N is derived from BNF, through the addition of manures and fertilisers, and recycled through the deposition of animal excreta. In many countries, including Australia, New Zealand and parts of North America and Europe, the use of legume-based pasture is the most common grazing management practice. In BNF, the N_2 atom is biochemically reduced from its most oxidised state (N_2) to its most reduced form (NH_4^+):

$$N_2 + 8H + 8e^- + 16\ ATP\ (energy) \xrightarrow{\textit{Nitrogenase}} 2NH_3 + H_2 + 16ADP + 16\ P_i \qquad \textbf{(2.1)}$$

[2] EcoN: Ravensdown Fertiliser Co-operative Ltd.; N-Care: Ballance AgriNutrients Ltd.; SustaiN: Summit-Quinphos Ltd.

This biochemical reaction is performed exclusively by prokaryotes (a large range of nitrogen-fixing bacteria such as *Rhizobium* and cyanobacteria), using an enzyme complex termed **nitrogenase**.

The amount of BNF in legume-based pastures depends on a number of factors including legume species, soil and climatic conditions, nutrient supply and grazing management. High levels of available phosphorus in soils are essential for maintaining both the presence and N_2-fixing activity of legumes in pastures and for supplying N to these pastures. Similarly, adequate levels of other nutrients, such as sulphur and molybdenum in particular, are required. For example, the largest annual estimates of 680 kg N ha^{-1} for white clover/ryegrass pasture were obtained using soils of low N status and under optimum conditions for N fixation (Ledgard 1995). Work by Sears and co-workers in the 1950s and 1960s from mown pastures suggested that white clover-based pastures were capable of fixing 500–700 kg N ha^{-1} y^{-1} (Sears *et al.* 1965), whereas measurements from the grazed pastures from 1970s onwards suggest annual BNF rates in the range of 65-392 kg N ha^{-1} are more common for grass/clover pastures in New Zealand (Crush 1987; Ledgard *et al.* 1990; Ledgard & Steele 1992; Ledgard *et al.* 1996; Goh & Williams 1999). These measurements also indicate annual BNF averaging about 185 kg N ha^{-1} for sheep farms to 200–250 kg N ha^{-1} for more intensively managed dairy farms (White 1989). BNF rates in the range of 100–300 kg N/ha/yr are therefore, common for grass/clover pastures in New Zealand (Ledgard *et al.* 1990). It is estimated that New Zealand agricultural systems receive an annual N input of 0.9–1.1 million tonnes through BNF (Saggar 2004).

Legumes tend to use soil N when the concentration of inorganic N is high, which results in less BNF. Consequently, application of fertiliser N to legume crops or pastures generally reduces the amount of BNF in soils (Table 2.1). Addition of fertiliser N causes an initial decrease in BNF, as the legume is suppressed and uptake of fertiliser N occurs. While reviewing the data on the effect of fertiliser N on BNF from various clover-based field experiments, Ledgard *et al.* (2001) reported an annual decrease of up to 75%. For each kg of fertiliser N applied, BNF decreased by between 0.3 and 0.7 kg N. The data in Table 1 suggest, with increasing N addition, BNF by clover continues to decrease, and the percent decrease varies between 20 to 75% depending on the time of application and grazing management. The impact of increasing fertiliser application on BNF is depicted in Figure 2.1. Furthermore, in intensively grazed systems, most of the

ingested N (75–95%) is excreted, mostly in urine. This has a major direct effect on BNF by altering soil N status.

Table 2.1 Effect of N fertiliser in reducing biological N fixation (BNF) in New Zealand pastures

Fertiliser N ($kg\ N\ ha^{-1}\ y^{-1}$)	Biological N fixation ($kg\ N\ ha^{-1}\ y^{-1}$)	Decrease (%)	Reference
0, 390	111, 47	58	Ledgard *et al.* (1996)
0, 100	100, 70	30	Crush (1987)
0, 200, 400	210, 170, 70	19, 67	Ledgard (1995)
0, 200, 400	154, 99, 39	36, 75	Ledgard *et al.* (2001)

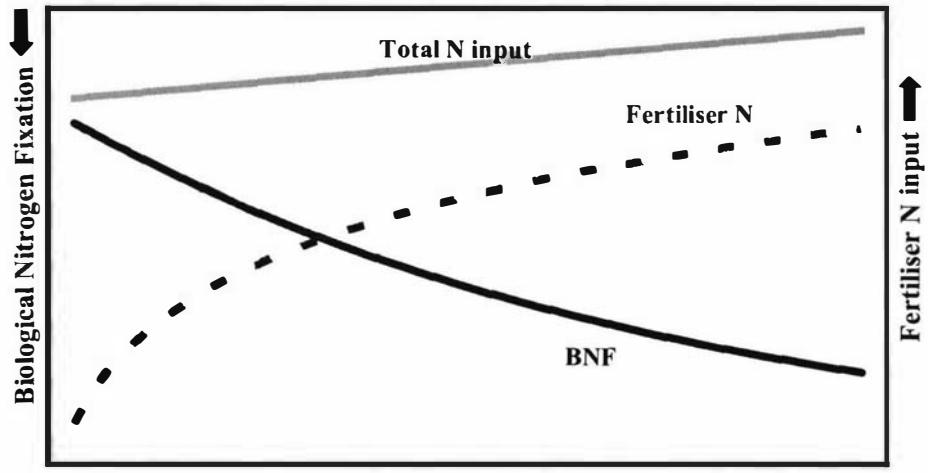


Figure 2.1 The influence of increased nitrogen fertiliser application on biological N fixation (BNF) in legume-based pastures.

Nitrogen fertilisers are used widely in the grass-based intensive pasture production systems of Europe and North America. Pure grass pasture often responds linearly up to 200–400 kg N/ha/yr, and application rates in this range are common (Whitehead 1995). Where pastures are cut for conservation, large quantities of nutrients are removed and the optimum N rate can be greater than that under grazed swards, where N is returned to pasture in the form of animal excreta. In legume-based pastures, small amount of N fertiliser has traditionally been added during the winter/spring period, mainly to offset the low level of biological N fixation during this period.

However, there has recently been a sharp increase in the use of N fertilisers in grazed pastures. This is attributed to a number of reasons including: (i) extra feed can be produced throughout the year to increase the stocking rate, achieve early calving, extend lactation later into autumn and make more high-quality silage to feed later in the lactation; (ii) feed obtained from N fertiliser application can be used to replace more expensive feed supplements; and (iii) the productivity and the profitability of the farm can be increased by fertiliser N application. The recent arrival of the clover weevil in New Zealand will also induce more fertiliser N use unless effective control measures can be found. More recently, there has been increasing interest in the use of N fertilisers in the hill country.

In grazed pastures, a substantial amount of N is decoupled and recycled through the direct deposition of animal excreta. Usually between 5 and 35% of the N in pasture protein is converted into animal protein (i.e. milk and meat), and the remaining N is excreted in dung and urine. The proportion of total N intake excreted and its partition between urine and faeces are dependent on the type of animal, the intake of dry matter, and the N concentration of the diet. For sheep and cattle, faecal excretion of N is usually about 0.8 g N 100 g^{-1} of dry matter consumed, regardless of the N content of the feed (Whitehead 1995). The majority of the N is excreted in urine and the proportion of N in the urine increases with increasing N content of the diet.

The concentration of N in urine may vary from 1 to 20 g N L^{-1} because of factors such as N content in the diet and the volume of water consumption, but it is normally in the range of 8–15 g N L^{-1}. The proportion of urine N present as urea increases with an increase in N intake. Typically, over 70% of the N in urine is present as urea; the rest consists of amino acids and peptides (Haynes & Williams 1993). The bulk of the N in faeces is in organic forms. About 20–25% of faecal N is water-soluble, 15–25% is undigested dietary N, and the remaining 50–65% is present in bacterial cells (Oenema *et al.* 1997). New Zealand's 5.32 million dairy cows and 4.5 million beef cattle daily excrete around 300 000 m^3 of dung and 180 million m^3 urine. It is estimated that annually in New Zealand about 70 million m^3 of effluent are being generated from dairy sheds, 4 million from piggery farms, and 50 million m^3 from meat processing plants (Saggar *et al.* 2004b).

The estimated global amount of N voided by animals ranges between 80 and 130 million tonnes per year, and is as large as, or larger than, the global annual N fertiliser

consumption of about 90 million tonnes. In New Zealand, however, the animals void almost 5 times more N (1.5 million tonnes of N) than the N fertiliser input (0.34 million tonnes).

2.4 Nitrogen dynamics in pasture soils

To understand the biochemical mechanisms involved in mitigating N losses through the use of inhibitors, it is important to understand the dynamics of N in soils. A detailed description of the biotic and abiotic N transformations is given in Bolan *et al.* (2004b). A simplified version of the transformation of N in a legume-based pasture is presented in Figure 2.2. The N transformations in soil include: mineralisation, immobilisation, nitrification, denitrification, NH_3 volatilisation, NH_4^+ fixation and NO_3^- leaching. While the first four reactions involve soil micro-organisms (biotic), the last three involve only chemical/physical processes (abiotic).

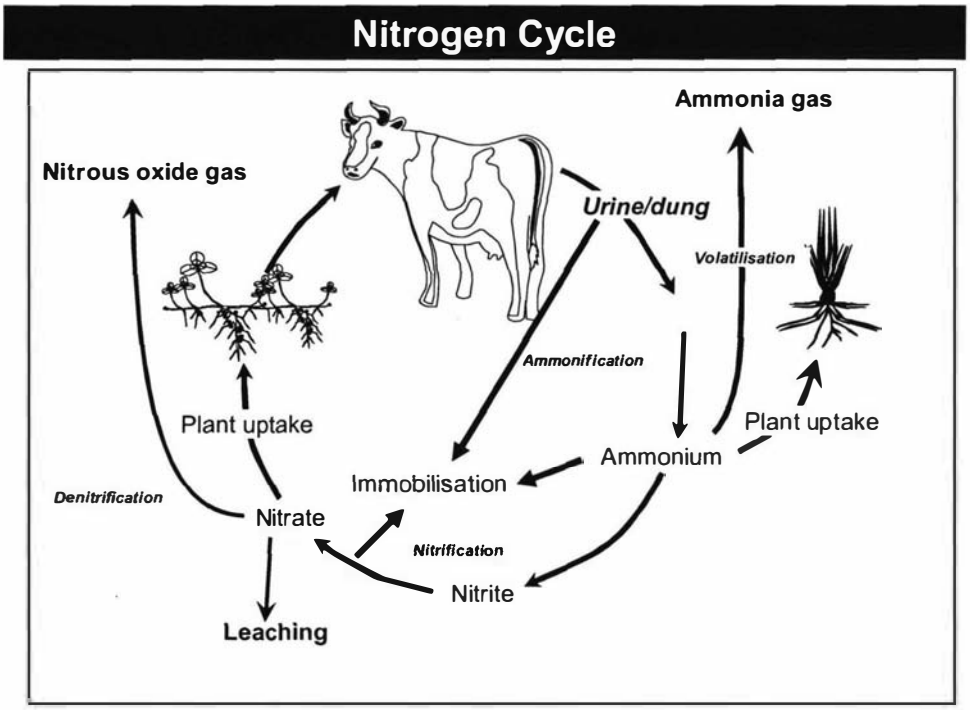


Figure 2.2 Schematic representation of nitrogen transformations in legume-based pastures (Dr. M.J.Hedley, Personal communication).

2.4.1 Mineralisation

The mineralisation process involves the conversion of plant-unavailable organic forms into plant-available inorganic forms by soil micro-organisms. The process includes aminization and ammonification reactions. Aminization is a microbial process in which heterotrophic micro-organisms first hydrolyse the macromolecules of organic N compounds, (e.g., proteins, into simple N compounds, such as amines and amino acids). For example, when blood and bone fertiliser, which contains protein as the major N compound, is added to pasture soils, it first undergoes aminization reactions (Eq. 2.2).

Proteins (in Blood and Bone) → amines ($R\text{-}NH_2$) + CO_2 **(2.2)**

Ammonification is a biological process in which a group of micro-organisms converts amines and amino acids into NH_4^+ ions. For example, urea ($CO(NH_2)_2$) in animal urine and fertilisers undergoes the ammonification reaction releasing NH_4^+ ions (Eq. 2.3). This process is also known as 'urea hydrolysis' and is carried out in the presence of the urease enzyme in the soil. Urease is a powerful enzyme produced by practically all microbial and plant species. The ammonification process also releases hydroxyl (OH^-) ions and hence the pH around the urea granules or urine spots in soil increases resulting in alkaline conditions. The build up to high NH_4^+ ion concentration and the elevation of pH during the ammonification reaction provide ideal conditions for ammonia volatilisation to occur. Thus, the addition of urease inhibitors, which retard urea hydrolysis, is likely to reduce ammonia volatilisation (see below).

$$CO(NH_2)_2 \xrightarrow{\textit{urease}} 2NH_4^+ + 2OH^- + CO_2 \qquad \textbf{(2.3)}$$

2.4.2 Nitrification

The biological conversion of NH_4^+ to NO_3^- is known as nitrification (Eq. 2.4). Broadly, the nitrification reaction involves a two-step process in which the NH_4^+ ions are first converted (oxidised) into nitrite (NO_2^-) and then to NO_3^-. A sequence of reactions are involved in the first step of ammonium oxidation to nitrite. Since the rate of conversion

of NO_2^- to NO_3^- is faster than the conversion of NH_4^+ to NO_2^-, it is unlikely that NO_2^-, which is toxic to plants, accumulates under most soil and climatic conditions. The nitrification process produces H^+ ions, thereby decreasing the pH. The NO_3^- ions produced through the oxidation of NH_4^+ ions are subject to leaching and gaseous emission losses. Thus, the addition of NIs that retards the NO_3^- production is likely to reduce the loss of N through these two processes (Eq. 2.4).

$$2NH_4^+ + 4O_2 \rightarrow 2NO_3^- + 4H^+ + 2H_2O \quad \textbf{(2.4)}$$

2.4.3 Immobilisation

Immobilisation is a microbial process in which the plant-available NH_4^+ and NO_3^- ions are converted to plant unavailable organic N. For example, the addition of carbon (C)-rich substances such as maize stubble and cereal straw in arable soils promotes immobilisation and reduces N availability to plants. Of major concern from a practical point of view is the amount of C relative to N (i.e. C:N ratio) in the decomposing organic matter. Problems arise when the N content of the decomposing organic matter is small, because microbes may become deprived of N and compete with plants for the available N in soil. Thus, the addition of plant residues with a high C:N ratio induces immobilisation of soil N by micro-organisms, thereby decreasing the amount of plant-available soil N.

2.4.4 Denitrification

In waterlogged soils, some micro-organisms obtain their oxygen from NO_3^-, resulting in the reduction of NO_3^-. The reduction of NO_3^- proceeds in a series of steps, producing NO_2^-, nitric oxide (NO), N_2O and N_2 gas (Eq. 2.5). Denitrification results not only in the loss of a valuable plant nutrient but also in the release of N_2O (a potent greenhouse gas), which is also implicated in the destruction of atmospheric ozone.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad \textbf{(2.5)}$$

2.4.5 Ammonium fixation

Ammonium ions are retained on inorganic and organic soil particles by cation exchange reactions and also fixed in the interlayers of 2:1 phyllosilicate clay minerals, such as mica, vermiculite, and illite (Nommik & Vathras 1982). When other cations are added through fertiliser application, the NH_4^+ ions on the cation exchange sites are released into the soil solution through a cation exchange process. Potassium ions, which are similar in size to NH_4^+ ions, have often been shown to replace the fixed NH_4^+ ions, thereby releasing NH_4^+ into the soil solution (McBride 1994).

2.4.6 Nitrate leaching

Ammonium, being a cation, is strongly retained on cation exchange sites, whereas NO_3^-, being an anion, is very weakly adsorbed onto the soil particles. Nitrate moves with water, and subsequent NO_3^- leaching not only results in the loss of a valuable nutrient but also causes ground water pollution. A high NO_3^- concentration in drinking water is toxic, especially to infants, and has been linked with "blue baby syndrome" (methamoglobinemia). The World Heath Organisation has therefore stipulated a safe upper limit (11.3 mg NO_3-N L^{-1} or 50 mg NO_3 L^{-1}) in drinking water.

Although leaching losses occur from both fertiliser N and urine N, a number of studies have shown that in grazed pastures, the latter provides the major pathways for NO_3^- leaching (Di & Cameron 2002a, b) The release of mineral N from faeces results in elevated concentrations of mineral N in the soil below the dung patch. The high concentrations of NO_3^- in dung patches (e.g., 90-130 mg N kg^{-1}) (Ryden 1986) can also be a significant source of both NO_3^- leaching and gaseous losses of N_2O and N_2 from grazed pastures.

2.4.7 Ammonia volatilisation

Ammonium ions in an alkaline medium dissociate into gaseous NH_3, which is subjected to volatilisation losses (Eq. 2.6). Ammonia volatilisation occurs when the soil pH is high (>7.5). In the case of urea application and urine deposition, the initial

increase in soil pH through the ammonification process (Eq. 2.6) is likely to result in NH_3 volatilisation.

$$NH_4^+ + OH^- \rightarrow NH_3 \uparrow + H_2O \text{ (pKa 7.6)} \quad \textbf{(2.6)}$$

2.5 Environmental impact of N losses

Loss of N, occurring mainly through NH_3 volatilisation, biological denitrification and NO_3^- leaching, has both economic and environmental implications (Bolan *et al.* 2004). In the context of environmental pollution and global climate change, while NO_3^- leaching attracts attention because of its potential human and animal health effects and water pollution, gaseous N, such as NH_3, N_2O and NO, cause concern because of their radiative or chemical effects on the atmosphere. Since 1900, the global anthropogenic use of reactive forms of N has increased from less than 5 to approximately 20 million tonnes N in 1950 to almost 150 million tonnes N in 1996, and is expected to approach 190 million tonnes N by 2020. This newly reactive N is derived from production of synthetic fertilisers, from the increased production of crops that fix N biologically, and from fossil fuel consumption (Mosier & Kroeze 2000). This increased use of reactive-N benefits the society, but it also represents a significant cost to society through increased NO_3^- leaching and enhanced emissions of NO_x (pronounced 'knox', sum of NO and NO_2), NH_3, N_2O and deposition of NO_y (sum of knox plus all other oxidised forms of N such as HNO_3 and peroxyacetyl nitrate in the atmosphere) and NH_x (Mosier *et al.* 2001).

In New Zealand, both NO_3^- leaching and gaseous emissions are considered more important issues for dairy farms than for sheep and beef farms. The environmental effects of NO_3^- leached to groundwater and other waterways and the potential damage to soils are a major concern to the farming industry, the scientific community, and the society. The accumulation of NO_3^- in the environment results mainly from non-point source leaching and runoff from the over-application of N fertilisers, voided urine and dung, and from poorly or untreated effluents and sewage. High concentrations of NO_3^- in lakes, rivers and estuaries can result in eutrophication and algal blooms, and links

have also been made between high NO_3^- and toxicity in fish eggs, amphibian eggs, and tadpoles (Agriculture and Agri-Food Canada 2003).

Agriculture is also one of the major sources of gaseous N emissions that result from increased N fertiliser use, animal excreta and organic manures, or N fixed by legumes, thereby polluting the environment. For example, NH_3 affects visibility, aerosol chemistry, health and climate, as it causes acidification and eutrophication when deposited in soil and water. It also acts as a neutralising agent for acidic aerosols, besides affecting vegetation and forming NO_3^-. Ammonia has a short lifetime in the atmosphere but it can act as a secondary source of NO and N_2O, which are directly or indirectly involved in global warming. Nitrous oxide accounts for 2–4% of total Global Warming Potential (GWP) (Watson *et al.* 1992). In the last few decades, the concentration of N_2O in the atmosphere has progressively increased at an annual rate of 0.2–0.3% as a result of human activities (Rasmussen & Khalil 1986; Prinn *et al.* 1990), and about 70% of the anthropogenic N_2O increase is attributed to agriculture (Watson *et al.* 1992).

It is generally recognised that the use of chemical N fertiliser is the most important contributor to N_2O emissions from agricultural soils worldwide. It is estimated that about 1.5 million tonnes of N is injected annually into the atmosphere as N_2O as a result of fertiliser application, which represents about 44% of the anthropogenic input and about 13% of the total annual input of N_2O to the atmosphere (Watson *et al.* 1992). Biologically fixed N and animal manures are the other major contributors to N_2O atmospheric input. Biologically fixed N can be nitrified and denitrified in the same way as fertiliser N, thus resulting in N_2O emissions. Legumes can thus increase N_2O emissions by a factor of 2 to 3 compared with non-legume pastures (Duxbury *et al.* 1982). Addition of animal waste/manure to soil supplies additional quantities of C and N, promotes microbial activity, and may release substantial amounts of N_2O (Beauchamp 1997). A 5- to 10-fold increase in N_2O was observed in grazed pasture compared with ungrazed pasture (Saggar *et al.* 2004b), suggesting, in grazed pastures it is animal excreta deposited in the form of dung and urine that provides high concentrations of available N and C, and is the principal source of N_2O production.

2.6 Inhibitors in nitrogen cycle

Nitrogen inhibitors are the compounds used in controlling N transformations in soils to reduce N losses. These can be grouped into two categories: (i) urease inhibitors (UIs); and (ii) nitrification inhibitors (NIs). The general theory for using NIs and UIs is they will slow N turnover by slowing the oxidation of N to NO_3^-, causing N to stay in the more immobile form of NH_4^+. The UIs are used to control urea hydrolysis and the subsequent ammonification process through their effect on urease enzyme. The NIs are used to control the oxidation of ammonium (NH_4^+) ions to nitrite (NO_2^-) ions.

Inhibitors do not inhibit nitrification indefinitely, but usually between 4 to 10 weeks depending upon soil temperature and pH. These include both specific and non-specific inhibitors. The specific inhibitors tend to control micro-organisms/enzymes involved in specific biochemical reactions e.g., enzymes involved in the ammonification (UIs) and nitrification (NIs) processes, whereas the non-specific inhibitors tend to have a blanket effect on microbial community in soils. Non-specific inhibitors include many agricultural pesticides such as herbicides (e.g., Monuran), fungicides (e.g., Terrazole), insecticides (e.g., BHC) and fumigants (e.g., Telone) that affect the activities of soil micro-organisms, including ammonification and nitrification processes. Martens & Bremner (1984, 1993) studied the effects of 46 herbicides and 15 insecticides applied at the rate of 5 $\mu g\ g^{-1}$ soil and 17 fungicides applied at the rate of 1 $\mu g\ g^{-1}$ soil, on urea hydrolysis and nitrification of urea N in two fine-textured and two coarse-textured soils. They found none of the herbicides and fungicides retarded urea hydrolysis in any of the four soils. However, ten herbicides (2,4-D amine, Acifluorfen, Amitrole, Chlorpropham, Diclopfop methyl, Dinoseb, Fenoxaprop ethyl, Propanil, Propham, and Tridiphane) and five insecticides (Carbaryl, Diazinon, Fenitrothion Lindane and Trimethacarb,) retarded nitrification of N in the two coarse-textured soils. Among the 17 fungicides studied, one (Maneb) retarded urea hydrolysis in all four soils, and seven (Anilazine, Benomyl, Chloranil, Captan, Maneb, Mancozeb and Thiram) in two coarse-textured soils. The fungicides, with the exception of Benomyl, Fenaminosulf, Folpet, Metalaxyl, Metham-sodium, PCNB and Teraazole, retarded nitrification in one of the coarse-textured soils.

Most of the chemical compounds used as inhibitors affect the growth and proliferation of micro-organisms, thereby inhibiting the nutrient cycling processes in

soils. A major concern, therefore, is the environmental fate of such inhibitors when repeatedly applied to soil. Some inhibitors are inhibitory to plant growth at concentrations that effectively inhibit nitrification.

Although many specific inhibitors are found to specifically block a particular enzyme system in the N transformation reaction sequence, when used in high concentration these compounds may act as general biocides, indicating specificity is often linked to concentration. Furthermore, depending on the concentration, these chemicals can either kill micro-organisms (i.e. biocidal action) or temporarily inhibit microbial function (i.e. biostatic action). However, complete inhibition of any N transformation process is seldom achieved with the use of these chemicals.

2.6.1 Urease Inhibitors

Urease inhibitors slow the conversion of urea to NH_4^+ by inhibiting the urease enzyme, which reduces NH_4^+ concentration in the soil solution and hence lowers the potential for NH_3 volatilisation and seedling damage. Slowing the hydrolysis of urea allows more time for it to diffuse away from the application site or for rain or irrigation to dilute urea and NH_4^+ concentration at the soil surface and increase its dispersion in the soil subsequently retaining NH_3 in the soil.

2.6.1.1 Urease activity

The urea in cattle urine and in fertiliser is usually hydrolysed within a few days by an enzyme termed urease, which is present in many plants and plant litter (Freney & Black 1988) and in most species of bacteria, yeast and fungi. The enzyme catalyses the hydrolysis of urea to NH_4^+ (Eq. 2.3) and carbamate ions which decompose to CO_2 and NH_3.

The active site of urease contains two nickel (II) atoms linked by a carbamate bridge. Two imidazole N atoms are bound to each Ni atom; a carboxylate group and a water molecule fill the remaining coordination site of the metal ion. The ability to hydrolyse urea is found to vary from 17 to 70 % for soil bacteria and from 78 to 98% for soil fungi (Lloyd & Sheaffe 1973). Although soil urease is considered to be of microbial origin there is evidence that some soil urease activity may be derived from

plants (Frankenberger & Tabatabai 1982). However, there is no direct evidence for the production of urease by plant roots.

The urease activity of soils is associated with organic matter (O'Toole *et al.* 1982; Reynolds *et al.* 1985; Kissel & Cabrera 1988): as the organic matter content of soil decreases with depth, so too does urease activity (Bremner & Mulvaney 1978; Mulvaney & Bremner 1981). Urease activity is greater in grassland than in cultivated soils (O' Toole *et al.* 1985; Reynolds *et al.* 1985; Whitehead & Raistrick 1993), which probably relates to differences in organic matter and microbial activity.

Hydrolysis of urea is temperature dependent and increase with soil temperature over the range of 0-40^{0}C (Vlek & Carter 1983), though slight hydrolysis has been detected at sub-zero temperatures (Bremner & Mulvaney 1978). It is also affected by urea concentration, soil water and soil pH. The optimum pH for urea hydrolysis is between 6.0–7.0 (Kissel & Cabrera 1988).

2.6.1.2 Mechanism of Inhibition of Urease

Thousands of chemicals have been tested as potential inhibitors of soil urease activity, for use with urea fertilisers. These can be classified according to their structures or according to their binding modes with urease, and mostly fall into three groups: (i) reactive organic or inorganic compounds (e.g., alk(en)yl thiosulfinate, hydroquinone, p-Benzoquinone) that react with sulfhydryl (mercapto) groups in the urease enzyme; (ii) metal chelating compounds (e.g., caprylohydroxamic acid, acetohydroxamic acid) that cause inhibition due to complex formation with one of the Ni atoms at the active site of urease; and (iii) competitive inhibitors (e.g., hydroxyurea, phosphoroamides, phenyl phosphorodiamidate PPDA, N-(n-butyl)phosphorothioic triamide NBPT) that resemble urea molecule (structural analogue), and bind to the active site of urease enzyme. Amtul *et al.* (2002) divided UIs into (i) substrate-analogue inhibitors, and (ii) non-substrate-like or mechanism-based inhibitors, depending on their binding modes.

Substrate-analogue inhibitors have structural similarities to urea and inhibit urease by competing for the same active site on the enzyme. Thiourea, methylurea, hydroxyl urea, and numerous hydroxamic acids are the main examples of the substrate-analogue UIs.

Non-substrate analogue inhibitors do not have any close structural similarity with urea, but they interfere with the enzyme's catalysis mechanism leading to enzyme inactivation. These compounds are also called "mechanism-based" inhibitors, e.g., imidazoles and sulphydryl reagents like p-chloromercuribenzoate, polyhydric phenols, aminocresols and quinones (e.g. p-benzoquinone, 2-5 demethylbenzoquinone).

A number of UIs have been studied and tested over the last 30 years, but the following two groups have gained importance during the last few years as potent UIs:

Hydroxamic Acids:

Hydroxamic acid [R-CONH-OH, R-C(OH)=NOH] (HXA) derivatives characterized by a terminal O=C-NHOH functionality were discovered by (Kobashi *et al.* 1962). Since then a range of hydroxamic acids have been designed and examined (Gale & Atkins 1969; Nervig & Kadis 1976; Kobashi *et al.* 1980). The best studied hydroxamate and the prototype of this class of inhibitors is acetohydroxamic acid (AHA), which inhibits ureases from *Clostridium sordelli, E. coli, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia rettgeri, Staphylococcus aureus* (Rosenstein *et al.* 1981) and many other micro-organisms, as well as ureases from soil (Pugh & Waid 1969). AHA is a stable synthetic lead molecule, weakly acidic and highly soluble in water, which structurally resembles urea. Hydroxamic acids are effective metal chelates and their mechanism of inhibition involves binding to the metal ions of the active site of enzyme.

Phosphorodiamidates:

The synthetic phosphorodiamidates are more potent than HXA and can be successfully used to inhibit the urease activity of ureolytic bacteria in soil (Byrnes *et al.* 1983; Martens & Bremner 1984; Kobashi *et al.* 1985; Liao & Raines 1985; Bremner *et al.* 1986; Rao & Ghai 1986). The strong interaction between urease and phosphoroamide compounds may result from the electrostatic stabilization and structural similarity of phosphoroamide (tetrahedral geometry) that may mimic an intermediate state in enzymatic catalysis. Many compounds have been studied and evaluated (Mulvaney & Bremner 1981; Martens & Bremner 1984; Broadbent *et al.* 1985; O' Connor & Hendrickson 1987), though most have shown limited potential as fertilizer amendment due to problems of low effectiveness, lack of sustained action, or

lack of stability in fertilizer. N-(n-butyl) thiophosphoric triamide (NBPT) is currently the most promising and effective at low concentrations when mixed with urea (Bremner & Chai 1986; Joo *et al.* 1987). NBPT is not an active UI and must be converted in the soil to its oxygen analogue N-(n-butyl) phosphoric triamide (BNPO), which is the actual UI (Christianson *et al.* 1990). The conversion of NBPT to its oxygen analogue N-(n-butyl) phosphoric triamide (NBPTO) is rapid, occurring within minutes/hours in aerobic soils (Byrnes & Freney 1995), but it can take several days in the floodwater of tropical soils. NBPTO forms a tridentate ligand with the urease enzyme, blocking the active site (Manunza *et al.* 1999).

2.6.2 Nitrification Inhibitors

Nitrification inhibitors have been used in agriculture to improve fertiliser efficiency and crop yields and to minimise denitrification and/or leaching losses of NO_3^- by maintaining applied fertiliser N in the soil as NH_4^+-N (Smith *et al.* 1989; Yadvinder Singh & Beauchamp 1989; Bronson *et al.* 1991). They can reduce emissions of N_2O directly by reducing the fraction of NH_4^+-N oxidised to NO_3^- and therefore the N_2O loss associated with nitrification before crop uptake, or indirectly by reducing the amount of NO_3^- substrate available for denitrification (Aulakh *et al.* 1984; Bronson *et al.* 1992).

2.6.2.1 Nitrification process

Nitrification usually refers to chemolithotrophic nitrification, but heterotrophic nitrification also exists. Heterotrophic nitrification is the oxidation of any reduced form of N, including organic N. Fungi are considered to be most efficient heterotrophic nitrifiers (Killham 1986). Chemolithotrophic nitrification is mediated by micro-organisms belonging to the family *Nitrobacteriaceae* (Watson *et al.* 1989). These organisms drive energy from the oxidation of NH_4^+ and NO_2^-, and can use CO_2 as a sole C source (Hooper *et al.* 1997). The oxidation of NH_4^+ and NO_2^- is mediated primarily by two separate groups of autotrophic bacteria: (i) ammonia-oxidising bacteria, belonging to the genera *Nitrosomonas, Nitrosococcus, Nitrosospira and Nitrosolobus* (Bock *et al.* 1991); and (ii) nitrite-oxidising bacteria, belonging to the genera *Nirobacter, Nitrococcus, Nitrospira and Nitrospina.* Of the nitrite-oxidising bacteria,

only *Nitrobacter* has been detected in soils (Bock *et al.* 1991). Ammonium oxidation is often thought to be the rate-limiting step in autotrophic nitrification (De Boer & Kowalchuck 2001). The main factors that affect nitrification are soil temperature, moisture, pH, and the substrates NH_4^+, O_2, and CO_2 (Stevenson 1982). *Nitrosomans europea* has been used in most physiological research regarding chemolithotrophic ammonia-oxidation. Ammonia oxidation is mediated by two enzymes, ammonia monooxygenase and hydroxylamine oxidoreductase (Hooper *et al.* 1997). Ammonia monooxygenase is located in the cytoplasimic membrane and converts NH_4^+ to hydroxylamine, and hydroxylamine oxidoreductase is located in the periplasm and converts hydroxylamine to NO_2^- (Wood 1986). It is evident from molecular techniques that representatives of genus *Nitrosospira*, and especially of cluster 3, are dominant ammonia-oxidising bacteria in fertilised soils (Kowalchuck & Stephen 2001). The heterotrophic nitrifying bacteria (*Paracoccus dentrificans*, *Thiosphaera pantotropha*, *Psedomonas putida* and *Alcaligenes faecalis*) possess ammonia- and hydroxylamine-oxidising enzymes that have strong similarities with those of autotrophic nitrifiers (Kuenen & Robertson 1994; Moir *et al.* 1996).

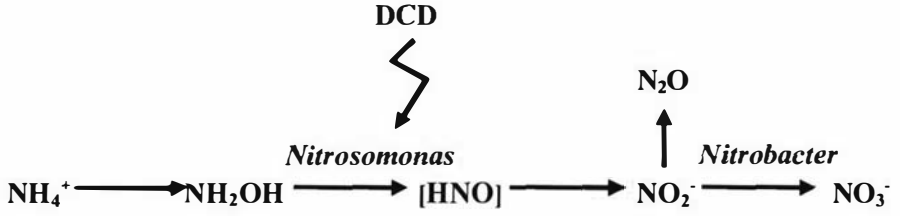
Nitrification inhibitors are chemicals designed to slow this process, reducing the risk that N will be lost through leaching and denitrification. Most of the NIs inactivate the ammonia mono-oxygenase. Many substances can potentially inhibit the nitrification reactions. Metals are particularly strong inhibitors of the reactions: when exposed to more than one inhibitor, the extent of inhibition increases greatly.

Among the large number of chemicals reported as NIs only eight (NP, nitrapyrin or N-Serve [2-chloro-6-(tri-chloromethyl)pyridine]; AM [2-amino-4-chloro-6-methylpyrimidine]; DCD [dicyandiamide]; ST [2-sulfanil-amodo thiazole]; TU [thiourea]; Dwell [5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole]; MBT [2-mercaptobenzothiazole]; and acetylene [C_2H_2]) have been widely tested. So far only two (NP, DCD) have gained acceptance for practical use. NP is seldom effective because of sorption on soil colloids, hydrolysis to 6-chloropicolinic acid, and loss by volatilisation; it is also corrosive, explosive and toxic to plants. DCD is expensive for large-scale use in agriculture, and high application rates (25 kg DCD ha^{-1}) are required for significant inhibition (Merino *et al.* 2002). A new NI, DMPP or ENTEC (3,4-dimethylpyrazol phosphate), effective at low concentrations of 0.5 to 1.0 kg active compound ha^{-1}, has recently been developed in Germany (Zerulla *et al.* 2001).

2.6.2.2 Mechanism of inhibition of nitrification

In general, specific NIs are the compounds that retard oxidation of NH_4^+ to NO_2^- without affecting subsequent oxidation of NO_2^- to NO_3^-. For example, a specific inhibitor such as DCD acts through its affect on cytochrome oxidase involved in the oxidation of hydroxylamine to NO_2^- during the nitrification process. The length of this effect is a function of the concentration of DCD in the product and the frequency of application. The non-specific inhibitors affect all enzymes in the same way. Non-specific methods of inhibition include any physical or chemical changes that ultimately denature the protein portion of the enzyme and are therefore irreversible, e.g., Benzotriazoles (Bz), used as a corrosion inhibitor for decades, is an effective NI in soils under warm climate.

DCD, the dimeric form of cyanamide with relatively high water solubility (23 g/L at 13^0 C) is receiving renewed interest, as it can move with fertilisers in the soil and can be dissolved in liquid manures (Amberger 1989). It also contains about 65% N, is non-volatile, degrades to CO_2, NH_3 and H_2O, and thus acts as a slow release N fertiliser. It is a bacteriostatic, non-toxic, chemical with LD_{50} of 10 g kg^{-1} body weight, which is about 3 times higher than NaCl (Amberger 1989). DCD inhibits the first stage of nitrification, the oxidation of NH_4^+ to NO_2^- (Eq. 2.7) specifically affecting *Nitrosomonas europea* (Zacherl & Amberger 1990). Presumably this effect is due to reaction of the CN group of DCD with sulfhydrl or heavy metal groups of the bacteria's respiratory enzyme.



(2.7)

The bioactivity and effectiveness of NIs may depend on many factors such as soil organic matter, soil pH and soil temperature (Rodgers *et al.* 1985; Prasad & Power 1995; Irigoyen *et al.* 2003; Di & Cameron 2004a). Generally organic matter reduces the effectiveness of NIs (McCarty & Bremner 1989, 1990) either by stimulating microbial activity that results in faster degradation of inhibitors (Slangen & Keerkhoff

1984) or by reducing the bioactivity of inhibitors through absorption on the organic matter. DCD applied at 100 μg DCD-N g^{-1} soil was only moderately effective in an organic soil (40% organic C) (Sahrawat *et al.* 1987). DCD is influenced by humic and fulvic acid in the organic matter, e.g., when shaken with purified humic and fulvic acid for 24 hours, both the amino and nitrile ends of DCD were sorbed to the humic materials in organic matter (Jacinthe & Pichtel 1992). The addition of undecomposed organic matter drastically reduced the effectiveness of NIs (Puttanna *et al.* 1999).

DCD decomposes more slowly in strongly acidic soils than in slightly acidic soils. Only 4.1% of DCD was mineralised in acid soils (pH 4 to 4.3) in 60 days, compared with 48% in a near-neutral (pH 6.8) soil (Rodgers *et al.* 1985). The addition of lime (increasing the soil pH from 5.4 to 8.3) generally decreases NIs effectiveness (Puttanna *et al.* 1999), due to increased nitrifier activity and increased general microbial activity (Slangen & Keerkhoff 1984) that rapidly biodegrades NIs. However, Bz and DCD showed resistance to degradation compared with the other two inhibitors (o-nitrophenol and n-nitroaniline).

One of the most important factors controlling the persistence of NIs is temperature (Keeney 1980; Zourarakis & Killorn 1990). NIs are more effective in laboratory incubations at temperatures well below optimal for nitrification (Bundy & Bremner 1973). This effect is likely to be the result of a combination of greater inhibitor persistence due to slow degradation and/or slow volatilisation and low nitrification activity. Vilsmeier (1980) found that 0.67 mg DCD-N 100 g^{-1} soil was degraded in 60 days to 0.6 mg at 8^0 C and to 0.1 mg at 20^0 C. Rapid reduction in nitrification inhibition by several inhibitors including DCD with increased temperature from 20 to 30^0C is common (McCarty & Bremner 1989; Puttanna *et al.* 1999; Di & Cameron 2004a), due to their faster degradation.

2.7 Changes in availability of N with inhibitors

Plants take N both as NH_4^+ and NO_3^-. Synchronizing plant N uptake with the released NH_4^+or NO_3^- by controlling the rate at which urea (in applied urine or fertiliser)

is hydrolysed to NH_4^+ and its subsequent oxidation to NO_3^- and the temporary rise in soil pH is critical to minimize gaseous and leaching losses of N. Most plants prefer NO_3^- over NH_4^+; however, the rate of uptake of NH_4^+ is often found to be greater than that of NO_3^-, especially at low temperatures.

Since plant roots can absorb both NH_4^+ and NO_3^- ions, ammonification and nitrification processes markedly influence the N absorption efficiency by plants, mainly by controlling the concentrations of these ions in soil solution. It has often been shown that while UIs decrease the concentration of NH_4^+ ions, NIs increase the concentration of NH_4^+ ions and decrease NO_3^- ions (Eq. 2.7). Thus by controlling nitrification, it is possible, not only to increase the N absorption efficiency by plants but also to minimize the N loss by leaching and volatilisation. To decrease the N loss, chemical fertilisers with NIs have been developed, but their application is very limited in developing countries because of their high cost. Certain tropical grass species such as *Brachiaria humidicola* have been shown to inhibit the nitrification process by suppressing the growth of ammonium-oxidising bacteria, accumulating NO_3^- in the soil, and enhancing nitrogen absorption. See http://ss.jircas.affrc.go.jp/kanko/newsletter/nl1999/No.18/03ishikawa.htm.

The application of DCD with urine has been found to reduce soil NO_3^- production but increase the NH_4^+ concentration at all the soil depths (0 to 500 mm) compared with urine alone (Cookson & Cornforth 2002). The capacity of NIs to preserve N in NH_4^+ form depends on several factors, such as soil temperature (Guiraud & Marol 1992; Zerulla *et al.* 2001), soil humidity (Grundmann *et al.* 1995), and treatment doses (Rajbanshi *et al.* 1992). The metabolic degradation of NI-DCD follows a linear kinetic equation (Rajbanshi *et al.* 1992) so does the increase in soil NH_4^+ concentration (Irigoyen *et al.* 2003), and its bioavailability. The decreased NO_3^- concentration found in lettuce leaves with the addition of DCD (Montemurro *et al.* 1998) has been attributed to high levels of NH_4^+ in soil. Application of NIs like NP and DCD caused a significant increase in NH_4^+ through mineralisation when applied alone to grasslands (Rodgers & Ashworth 1982). The alkaline N fertilisers such as NH_3 and urea have been found to generally nitrify faster than acid forming fertilisers such as ammonium sulphate (Keeney 1980; Abbasi *et al.* 2003). Hence the relative inhibition by NIs could be lower with the alkaline-forming fertilisers, at least in acid and neutral soils.

Urease inhibitor (NBPT) delays urea hydrolysis and thus keeps the N in urea form (Bremner *et al.* 1991; Wang *et al.* 1991; Watson *et al.* 1994b). Although UIs have little impact on nitrification (Bundy & Bremner 1973; Bremner *et al.* 1986), NBPT has shown the decrease in NO_2^- and NO_3^- accumulation in soil compared with unamended urea (Bremner & Chai 1989), suggesting an association with reduced rates of nitrification (Watson *et al.* 1994b).

The assimilation of N by plants is a complex biochemical process involving a series of N assimilatory enzymes, and is beyond the scope of this chapter. For a detailed review on these processes please refer to (Stewart *et al.* 1980) and (Lea 1993).

Briefly, in the presence of sufficient sun light as a source of energy, N assimilatory enzymes (NO_3^- reductase) in plants rapidly reduce NO_3^- to NH_3, which is then assimilated into glutamine and glutamate (Figure 2.3). Glutamine, glutamate and organic acids arise from carbohydrate metabolism then serve as N donors in the biosynthesis of amines, amides and essentially all amino acids and nucleic acids. The amino acids thus serve as building blocks for synthesis of proteins. Thus NO_3^- reduction occurs both in aerial portions (shoots and leaves) and in roots of plants; however, most reduction occurs in shoot. The relative importance of these two sites of NO_3^- conversion is considered most important.

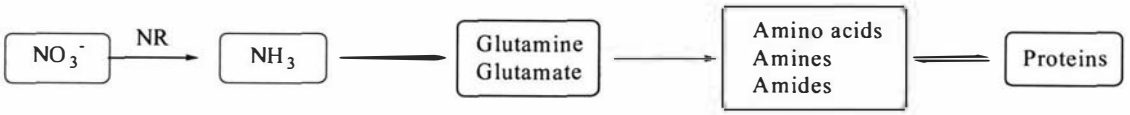


Figure 2.3 Schematic representation of nitrogen metabolism in plants. (NR denotes *nitrate reductase*).

The rate of NH_4^+ assimilation is faster than that of NO_3^- as the former is directly incorporated into organic compounds (Figure 2.3). In order to maintain a charge balance, plant uptake of NH_4^+ and NO_3^- affects the pH of rhizosphere by releasing either hydrogen (H^+) or hydroxyl (OH^-) ions. The release of such ions by plants also affects the uptake of other anions and cations. For example, NH_4^+ absorption reduces the uptake of cations such as calcium (Ca^{+2}), magnesium (Mg^{+2}) and potassium (K^+) and increases uptake of anions such as phosphate and sulphate. NO_3^- uptake reduces the absorption of anions.

A number of studies carried out in different agricultural systems reported that the application of N fertiliser with UIs or NIs improves the bioavailability of N, resulting in increased dry matter yield and N uptake (Watson *et al.* 1998; Xu *et al.* 2002a; Zaman *et al.* 2005). Such increases are always attributed to the delayed urea hydrolysis by UIs and to NH_4^+ retention by NIs. However, there is little information in the literature on the type of N taken up by plants after the application of N inhibitors and their effects on the biochemical processes of N assimilation. Recently, in field-plot studies Zaman *et al.* (2005), reported 20%, 17% and 15% increase in pasture production from urea applied with UI (NBPT), NI (DCD) and UI+NI, respectively. Under lysimeter studies, application of DCD (15 kg ha^{-1}) with urine increased pasture yield by an average of 49% in the autumn and by 18% in the spring urine treatment (Di & Cameron 2002b). DCD applied twice with urine plus urea resulted in a 15% to 33% increase in pasture yields and a 24% increase in N uptake (Di & Cameron 2004c). Cameron *et al.* (2005) also reported that treating urine patches with DCD (lysimeters) may increase pasture production by 15%. This higher dry matter yield and N uptake with inhibitors can be attributed to the retention of applied N as mineral N or organic N in the soil profile, which subsequently becomes available for pasture plants.

Table 2.2 Selected references on the effect of urease inhibitors (UIs) in nitrogen economy

Inhibitor (dose)	N source (kg N/ ha^{-1})	Reduction in N losses	Type of crop	Effect on dry matter yield	Country	Reference
UI – NBPT (0.05% w/w)	Urea (100)	83% reduction (NH_3)	Perennial ryegrass pasture	9 % increase	Ireland	Watson *et al.* (1994b)
UI – NBPT (0.05%, 0.10%, 0.15% w/w)	Urea (100)	75–81% (sandy loam) 75–85% (clay loam) reduction (NH_3)	-	-	Canada	Rawluk *et al.* (2001)
UI – NBPT (0.25% w/w)	Urea (120)	89% (sandy loam) 47% (clay loam) reduction (NH_3)	Wheat crop	No significant effect	Italy	Gioacchini *et al.* (2002)
UI –Agrotain (0.1% w/w)	Urea Urine (600)	27% 23% reduction (NH_3)	Ryegrass-clover pasture	Not monitored	New Zealand	Singh *et al.* (2003) Singh *et al.* (2004)
UI –HQ (0.3% w/w) + DCD-NI (5% w/w)	Urea	62% decrease in N_2O emissions	Rice crop	35-37% increase	Belgium	Xu *et al.* (2002b)

Table 2.3 Selected references on the effect of nitrification inhibitors (NIs) in nitrogen economy

Inhibitor (dose)	N source (kg N ha^{-1})	Reduction in N losses	Type of crop	Effect on dry matter yield	Country	Reference
NI –DCD (25 kg/ha)	CAN (80) Cattle slurry (85)	42% decrease in N_2O 60% decrease in N_2O	Perennial ryegrass pasture	-	Spain	Merino *et al.* (2002)
NI –DCD (12 kg/ha)	Dairy farm effluent (1100)	18% decrease in NO_3^--N leaching	Ryegrass pasture	19.2% increase	New Zealand	Williamson *et al.* (1998)
NI –DCD (25 kg/ha)	Cattle urine (450)	36% decrease in NO_3^--N content (0-100mm)	Ryegrass-clover pasture	No significant effect	New Zealand	Cookson & Cornforth (2002)
NI –DCD	Cattle urine (1000)	76% (autumn) 42% (spring) decrease in NO_3^--N leaching	Ryegrass- clover pasture	30% increase	New Zealand	Di & Cameron (2002b)
NI –DCD (6 kg/ha)	Urea (60)	-	Wheat crop	22–25% increase	USA	Rao & Popham (1999)
NI–DCD (12.5 kg /ha)	Urea (120)	56–58%	Perennial rye grass-spring barley	-	Scotland	McTaggart *et al.* (1997)
Nitrapyrin (7.5 kg/ha)	Urea (120)	40%				

2.8 Effect of inhibitors on N losses

Many research trials have confirmed inhibitors are effective in delaying the conversion of either urea to NH_4^+ (UIs) or NH_4^+ to NO_3^- (NIs). The majority of research indicates the application of UIs to soils with fertiliser urea or urine reduces NH_3 volatilisation while the application of NIs reduces NO_3^- leaching and N_2O emissions. Some studies also show NIs increase NH_3 volatilisation (Davies & Williams 1995; Nastri *et al.* 2000).

Treating urea with UI (NBPT) reduces NH_3 loss from surface applications (Clay *et al.* 1990; Bremner *et al.* 1991). Laboratory (Carmona *et al.* 1990; Vittori-Antisari *et al.* 1996) and field studies (Watson *et al.* 1994a; Rawluk *et al.* 2001) have shown increased inhibition of urease activity with an increasing rate of UI that followed the law of diminishing returns (Watson *et al.* 1994b). NBPT can reduce NH_3 volatilisation significantly in urea, with concentrations as low as 0.005 % (w/w) (Carmona *et al.* 1990). Christianson *et al.* (1990) observed 68% inhibition of urea hydrolysis at 0.01% NBPT (w/w) and NH_3 losses 1.5 to 3 times lower when the rate was increased to 0.1%. The optimum concentration of NBPT for temperate grassland soils to inhibit urea hydrolysis is 0.1% of urea (w/w) (Watson *et al.* 1994b). However, it has been observed that NBPT is less effective at higher temperatures (Bremner *et al.* 1991) and in soils with high levels of organic carbon (Carmona *et al.* 1990; Wang *et al.* 1991). A soil incubation study, using a wide range of soil types, indicated the effectiveness of NBPT in lowering NH_3 volatilisation was the greatest in soils with a high pH and low buffering capacity (Watson *et al.* 1994a). As these were the soil conditions leading to high NH_3 loss from unamended urea, NBPT has the potential to improve the efficiency of urea for temperate grassland. There is little evidence of any long-term adverse effect on grass production or reduced efficacy with repeated applications of NBPT amended urea over a period of 3 years (Watson *et al.* 1998). Results of more recent studies on the effect of UIs on NH_3 volatilisation are summarised in Table 2.2.

Urease inhibitors have little effect on nitrification. Although NBPT has been shown to decrease NO_2^- and NO_3^- accumulation in soil (Bremner & Chai 1989; Watson *et al.* 1994a), this is probably due to the slow formation of exchangeable NH_4^+ caused by the inhibition of urea hydrolysis (Vittori-Antisari *et al.* 1996).

In New Zealand, there has recently been increasing interest in the use of NIs to mitigate environmental impacts of N losses through leaching and gaseous emissions from animal excreta and effluent application (Table 2.3). Di & Cameron (2002b) found the application of DCD following two urine applications (1000kg N/ha) reduced N_2O emissions by 82%. Williamson & Jarvis (1997) obtained 74% reduction in N_2O emissions in a short-term study (37 days) where DCD was applied to urine (60 kg N/ha). The new inhibitor DMPP (1 kg ha^{-1}) reduced N_2O emissions by 60% in autumn and by 48% in spring when applied to a grassland after slurry application (Merino *et al.* 2005).

About 60% reduction in NO_3^- leaching from grazed pasture soils, including animal urine patches with DCD, has been reported with soil lysimeters (Di & Cameron 2004c) using a free-draining shallow stony soil. As NO_3^- leaching is accompanied by counter cations, e.g., calcium, potassium, and magnesium, the leaching of these cations was also reduced by the NI (Di & Cameron 2004b).

There is some evidence that both UIs and NIs may have detrimental effects on plant leaves, e.g., transient leaf tip scorch with UIs, and DCD phytoxicity under certain weather condition (Bremner 1995; Prasad & Power 1995; Watson 2000; Belastegui Macadam *et al.* 2003). However, the benefits of inhibitors in reducing N losses and increasing pasture production would appear to outweigh these short-term detrimental effects. These same trials showed a wide range of economic returns, depending upon soil type, drainage, time of application and environmental conditions. The greatest likelihood of N losses is from coarse-textured or poorly drained soils; it is in these situations that the use of inhibitors would be most economical. However, inhibitors do not work as well in coarse-textured soils as in these soils urea and NH_4^+ ions have a tendency to move away from the inhibitor with rainfall or irrigation (University of Illinois).

Studies on the effect of nitrification inhibitors on N economy (Table 2.3) have shown that the inhibitory action of these chemicals depends on their persistence and bioactivity in soils, which in turn are affected by the intrinsic properties of the compound, soil properties and climatic conditions. The half-lives of inhibitors may vary from a few days to several weeks, depending on the nature of the compound, rate of application, soil type, pH and season (soil temperature). The ideal inhibitor for use in agriculture should:

- specifically block an enzymatic reaction (e.g., NIs should block ammonium oxidation to nitrite, but not nitrite oxidation to nitrate, during the nitrification process)
- remain in close contact with N compounds (e.g., UIs must move with urea molecules that are not readily absorbed by soil; whereas NIs must be close to NH_4^+ ions that are readily retained by soil)
- not adversely affect other beneficial soil organisms and higher plants
- remain effective in the soil for several weeks after N input through fertiliser addition and excretal deposition
- not to be toxic to animals and humans at the levels used to inhibit nitrification effectively
- cost effective to use.

The ultimate goal of any inhibitor is to increase the efficiency of N use. For an economic benefit to occur, the N saved from leaching and gaseous losses by using the inhibitors would have to result either in an increase in pasture production, with a value greater than the cost of the inhibitors, or in a reduction in fertiliser input. The economic benefits of reduced environmental pollution and future damage to our environment from leaching and gaseous emissions are of higher significance over the long-term than the productivity gains. The value of inhibitors in reducing N losses from N fertilisers and increasing crop yields is well established in arable soils. The inhibitors are also reported to increase pasture production. The increase in stocking rates needed to utilise this extra pasture may, however, enhance emissions of other greenhouse gases. Results of a recent desktop study (de Klein & Monaghan 2005), demonstrated the use of NIs had a limited effect on total greenhouse gas emissions reduction, and compared with the reduction in N_2O emissions, due to an increase in both CH_4 and CO_2 emissions from the farm system.

Lysimeter studies by (Di & Cameron 2002a,b, 2003, 2004b, c), showed DCD reduced NO_3^- leaching and N_2O emissions from urine and urea applications. Understanding the soil and plant processes controlling DCD decomposition, the variable response in different soils, and the impact DCD has in causing changes in the N transformations and N cycle is the focus of our current research. This will allow us to develop simple assays and models to monitor and simulate the degradation of these

inhibitors in various soil types, and enhance our understanding of the impact of these inhibitors on the bioavailability of N in managed grassland ecosystems.

2.9 Conclusions

Application of UIs or NIs with N fertiliser, animal urine and animal slurries has been shown to improve the bioavailability of N, resulting in increased dry matter yield and N uptake. Such increases result from the delayed urea hydrolysis by UIs and NH_4^+ retention by NIs. Loss of N, occurring mainly through NH_3 volatilisation, biological denitrification, and NO_3^- leaching, has both economic and environmental implications. Therefore, the economic benefits of reduced environmental pollution and future damage to our environment as a result of the use of N inhibitors are of higher significance to the productivity gains over the long-term.

Most studies have examined the value of N inhibitors in reducing N losses from N fertilisers under cropping conditions, thereby enhancing the N use efficiency of these fertilisers. In New Zealand, the majority of N loss occurs from animal excretal deposition in grazed pasture. Recently, there has been increasing research interest in the evaluation of N inhibitors in reducing N losses from grazed pastures. However only limited work has been carried out examining the relative value of the two groups of N transformation inhibitors (urease and nitrification inhibitors) in enhancing the utilisation of N from urine and urea fertilizers, which is one of the main focuses of the study reported in this thesis.

The value of inhibitors in mitigating N losses would depend on their rate of degradation and persistence in soils. Currently, there is a strong debate in New Zealand on the effectiveness of the NI (DCD), in mitigating N loss. However, it is difficult to devise mitigation strategies from the existing information because the key soil and environmental factors influencing DCD efficiency are poorly understood. In this thesis an attempt has been made to quantify the rate of degradation of DCD in various soil types and to relate the rate of degradation of DCD to its effect on nitrification inhibition. Furthermore, there is little information on the long-term impact these inhibitors will have in altering the N cycle of grazed pasture systems, and on the issues of toxicity.

The understanding of the interrelations between N_2O and NH_3 emissions, and NO_3^- and NH_4^+ leaching is central to the understanding that how pasture systems behave

and respond to inhibitors and to determine the effectiveness of land-management strategies to reduce overall N losses. Mitigation strategies neglecting these interrelations may be suboptimal. For example, there are already claims that NIs lead to increased NH_3 volatilisation and have the potential to enhance NH_4^+ leaching. However, there is limited quantitative data available to assess accurately the NH_3 volatilisation and NH_4^+ leaching contribution of NIs, which has been covered in this thesis.

Chapter 3

Development of methodology for simultaneous measurement of ammonia and nitrous oxide emission from soil cores

3.1 Introduction

An upsurge of interest in gaseous losses of N (NH_3 and N_2O) from the soil has occurred during the last decade mainly because of their implication in environment degradation (discussed in the previous chapter). Quantitative information is required to assess the impact of these losses on the environmental pollution.

Various indirect (i.e., mass balance approach) and direct methods are available for measuring NH_3 volatilisation from soils. Direct measurement of NH_3 volatilisation can be carried out by using either enclosure (e.g., closed chamber) (Kissel *et al.* 1977; Ball *et al.* 1979), micrometeorological or wind tunnel methods. Micrometeorological methods are available for accurately measuring NH_3 volatilisation in the field (Denmead 1983; Schjoerring *et al.* 1992; Sherlock *et al.* 1995; Wood *et al.* 2000). These methods have an advantage as they do not disturb the natural environmental conditions that influence NH_3 volatilisation, but at the same time are costly in instrumentation, laborious, weather dependent in their application and need very large experimental plots – more than 1 ha for the micrometerological method and about 1 ha for the wind-tunnel method. Closed chamber procedures involving passive sampling for NH_3 have been used in the field, but these can introduce experimental artefacts that alter the dynamics and extent of NH_3 volatilisation (Freney *et al.* 1983; Black *et al.* 1985; Matson & Harriss 1995). In the last decade, significant improvement has been achieved in measuring N_2O emissions, and methods (Bolan *et al.* 2004) (e.g., static chamber technique) have been developed that are internationally acceptable and capable of

quantifying N_2O emissions quite accurately under field conditions (Saggar *et al.* 2002; Hedley *et al.* 2006).

Ammonia and N_2O gases have common sources in agriculture, and in order to understand the processes involved in their production in soils and subsequent release to the atmosphere, their simultaneous measurement is required. However, methods adopted for measuring these gases are quite different from each other, and there has been no attempt to measure these gases simultaneously. Currently, the static chamber technique is the most common method used for monitoring surface fluxes of N_2O since this gas is less reactive than NH_3 with water (Liss & Slater 1974), and is much less affected by an increase in chamber head space concentration (Mosier *et al.* 1991). However, in contrast to NH_3, N_2O is generally very difficult to detect at a small concentration gradient above the soil surface when it is being emitted. Nitrous oxide fluxes, therefore, are determined from headspace samples collected periodically from closed chambers, whereas NH_3 flux measurements require an active flow-through system. In a static chamber, an elevated NH_3 concentration will affect subsequent NH_3 emission from the soil covered by the chamber.

The main objective of this study was therefore to design and test a novel chamber technique that could be used to measure NH_3 volatilisation and N_2O emission simultaneously. To further assess the effectiveness of this chamber technique, simultaneous emission of NH_3 and N_2O was determined from pasture soil cores receiving urea and urine under controlled conditions. Another, passive sampling technique (Carran *et al.* 2000) was also compared against the developed technique to capture NH_3 under field conditions.

3.2 Materials and Methods

3.2.1 Standards and reagents

All the chemicals used were of analytical reagent grade and the solutions were prepared using reverse osmosis (RO) water. The different absorbents used for NH_3 absorption were 2% (w/v) boric acid (H_3BO_3) (0.32 M H_3BO_3), 0.05 M sulphuric acid

(H_2SO_4) and 10% (w/v) oxalic acid ($C_2H_2O_4$) (1.1 M $C_2H_2O_4$) in acetone. The NH_3 absorbed by 2% H_3BO_3 and 0.05 M H_2SO_4 was determined by back titrating excess of these acids against 0.01 M H_2SO_4 and 0.05 M NaOH, respectively. Further, the absorbed NH_3 in 0.05 M H_2SO_4 and 10% $C_2H_2O_4$ was also measured as NH_4^+-N concentration by standard colorimetric method on Technicon auto analyser using 0.25, 0.5, 1, 2, 4, 8 and 12 mg NH_4^+-N L^{-1} standards prepared in the respective matrix solutions.

3.2.2 Description of sampling system / instrumentation

A simplified schematic diagram of the chamber system developed in this study to measure both the active flux for NH_3 and passive flux for N_2O measurements simultaneously is shown in Figure 3.1. The system consisted of 12 air tight closed chambers, a constant air supply and chemical traps to capture NH_3. The chambers were modified PVC 'Sewer-hatches' attached to 250 mm diameter and 150 mm deep sections of PVC pipe with a sealed base. The 'Sewer-hatch' rim had an internal half-turn locking system and a rubber 'O'-ring which form a gas-tight seal. The system included two types of removable lids having one or two ports for sampling N_2O or NH_3 fluxes, respectively. The lid with one port had a 10 cm long tubing (3.2 mm diameter) with a three-way stopcock attached to it to take N_2O gas samples using 60 ml polypropylene syringes. The lid with two ports had one input port connected to a compressed air supply and other exhaust port connected to a chemical trap to absorb NH_3. Fifty ml of 0.05 M H_2SO_4 (Wulf *et al.* 2001) or 2 % (w/v) H_3BO_3 were used to trap NH_3. A manifold with 12 air valves supplied compressed air to the chambers (Plate 3.1). All the connections were made with appropriate PVC tubings of 4.8 mm diameter. A constant air flow of 1 dm^3 min^{-1} was maintained in each chamber using the valves in the manifold.

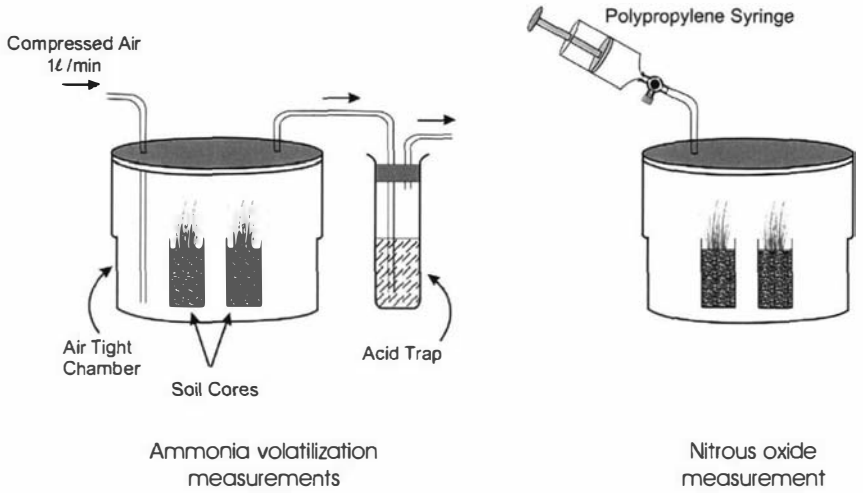


Figure 3.1 Schematic diagram of the basic component used in the method developed for simultaneous measurement of NH_3 and N_2O.

Plate 3.1 Chambers used for simultaneous measurement of NH_3 and N_2O emissions

3.2.3 Efficiency of ammonia absorption

The methodology for measuring N_2O using the static chamber technique had already been established and tested (Saggar *et al.* 2002), so this study was conducted to find a method appropriate for measuring NH_3 emissions along with N_2O measurement.

The first step towards this was to select the appropriate absorbent for NH_3 emissions. The efficiency of 2% H_3BO_3 (w/v) and 0.05 M H_2SO_4 as absorbents for NH_3 gas was tested under two conditions: passive and active. Passive sampling implies that there is no air flow in the chamber, whereas during active sampling there is continuous flow of air through the chamber. The chambers used are discussed above in section 3.2.2. For both passive and active sampling, two sets with three chambers each were used. One set was used for testing 2% H_3BO_3 (w/v) and the other for 0.05 M H_2SO_4. A Petri dish containing 20 ml H_3BO_3 or 10 ml H_2SO_4 was placed in each of the three chambers. In passive sampling, the chambers were closed with the lid containing one port and then a known quantity of NH_3 gas was introduced. Ammonia gas was formed by adding 1 M sodium hydroxide (NaOH) to a known quantity of ammonium chloride (NH_4Cl) in a closed Agee jar. The chambers were closed for two hours and the NH_3 trapped by the absorbents was determined by titration (Section 3.2.1).

In the case of active sampling, chambers were closed with the lids having two ports and the acid traps were connected to the respective chambers using plastic tubing. In three chambers of each set, a known quantity of NH_3 gas was introduced, compressed air was passed through the chambers via one port @ 1 dm^3 min^{-1} and NH_3 in the compressed air from the other port was trapped using 2% H_3BO_3 and 0.05 M H_2SO_4. The NH_3 trapped in the acid solutions was then analysed by titration and auto analyser methods for H_3BO_3 and H_2SO_4, respectively.

The recovery of NH_3 (A_R) was calculated by the following formula:

$$A_R\ (\%) \;=\; (A_C \,/\, A) \;\; X \;\; 100 \qquad \textbf{(3.1)}$$

where, A_C = NH_3 (mg N) captured by the active or passive method; A = NH_3 (mg N) added before the experiment was started.

The efficiency of H_2SO_4 (0.05 M) as an absorbent of NH_3 was further tested for a range of NH_3 concentrations (1.26 µg to 5.0 mg NH_3-N) under both passive and active sampling.

3.2.4 Evaluation of the developed chamber equipment for simultaneous measurement of ammonia and nitrous oxide

The chambers adapted above were evaluated by monitoring NH_3 and N_2O emissions from urea and urine applied to intact soil cores under glass house conditions. This experiment hereafter is called glasshouse experiment -A

3.2.4.1 Experimental set up (Glasshouse experiment A)

Intact soil cores (100 mm diameter, 100 mm depth) were collected from a sheep-grazed permanent pasture site at Massey University. The soil at this site was Manawatu fine sandy loam, classified as a weathered fluvial recent soil. These cores were first saturated overnight with deionised water and then kept on pressure plates at -10 kPa pressure for 2 days to bring them to field capacity. Cores were weighed and then maintained at field-capacity moisture content throughout the experiment. The experiment was set up in April 2003 by placing three cores in each chamber in a glasshouse maintained at a temperature ranging from 15-20° C and collecting initial gas samples for background emissions of NH_3 and N_2O. The treatments with three replications were applied the following day. The treatments were: T1- Control (water); T2 – Urine; T3 – Urea (applied as granules to the soil surface). Nitrogen in the form of urine and urea was added @ 476 kg N ha^{-1} and 600 kg N ha^{-1}, respectively. During the 10-week experimental period gas samples were collected every day for the first 30 days for NH_3 using the active sampling technique, and for six weeks in case of N_2O emissions. This experiment was part of another major experiment which was undertaken to study the effect of urease inhibitor on N transformations from N inputs of urine and urea, and is discussed in Chapter 4.

3.2.4.2 Ammonia measurements using the active sampling technique

Active sampling was used to monitor NH_3 volatilisation and passive sampling for N_2O emissions. Ammonia emissions were determined by flushing air @ 1 dm^3 min^{-1} continuously through each chamber for 10-12 hrs. This flow rate corresponded to 0.2 exchange volumes min^{-1}. During recovery tests performed for NH_3, 95-100% NH_3 was recovered with this flow rate, and no attempt was therefore made to determine if a change in air flow rate would affect the NH_3 loss. The NH_3-charged air was then passed through 50 ml of 0.05M H_2SO_4 to trap NH_3. The sulphuric acid solution was then analyzed for total NH_4^+-N using a Technicon auto analyser. After taking NH_3 measurements, the lids were removed and the chambers were kept open to achieve equilibrium with ambient conditions.

The NH_3 flux (mg N m^{-2} hr^{-1}) was then calculated using the following equation:

$$N_{(NH_3\ flux)} = \frac{C \times V}{a \times D} \qquad \textbf{(3.2)}$$

where, C = NH_3 concentration in the acid trap (mg dm^{-3}); V = is the volume of the acid (dm^3); a = total cross section area (m^2) of soil cores in the chamber; D = duration (hours) of each sampling.

3.2.4.3 Nitrous Oxide measurements using the closed chamber technique

Nitrous oxide samples were collected using the closed chamber technique (Hedley *et al.* 2006). The method is briefly discussed here. Gas samples were taken for a period of one hour with 60 ml polypropylene syringes fitted with 3-way stopcocks after sealing the chambers with the lid having one port. Three gas samples were taken from each chamber at times t0, t30, t60 (i.e., 0 min, 30 min and 60 min respectively, after closing the chamber). The gas samples collected were transferred to evacuated vials and then analyzed using a Shimadzu GC – 17A gas chromatograph with a ^{63}Ni-Electron capture detector; N_2O (mg m^{-2} hr^{-1}) flux was estimated from the measurements

made at the three time periods (t0, t30 and t60). The sample of ambient air taken just after closing the chamber (t0) was used as a reference for calculating N_2O gas fluxes. Accuracy of the gas chromatographic data at ambient concentrations was ± 1% or better. The increases in N_2O concentrations within the chamber headspace were generally linear ($R^2 > 0.90$) with time. Therefore, N_2O flux (mg m^{-2} hr^{-1}) was calculated (Mosier & Mack 1980) from Eq. 3.3 using linear regression and the ideal gas law.

$$F = \rho \times \frac{V}{A} \times \frac{\Delta c}{\Delta t} \times \frac{273}{(T + 273)} \qquad \textbf{(3.3)}$$

where;

F = flux (mg m^{-2} hr^{-1})

ρ = density of gas (mg m^{-3})

V = Volume of chamber (m^{-3})

A = Base area of chamber (m^{-2})

$\Delta c / \Delta t$ = Average rate of change of N_2O concentration with time (ppmvh^{-1})

T = Temperature in the chamber (°C)

3.2.5 Comparison of active vs passive samplers for ammonia volatilisation

3.2.5.1 Glasshouse experiment B

A second glasshouse experiment was conducted in which a semi-quantitative passive NH_3-trapping apparatus containing $C_2H_2O_4$ as an absorbent (Carran *et al.* 2000) was compared with the active sampling method developed and discussed above. These passive traps are easy to use under field conditions compared to the active sampling technique developed in this study. The aim of this experiment was to establish a relationship, if any, between the semi-quantitative passive and quantitative active sampling techniques, which could then be used to quantitatively predict NH_3 losses under field conditions. This experiment, hereafter, is called glass-house experiment-B.

The passive sampling apparatus consisted of a polycarbonate vial, with internal dimensions of 21 x 90 mm. Inside the polycarbonate vial three layers of stainless steel

mesh (0.5 mm) were secured against the bottom of the tube. The mesh was loaded with $C_2H_2O_4$ by dipping in a solution of 10% $C_2H_2O_4$ (w/v) in acetone followed by drying at room temperature.

To develop the relationship between the two methods, NH_3 emissions were measured from soil cores treated with three levels of urine-N. The cores were collected from the same site, and prepared in the same way, as discussed in Section 3.2.4.1 of this chapter. The experiment included the following treatments with three replications each: T1 – Control (water); T2 – Urine @ 144 kg N ha^{-1}; T3 - Urine @ 290 kg N ha^{-1}; T4 - Urine @ 570 kg N ha^{-1}. This experiment was conducted by placing three cores in each chamber under controlled glasshouse conditions. Ammonia emissions were measured daily for the first 15 days after the application of treatments, by both the active and passive sampling apparatus. One passive sampler ($C_2H_2O_4$-coated polycarbonate vial) was placed in the middle of each core to absorb emitted NH_3 (Plate 3.2). These samplers were kept open for an average of 8 hours a day. Each sampler traps a maximum of 2.6 mg NH_3-N (Bowatte 2003). The samplers were replaced every six days and the removed samplers were recapped until analysis. Active sampling was done during the night in this experiment. The diurnal variation in temperature was not taken into account, as the temperature was controlled in the glasshouse and varied only by ± 5^0C. The NH_3 absorbed by 10% $C_2H_2O_4$ in the sampler was dissolved in 10 ml distilled water and the concentration of NH_4^+-N in the solution was then measured using a Technicon auto analyser.

Plate 3.2 Three passive samplers placed on three cores in the chamber

3.2.5.2 Field experiment

The two methods i.e., the active sampling method and the passive sampler were further compared in a field-plot study which was part of a Tech NZ-funded collaborative project undertaken by various CRIs (Landcare Research, HortResearch and AgResearch), Massey University and Summit Quinphos Ltd with the aim of evaluating the value of inhibitors in mitigating N losses in grazed pastures. In this experiment, 15 (1.0 m x 1.5 m) plots were established and arranged into three blocks of 5 plots. Five treatments were assigned at random within each of the three blocks to give a randomized complete block design. The treatments, with three replications, included in this plot study were: T1 = Control (water), T2 = Urine, T3 = Urine + DCD, T4 = Urine + Agrotain, T5 = Urine + DCD + Agrotain. Urine was applied @ 600 kg N ha^{-1}, and DCD (25 kg ha^{-1}) and Agroatin (3 l ha^{-1}) were the respective nitrification and urease inhibitors used. Further details of the experiment are described in Chapter 6 of this thesis. Ammonia emissions were measured daily by both the active and passive sampling apparatus for the first 15 days after application of the treatments. Four passive samplers were placed diagonally in each plot. After exposure in the field for 6 days, samplers were recapped until analysis. The NH_3-N loss from each plot was estimated as the sum of NH_4^+-N in the four vials of each plot. In the case of active sampling, NH_3 emissions were measured by modifying the original method of Kissel *et al.* (1977). PVC chambers, each of 0.040 m^2 area, were inserted 30 to 40 mm into the ground. After treatment application in the confined chamber area, the chambers were covered with a transparent lid. Ambient air from each chamber was drawn at 4 to 5 dm^3 min^{-1} by using a specially designed manifold and ammonia trapping system. This was achieved by making two holes opposite each-other in every chamber. The main flow was then split into 0.3 to 0.4 dm^3 min^{-1} and passed through 50 ml of 0.05 M H_2SO_4 in tightly sealed plastic bottles. The acid solution was replaced every 24 hr and the NH_4^+-N concentration was measured using an auto analyser.

Plate 3.3 Passive samplers placed in a plot during the field-plot study

3.2.6 Statistical methods

Analysis of variance was carried out using SAS for Windows (Version 8). Analysis of variance for data on NH_3 volatilisation and N_2O emission was carried out using the General Linear Model (GLM) procedure. Means were compared using the Least Mean Square Multiple Comparison method at 5% significant level. The regression analysis between total amount of NH_3-N absorbed by active and passive sampling was conducted using SAS software.

3.3 Results

3.3.1 Recovery of ammonia

The recovery test indicated that at a high level of NH_3-N input (5 mg), both 0.05 M H_2SO_4 and 2% H_3BO_3 showed 100% recovery by active sampling, whereas under passive sampling 0.05 M H_2SO_4 achieved a higher recovery (98.5%) than H_3BO_3 (85%) (Figure 3.2). Therefore, 0.05 M H_2SO_4 was used for further testing, by both passive and active sampling of NH_3 absorption over a range of NH_3 input (1.26 to 10 μg NH_3-N) into the chamber. In both the active (R^2 = 0.99) and passive (R_2 = 0.96) sampling techniques there was a linear positive relationship between the amounts of NH_3 introduced in the chambers and the amounts recovered (Figure 3.3). However, in general, the recovery of NH_3 was significantly ($P<0.05$) lower under passive sampling than under active sampling, and the difference was more pronounced at the low level of NH_3 input.

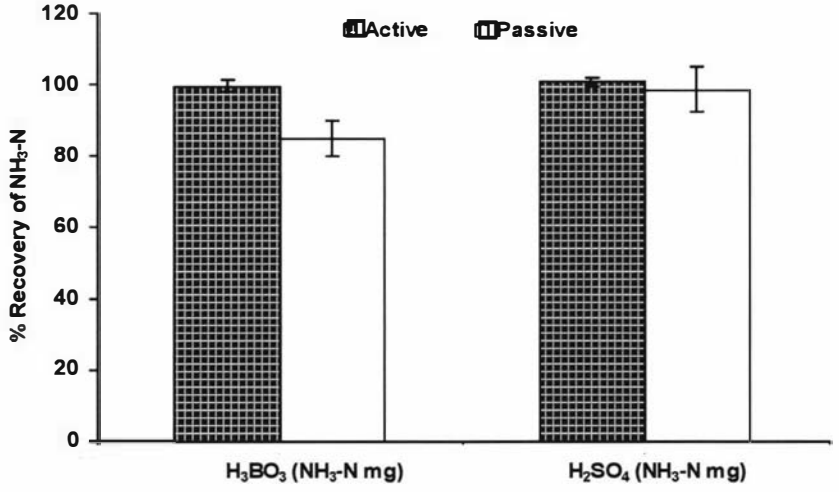


Figure 3.2 Recovery of NH_3 with both active and passive sampling using 2% H_3BO_3 (w/v) and 0.05 M H_2SO_4 as absorbents

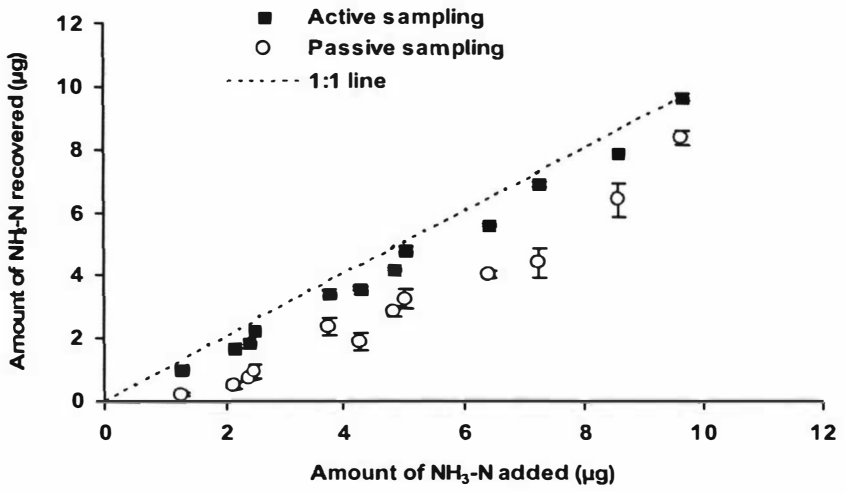


Figure 3.3 Ammonia recovered in active and passive sampling techniques from various levels of NH_3 addition.

3.3.2 Technique evaluation

The quantitative information on the extent of NH_3 and N_2O emissions from urine and urea observed in this study is discussed in detail in Chapter 4, in which the effect of urease inhibitor on the dynamics of N transformations from these two sources is examined. In this section, the effectiveness of the technique developed in this study in the simultaneous monitoring of NH_3 and N_2O emissions from urine and urea sources is discussed.

3.3.2.1 Ammonia emissions

In the glasshouse experiment-A, the NH_3 emission flux from the control treatment ranged from 1.76 to 8.20 mg NH_3-N m^{-2} d^{-1} (Figure 3.4). The addition of N as urine and urea resulted in significant ($P < 0.05$) increases in NH_3 emissions as compared to the control. Urea resulted in a higher rate of NH_3 emission than urine (Figure 3.4). The peak NH_3 emission flux (999 mg NH_3-N m^{-2} d^{-1}) from the urine treatment was obtained within 24 hours of application whereas for urea (4892 mg NH_3-N m^{-2} d^{-1}) it was observed after two days. The total amount of NH_3 emitted from the added N was estimated from the difference in the amount of NH_3 emitted between soil cores with and without added N. These calculations indicate that the cumulative NH_3 emitted from

added urine and urea treatments measured by the active sampling method accounted for 9% and 32%, respectively, of the applied N (Figure 3.5). The amount of urine N emitted as NH_3, falls within the range of 4% and 27% obtained by Lockyer & Whitehead (1990) from dry and wet soil, respectively, as measured by a wind-tunnel method, but is lower than the 17% measured by an active sampling technique from soil at field capacity (Carran *et al.* 1982). The results obtained with urea by the active sampling technique (32% emission from added N) were also consistent with those obtained by micrometeorological methods, where percentage NH_3 losses were 20, 22 and 28% when urea was applied @ 115, 100 and 120 kg N ha^{-1}, respectively (Marshall & DeBell 1980; Black *et al.* 1985; Prasertsak *et al.* 2001).

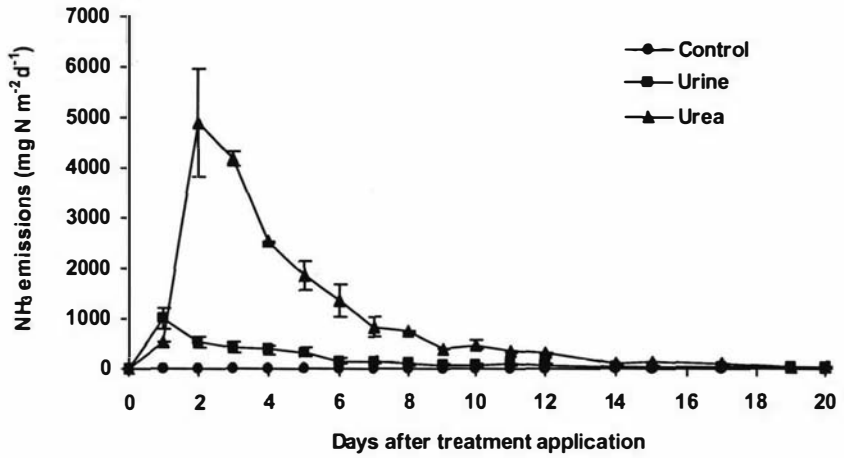


Figure 3.4 Ammonia emissions from urine and urea applications.

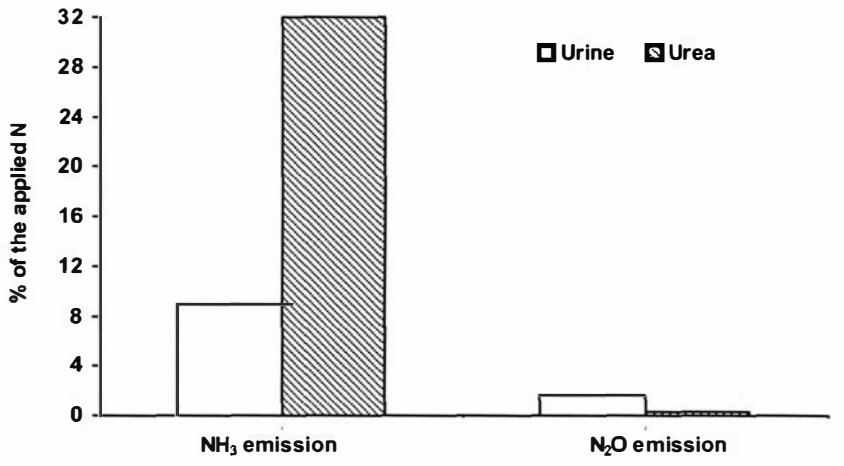


Figure 3.5 The percent losses as NH_3 and N_2O-N of applied N as urine and urea.

3.3.2.2 Nitrous Oxide emissions

The results show significantly higher emissions from both the urea and urine treatments as compared to the control (0.09 to 0.70 mg N_2O-N m^{-2} d^{-1}) over the experimental period of six weeks (Figure 3.6). The overall emissions from the urea and urine treatments were 13 and 53 times higher respectively, than those from the control. Unlike NH_3 emissions, N_2O emissions were higher in the urine treatment as compared to the urea treatment. When expressed as a percentage of the applied N, the N losses as N_2O from urine and urea were 1.6% and 0.32%, respectively (Figure 3.5). The highest N_2O flux in the urine treatments (150 mg N_2O-N m^{-2} d^{-1}) was found within 24 hours of application, whereas the peak in the urea treatment (11 mg N_2O-N m^{-2}) was observed after 4 days (Figure 3.6). Sherlock & Goh (1984) measured a loss of 7% of applied urine-N as N_2O when simulated urine was added to the soil; this was greater than the N_2O loss from aqueous urea, and much higher than the value (1.6%) obtained in the present study (Figure 3.5). The amount of N emitted as N_2O in the urea treatment (0.32%) was quite close to the 0.05 – 0.1% found by Peterson *et al.* (2004) when urea (200-400 kg N ha^{-1}) was applied in aqueous form, and to the 1.4% of applied urea-N (360 kg N ha^{-1}) in a field study conducted by Clayton *et al.* (1997).

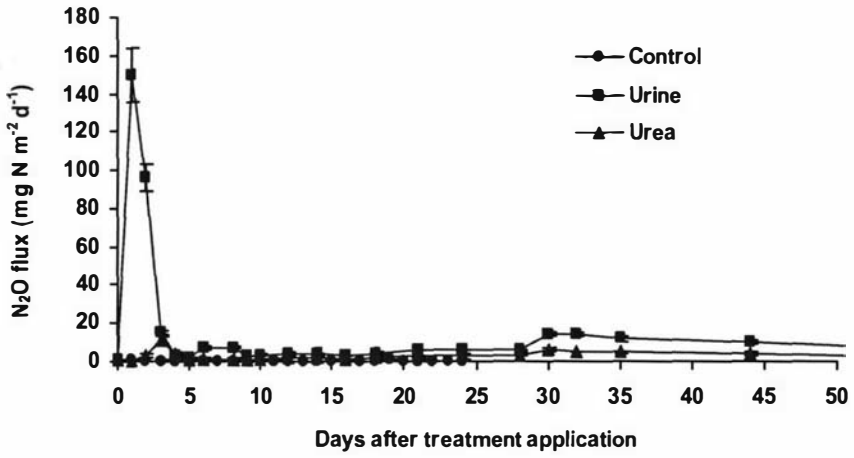
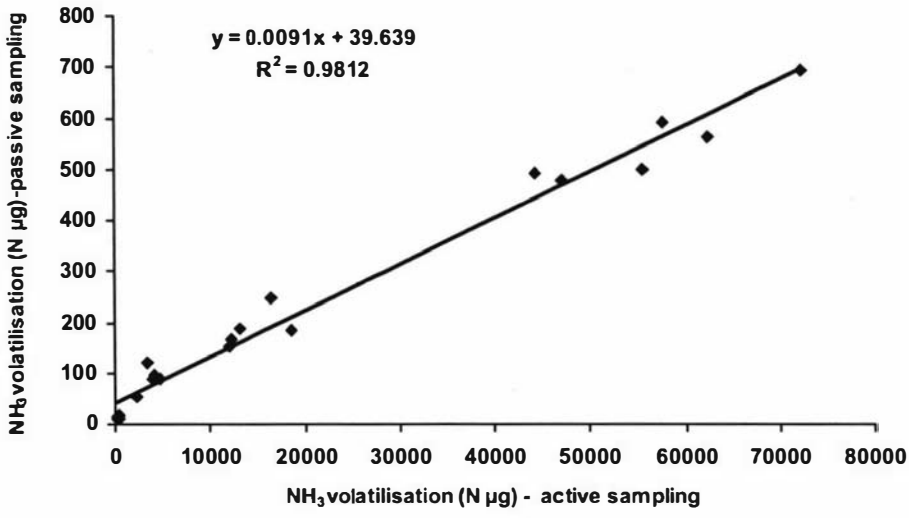


Figure 3.6 Nitrous oxide losses from urine and urea applications.

3.3.3 Comparison of Active vs Passive samplers

In the glasshouse experiment B, a comparison of NH_3 emitted from a range of urine applications using the passive and active sampling techniques was attempted (Figure 3.7a). The amount of NH_3-N absorbed by the passive samplers ranged from 10.6 to 692 μg NH_3-N per chamber whereas, for active sampling, the absorbed amount varied from 227 to 72,325 μg NH_3-N per chamber. Although the passive sampling technique grossly underestimated the amount of NH_3 emission, there was a significant linear relationship between these two methods ($y = 0.0091x + 39.639$, $r^2 = 0.98$). The regression relationship therefore indicates that the passive samplers can be used to predict NH_3 emissions under controlled conditions. However, in the field study, values of NH_3 emitted from plots for the first 15 days after treatment applications differed even more markedly when monitored with the active and passive sampling techniques (Figure 3.7 b). The NH_3 losses measured by active sampling for the different N treatments varied from 0.5 to 27 kg N ha^{-1} (Figure 3.7 b), but the passive sampling technique did not account for this variation., with the regression relationship between these two techniques being non- significant ($P > 0.05$).

(a)



(b)

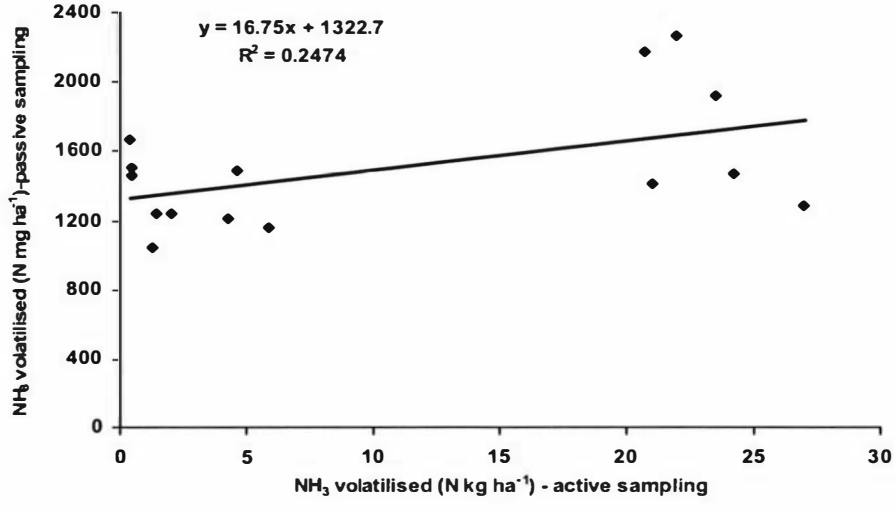


Figure 3.7 **Relationship between NH_3 emissions measured by active and passive sampling techniques (a) under glass house (b) under field conditions (please note different units used for two axis).**

3.4 General Discussion

The study indicated that: (i) the newly developed chamber technique can be efficiently used for active sampling of NH_3 and passive sampling of N_2O simultaneously by altering the lids used for these chambers; (ii) sulphuric acid is more efficient in absorbing NH_3 than boric acid; (iii) the efficiency of NH_3 absorption by H_2SO_4 under passive sampling was less at low levels of NH_3 concentration; (iv) the methods developed in this study are proficient in achieving simultaneous measurement of NH_3 and N_2O emissions from soil cores treated with urine and/or urea; (v) there was a linear relationship between NH_3 emissions measured by active and passive sampling methods under controlled glass-house conditions, while the relationship did not hold true under field conditions; (vi) while the NH_3 emissions were higher from urea than from urine, the reverse was noticed for N_2O emission. In this section, some of these observations will be discussed in relation to the results obtained by others in the literature.

The direct measurement of NH_3 volatilisation by enclosing a volume of air above the soil surface and then collecting the NH_3 from that air using various types of absorbents (acids), has been studied by several workers (Kissel *et al.* 1977; Schjoerring *et al.* 1992; Zia *et al.* 1999; Wulf *et al.* 2001). Among various acids, the most commonly used are H_2SO_4 and H_3BO_3. So, a preliminary study was conducted to examine the efficiency of these two acids as absorbents for NH_3. At a high concentration of 5 mg NH_3-N, both absorbents showed 100% recovery under active sampling conditions, but 0.05 M H_2SO_4 was found to be the better absorbent under passive sampling conditions (98.5% recovery). Thus, 0.05 M H_2SO_4 was used for further evaluation of the sampling technique and for all the subsequent experiments. The recovery of NH_3 by 0.05M H_2SO_4 using passive sampling was equally as good as active sampling (100%) at high concentrations of NH_3-N (0.7 to 5.0 mg NH_3-N) (Table 3.1), but at the lower concentration (1.26 to 10 μg NH_3-N), the recovery by passive sampling (52%) was significantly lower ($P<0.05$) compared to active sampling (88%) (Figure 3.3). Under passive sampling, the movement of NH_3 from the source to the absorbent was solely dependant upon diffusion; low concentrations of NH_3 would have

decreased the diffusion gradient significantly, thereby resulting in the low recovery of NH_3 by the passive sampling technique.

Table 3.1 Ammonia recovered under active and passive sampling at high levels of NH_3-N addition

Amount added (mg NH_3-N)	Active sampling		Passive sampling	
	Amount recovered (mg NH_3-N)	% Recovery	Amount recovered (mg NH_3-N)	% Recovery
5.00	5.04	101	4.92	98.4
1.57	1.54	98.1	1.54	98.1
0.80	0.82	102	0.84	105
L.S.D (0.05%) for sampling methods	0.18			

Under field conditions, a necessary prerequisite for NH_3 volatilisation is a supply of free NH_3 near the soil surface. Ammonia volatilisation is driven by the difference in partial pressure of NH_3 between the air and soil atmosphere. The partial pressure of NH_3 in the soil is controlled by the rate of removal of NH_3 in solution. A number of workers (Marshall & DeBell 1980; Wang *et al.* 2004) have observed that, in general, methods involving diffusion in closed static systems (i.e. passive sampling) lead to lower estimates of NH_3 loss than closed dynamic or semi-open methods (i.e, active sampling). The higher recovery of NH_3 in the active sampling technique probably resulted from mass movement of NH_3 in the compressed air. In our study, for a range of NH_3-N concentrations (1.26 µg to 5 mg NH_3-N), recovery of the emitted NH_3 was 94.4% with the active sampling method, which is similar to the 99% recovery observed by Wang *et al.* (2004) using a vented-chamber technique. van Der Weerden *et al.* (1996) evaluated the much used wind-tunnel method to measure NH_3 volatilisation and found the percentage recovery for French and UK wind-tunnels was 90% and 86% respectively. The results obtained with the active sampling method were consistent with those obtained by micrometeorological method, where N application rates were 100 and 134 kg N ha^{-1} and the percentage NH_3 loss was 28% and 30% of the applied N (Black *et al.* 1985; Fox *et al.* 1996). Both the recovery test and glasshouse experiments showed that the active sampling method gave results similar to those obtained by the

micrometeorological and wind tunnel methods discussed above and could therefore be successfully coupled with N_2O measurements.

In the glasshouse experiment A, NH_3 volatilisation rates were higher from the urea than from the urine treatment. Urea application resulted in 32% volatilisation losses of the applied N as compared to 9% from the urine treatment (Figure 3.5). In contrast, N_2O emissions were higher in the urine treatment (1.6%) than in the urea treatment (0.32%) (Figure 3.5). The reason for this difference is attributed to the difference in rate of hydrolysis of urea and is discussed in detail in Chapter 4.

When both the active sampling technique and passive samplers for NH_3 flux measurements were compared, under glasshouse conditions within enclosed chambers, the methods were highly correlated ($r^2 = 0.98$). However, under field conditions, a poor relationship was obtained between the two methods ($r^2 = 0.25$). Wind has often been shown to be an important factor influencing NH_3 emission rates (Thompson *et al.* 1990; Sommer *et al.* 1991; Misselbrook *et al.* 2005). In the field, these passive samplers were open to the wind from one direction only; no information on wind velocity was available, and no consideration was taken for the wind direction. Therefore, it was impossible to establish the area influencing for the amount of NH_3 absorbed. Furthermore, plots with different treatments were not isolated as in the glasshouse experiment, where passive samplers were confined in chambers, and hence a relationship between the passive samplers and active sampling could not be established for each treatment in the field-plot study.

Although studies have been done to compare different methods of measuring NH_3 emissions (Wang *et al.* 2004; Misselbrook *et al.* 2005), so far no study has been found where a relationship was developed between two methods, except for one by Carran (personal communication) who developed the following relationship between active and passive sampling techniques:

$$NH_3\text{-N volatilised (kg N ha}^{-1}) = (0.8)\ (\mu g\ NH_3\text{-N / sampler})\ (r^2 = 0.6) \qquad \textbf{(3.4)}$$

Bowatte (2003) used passive samplers to trap NH_3 emissions from small experimental plots and then subsequently used the above equation to estimate total NH_3 volatilisation. Wang *et al.* (2004) found that the active vented-chamber method achieved a higher

(99.0%) recovery of NH_3 emission than the passive method (70.8%) and the former method was found to be more suitable for *in situ* determination of NH_3 volatilisation in the field.

3.5 Summary and Conclusions

Nitrogen transformation in soils is a dynamic process, which makes it difficult to estimate gaseous N emissions through mass balance methods because these processes occur simultaneously. A main concern in the development of strategies to reduce NH_3 and N_2O emissions from agricultural land is that reduction in the emission of one gas may affect the emission of the other. Consequently, it is necessary to measure the emission of these two gases simultaneously using a system such as thatdeveloped in this study. The main advantages of the method described in this chapter are that it allows the simultaneous measurement of NH_3 volatilisation and N_2O emission from pasture soil under various management options.

The main conclusions that can be drawn from this methodological study are as follows:

- The active sampling technique showed an NH_3-N recovery of 94.4%.
- The adapted chamber technique using 0.05 M H_2SO_4 as an absorbent for NH_3 is a robust technique that can be successfully used to measure NH_3 (active sampling) and N_2O (passive sampling) emissions simultaneously.
- The passive sampling technique of Carran *et al.* (2000) can be used to predict NH_3 volatilisation under controlled glasshouse conditions, but is not efficient in predicting NH_3 volatilisation under field conditions.
- The simultaneous measurement technique developed in this study indicated that the amount of NH_3 emitted by surface-applied urea was higher than that of urine and vice versa in the case of N_2O.

The results in this chapter have demonstrated that the developed technique can successfully be used to measure gaseous emissions of NH_3 and N_2O under different management practices. This technique was, therefore, used to quantify the effect of

urease and nitrification inhibitors on the emission of these gases, and these studies are described in the next series of chapters.

Chapter 4

Effect of urease inhibitor (Agrotain) on gaseous emissions of nitrogen from cattle urine and urea fertiliser

4.1 Introduction

Nearly half (13.6 M ha) the usable land in New Zealand is grassland used for livestock farming. These managed grasslands, receive nitrogen (N) from biological N fixation by clovers and fertiliser and N recycled through farm effluent application and the uneven deposition of animal excreta. Nitrogen is extremely dynamic and is the major nutrient element that most strongly regulates pasture production, but N is also a major contributor to environmental degradation. As already discussed in the last chapter, loss of N occurs mainly through ammonia (NH_3) volatilisation, nitrous oxide (N_2O) emission and nitrate (NO_3^-) leaching. This has both economical and environmental implications. Quantitatively, NH_3 is the major form of gaseous N loss. The efficient use of urea fertiliser in arable and pastoral agriculture is often prejudiced by the loss of a large portion of the applied N by NH_3 volatilisation, especially under tropical conditions (Vlek & Craswell 1979; Freney *et al.* 1981). Losses up to 30% are associated with urea-containing fertilisers (Terman 1979; Fenn & Hossener 1985; Lightner *et al.* 1990) and with ammonium-based fertilisers applied to high pH soils (Hargrove *et al.* 1977; Whitehead & Raistrick 1990). Similarly, enclosure measurements indicate that 4-41% of the N from applied cattle urine may be lost through NH_3 volatilisation (Lockyer & Whitehead 1990; Whitehead & Raistrick 1993). Ammonia affects visibility, aerosol chemistry, health, and climate as it causes acidification and eutrophication when deposited on soils and waters. Ammonia can also act as a secondary source of NO and N_2O (Bouwman 1990).

The use of nitrogen transformation inhibitors is one of the approaches that is gaining fast momentum as a mitigation option to reduce N losses and to control N dynamics in soils. Nitrogen transformation inhibitors can be grouped into two categories: (i) urease inhibitors (UIs); and (ii) nitrification inhibitors (NIs). The UIs are used to control the conversion of amide N ($R\text{-}NH_2$) in urea-based fertilisers and urine to ammonium (NH_4^+) ions (i.e ammonification or urea hydrolysis reaction). The NIs are used to control the oxidation of NH_4^+ ions to nitrite (NO_2^-) ions (i.e. nitrification reaction). So, the first step towards mitigating N losses from urea-based N sources, such as urea fertilisers and urine, is to control the first chemical process in the N cycle i.e., urea hydrolysis. As UIs control urea hydrolysis and the subsequent ammonification process through their effect on enzyme urease, the present study was aimed at examining the effect of UI on N gaseous emissions from urea and urine. Among the numerous UIs that have been tested, one of the most efficient is N-(n-butyl) thiophosphoric triamide (NBPT, known by the commercial name of Agrotain). There is limited information on the effect of this inhibitor in pasture soils in New Zealand in contrast to arable soils in U.S.A and Europe where it has been evaluated on a range of crops including corn (Schlegel *et al.* 1986), rice (Buresh *et al.* 1988), Kentucky bluegrass (Joo *et al.* 1992) and perennial rye grass (Watson *et al.* 1994b; Watson *et al.* 1998). Recently in New Zealand, NBPT-amended urea products called Sustain Green (urea plus Agrotain), Sustain Yellow (urea plus Agrotain and 4% sulphur coating) have become commercially available (http://www.summitquinphos.co.nz). Research work is required to evaluate these formulations under temperate conditions in New Zealand to assess the potential value of Agrotain inhibitor in mitigating N losses. The overall objective of the study was thus to assess the inhibitory effect of UI Agrotain on the gaseous losses of N from pasture soil when N is added in the form of urea and urine, and to examine its effect on N transformations in the soil. The specific objectives of the study reported in this chapter were:

- To examine the effect of Agrotain on NH_3 volatilisation losses from soil cores receiving different rates of urea and urine-N.
- To examine the effect of Agrotain on N_2O emission.
- To examine the transformation of mineral N with the application of Agrotain.
- To examine the effect of Agrotain on herbage yield.

- To examine the relative effect of Agrotain and elemental sulphur coating on the N transformations and losses from applied urea.

4.2 Materials and Methods

4.2.1 Experimental details

Two experiments were conducted using in-situ soil cores collected from Massey University sheep farm. In the first experiment, emissions of NH_3 and N_2O were compared between urea and Sustain Yellow (urea plus Agrotain and elemental S coating) and between urine and urine plus Agrotain. In the second experiment, emissions of NH_3 and N_2O were compared among urea, Sustain Yellow, Sustain Green (urea plus Agrotain) and S-coated urea. The second experiment was aimed at separating the effects of UI and S coating on gaseous emissions of NH_3 and N_2O.

Plate 4.1 Three intact cores placed in each chamber during the experiment

4.2.1.1 Collection of cores

Intact soil cores (100 mm diameter, 100 mm depth) from three representative sites were collected from a sheep-grazed permanent legume-based pasture at Massey University Frewens Research Block, Turitea campus. The soil cores were prepared in the same way as in Section 3.2.4.1 of Chapter 3. The soil at this site is Manawatu fine sandy loam, classified as a weathered fluvial recent soil (a Fluvent). Some pertinent soil chemical and physical properties are given in Table 4.1.

Table 4.1 Chemical and physical properties of the soil from 0-50 and 50-100 mm depths

	Depth (mm)	Bulk Density ($Mg\ m^{-3}$)	Clay content (%)	Total N (%)	Total C (%)	pH (soil:water 1:2.5)	C.E.C ($cmol_c kg^{-1}$)
Manawatu fine sandy loam	0-50	1.2	12	0.30	3.5	5.7	10
	50-100	1.2	12	0.15	1.5	6.2	nd

nd = not determined

4.2.1.2 Experimental set-up

Experiment 1

The experiment was set up by placing three cores in each chamber in a glasshouse maintained at a near constant temperature ranging from 15-20°C. The experiment comprised six treatments with three replications: Control (water) without UI; Control (water) with UI; Urine without UI; Urine with UI; Urea without UI; Urea with UI (Sustain Yellow). Sustain Yellow is a commercial fertiliser product manufactured by Summit Quinphos Ltd. in which sulfur-coated (4%) urea granules are mixed with Agrotain applied @ 1 l t^{-1} urea i.e., 0.1% (w/w) of urea. The background NH_3 and N_2O emissions were measured a day before applying the treatments. Nitrogen in the form of urine and urea was added at the rate of 47.6 g N m^{-2} (476 kg N ha^{-1}) and 60.0 g N m^{-2} (600 kg N ha^{-1}), respectively. The UI was mixed with urine and then added to the soil cores. The amount of UI Agrotain (liquid form) added in the urine treatment was similar on a total N basis, to that present in Sustain Yellow (@1 l t^{-1} urea). The fertiliser-grade urea and Sustain Yellow granules (2-4 mm) were applied to the soil

surface. The gaseous emissions were monitored for 10 weeks (5 weeks for NH_3) using the methods described in Section 3.2.4.2 and 3.2.4.3. High rates of N were applied to simulate urine patches in a pasture where N loading rate can be very high (600-1000 kg N ha^{-1}) for dairy cattle, with 80-90% being urea N (Haynes & Williams 1993).

Experiment 2

This experiment comprised the following six treatments with three replications; Control without UI, Control with UI, Urea alone, Urea with UI (Sustain Green - another commercially available fertiliser with just Agrotain coating @ 1 l t^{-1} urea); Urea with UI (Sustain Yellow) and S-coated urea (32% N, 27% S). Background NH_3 and N_2O fluxes were measured one day prior to the introduction of the treatments. Nitrogen in the form of urea and amended forms of urea was applied @ 10 g N m^{-2} (100 kg N ha^{-1}). Ammonia emissions were monitored for 15 days and N_2O emissions for six weeks. In this experiment, a low rate of N application (100 kg N ha^{-1}) was used to overcome the urea scorch observed at the high rate of N application (600 kg N ha^{-1}) in Experiment 1. The temperature in the glass house was maintained at 25-30^0 C, which was higher than that of Experiment 1.

Table 4.2 Characteristics of N treatments used in Experiments 1 and 2

N treatments	Components	pH	Total N (%)	Total C (%)	Total S (%)
Urine	-	7.8	7.9	3.5	-
Urea	-	-	46	20*	-
Sustain Yellow	Urea+Agrotain + S coating	-	44	~19*	4
Sustain Green	Urea+ Agrotain	-	46	~20*	-
S-coated Urea	Urea+S coating	-	32	~14*	27

* Estimated from the chemical make up

4.2.2 Gaseous measurements

The gaseous emissions were measured simultaneously, using an active flux for NH_3 and passive flux for N_2O emissions as discussed in Chapter 3. Ammonia collected in 0.05 M H_2SO_4 for 12 hours during the night was analysed for total NH_4^+-N using an

auto analyser. Ammonia measurements were taken daily, and the NH_3 flux was calculated as discussed in section 3.2.4.2. Nitrous oxide measurements were taken daily for the first week to capture immediate changes in gas fluxes, followed by alternate days for two weeks and then twice a week for the following 2 weeks. For the remaining 3 weeks, measurements were taken once a week only, as the fluxes approached background levels. To minimise the variation in the flux pattern, sampling was always carried out between 10 a.m. and 1 p.m. The N_2O flux was calculated as discussed in section 3.2.4.3. The cumulative N_2O emissions during the sampling period were estimated by averaging the rate of emission between two successive determinations, multiplying that average rate by the length of the period between the measurements and adding that amount to the previous cumulative total.

4.2.3 N recovery

The N recovery in various components (NH_3 emission, N_2O emission and mineral N contents in soil, plant N uptake) was calculated as follows:

$$N_{\text{recovery}} = \left[\frac{N_P - N_C}{N_T}\right] \times 100 \qquad \textbf{(4.1)}$$

Where, N_P refers to various N components (NH_3 emissions, N_2O emissions, mineral N present in soil and N taken up by plants) in the urea and urine treatments; N_C refers to the corresponding N components in the control treatment; N_T refers to N added to soil as urine or urea.

The percentage reduction in total N as NH_3 or N_2O emitted with addition of UI to N input (urea/urine) was calculated using the following equation:

$$\%\ reduction\ in\ NH_3 / N_2O - N = \frac{[(A-C)-(B-D)]}{(A-C)} X100 \qquad \textbf{(4.2)}$$

where, A = total NH_3/N_2O-N emitted from N only treatment

B = total N_2O-N emitted from N+DCD treatment

C = total N_2O-N emitted from the control (nil N) without inhibitor treatment

D = total N_2O-N emitted from the control with inhibitor treatment

4.2.4 Analysis

4.2.4.1 Urine analysis

The urine was collected from Friesian cows during a milking session at the Massey University No.1 Dairy farm. Individual fresh urine samples were bulked and frozen (-18 ^{0}C) within an hour of collection. They were then analyzed for total N (Ebina *et al.* 1983) and total C (Bremner & Tabatabai 1971). The urine applied had a pH of 7.8, an average total N and total C content of 7.9 g l^{-1} and 3.5 g l^{-1}, respectively.

4.2.4.2 Soil analysis

Mineral N, Total C and N and Soil pH analysis

At the end of the experiment, soil cores were cut into 0-50 mm and 50-100 mm depth sections. A sub-sample (5g oven dry equivalent) was extracted with 2 *M* KCl solution by shaking for 1 hr (1:6 soil: extractant ratio). The extracts were analysed for nitrate (NO_3^-) and ammonium (NH_4^+) contents colorimetrically by a Technicon auto-analyser (Blakemore *et al.* 1987). Total C and N in the soil were measured by combustion in a Leco FP-2000 CNS (LECO Corp., St Joseph, MI, USA).

Soil pH was measured at a 1: 2.5 soil: water ratio using a combined electrode pH meter (Blakemore *et al.* 1987).

Soil cation exchange capacity

The cation exchange capacity (C.E.C.) of the soil was measured using a method of Hesse (1971). Five g of soil was shaken and centrifuged with 1M ammonium acetate twice and then washed three times with 95% ethanol. The NH_4^+ ions adsorbed by the soil were extracted with 0.25 M of barium chloride and, after adding 40% neutralised formalin, the extract was titrated against 0.02 M sodium hydroxide. The C.E.C. of the soil was calculated from the number of moles of sodium hydroxide required to neutralise the H^+ ions generated in the reaction between formalin and the NH_4^+ ions.

4.2.4.3 Herbage yield and analysis

No pasture yield data was available from Experiment 1 as the pasture died from the injury caused by the very high levels of applied urine and urea. In Experiment 2, the herbage on the cores was cut at 2 cm height from the soil surface twice (after 20 and 41 days of treatment application) during the experimental period, dried at 65^0C for 24 hours and weighed for total dry matter (DM) yield. The herbage samples were analysed for total N by the Kjeldahl digestion method (McKenzie & Wallace 1954).

4.2.5 Statistical Methods

An analysis of variance using SAS software (version 8) was performed on the results for total NH_3 and N_2O emitted, mineral N, herbage DM and herbage N uptake using the General Linear Model (GLM) procedure. Mean comparisons were done using Fisher's Least Significant Difference (LSD) at 5% significance.

4.3 Results

4.3.1 Experiment 1

4.3.1.1 Ammonia emissions

The NH_3 emission flux from the control treatment ranged from 1.76 to 8.20 mg NH_3-N m^{-2} d^{-1}. There was no significant change in NH_3 emissions when UI was applied to the control. The addition of urine and urea treatments @ 47.6 and 60 g N m^{-2} respectively, resulted in increased NH_3 emissions within a short period of their application (Figure 4.1). The urine treatment increased the NH_3 flux within 24 hours of application and reached a maximum value of 999 mg NH_3-N m^{-2} d^{-1}. Application of urea to the soil cores resulted in the highest flux value of 4890 mg NH_3-N m^{-2} d^{-1} after two days of application. In both treatments, NH_3 emissions dropped quickly for the first

five days and then gradually to background levels. Addition of UI resulted in a significant reduction ($P< 0.05$) and delay in NH_3 emissions from both urine and urea. In the urine treatment with added UI, the peak NH_3 emission flux was delayed by a day and reduced to 375 mg NH_3-N m^{-2} d^{-1}, and subsequent NH_3 emissions for six days remained significantly lower than those in urine only treatment. After day 6, the emissions from urine+UI were slightly, but non-significantly higher than from urine only treatment until they reached background levels after 12 days. The total NH_3-N released from urine decreased from 4.36 to 3.39 g NH_3-N m^{-2} with the addition of UI, resulting in a 22.4% reduction in NH_3 emissions (Table 4.3). Ammonia emissions from Sustain Yellow were also significantly lower than from the urea treatment for the first 6-7 days after application, and the peak emission was reduced from 4890 mg NH_3-N m^{-2} d^{-1} (urea) to 1470 mg NH_3-N m^{-2} d^{-1} (Sustain Yellow) (Figure 4.1). However, subsequent NH_3 emissions after day 6 remained significantly higher in the Sustain Yellow than in the urea treatment until they gradually reached the background level (2.99 NH_3-N m^{-2} d^{-1}). Application of Sustain Yellow reduced the total NH_3 losses from 19.7 to 14.3 g NH_3-N m^{-2}, resulting in a 27.5% reduction in NH_3 losses (Table 4.3).

Total N losses as NH_3 from the soil cores were 8.88, 6.89, 32.6 and 23.6% for applied urine, urine+UI, urea and Sustain Yellow, respectively (Table 4.3). Most of the NH_3 loss (68 to 78%) occurred within 6 days of application of both urine and urea. The emissions were negligible from all the treatments after 20 days of application.

4.3.1.2 Nitrous oxide emissions

The N_2O emission rate in the control treatment remained steady between 0.09 and 0.70 mg N_2O-N m^{-2} d^{-1}. There was no significant change in emission rate with the addition of UI to the control. Urine application resulted in peak N_2O emission of 158 mg N_2O-N m^{-2} d^{-1} within 24 hours, followed by a progressive decline with time (Figure 4.2). With urea, the highest peak of 11.2 mg N_2O-N m^{-2} d^{-1} was observed on the third day after application. Another small peak was obtained in all the treatments, except in the control and control+UI, after 30 days. Nitrous oxide emissions remained higher in all the treatments than in the control until 70 days after application. Addition of UI to urine reduced the peak N_2O emission on day 1 from 158 (urine) to 83.8 mg N_2O-N m^{-2} d^{-1} (urine+UI), and the subsequent N_2O emissions remained lower than in the urine-only

treatment throughout the experiment. The total N_2O emission from urine was 0.80 g N_2O-N m^{-2} and decreased to 0.31 g N_2O-N m^{-2} with the addition of UI, resulting in a 62% reduction in N_2O emissions (Table 4.3).

In the urea treatment, the highest emission of 11.2 mg N_2O-N m^{-2} d^{-1} was obtained on day 3 but then decreased markedly within a day. This declining phase was followed by a small but steady increase in emissions, giving a peak value of 6.21 mg N_2O-N m^{-2} d^{-1} on day 30 (Figure 4.2). Addition of Sustain Yellow resulted in a smaller initial peak (2.20 mg N_2O-N m^{-2} d^{-1}) than from the urea-only treatment. However, the emission trends were similar in both treatments. Nitrous oxide emission fluxes in the Sustain Yellow treatment were however, higher than in urea-only treatment between days 30 and 70. Overall there was no significant difference in total N_2O emissions in the urea and Sustain Yellow treatments (Table 4.3). Nitrogen loss as N_2O emissions was 1.65, 0.62, 0.33 and 0.37% for applied urine, urine+UI, urea and Sustain Yellow, respectively.

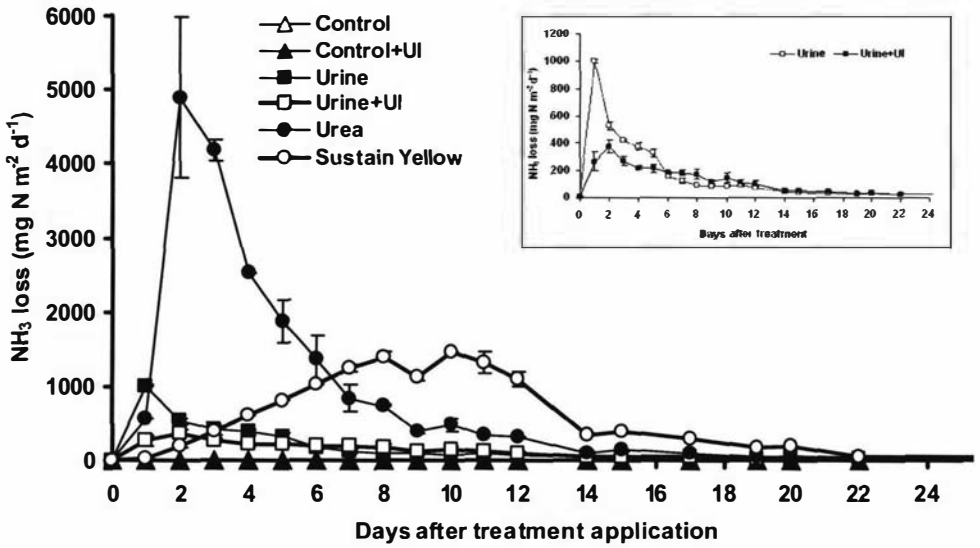


Figure 4.1 **Ammonia emissions from urine and urea applications, with and without UI. The inset gives the enlarged graph for NH_3 emissions in the urine and urine+UI treatments. Each value represents a mean of three replicates with standard deviation shown by vertical bars**

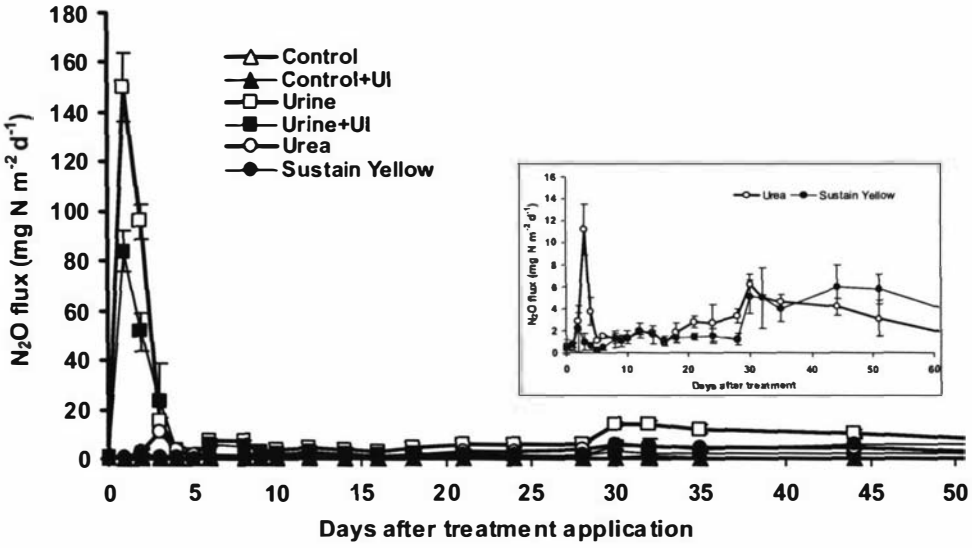


Figure 4.2 **Nitrous oxide emissions from urine and urea applications, with and without UI. The inset gives the enlarged graph for N_2O emissions in the urea and Sustain Yellow treatments. Each value represents a mean of three replicates with standard deviation shown by vertical bars**

Table 4.3 **Total N applied and N emitted as NH_3 and N_2O (g N m^{-2}) over the experimental period from various treatments with and without UI**

Treatments	N added (g N m^{-2})	NH_3-N (g N m^{-2})	% of added N emitted as NH_3	N_2O-N (g N m^{-2})	% of added N emitted as N_2O	Total % of N emitted
Control	-	0.134 e*	-	0.014 c	-	-
Control+UI	-	0.113 e	-	0.015 c	-	-
Urine	47.6	4.36 c	8.88	0.80 a	1.65	10.6
Urine+UI	47.6	3.39 d	6.88	0.31 b	0.62	7.50
Urea	60.0	19.7 a	32.6	0.21 b	0.33	32.9
Sustain Yellow	60.0	14.3 b	23.6	0.24 b	0.37	24.0
L.S.D. (0.05%)		1.48		0.16		

* Values followed by the same letter in a given column do not differ significantly at the 0.05 level

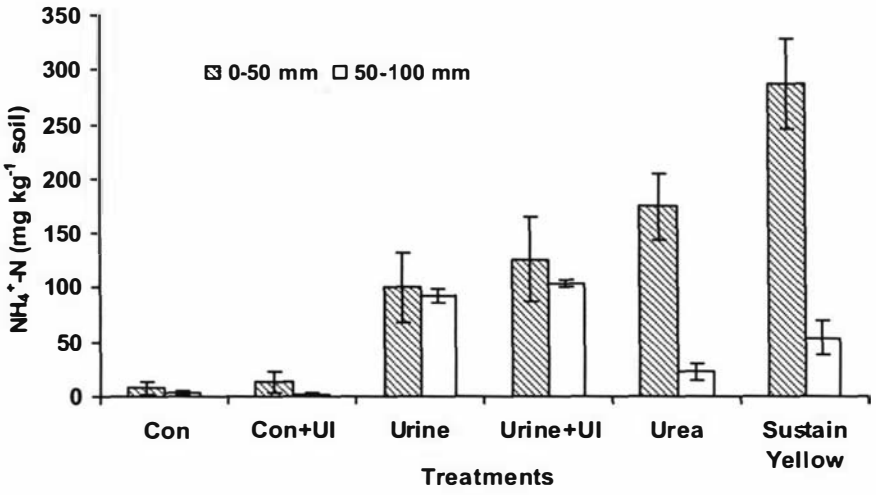
4.3.1.3 Mineral nitrogen

The soil cores analysed at the end of the experiment contained higher concentrations of NO_3^- than NH_4^+-N in all the treatments at both 0-50 and 50-100 mm depths (Figure 4.3 a & b).

In the control soils, the NH_4^+-N concentrations ranged from 7.7 to 13.3 mg kg^{-1} soil at 0-50 mm depth and from 2.3 to 3.2 mg kg^{-1} soil at 50-100 mm depth. Nitrate-N concentration in the control soils ranged from 61.4 to 79.7 mg kg^{-1} soil at 0-50 mm depth and from 36.6 to 54.4 mg kg^{-1} soil at 50-100 mm depth (Figure 4.3 a & b). Soil NH_4^+ and NO_3^- concentrations were significantly higher ($P < 0.05$) in the cores receiving urine and urea (with and without UI) than in the control soils at both the depths. In the urine treatment, there was no significant difference in NH_4^+-N concentrations between 0-50 mm (100 mg N kg^{-1} soil) and 50-100 mm (92 mg N kg^{-1} soil) depths (Figure 4.3 a), indicating that urine was uniformly distributed down the depth of the cores, which were kept at field-capacity moisture content throughout the experiment. Nitrate-N concentrations in the urine treatment were however, significantly higher at 0-50 mm (324 mg N kg^{-1} soil) than at 50-100 mm (210 mg N kg^{-1} soil) depths (Figure 4.3 b). With the addition of UI to the urine treatment there was a significant ($P < 0.05$) increase in NH_4^+-N concentration at both 0-50 mm depth (from 100 to 126 mg N kg^{-1} soil) and 50-100 mm depth (from 92 to 104 mg N kg^{-1} soil) (Figure 4.3 a). However, UI addition to the urine treatment had no significant effect on NO_3^--N concentrations at either depth (Figure 4.3 b).

In the urea treatment, significantly higher NH_4^+-N concentrations were found at 0-50 mm depth (175 mg kg^{-1} soil) than at 50-100 mm (22.5 mg kg^{-1} soil) depth (Figure 4.3 a). As urea was surface applied, this difference was expected because NH_4^+ would be confined to the top soil layer. A similar trend was found in NO_3^--N concentrations at 0-50 and 50-100 mm depths, with concentrations of 268 and 123 mg NO_3^--N kg^{-1} soil, respectively (Figure 4.3 b). In the soil cores receiving Sustain Yellow, the trend of NH_4^+ and NO_3^- distributions were same as in the urea treatment, but the concentrations at both soil depths increased significantly. The average NH_4^+-N concentration in the soil cores increased from 99 to 171 mg kg^{-1} soil and NO_3^--N concentration increased from 196 to 243 mg kg^{-1} soil, with Sustain Yellow as compared to urea alone (Figure 4.4).

(a)



(b)

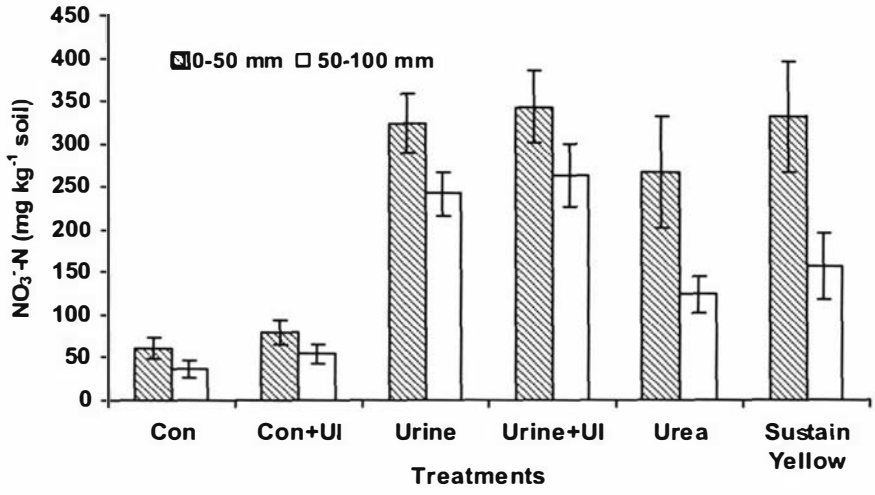


Figure 4.3 **Distribution of (a) NH_4^+ and (b) NO_3^- concentrations in soil cores at 0-50 mm and 50-100 mm depths in various treatments with and without UI. Each bar value represents a mean of nine replicates with standard deviation shown by vertical bars (a) 0-50 mm (LSD (0.05) = 19.2); 50-100 mm (LSD (0.05) = 5.36) (b) 0-50 mm (LSD (0.05) = 36.4); 50-100 mm (LSD (0.05) =20.7)**

4.3.1.4 Nitrogen recovery

Total N added in the urine and urea treatments was 47.6 g N m^{-2} (412 mg N kg^{-1} of soil) and 60 g N m^{-2} (526 mg N kg^{-1} soil), respectively. The total N recovery in NH_3 and N_2O emissions and soil NH_4^+ and NO_3^--N concentrations in all the treatments is given in Figure 4.4. The fraction of added N recovered was significantly higher with added UI in the urine (89%) and slightly higher in the urea (87%) treatments as compared to urine (86%) and urea alone (75%) treatments. In the urine treatment, 26% and 62% N, respectively, of the total N recovered was in NH_4^+ and NO_3^- forms (Figure 4.4). With adding UI, recovery of N was similar, 28% as NH_4^+ and 64% as NO_3^-. In the urea treatments, the fraction of N recovered as NH_4^+ increased from 23% to 34% and as NO_3^- from 33% to 38% with the addition of UI. As discussed earlier, addition of UI to urine and urea decreased NH_3 emissions from 10.3 to 7.62% and from 43.4 to 27.3%, respectively of the recovered N. Nitrous oxide emissions accounted for 1.90% of the total N recovered in the urine treatment and for 0.71% in the treatment with added UI. There was no effect of UI on N_2O emissions (0.43% of the recovered N) in the urea treatment (Figure 4.4).

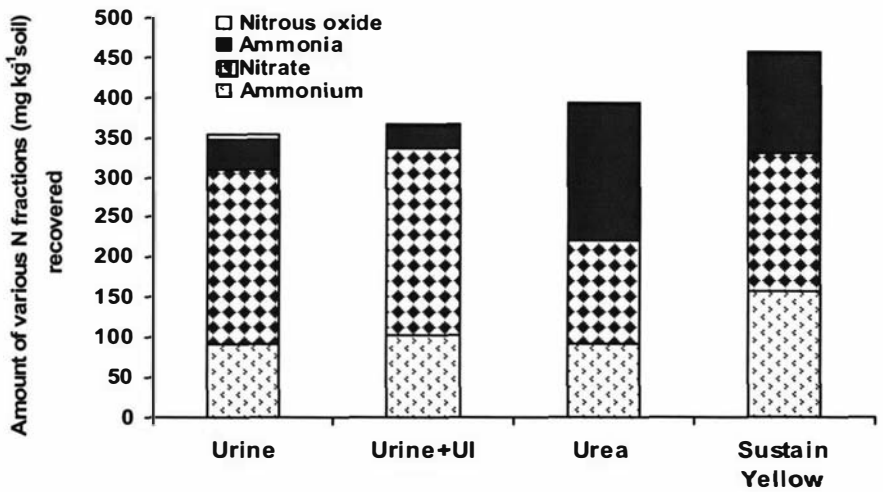


Figure 4.4 Recovery of N fractions (NH_3 and N_2O emitted during the experiment and N present as NH_4^+ and NO_3^--N in the soil at the end of the experiment) from N applied as urine and urea, with and without added UI.

4.3.1.5 Soil pH

The mean pH of the soil receiving N in the form of urine or urea, with and without UI, was significantly higher than that in the control soil (Figure 4.5). When urea or urine is applied to soils, the urease enzyme hydrolyzes the urea to NH_4^+. This process releases alkali ions (OH^-), thereby increasing the pH.

In the urine treatment, pH increased from 5.7 to 6.9 within a day of application and remained significantly higher than in the urine+UI treatment. Addition of UI to urine delayed the pH increase by a day (Figure 4.5) and the maximum pH value obtained was 6.7. In the urea treatment, pH increased from 5.7 to 6.9 two days after application, while the increase with Sustain Yellow was from 5.7 to 6.3. The pH in the Sustain Yellow treatment remained significantly lower than that in the urea treatment throughout the measurement period. This may be attributed to the effect of UI on the rate of urea hydrolysis and the associated release of OH^- ions.

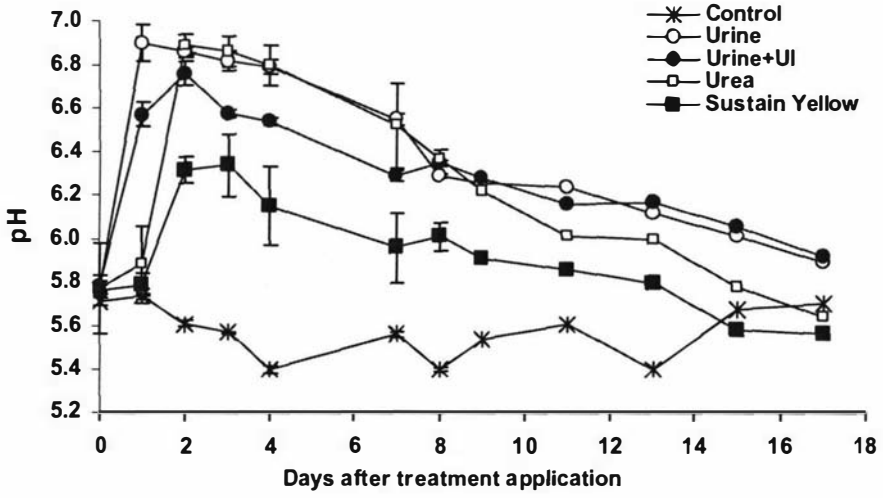


Figure 4.5 pH distribution in Manawatu sandy loam soil receiving added urine and urea, with and without UI

4.3.2 Experiment 2

In Experiment 1, the effect of UI on N transformations from urea was examined by comparing commercial urea with Sustain Yellow. However, Sustain Yellow contains both UI and S coating, both of which have inhibitory properties. Therefore, it was not possible to separate out the effect of the UI from that of the S coating on N transformations. Thus, the main objective of Experiment 2 was to differentiate the effect of the S coating and UI on gaseous N emissions. Sulphur-coated urea is a typical slow release fertiliser and was included as one of the treatments to compare its effect on gaseous N emissions with that of urea amended with UI and S (Sustain Yellow). Although some of the treatments (Control, urea and Sustain Yellow) were common in Experiments 1 and 2, it was not possible to compare N transformations between these two experiments because of the differences in the rates of N used (600 and 100 kg ha^{-1} in Experiment 1 and 2, respectively) and glasshouse conditions (temperature).

4.3.2.1 Ammonia emissions

Results from the control and from urea and urea-amended treatments (applied @ 10 g N m^{-2}) (Figure 4.6) showed that NH_3 emissions in the control did not vary much during the experiment and ranged between 0.06 to 2.25 mg NH_3-N m^{-2} d^{-1}. Ammonia emissions in the urea treatment increased rapidly during the first 4 days, peaking on second day (260 mg NH_3-N m^{-2} d^{-1}). The total NH_3 emission in the urea treatment was 4.75% of the N applied, compared to 2.75, 2.75 and 2.45% in the Sustain Green, Sustain Yellow and S-coated urea treatments, respectively (Table 4.4). This accounted for a 42-48% decrease in NH_3 emissions from urea fertiliser amended with UI and S compared to those from urea alone (Table 4.4). The time to reach the peak NH_3 emission (T_{max}) (48 to 50 mg NH_3-N m^{-2} d^{-1}) from the amended-urea products was delayed by one day. Delaying T_{max} increases the chance that water/irrigation washes the urea into the soil, resulting in lower total NH_3 volatilisation.

However, a second NH_3 peak was obtained after 9 days in the Sustain Yellow and S-coated urea (51.3 and 31.4 mg NH_3-N m^{-2} d^{-1}, respectively) treatments and after 10 days in the Sustain Green treatment (52.4 mg NH_3-N m^{-2} d^{-1}). This may be

attributed to an increased concentration of NH_4^+ ions released later in the experimental period due to their slow-release characteristics.

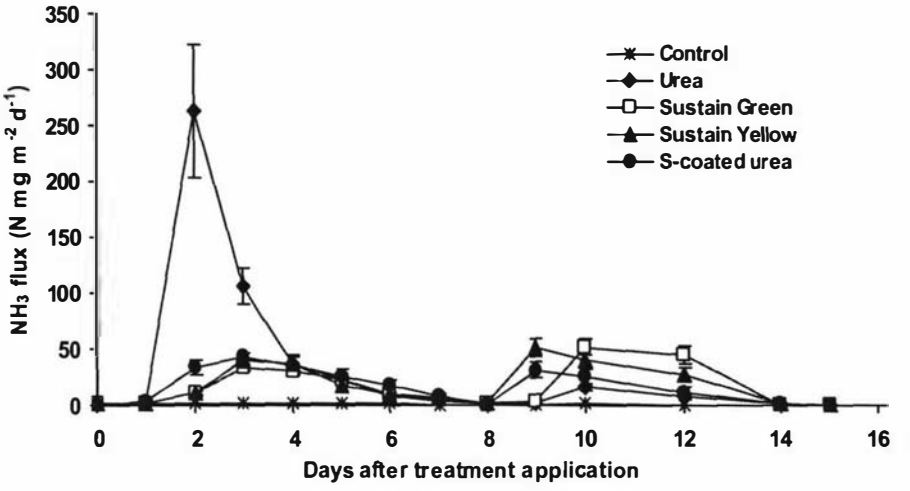


Figure 4.6 Ammonia emissions from the urea and amended-urea treatments. Each value represents a mean of three replicates with standard deviation shown by vertical bars

4.3.2.2 Nitrous oxide emissions

The N_2O emissions from the control soil remained nearly constant and ranged from 0.12 to 0.66 mg N_2O-N m^{-2} hr^{-1} during the experimental period. The application of urea, Sustain Green, Sustain Yellow and S-coated urea, resulted in an increase in N_2O emission within a day (Figure 4.7). There were two peaks observed later during the experimental period in all the treatments. In the urea treatment, the highest N_2O emission rate (72 mg N_2O-N m^{-2} hr^{-1}) was obtained 4 days after application; a subsequent smaller peak (23 mg N_2O-N m^{-2} hr^{-1}) was observed after 19 days. Similar trends were found in all the other treatments. The peak emissions obtained at the 4^{th} and 9^{th} days for Sustain Green (26.5 and 11.8 mg N_2O-N m^{-2} hr^{-1}, respectively) and Sustain Yellow (17 and 16.1 mg N_2O-N m^{-2} hr^{-1}, respectively) were significantly lower compared to those in the urea treatment. In the S-coated urea treatment, the peak on the 4^{th} day (37.8 mg N_2O-N m^{-2} hr^{-1}) was significantly lower than that in the urea treatment, but the peak on the 19^{th} day was significantly higher (45 mg N_2O-N m^{-2} hr^{-1}), with subsequent emissions also remaining higher. The total amount of N_2O-N emitted was

0.44, 0.20, 0.23 and 0.62 g N_2O-N m^{-2} in the urea, Sustain Green, Sustain Yellow and S-coated urea treatments, respectively. This indicates that while Sustain Green and Sustain Yellow had significantly decreased N_2O emissions by 56% and 49%, respectively over urea, S-coated urea had increased emission by 42% (Table 4.4).

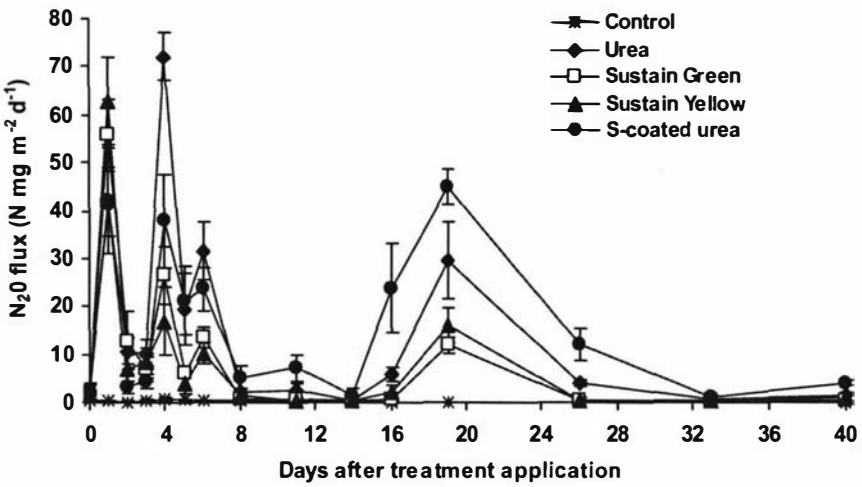


Figure 4.7 **Nitrous oxide emissions from the urea and amended-urea treatments. Each value represents a mean of three replicates with standard deviation shown by vertical bars**

Table 4.4 **Total N applied and N emitted as NH_3-N and N_2O-N (g N m^{-2}) over the experimental period from urea treatments with and without UI**

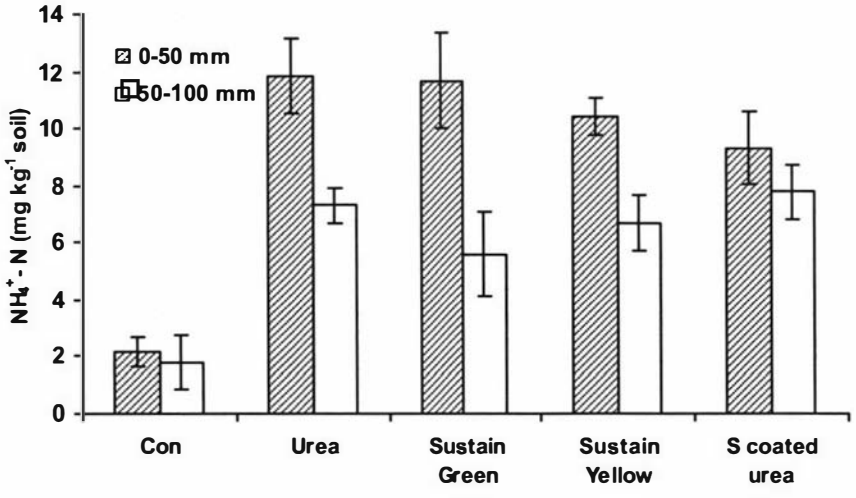
Treatments	N added (g N m^{-2})	NH_3-N (g N m^{-2})	% of added N emitted as NH_3	N_2O-N (g N m^{-2})	% of added N emitted as N_2O	Total % of N emitted
Control	-	0.015 c*	-	0.009 d	-	-
Urea	10	0.49 a	4.74	0.44 b	4.27	9.01
Sustain Green	10	0.29 b	2.76	0.20 c	1.96	4.72
Sustain Yellow	10	0.29 b	2.79	0.23 c	2.17	4.96
S-coated urea	10	0.26 b	2.49	0.62 a	6.07	8.56
L.S.D (0.05%)		0.08		0.08		

* Values followed by the same letter in a given column do not differ significantly at the 0.05 level

4.3.2.3 Mineral nitrogen

The concentration of NO_3^--N was higher than that of NH_4^+-N at both 0-50 and 50-100 mm depths (Figure 4.8 a & b) for all the treatments at the end of the experiment. The NH_4^+-N concentration was significantly higher ($P<0.05$) in soil cores receiving urea or amended-urea products than in the control treatment (2.16 and 1.79 mg N kg^{-1} soil, respectively at different depths) (Figure 4.8 a). There was no significant difference in NH_4^+-N concentration at 0-50 mm soil depth among the urea (11.8 mg kg^{-1} soil), Sustain Green (11.7 mg kg^{-1} soil) and Sustain Yellow (10.4 mg kg^{-1} soil) treatments (Figure 4.8 a), but the soil cores receiving S-coated urea had a significantly ($P < 0.05$) lower NH_4^+-N concentration (9.31 mg kg^{-1} soil). No significant difference between the N-receiving treatments was found in NH_4^+-N concentrations at 50-100 mm depth. Similarly, NO_3^--N concentrations were also significantly higher ($P < 0.05$) in the urea or amended-urea treatments than in the control treatment (2.16 and 0.95 mg N kg^{-1} soil, respectively at different depths) (Figure 4.8 b). The NO_3^--N concentrations in the Sustain Green (84.9 mg kg^{-1} soil) and Sustain Yellow (81.1 mg kg^{-1} soil) treatments were higher than those in urea (80.8 mg kg^{-1} soil) and S-coated urea (77.5 mg kg^{-1}) treatments, but the differences were not significant. The concentration of NO_3^--N at 50-100 mm depth in the S-coated urea (50.6 mg kg^{-1} soil) was significantly higher than in the urea treatment (38.1 mg kg^{-1} soil) (Figure 4.8 b).

(a)



(b)

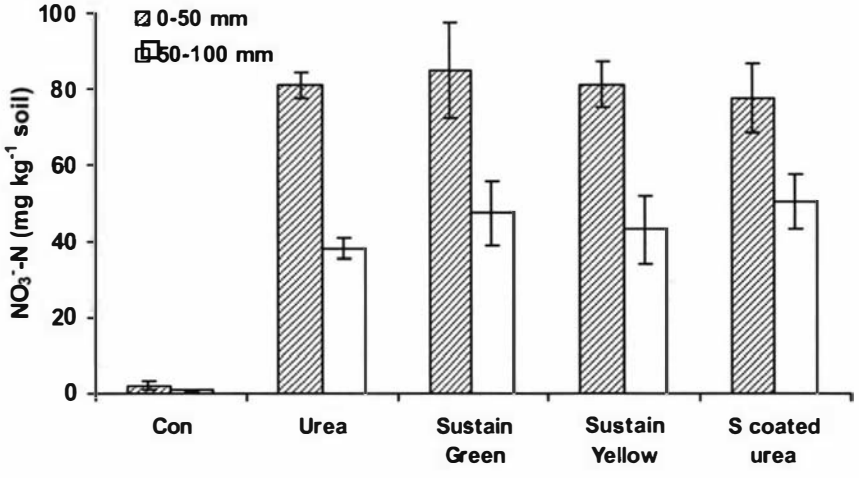


Figure 4.8 **Distribution of (a) NH_4^+ and (b) NO_3^- concentrations in soil cores at 0-50 mm and 50-100 mm depths receiving urea and amended-urea treatments. Each bar value represents a mean of nine replicates with standard deviation shown by vertical bars (a) 0-50 mm (LSD (0.05) = 2.12); 50-100 mm (LSD (0.05) = 1.88) (b) 0-50 mm (LSD (0.05) = 13.9); 50-100 mm (LSD (0.05) =11.8**

4.3.2.4 Soil pH

The soil pH for the control treatment fluctuated between 5.4 and 5.8 during the study period of 15 days (Figure 4.9). The addition of urea resulted in the highest pH of 6.32 after 48 hours, with the pH decreasing rapidly after day 4. The pH in the Sustain Green and Sustain Yellow treatments showed a gradual increase, reaching a maximum of 6.1 after day 4 and day 5, respectively. This increase in pH was then followed by a gradual decrease until day 14, when it was similar to that of the control. The S-coated urea treatment resulted in the lowest rise in pH (6.0) after five days; the pH then decreased rapidly and was lower than that in the urea treatment by end of 13 days.

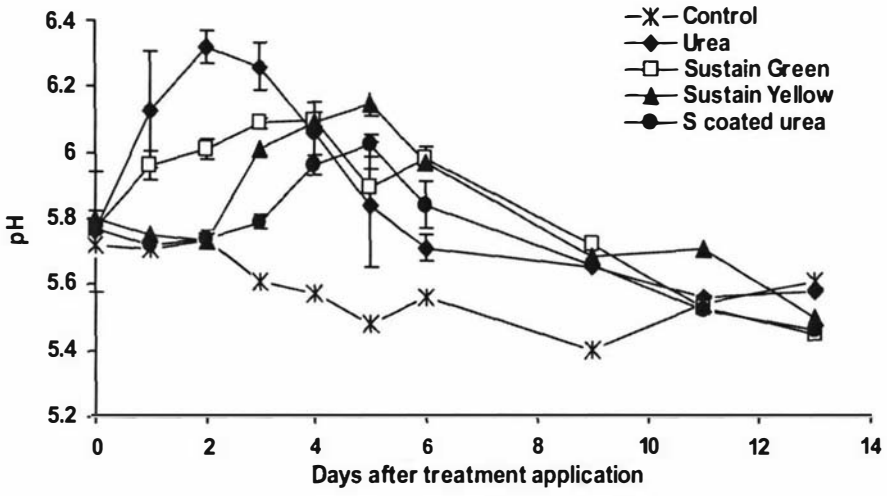


Figure 4.9 pH distributions with and without added urea and amended-urea in Manawatu sandy loam soil.

4.3.2.5 Herbage dry matter yields

The herbage DM yield and N uptake in the control treatment, receiving no N, were significantly lower than those in the treatments receiving N (Table 4.5). The highest yield of 232 g m^{-2} was found in the Sustain Green, followed by the Sustain Yellow (223 g m^{-2}) and urea (194 g m^{-2}) treatments; however, these treatment differences were not significant. The presence of UI in Sustain Green and Sustain Yellow significantly ($P<0.05$) increased the N uptake in herbage i.e., 7.61 g N m^{-2} and 6.94 g N m^{-2}, respectively, compared to that in the urea treatment (5.27 g N m^{-2}). Herbage yields (150 g m^{-2}) and N uptake (4.36 g N m^{-2}) in the S-coated urea treatment

were significantly lower than those in the Sustain Green and Yellow treatments, but did not differ significantly from those in the urea treatment (Table 4.5).

Table 4.5 Total dry matter (DM) yield, percent of added N in DM and N uptake in the response to applied N to the soil cores

Treatments	Total DM ($g\ m^{-2}$)	N in DM (%)	N uptake ($g\ m^{-2}$)
Control	95.6 c*	1.99	1.90 c
Urea	194 ab	2.72	5.27 b
Sustain Green	232 a	3.29	7.61 a
Sustain Yellow	223 a	3.11	6.94 a
S coated urea	150 b	2.90	4.36 b
L.S.D. (0.05%)	48.5		1.39

* Values followed by the same letter in a given column do not differ significantly at the 0.05 level
All the treatments were applied @ 10 g N m^{-2}, except for the control where no N was applied

4.3.2.6 Nitrogen recovery

The total N added through urea and amended-urea products was 10 g N m^{-2} (86 mg N kg^{-1} of soil). In the urea treatment, the percentage of the N recovered in the form of NH_4^+ and NO_3^- was 7% and 56%, respectively. The urea-amended fertiliser treatments having UI and S coating showed a similar NH_4^+ and NO_3^- distribution as that in the urea treatment (Figure 4.10). However, a clear-cut difference in N lost as NH_3 and N_2O was observed between these treatments. In the urea treatment, 4.2% and 3.7% of recovered N was lost as NH_3 and N_2O, respectively, whereas in the Sustain Green and Yellow treatments the loss of NH_3 accounted for 2.1% and 2.3%, and N_2O accounted for 1.5% and 1.7%, respectively of recovered N. In the S-coated urea treatment the loss of NH_3 accounted for 2.3% of the recovered N while the loss of N_2O accounted for 5.6%, which was higher than in the urea treatment (3.7%). The decrease in N lost through NH_3 and N_2O in Sustain Green and Yellow was compensated by an increase in percentage of N taken up by plants. Nitrogen uptake accounted for 35% and 34% of the recovered N in the Sustain Green and Yellow treatments compared with 29% in the urea treatment and only 20% in the S-coated urea treatment.

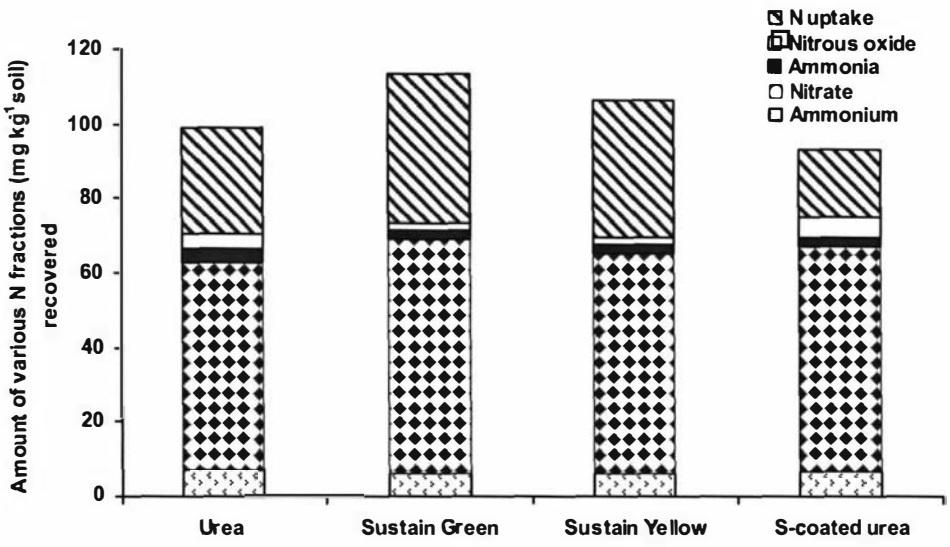


Figure 4.10 Recovery of N fractions (NH_3 and N_2O emitted during the experiment and N present as NH_4^+ and NO_3^--N in the soil at the end of the experiment) from N applied as urea and amended-urea.

4.4 General discussion

The results in this chapter have indicated that: (i) while NH_3 volatilisation was higher from urea fertiliser than urine, the trend was the opposite for N_2O emissions; (ii) urease inhibitor decreased NH_3 losses from both urine and urea, with the effect more pronounced for urea; (iii) urease inhibitor decreased N_2O losses from urine application, though no reduction in losses was observed from urea applied @ 60 g N m^{-2}; under the higher temperature conditions in Experiment 2, UI significantly reduced N_2O emissions from urea applied @10 g N m^{-2}; (iv) coating of urea with elemental S resulted in a decrease in NH_3 emissions but an increase in N_2O emissions; however, it did not influence the effect of Agrotain on gaseous emissions from urea (Sustain Yellow); (iv) plant uptake of N was higher from urea+UI (Sustain Green and Yellow) than from urea. In this section, these results are discussed in detail in relation to the results obtained in other studies, and an attempt will be made to provide possible reasons for these observations.

The NH_3 emissions from urine and urea applied @ 60 g N m^{-2} in this study are comparable in magnitude to results reported by other workers. As the urea N

($CO(NH_2)_2$) in fertiliser and urine, hydrolyses in the presence of urease enzyme in the soils (Eq. 4.3), NH_4^+ ions accumulate. The urea hydrolysis also results in the release of alkali ions (OH^-), thereby increasing soil pH. The NH_4^+ ions dissociate into NH_3 in the presence of OH^- ions (Eq. 4.4), resulting in the release of NH_3 gas.

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + 2OH^- + CO_2 \quad \textbf{(4.3)}$$

$$NH_4^+ + OH^- \rightarrow NH_3 + H_2O \quad \textbf{(4.4)}$$

In this study, following the hydrolysis of urea-N, the soil pH increased to 7 in Experiment 1 and 6.4 in Experiment 2. The lower increase in pH in Experiment 2 may be attributed to the relatively low amount of urea applied (10 g N m^{-2}) compared to the very high amounts of urea (60 g N m^{-2}) and urine (47.6 g N m^{-2}) in Experiment 1.

The highest NH_3 flux occurred earlier (within 24 hours) in the urine than in the urea treatment. The difference in the NH_3 flux pattern between these N sources was probably due to the difference in the rate of urea hydrolysis. These results are supported by the findings of Sherlock & Goh (1984) who reported more rapid hydrolysis of urine urea than of urea fertiliser. The presence of hippuric acid in animal urine has been shown to have a stimulatory effect on urea hydrolysis (Whitehead *et al.* 1989). Sherlock & Goh (1984) calculated half-lives of urine urea as 3.0 and 4.7 h respectively, under summer and autumn conditions in New Zealand. The high urine pH (8.0) would also directly favour the hydrolysis of urea because this is the optimum pH for urease activity (Vlek *et al.* 1980). Vallis *et al.* (1982) found that more than 80% of urea in urine voided onto a subtropical pasture was hydrolysed within 2 h, as a result of urease activity in soil and/or plant residues. In Experiment 1 of this study, where urea was surface applied, the amount of NH_3 emitted (19.7 g N m^{-2}) and the peak flux was higher in the urea than in the urine treatment (4.36 g N m^{-2}). This provioded more chances for the release of NH_4^+ ions as NH_3 from urea in contrast to NH_4^+ ions from urine that may have moved down the soil core thus reducing the NH_4^+ concentration at the soil surface and subsequent NH_3 emissions. Volatilisation is essentially a physicochemical process which is governed by the equilibrium relationship between gaseous phase $NH_3(g)$ and $NH_3(aq)$ in solution (Eq 4.5); NH_3 in solution is in turn maintained by NH_4^+ - NH_3 equilibrium (Eq. 4.6) as follows.

$$NH_3\,(aq) \leftrightarrow NH_3\,(g) \quad \textbf{(4.5)}$$

$$NH_4^+\,(aq) \leftrightarrow NH_3\,(aq) + H^+\,(aq) \quad \textbf{(4.6)}$$

As might be expected from the NH_3 (aq)-pH relationship, maximum soil pH coincided with maximum NH_3 (g) flux and as soil pH declined so did the observed NH_3 (g) fluxes in both the experiments. The amount of N added as urea (60 g N m^{-2}) was more than that in urine (47.6 g N m^{-2}), which also might have contributed to the higher NH_3 losses in the urea treatment.

The addition of UI in the urine and urea (Sustain Yellow and Sustain Green) treatments was highly effective in reducing NH_3 emissions and delaying the time of peak NH_3 emission (T_{max}) in both the experiments. The delay in T_{max} was one day in the urine and 8 days in the urea treatments (Figure 4.1 and 4.6). The significant factors that contribute to the effectiveness of UIs in controlling NH_3 emissions include the capacity to inhibit urea hydrolysis and the diffusion of urea away from the zone of high soil pH associated with urea hydrolysis. Christianson *et al.* (1990) observed that, although UIs had decreased urea hydrolysis in soils by only 25% over six days, NH_3 losses from the inhibitor-treated samples were reduced by 90% during the same period. This indicates that the mode of action of these inhibitors was not simply one of delaying urea hydrolysis *per se.* Soil column studies by Clay *et al.* (1990) have shown that urea treated with UIs diffuse to greater depth than untreated urea. This could be attributed to the greater diffusion of non-ionic urea molecules in the presence of UIs and the reduced diffusion of NH_4^+ ions produced in the absence of UIs. This enhanced diffusion of N reduces subsequent NH_3 volatilisation. Thus the mode of action of NBPT (Agrotain) appears to be one of slowing urea hydrolysis long enough for urea to diffuse away from the placement zone. Treating urea with UIs also reduced the rise in soil pH (Figure 4.5 and 4.9) that normally occurs concurrently with urea hydrolysis. The increase in the concentration of urea in the soil allows enhancement of the rate of nitrification, thereby lowering both the concentration of NH_4^+ and pH at the site of placement.

In Experiment 1, more NH_4^+ was found in the soil cores treated with UI than in the cores receiving urine and urea alone; however, this did not apply in Experiment 2. The addition of N sources such as urine and urea to the soil result in the accumulation of exchangeable NH_4^+ in the first 4-5 days with a strong concentration gradient being developed from the placement site (top surface layer) down the soil core depth (Christianson *et al.* 1993; Vittori-Antisari *et al.* 1996). This high concentration of NH_4^+ near the soil surface is subject to volatilisation, immobilization and nitrification

reactions. As already discussed, the presence of UIs slows down the formation of NH_4^+ ions due to inhibition of urea hydrolysis and results in their diffusion down the soil depth thereby lowering the concentration gradient from the surface to deeper depths of the soil core. The slow released NH_4^+ ions are thus less susceptible to NH_3 loss, thereby providing a greater chance for uptake by plants. This is quite clear in Experiment 2, as more N was taken up in soil cores receiving the Sustain Green and Yellow treatments than in cores with urea alone. As there were no plants to take up exchangeable NH_4^+ in Experiment 1, the build-up in NH_4^+ was seen in the cores receiving UI along with the N input. These NH_4^+ ions would have been nitrified or immobilized with increasing time after application. Urease inhibitors have been shown to prevent the apparent intial immobilization of urea but hydrolysis then proceeds at a rate comparable to that without inhibitor (Hendrickson *et al.* 1987).

In the urea treatment, the amount of N_2O emitted was 0.3% and 4.3% of the applied N @ 60 and 10 g N m^{-2} in Experiment 1 and 2, respectively. The N_2O emission from the urine treatment in Experiment 1 (1.6%) was found to be consistent with the general range of N_2O emissions of 0.1 - 3.8 % of applied urine-N (Oenema *et al.* 1997; van Groenigen *et al.* 2005). In Experiment 1, N_2O emissions were higher in the urine than in the urea treatment, and also the peak flux was obtained earlier (within 24 hours) after urine application. Sherlock & Goh (1983) measured greater losses of N_2O from simulated urine than aqueous urea, also, peak emissions were observed within a few hours after urine application, compared to 24-48 hours after urea application. Increases in N_2O emission within 24 hour of urine application were also observed by (de Klein & van Logtestijn 1994; Koops *et al.* 1997). In Experiment 1, urine was added to the soil cores at field-capacity moisture content and may have created water-saturated conditions, thus stimulating denitrification. Saggar *et al.* (2002, 2003, 2004b) have also shown that, under field conditions, sites having water-filled pore space (WFPS) above field capacity had higher N_2O emissions because of the formation of anaerobic sites, which is a fundamental requisite for denitrification. Sherlock & Goh (1983) also explained this initial stimulation of N_2O production, either because of chemodenitrification or by anaerobiosis in microsites as a result of CO_2 generated from the rapid hydrolysis of urine urea. The addition of UI decreased the N_2O emissions in the urine (Experiment 1) and urea (Experiment 2) treatments. In Experiment 1, the

Sustain Yellow treatment caused low N_2O emissions compared to those in the urea treatment for first 20 days (Figure 4.2) but, as the effect of UI started diminishing, there was a gradual increase in N_2O emissions; these showed a peak after 30 days and remained higher than in the urea treatment until the termination of the experiment. In Experiment 2 in contrast, N_2O emissions in the amended-urea treatments remained lower throughout the experimental period. This difference in the effect of UI on N_2O emission between these two experiments could be attributed to continuous removal of N through plant uptake resulting in a low concentration of NO_3^- for denitrification. Plants also affect NH_3 volatilisation by decreasing the concentration of NH_4^+ in the soil solution through N uptake and by altering the pH of the rhizosphere soil (Saggar *et al.* 2004b). Moreover, in Experiment 2, the temperature was higher in the glasshouse (25-30 ^{0}C), which might have resulted in faster nitrification in the soils. As the amount of NH_4^+ ions for nitrification would initially be lower in the amended-urea treatments than in the urea-alone treatment, thereby resulted a decrease in N_2O emissions produced during nitrification. The reduced N_2O emissions resulted slightly higher NO_3^--N content in the soil cores (Figure 4.8 b) receiving amended-urea compared to the urea only treatment which might be liable to leaching. This increase in NO_3^--N content was also found in urine and higher rate of urea treatments in Experiment 1.

With S-coated urea, the reduction in NH_3 emissions showed that it was effective in decreasing NH_3 losses (Table 4.3). However, this treatment resulted in decreased N uptake and higher N_2O losses compared to the urea-alone treatment. Nitrogen release in the S-coated urea treatment appeared to be slower than in the other urea treatments, with plant growth being adversely affected as not enough N was available for the plants. The resulting continuation of N release from S-coated urea later in the experimental period when plant growth was reduced could explain the relatively high N_2O fluxes. However, there was no difference in gaseous emissions and N transformation between Sustain Yellow (urea+Agrotain+elemental S) and Sustain Green (urea+Agrotain) treatments indicating that S coating did not influence the effect of Agrotain on N transformation of urea. It has to be pointed out, however, that while S-coated urea contained 27% S, Sustain Yellow has only 4% S.

4.5 Conclusions

- Urease inhibitor was effective in reducing NH_3 volatilisation losses both from urine and urea as N sources.
- Urease inhibitor was effective in reducing N_2O emissions from urine and urea but this reduction varied depending upon the plant uptake and nitrification rate in the soil.
- Urease inhibitor increased herbage dry matter yields and N uptake from urea fertiliser.
- Urease inhibitor caused a slight increase in NO_3^--N concentration in the soil in both the urine and urea treatments; this nitrate may be susceptible to leaching.
- Elemental S coating on urea reduced N lost as NH_3 but increased N_2O emissions and reduced N uptake by plants.
- The S coating in the Sustain Yellow did not show any additional effect on N gaseous emissions and N transformations compared with those in the Sustain Green treatment.

Chapter 5

Influence of nitrification inhibitor (DCD) on the gaseous and leaching losses of nitrogen from urea and cattle urine in pasture soil

5.1 Introduction

An upsurge of interest in gaseous losses of N as ammonia (NH_3) and nitrous oxide (N_2O) and nitrate (NO_3^-) leaching from the soil has occurred during the last decade mainly because of the environmental impacts of these losses discussed in previous chapters. High concentration of NO_3^- in ground water can cause contamination of ground and surface waters which is linked to increasing incidences of NO_3^- toxicity in human and livestock (i.e. methaemoglobinaemia). To safeguard human health, the New Zealand Ministry of Health has introduced drinking water guidelines limiting NO_3^--N concentration to 11.3 mg N l^{-1}. Regional authorities have developed regional resource plans that may restrict land uses to protect water resources. To minimise adverse environmental impacts by farming activities, the New Zealand dairy industry introduced its own environmental quality assurance programme for farmers to follow. Although these measures will no doubt help farmers to adopt best management practices, they alone may not be sufficient to result in a dramatic reduction in N losses as gaseous emissions and NO_3^- leaching. The single largest source of N_2O emission in New Zealand is animal excreta deposited during grazing (80% of agricultural N_2O emission), while N fertiliser use currently contributes only 14% of agricultural emissions. Nitrogen fertiliser use has, however, increased 4-fold since 1990 (de Klein & Ledgard 2005). The resurgence and intensification of the dairy industry (MAF 2003) is one of the major reasons contributing to the increase in N use in New Zealand.

As discussed in previous chapters, various approaches have been attempted to mitigate the economic and environmental impacts of N losses and the approach which is gaining fast momentum is the use of inhibitors. These N transformation inhibitors i.e, both urease inhibitors (UIs); and nitrification inhibitors (NIs) have been shown to slow down the transformation of N in soils, thereby reducing the loss of N through leaching and gaseous emissions. Although the value of these inhibitors in enhancing the N use efficiency has been tested extensively under arable cropping (Freney 1997; Subbarao *et al.* 2006), only a limited number of studies has examined their value in grazed pastures. Results in Chapter 4 indicated that the use of the UI, Agrotain, was very effective in reducing N losses through NH_3 emission from both urine and urea fertiliser in pasture soils; however, its effect on N_2O emission was not consistent and it also increased the concentration of soil NO_3^- which could be subject to leaching losses.

Interest in nitrification inhibitors stems from the fact that they can reduce the leaching of NO_3^- and the emissions of N_2O directly by reducing the fraction of NH_4^+-N oxidised to NO_3^-, and therefore the N_2O loss associated with nitrification, or indirectly by reducing the amount of NO_3^- substrate available for denitrification (Aulakh *et al.* 1984; Bronson *et al.* 1992). A commonly used nitrification inhibitor is dicyandiamide (DCD) which inhibits the first stage of nitrification, the oxidation of NH_4^+ to NO_2^- (Amberger, 1989). It is not a broad-spectrum bactericide and does not affect other heterotrophs that are responsible for much of the soil biological activity (Amberger 1989).

DCD has been used in the past to increase the efficiency of N in fertilisers or manures with variable results (Amberger 1989; Wadman *et al.* 1993; Davies & Williams 1995; Williamson *et al.* 1998). Recent studies by Di & Cameron (2002b, 2003, 2004c, 2005) with DCD on urine patches in grazed pasture soils showed 56-73% reduction in N_2O emissions and 74-76% reduction in NO_3^- leaching. But limited studies have been done to see the overall effect of DCD on gaseous emissions of both NH_3 and N_2O and leaching of NH_4^+ and NO_3^-. Thus the present study (two experiments) was conducted with an overall objective of gaining a comprehensive understanding of the effect of DCD on the dynamics of urine-N and urea-N that could be used to devise strategies to mitigate N losses from grazed pastures. Further, the effect of DCD on the leaching of Ca^{+2}, K^+ and Mg^{+2} cations from urine application, which is not of environmental concern but an economic loss to the farmer, was also assessed.

The specific objectives of the study reported in this chapter include:

- To examine the effect of DCD on NH_3 and N_2O emissions from soil cores receiving different rates of urea and urine-N.
- To examine the transformation of mineral N with the application of DCD.
- To examine the effect of DCD application on NH_4^+, K^+, Mg^{+2}, Ca^{+2}, and NO_3^- leaching.
- To examine the effect of DCD on herbage yield.

5.2 Materials and Methods

5.2.1 Experimental details

5.2.1.1 Collection and preparation of cores

Intact soil cores (100 mm diameter, 100 mm depth) were collected from three representative sites of a sheep-grazed permanent legume-based pasture at Massey University Frewens Research Block, Turitea campus. The cores were prepared as described in Section 3.2.4.1 of Chapter 3. The chemical and physical properties of the soil are given in Table 4.1 of Chapter 4.

5.2.1.2 Treatments

Two glasshouse experiments were conducted to examine the effect of DCD on N losses at various levels of urea fertiliser (Experiment 1) and urine application (Experiment 2). The results from these two experiments were used to compare the effect of DCD on N losses between urine and urea fertiliser application to pasture soils. The experiments were conducted separately and for both the experiments three cores were placed in each chamber, maintained at a temperature ranging from 15-20°C and field capacity soil moisture content for the 50-day experimental period. The

background emissions of NH_3 and N_2O were taken from all the 24 chambers in each experiment before applying the treatments.

Experiment 1

In this experiment, eight treatments with different rates of urea (Ur) were applied with three replications. These were UrT1- Control without DCD, UrT2 – Control with DCD (2.5 g m^{-2}), UrT3 – Urea @ 2.5 g N m^{-2} (25 kg N ha^{-1}), UrT4 – Urea @ 2.5 g N m^{-2} + DCD, UrT5 – Urea @ 5.0 g N m^{-2} (50 kg N ha^{-1}), UrT6 – Urea @ 5.0 g N m^{-2} + DCD, T7 – Urea @7.5 g N m^{-2} (75 kg N ha^{-1}) , UrT7 – Urea @ 7.5 g N m^{-2} + DCD. These treatments are hereafter referred to as UrT1, UrT2, UrT3, UrT4, UrT5, UrT6, UrT7 and UrT8.

Experiment 2

In this experiment, eight urine treatments were used in triplicate. These include: T1- Control without DCD, T2 – Control with DCD (2.5 g m^{-2}), T3 – Urine @ 14.4 g N m^{-2} (144 kg N ha^{-1}), T4 – Urine @ 14.4 g N m^{-2} + DCD, T5 – Urine @ 29 g N m^{-2} (290 kg N ha^{-1}), T6 – Urine @ 29 g N m^{-2} + DCD, T7 – Urine @ 57 g N m^{-2} (570 kg N ha^{-1}), T7 – Urine @ 57 g N m^{-2} + DCD. These treatments are hereafter referred to as T1, T2, T3, T4, T5, T6, T7 and T8.

DCD was dissolved in water (19.08 mg in 10 ml of water) and applied separately after the surface application of urea in Experiment 1, whereas it was mixed in urine before application in Experiment 2. Emissions of NH_3 were monitored for 15 days in both the experiments and N_2O for 35 and 50 days for Experiment 1 and 2, respectively. Selected cores in Experiment 2 were leached at the end of the experiment.

5.2.1.3 Setting up of cores for leaching

At the end of gaseous measurements of Experiment 2, selected cores (one from each chamber) from T5, T6, T7 and T8 treatments receiving urine-N @ 29 and 57 g N m^{-2} were leached with 2.5 pore volumes of deionized water. These treatments were selected as the rate of urine applied was close to the actual rate in pasture under a cow urine patch (600 kg N ha^{-1}). One core from each chamber was taken to get three replicates from three

representative sites in the pasture. The herbage on the cores was trimmed next to the soil surface and water was added using a peristaltic pump @ 1 ml/min (Plate 5.1). A layer of silica sand was spread on the surface of each core and a filter paper was placed on the top to ensure the uniform distribution of water on the core surface. Leachate was collected initially in small volumes of 5, 10, 15 and 20 ml and then in 50 and 100 ml; the leachates were analysed for mineral N (NH_4^+ and NO_3^- ions) and potassium (K^+), magnesium (Mg^{+2}) and calcium (Ca^{+2}).

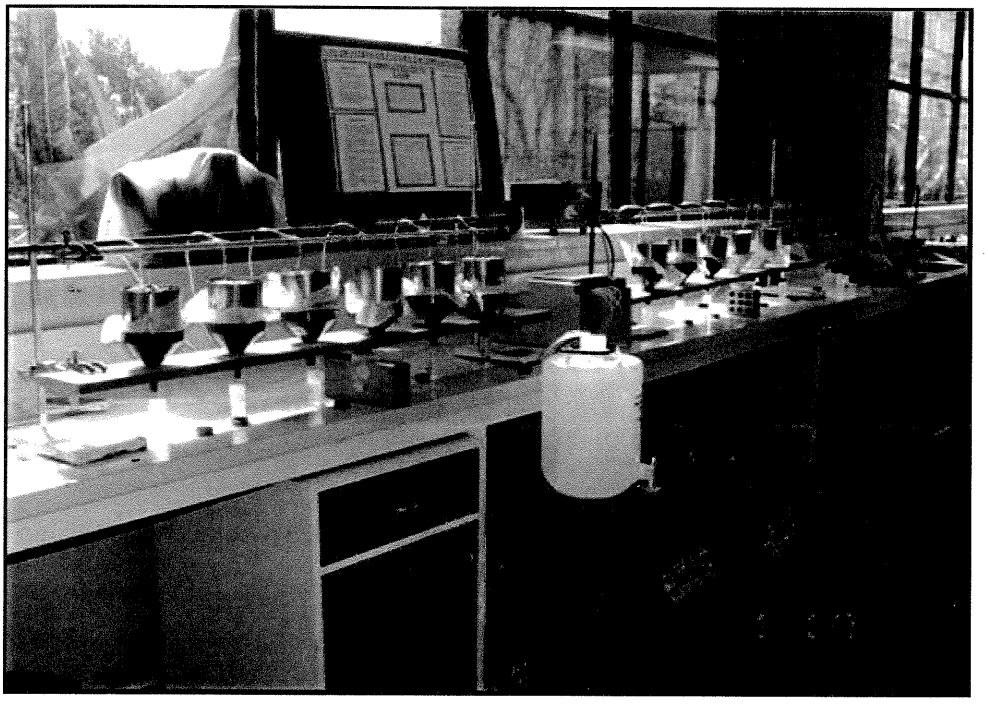


Plate 5.1 Apparatus used for leaching of soil cores.

5.2.2 Gaseous emissions

Ammonia and N_2O emissions were measured periodically using active and passive flux as described in Chapter 3. Ammonia collected in 0.05 M H_2SO_4 for 8 hours during the night was analysed for total NH_4^+-N using an auto-analyser. Ammonia measurements were taken daily and the NH_3 flux was calculated as discussed in section 3.2.4.2. Nitrous oxide measurements were taken daily for the first week to capture immediate changes in gas fluxes, followed by two weeks measurements on alternate days and then twice in the next week. For the remaining period, measurements were

taken once a week only as the fluxes approached the background levels. The N_2O flux was calculated as discussed in Section 3.2.4.3 of Chapter 3.

5.2.3 N recovery

The N recovery in various components (NH_3 emission, N_2O emission and mineral N contents in soil, N uptake) was calculated using the formula discussed in Section 4.2.3 of Chapter 4. The data on mineral N, NH_3 and N_2O emissions for the control treatment (i.e. without N addition) indicated that DCD did not make a significant contribution to the N budget, and hence, for calculation of N recovery from the treatments receiving DCD, the N input was considered only from the urine or urea application.

The percentage reduction in N lost as NH_3 and N_2O was calculated using Eq. 4.2 in section 4.2.3 of Chapter 4.

5.2.3 Analysis

5.2.3.1 Urine analysis

The urine was collected from Friesian cows during the milking session at the Massey University No.1 Dairy farm. Individual fresh urine samples were bulked and frozen (-18 ^{0}C) within an hour of collection. They were then analyzed for total N (Ebina *et al.*, 1983) and total C (Bremner & Tabatabai 1971). The urine had a pH of 7.8, and total N and C concentrations of 6.5 g l^{-1} and 2.3 g l^{-1}, respectively.

5.2.3.2 Soil analysis

At the end of the experiment, soil cores were cut into 0-50 mm and 50-100 mm depth sections and analysed for NH_4^+ and NO_3^- contents as discussed in Section 4.2.4.2.

5.2.3.3 Herbage analysis

The herbage was cut at 2 cm height twice during the 50 day period (22 and 48 days after treatment application), dried at 65^{0}C for 24 hours and weighed. The cumulative dry matter (DM) yield was recorded for each chamber. The dried herbage was ground finely and then digested and analysed as described in Section 4.2.4.3. The dry matter response and the recovery of added N through plant uptake were calculated using the following equations:

$$DM\ response = \frac{[DM\ (urine/urea \pm DCD)] - [DM\ (control \pm DCD)]}{N\ added\ (urine/urea)} \quad \textbf{(5.1)}$$

$$N\ recovery = \frac{[Herbage\ N(urine/urea \pm DCD)] - [HerbageN(control \pm DCD)]}{N\ added(urine/urea)} \quad \textbf{(5.2)}$$

5.2.3.4 Leachate analysis

Leachates collected from the soil cores were analysed for NH_4^+ and NO_3^- concentrations by a Technicon auto-analyser (Blakemore *et al.* 1987). The samples of leachate were diluted with deionised water to obtain K^+ and Ca^{+2} concentrations within 1-10 mg l^{-1} (ppm) and Mg^{+2} concentrations within 0.1-1 mg l^{-1}. The concentration of K^+ in these samples was determined by atomic emission in the presence of 0.2% caesium chloride. The Ca^{+2} and Mg^{+2} concentrations were determined by atomic absorption in the presence of 0.2% strontium nitrate.

5.2.4 Statistical Methods

An analysis of variance using SAS software (version 8) was performed on the results of total NH_3 and N_2O emitted, mineral N, total amount of cations (Ca^{+2}, K^+, Mg^{+2}, NH_4^+) leached, total NO_3^- leached, and herbage DM yield and N uptake using the General Linear Model (GLM) procedure. Mean comparisons were done using Fishers Least Significant Difference (LSD) at 5% significance. The regression analysis between total amount of cations and NO_3^- leached was conducted using SAS software.

5.3 Results

5.3.1 Experiment 1

5.3.1.1 Ammonia emissions

The NH_3 emissions in all the treatments peaked within a day after urea application, both with and without DCD, and then generally tailed off, reaching the background levels by day 14 (Figure 5.1). Ammonia fluxes increased with increasing rate of urea application. Ammonia emission from the control (±DCD) was consistently low throughout the experiment ranging from 0.26 to 1.70 and 0.01 to 1.14 mg NH_3-N m^{-2} d^{-1} in soil cores with and without DCD, respectively. In the absence of any N input through urea application (i.e. control treatment) there was no significant difference in total NH_3 emitted between the +DCD (5.76 mg NH_3-N m^{-2}) and –DCD (12.1 mg NH_3-N m^{-2}) treatments. With addition of DCD to the higher rates of urea application (5.0 and 7.5 g N m^{-2}), there was an increase in NH_3 fluxes as compared to treatments without DCD application. The NH_3 emission from UrT6 and UrT8 treatments (+DCD) showed higher peaks of 64.4 and 109 mg NH_3-N m^{-2} d^{-1} as compared to 54.7 and 91.3 mg NH_3-N m^{-2} d^{-1} in UrT5 and UrT7 treatments (-DCD), respectively. Thus, cumulative fluxes over the measuring period were significantly higher in UrT6 (117 mg NH_3-N m^{-2}) and UrT8 (230 mg NH_3-N m^{-2}) as compared to UrT5 (94.8 mg NH_3-N m^{-2}) and UrT7 (173 mg NH_3-N m^{-2}), which corresponded to a 34.5% and 39.3% increase in NH_3 emission, respectively (Table 5.1). However, this was not the case when urea was applied @ 2.5 g N m^{-2} (UrT3). The NH_3 emission rate was higher in UrT3 (- DCD) with a peak emission of 37.5 mg NH_3-N m^{-2} d^{-1} as compared to UrT4 (+DCD) (27.3 mg NH_3-N m^{-2} d^{-1}) treatment for the first three days, after which there was no significant difference in the emission rates. The cumulative NH_3 emission was higher (54.6 mg NH_3-N m^{-2}) in the UrT3 compared to the UrT4 (39.1 mg NH_3-N m^{-2}) treatment.

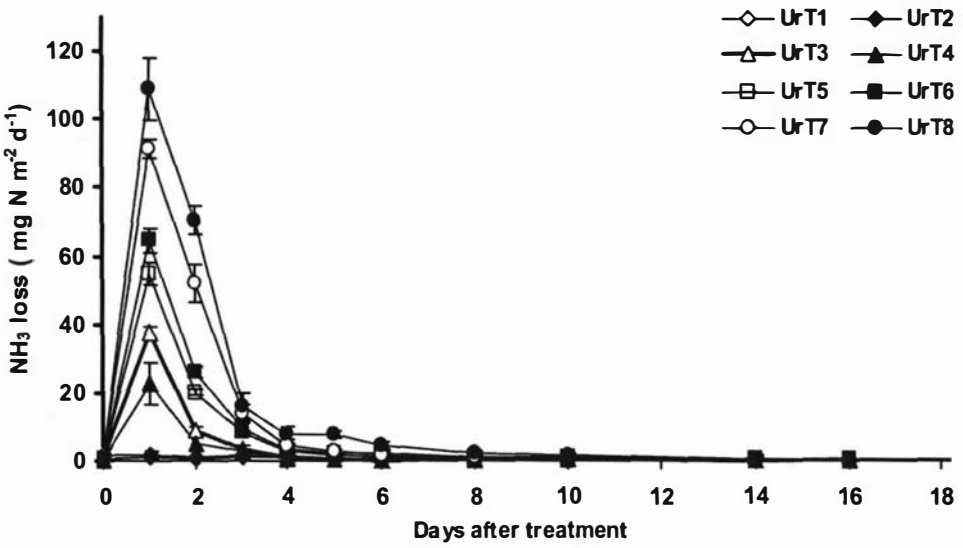


Figure 5.1 **Ammonia volatilisation losses from urea, with and without DCD, applied at different rates to Manawatu sandy loam soil. Each value represents a mean of three replicates with standard deviation shown by vertical bars**

5.3.1.2. Nitrous oxide emissions

In the control treatments (with no N input), the N_2O-N flux ranged from 0.09 to 2.20 mg N_2O-N m^{-2} d^{-1} and 0.12 to 3.10 mg N_2O-N m^{-2} d^{-1} in the presence and absence of DCD, respectively. No significant difference was observed in total N_2O-N emitted from the soil cores without DCD (20.3 mg N_2O-N m^{-2}) as compared to cores with added DCD (16.2 mg N_2O-N m^{-2}) in the control treatment. Similarly to NH_3, N_2O emission rate also increased with increasing level of urea application (Figure 5.2). Rates of N_2O emission from all the urea doses added without DCD were substantial and increased over time, showing peak emission at day 4 after which there was a progressive decline until day 16 (Figure 5.2). After day 16, the emissions became almost constant and were close to background emissions. A maximum N_2O emission flux of 7.8, 21.0 and 43.0 mg N_2O-N m^{-2} d^{-1} was detected at day 4 in the UrT3, UrT5, UrT7 treatments, respectively. Addition of DCD along with urea to soil cores in the UrT4, UrT6, UrT8 treatments showed no peak emissions of N_2O, and maintained N_2O emission rates significantly lower than those in the corresponding –DCD treatments (UrT3, UrT5, UrT6) until they reached background levels. Total loss of N as N_2O over the 35 day

period was 46.5, 116 and 183 mg N_2O-N m^{-2}, amounting to 1.04%, 1.91% and 2.16% of applied N in the UrT3, UrT5 and UrT7 treatments, respectively (Table 5.1). Application of DCD reduced N_2O emission by 34%, 73% and 93% in the UrT4, UrT6 and UrT8 treatments relative to the corresponding soil cores without DCD. The total loss of N as N_2O from DCD-treated cores in the UrT4, UrT6, UrT8 treatments was 33.4, 41.6 and 27.2 mg N_2O-N m^{-2}, respectively being equivalent to 0.69%, 0.51% and 0.15%, respectively of the urea N applied.

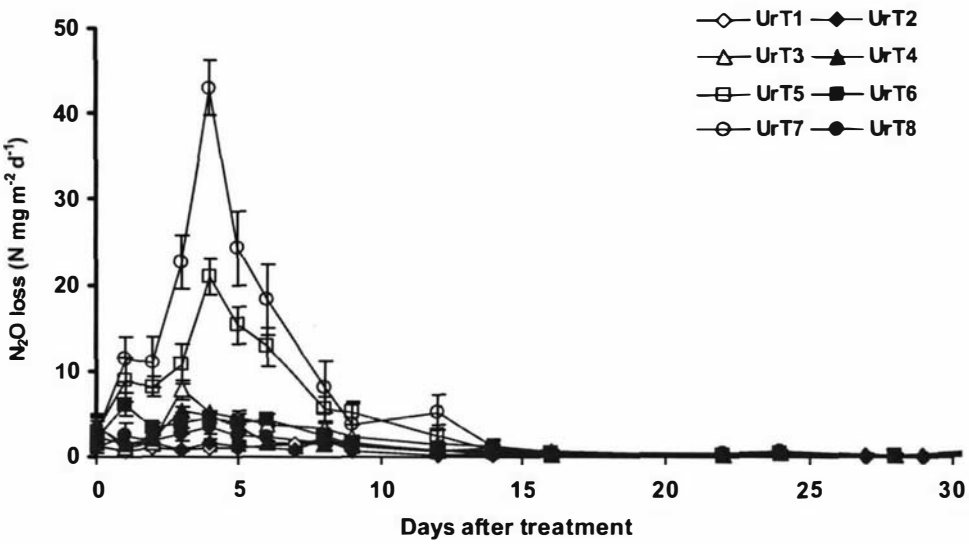


Figure 5.2 Nitrous oxide losses from urea, with and without DCD, applied at different rates to Manawatu sandy loam soil. Each value represents a mean of three replicates with standard deviation shown by vertical bars.

Table 5.1 **Total N applied (g N m^{-2}) and total N emitted as NH_3-N and N_2O-N (mg m^{-2} soil) over the experimental period from soil cores receiving varying urea rates with and without DCD**

Treatments	N added (g N m^{-2})	DCD added (g DCD m^{-2})	NH_3-N (mg N m^{-2})	% of added N emitted as NH_3	N_2O-N (mg N m^{-2})	% of added N emitted as N_2O
UrT1	-	-	12.1 g		20.3 cd	
UrT2	-	2.5	5.76 g		16.2 d	
UrT3	2.5	-	54.6 e	1.69	46.5 c	1.05
UrT4	2.5	2.5	39.1 f	1.33	33.4 cd	0.69
UrT5	5.0	-	94.8 d	1.65	116 b	1.91
UrT6	5.0	2.5	117 c	2.22	41.6 cd	0.51
UrT7	7.5	-	173 b*	2.14	183 a	2.16
UrT8	7.5	2.5	230 a	3.07	27.2 cd	0.15
L.S.D. (0.05%)			20.6		29.3	

* Values followed by the same letter in a given column do not differ significantly at the 0.05 level

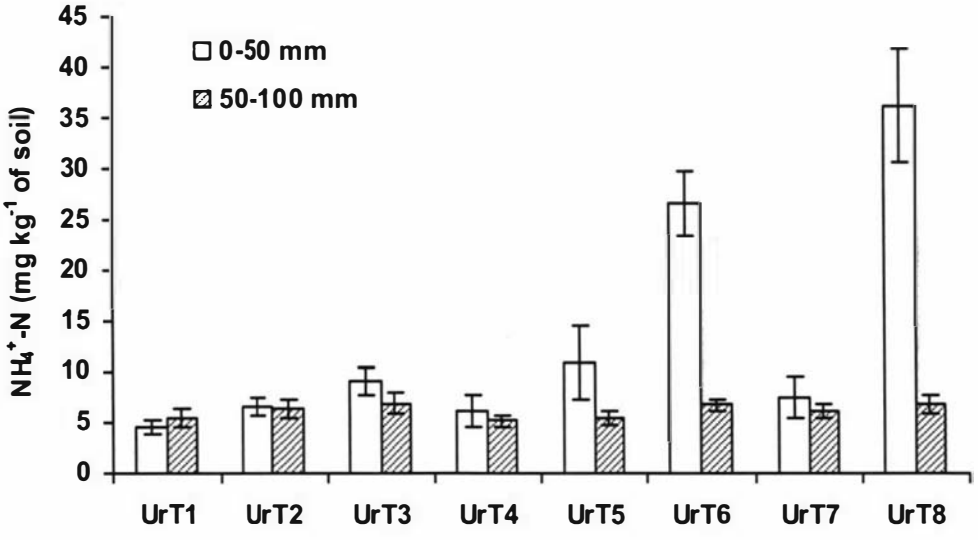
5.3.1.3 Mineral nitrogen

At the end of the experiment, NH_4^+-N levels in the control treatments (±DCD) were higher than NO_3^--N levels at both 0-50 and 50-100 mm depths (Figure 5.3). In treatments where urea was added, both with and without DCD, mineral-N was mainly present in the NO_3^- form. Application of urea (±DCD) significantly increased both NH_4^+ and NO_3^--N in soil cores as compared to control treatments at both the depths. Addition of DCD was able to maintain higher NH_4^+-N concentrations in the soil cores compared to the ones without DCD. With the addition of DCD to the control treatment, the NH_4^+-N concentration increased from 4.54 to 6.61 mg NH_4^+-N kg^{-1} at 0-50 mm depth and from 5.38 to 6.71 NH_4^+-N kg^{-1} at 50-100 mm, although the increases were not significant (Figure 5.3a). In the soil cores receiving urea without DCD (i.e., UrT5 and UrT7), NH_4^+-N concentration at the end of the experiment was, respectively, 10.9 and 7.48 mg NH_4^+-N kg^{-1} soil in the 0-50 mm layer and 5.46 and 6.10 mg NH_4^+-N kg^{-1} soil in the 50-100 mm layer. DCD seemed to be effective until day 35 as the NH_4^+-N concentration in UrT6 and UrT8 significantly increased, respectively, to 26.6 and 36.2 mg NH_4^+-N kg^{-1} soil at the 0-50 mm depth and 6.71 and 6.78 mg NH_4^+-N kg^{-1} soil at

50-100 mm depth (Figure 5.3a). The increase in NH_4^+-N with the addition of DCD was more apparent at 0-50 mm depth, as the amount of NH_4^+-N was then 3 to 10 times greater as compared to 1.12 to 1.24 times greater at 50-100 mm depth. However, the trend was opposite at the lowest level of urea N addition (2.5 g N m^{-2}). In this N treatment, NH_4^+-N content at both depths was significantly higher in the absence (UrT3) than the presence (UrT4) of DCD. In UrT3 and UrT4 treatments, NH_4^+-N concentrations were, respectively, 9.09 and 6.90 mg NH_4^+-N kg^{-1} soil at 0-50 mm depth, and 6.10 and 5.15 mg NH_4^+-N kg^{-1} at 50-100 mm depth (Figure 5.3a).

The concentration of NO_3^--N in soil cores with DCD was significantly lower at both 0-50 and 50-100 mm depths than in the soil cores without DCD, except in the control treatment where no significant difference in NO_3^--N concentration was found (Figure 5.3b). The NO_3^--N was 25.0, 50.0 and 66.4 mg NO_3^--N kg^{-1} soil at 0-50 mm depth and 19.3, 24.3 and 38.8 mg NO_3^--N kg^{-1} soil at 50-100 mm depth for UrT3, UrT5 and UrT7 treatments, respectively. With the addition of DCD, the NO_3^--N concentration reduced to, respectively, 15.3, 32.8 and 39.0 at 0-50 mm and 14.0, 17.9 and 21.8 at 50-100 mm for UrT4, UrT6 and UrT8 (Figure 5.3b). The total NO_3^--N reduction with the addition of DCD in treatments receiving urea-N ranged from 38 to 47% at 0-50 mm depth and 32 to 48% at 50-100 mm depth.

(a)



(b)

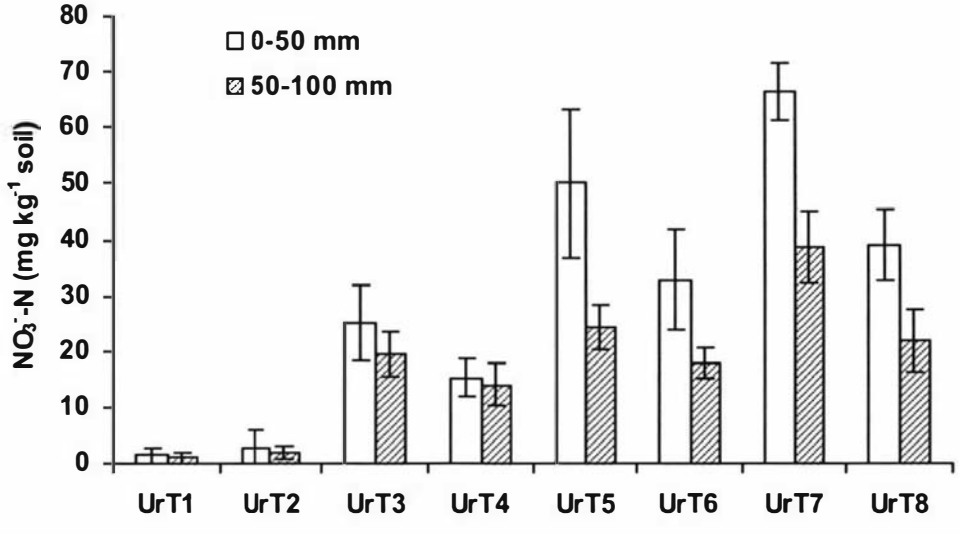


Figure 5.3 **Distribution of (a) NH_4^+ and (b) NO_3^- concentrations in soil cores at 0-50 mm and 50-100 mm depths receiving urea, with and without DCD, at varying rates . Each bar value represents a mean of nine replicates with standard deviation shown by vertical bars.**

5.3.1.4 Dry matter yield and N uptake

The effect of urea addition at different rates was evident in both total DM as well as dry matter response (DMR) to the added N. The application of increasing urea @ 2.5, 5.0 and 7.5 g N m^{-2} caused an increase in DM as compared to the control. The yield response was 4.6, 8.7 and 14.3 g DM g^{-1} N added in the UrT3, UrT5and UrT7 treatments respectively (Table 5.2). The highest herbage accumulation of 292 g m^{-2} was obtained in UrT7 (7.5 g N m^{-2}), followed by 228 g m^{-2} in UrT5 (5.0 g N m^{-2}) and then by 196 g m^{-2} in the UrT3 (2.5 g N m^{-2}) treatment. Except in the UrT6 treatment, the application of DCD increased herbage yields in the other two urea treatments, as compared to the respective non-DCD treatments, though these increases were not significant. No particular trend was observed in DMR with the addition of DCD. Dry matter response in the 2.5 g N m^{-2} treatment increased from 4.6 g DM g^{-1} N (UrT3) to 14.3 g DM g^{-1} N (UrT4) with the addition of DCD, but in the 5.0 g N m^{-2} treatment it decreased from 8.7 g DM g^{-1} N (UrT5) to 7.5 g DM g^{-1} N (UrT6). The rate of urea applied also had a significant effect on the percentage of N recovered in the herbage. Nitrogen recovered through plant uptake increased with the increasing rate of urea application. The addition of DCD increased N recovered from 15 to 41%, 19 to 31% and 44 to 57% in UrT4, UrT6 and UrT8 respectively.

Table 5.2 Total DM yield, percent of added N in DM and DM response to the N added as urea to the soil cores

Treatment	N added (g m^{-2})	DCD added (g DCD m^{-2})	Total DM (g m^{-2})	N in DM (%)	Added N recovered (%)	DM response (g DM g^{-1} N)
UrT1	-	-	184 bc	2.98	-	-
UrT2	-	2.5	181 c	2.94	-	-
UrT3	2.5	-	196 bc	2.98	14.4	4.59
UrT4	2.5	2.5	217 bc	3.06	41.2	14.3
UrT5	5.0	-	228 b	3.07	18.5	8.73
UrT6	5.0	2.5	219 bc	3.22	30.5	7.52
UrT7	7.5	-	292 a	3.01	44.0	14.3
UrT8	7.5	2.5	293 a*	3.27	56.8	14.9
L.S.D. (0.05%)			46.4			

*Values followed by the same letter in a given column do not differ significantly at the 0.05 level.

5.3.1.5 N recovery

The total N recovered for all the treatments varied from 87.1% to 120% (Table 5.3). For all the urea treatments without DCD (UrT3, UrT5 and UrT7), 70-84% of the recovered N remained as NO_3^--N, whereas when DCD was applied along with urea (UrT4, UrT6 and UrT8), the percent of recovered N as NO_3^--N reduced to 42-68% (Figure 5.4). In contrast, the percentage of recovered N as NH_4^+-N increased from 3-6% to 22%, exception for the UrT4 treatment where the percentage of recovered N as NH_4^+-N decreased from 12% to 5% with the addition of DCD. A similar trend was observed in the percentage of recovered N lost as NH_3. The percent of N recovered lost as NH_3 was highest in the DCD treated cores (UrT6 and UrT8), but in the UrT4 cores the % of N recovered as NH_3 showed a slight decrease from 1.44% in the UrT3 cores without DCD to 1.32%. In contrast, the percent of recovered N lost as N_2O decreased with the addition of DCD in all the treatments. Dicyandiamide addition to the urea treatments i.e., UrT4, UrT6 and UrT8 decreased the percent of recovered N lost as N_2O from 0.83 (UrT3) to 0.72%, 1.79% (UrT5) to 0.48% and 2.16 (UrT7) to 0.16%, respectively (Figure 5.4). The application of DCD to urea increased the percent of recovered N in herbage in all the applied urea rates (Figure 5.4).

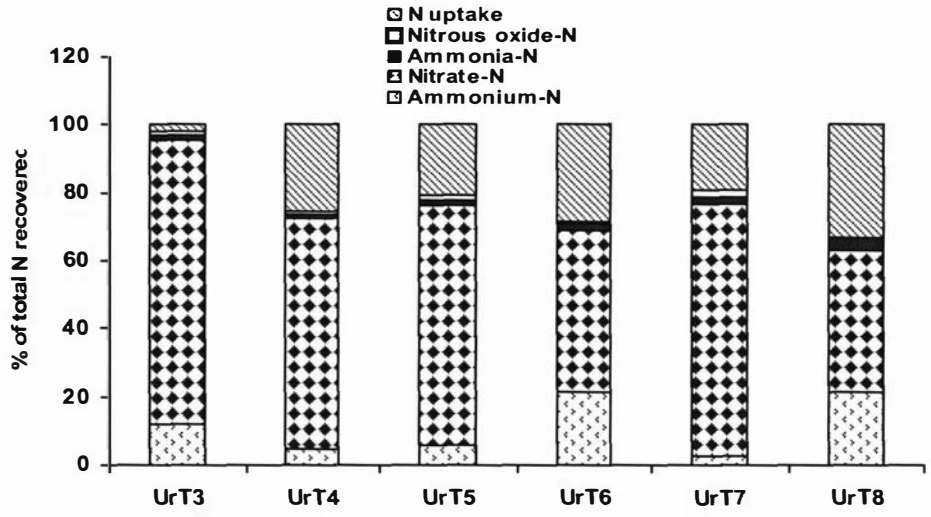


Figure 5.4 Percent of total N recovered in N fractions in various treatments at the end of the Experiment 1.

Table 5.3 The amount of N (mg kg^{-1} soil) as NH_4^+ and NO_3^- in the soil, N lost as NH_3 and N_2O and plant N measured following the application of varying rates of urea with and without DCD to intact soil cores

Treatment	NH_4^+-N	NO_3^--N	NH_3-N	N_2O-N	Herbage uptake	Total N recovered	N added	% recovery
UrT1	4.98	1.24	0.11	0.18	47.9	54.4	-	
UrT2	5.64	2.47	0.05	0.15	48.1	56.4	-	
UrT3	7.94	22.1	0.46	0.39	48.4	79.3	20.7	120
UrT4	6.49	14.7	0.29	0.28	52.7	74.5	20.7	87.1
UrT5	7.85	35.7	0.86	1.05	58.0	103	46.6	105
UrT6	15.8	24.7	1.05	0.37	61.7	104	46.6	101
UrT7	6.75	51.2	1.54	1.63	60.7	121	69.0	97.8
UrT8	19.5	29.2	2.12	0.25	69.5	121	69.0	93.0

5.3.2 Experiment 2

5.3.2.1 Ammonia emission

The NH_3 emissions increased with the increasing rates of urine application to the soil cores both with and without DCD, and were greater than in the control treatment (T1) (Figure 5.5). The NH_3 emission from the control treatment ranged from 0.08 to 2.25 mg NH_3-N m^{-2} d^{-1}. No significant difference was found in the NH_3 emissions with the addition of DCD to the control treatment (0.10 to 1.60 mg NH_3-N m^{-2} d^{-1}). Peak emissions in all the urine treatments with or without DCD were observed within 24 hours of application and were followed by a sharp decline. Emissions reached the background level in 14 days after commencing measurements. The highest emission peak (798 mg NH_3-N m^{-2} d^{-1}) was from the cores receiving the highest level of urine application (T7). Addition of DCD to this urine treatment (T8) resulted in a small reduction in the peak (698 mg NH_3-N m^{-2} d^{-1}), and the emission rate remained lower than in T7 treatment until day 3 after application. Similar differences in NH_3 emissions were observed between treatments T5 (223 mg NH_3-N m^{-2} d^{-1}) and T6 (173mg NH_3-N

m^{-2} d^{-1}), but not between T3 (56.6 mg NH_3-N m^{-2} d^{-1}) and T4 (56.3mg NH_3-N m^{-2} d^{-1}) treatments. However, after 3 days there was an increase in NH_3 emissions in all the treatments receiving DCD (T4, T6 and T8), compared to the urine alone treatments. This difference in NH_3 emissions remained until day 14 when emissions in all the treatments reached the background level. The total amount of NH_3 emitted was higher in all DCD treatments (T4, T6 and T8) compared to the treatments without DCD (T3, T4 and T7), but the difference was not significant ($P > 0.05$) (Table 5.4).

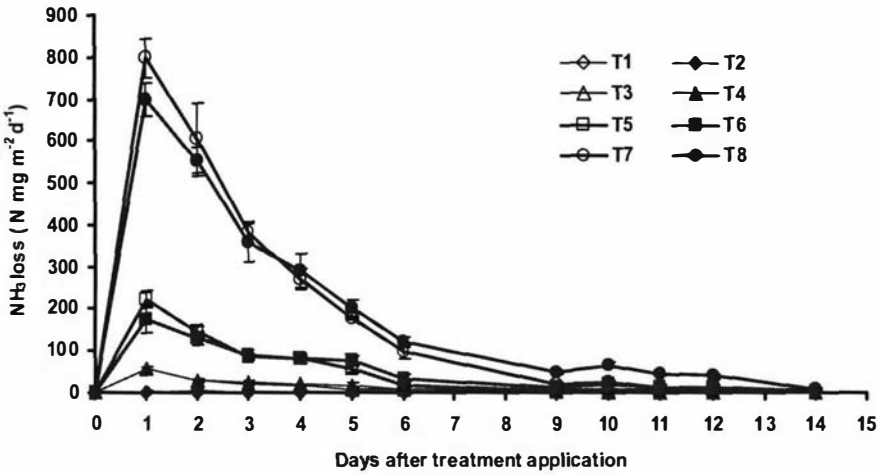


Figure 5.5 Ammonia volatilisation losses from urine applied with and without DCD at different rates to Manawatu sandy loam. Each value represents a mean of three replicates with standard deviation shown by vertical bars.

5.3.2.2 Nitrous oxide emission

Application of urine to soil cores with and without DCD increased N_2O emissions as compared to the control. The N_2O emissions from the control ranged from 0.07 to 2.30 mg N_2O-N m^{-2} d^{-1} and 0.07 to 0.66 mg N_2O-N m^{-2} d^{-1} with and without DCD, respectively. There was no significant difference in the total N_2O-N emitted in the control with (0.02 g N m^{-2}) and without (0.01 g N m^{-2}) DCD. The treatments without DCD ie., T3, T5 and T7 showed peaks of 57.5, 135 and 196 mg N_2O-N m^{-2} d^{-1}, respectively within 24 hours of application (Figure 5.6). Addition of DCD with urine (T4, T6 and T8), significantly reduced N_2O emission peaks (47, 98 and 174 mg N_2O-N

m^{-2} d^{-1}, respectively) and emissions remained lower throughout the measurement period than the corresponding urine treatments without DCD. Emissions reached the background level by day 50, except in the T7 treatment which still maintained higher emissions than the background emissions. Total N_2O emissions over the entire 50 day measurement period were reduced by 45%, 62% and 81% in the T4, T6 and T8 treatments, respectively, with the addition of DCD (Table 5.4).

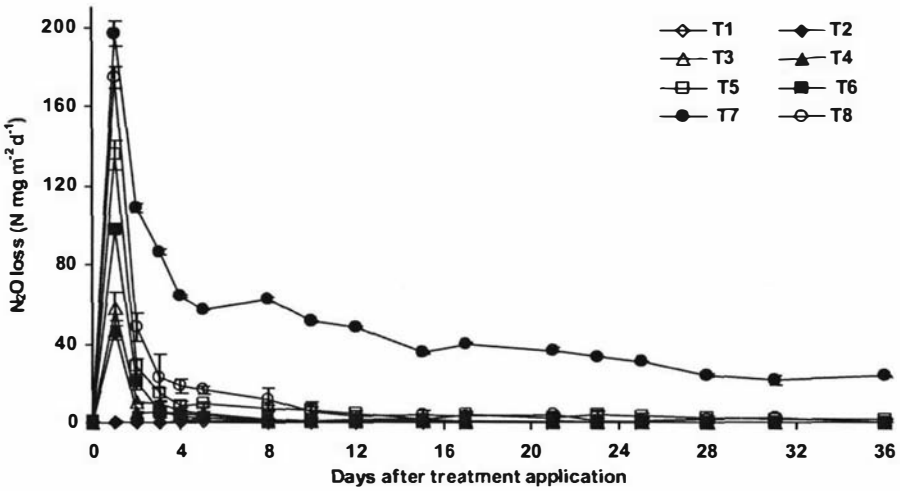


Figure 5.6 **Nitrous oxide losses with and without DCD from urine applied at different rates from Manawatu sandy loam soil. Each value represents a mean of three replicates with standard deviation shown by vertical bars.**

Table 5.4 Total N applied and N emitted as NH_3 and N_2O (g N kg^{-1} soil) over the experimental period (50 days) from soil cores receiving varying urine rates with and without DCD

Treatments	N added (g N m^{-2})	NH_3-N (g N m^{-2})	% of added N emitted as NH_3	N_2O-N (g N m^{-2})	% of added N emitted as N_2O
T1	-	0.01 c		0.01 f	
T2	-	0.01 c		0.02 f	
T3	14.4	0.15 c	0.95	0.12 d	0.74
T4	14.4	0.17 c	1.14	0.08 e	0.45
T5	29.0	0.64 b	2.15	0.33 c	1.09
T6	29.0	0.67 b	2.27	0.14 d	0.45
T7	57.0	2.43 a*	4.24	2.05 a	3.57
T8	57.0	2.51 a	4.38	0.40 b	0.67
L.S.D.(0.05%)		0.18		0.04	

*Values followed by the same letter in a given column do not differ significantly at the 0.05 level.

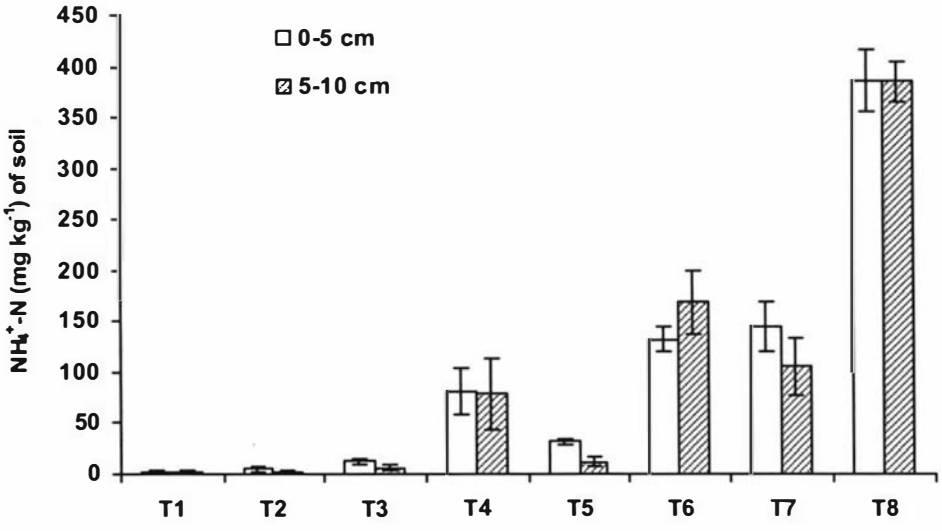
5.3.2.3 Mineral nitrogen

At the end of the experiment, between 75 to 93% of the applied N was still present in soil in all the treatments receiving urine with and without DCD. This N in the T3, T5 and T7 treatments receiving urine alone was mainly in the form of NO_3^--N (Figure 5.7). In the T4, T6 and T8 treatments receiving urine with DCD, mineral N was, in contrast, mainly present as NH_4^+-N. The addition of DCD increased the amount of NH_4^+-N significantly at both 0-50 and 50-100 mm depths (Figure 5.7 a), and the percent increase was greater at 50-100 mm depth. This indicates that either the applied urine N moved down the soil and was subsequently hydrolysed to NH_4^+ ions and/or that NH_4^+ ions moved down the soil and resulted in increased NH_4^+-N leaching with DCD application. These findings were supported by the concentrations of NH_4^+-N in the leachate collected from the selective cores (Figure 5.9 a). In the T4 treatment, the NH_4^+-N concentrations both at 0-50 mm (81.2 mg N kg^{-1} soil) and 50-100 mm (78.2 mg N kg^{-1} soil) depths were significantly higher than in the T3 (12.5 and 6.19 mg N kg^{-1} soil) treatment at the respective depths. Similarly, in the T6 and T8 treatments, NH_4^+-N at both depths was significantly higher than in the T5 and T7 treatments (Figure 5.7 a).

The NH_4^+-N concentration increased from, respectively, 31.1 and 11.9 (T5) to 169 and 133 mg N kg^{-1} soil (T6) at 0-50 mm and 50-100 mm depths. In the T8 treatment this increase was from 144 and 105 mg N kg^{-1} soil (T7) to 386 and 385 mg N kg^{-1} soil, respectively, at 0-50 and 50-100 mm. However, there was no significant difference in NH_4^+-N concentration at either depth between the T1 and T2 treatments.

The addition of DCD decreased the amount of NO_3^--N at both 0-50 and 50-100 mm depths at all the rates of urine applied. In the T4 treatment with added DCD, the amount of NO_3^- -N decreased from 161 (T3) to 55.0 mg N kg^{-1} soil and from 91.4 (T3) to 31.5 mg N kg^{-1} soil at 0-50 mm and 50-100 mm depths, respectively. Similarly, in the T6 treatment, application of urine with DCD decreased the NO_3^- -N concentration from 300 and 120 mg N kg^{-1} soil (T5 – urine only) to 69.2 and 44.4 mg N kg^{-1} at 0-50 and 50-100 mm, respectively. The decrease was from 418 (T7) to 98.4 mg N kg^{-1} soil (T8) and from 213 (T7) to 69.6 mg N kg^{-1} soil (T8) at 0-50 mm and 50-100 mm depths, respectively, in soil cores receiving urine @ 57 mg N kg^{-1} soil (Figure 5.7 b).

(a)



(b)

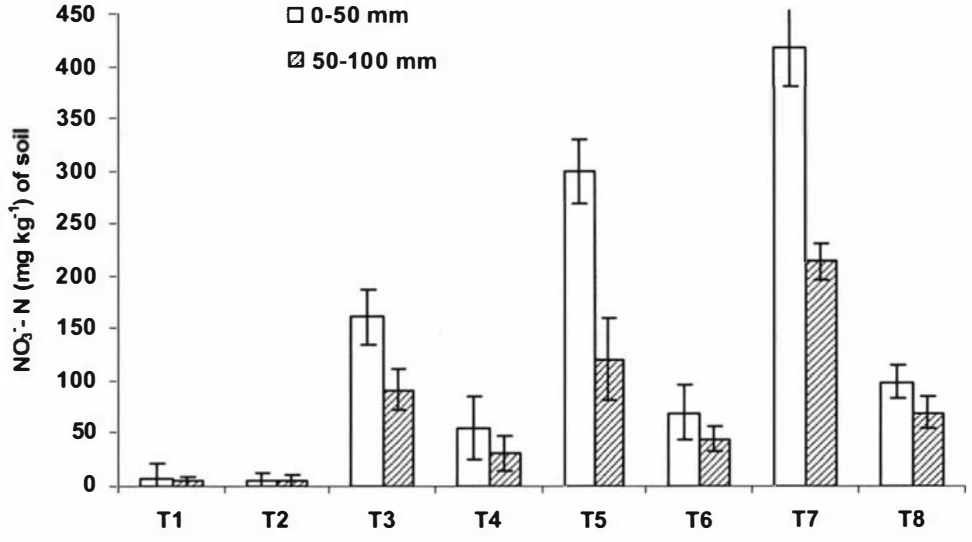


Figure 5.7 **Distribution of (a) NH_4^+ and (b) NO_3^- concentration in soil cores at 0-50 mm and 50-100 mm depths receiving urine, with and without DCD, at varying rates. Each bar value represents a mean of six replicates with standard deviation shown by vertical bars.**

5.3.2.4 Dry matter yield and N uptake

Urine application at different rates with and without DCD significantly increased pasture dry matter (DM) yield over the control (Table 5.5). However, at the highest rate of urine application (T7), DM yield was lower (224 g m^{-2}) than at T5 (268 g m^{-2}). The addition of DCD affected both DM yield and dry matter response (DMR, g DM g^{-1} N). With DCD addition, the DM yield decreased in the T4 and T6 treatments when compared to the T3 and T5 treatments (Table 5.5); however, this decrease was not significant for T4. The highest DMR was noticed in T3 (4.63 g DM g^{-1} N) followed by the T5 treatment (4.03 g DM g^{-1} N) and was lowest (1.27 g DM g^{-1} N) in the T7 treatment. Dry matter response was similarly reduced in T4 (2.74 g DM g^{-1} N) and T6 (2.80 g DM g^{-1} N) treatments with DCD addition, as compared to T3 (4.63 g DM g^{-1} N) and T5 (4.03 g DM g^{-1} N) treatments, respectively. However, the trend was opposite in the T8 treatment where DMR was higher (1.44 g DM g^{-1} N) with DCD as compared to the T7 treatment (1.27 g DM g^{-1} N).

The rate of urine applied had a significant negative effect on the percent of N recovered in the herbage (Table 5.5). The soil cores receiving the T3 treatment had the highest recovery of 21.5% of applied N in the DM followed by the T5 (16.4%) and T7 (5%) treatments. The addition of DCD reduced the percent of added N recovered in the herbage as compared to their counterparts, except in T8 and T7; this may be attributed to the slight increase in yield with DCD addition. However, the trend in percent of N recovered was similar to herbage yield, with the T4 (15.6%) being maximum, followed by the T6 (12.6%) and T8 (5.5%) treatments.

Table 5.5 Total DM yield, percent of added N in DM and DM response to the N added as urine to the soil cores

Treatment	N added ($g\ m^{-2}$)	Total DM ($g\ m^{-2}$)	N in DM (%)	N uptake ($g\ m^{-2}$)	Added N recovered (%)	DM response ($g\ DM\ g^{-1}\ N$)
T1 (Control)	-	151.4 c*	2.86	4.33	-	-
T2	-	143.4 c	2.89	4.14	-	-
T3	14.4	218.1 b	3.40	7.42	21.5	4.63
T4	14.4	182.9 bc	3.49	6.38	15.6	2.74
T5	29.0	268.4 a	3.40	9.13	16.4	4.03
T6	29.0	224.7 b	3.48	7.82	12.6	2.80
T7	57.0	224.1 b	3.22	7.22	5.0	1.27
T8	57.0	225.4 ab	3.23	7.28	5.5	1.44
L.S.D. (0.05%)		43.48				

*Values followed by the same letter in a given column do not differ significantly at the 0.05 level.

5.3.2.5 N recovery

The amount of N that was lost from the soil cores during the treatments as NH_3 and N_2O emissions and herbage uptake, and that remained in the soil as NH_4^+ and NO_3^- at the end of the experiment, are shown in Table 5.6. Figure 5.8 shows these data depicted as a percentage of the amount of N applied. The total N recovered for all the treatments varied from 89 to 117% (Table 5.6). For all the urine treatments without DCD (T3, T5 and T7), 54-87% of the applied N remained as NO_3^- -N in the soil as compared to 14 to 30% in the treatments receiving DCD (T4, T6 and T8). The trend was opposite for NH_4^+-N, with 5.5 to 22% of the applied N found as NH_4^+-N in the T3, T5 and T7 treatments not receiving DCD as compared to 56 to 70% in the T4, T6 and T8 treatments receiving DCD. The percent of applied N lost as NH_3 increased from 0.94 to 4.23% with increasing doses of urine. A similar trend was observed in the urine treatments receiving DCD (T4, T6 and T8), where the percent of applied N lost as NH_3 increased from 1.13 to 4.39%. The percent of applied N lost as NH_3 was slightly higher in treatments receiving DCD as compared to treatments without DCD. In contrast, the percent of N lost as N_2O was lower in the treatments receiving DCD i.e., T4 (0.45%), T6 (0.45%) and T8 (0.68%) as compared to the T3 (0.74%), T5 (1.11%) and T7

(3.57%) treatments without DCD. The percent of applied N taken up by herbage from the urine treatments T3 (22%) and T5 (17%) was higher than in the treatments T4 (15.3%) and T6 (13%) treatments receiving DCD. However, at the highest rate of urine (57 g N m^{-2}) there was no significant difference in the percent of applied N taken up by herbage between -DCD (T7) (5.1%) and +DCD (T8) (5.5%) treatments.

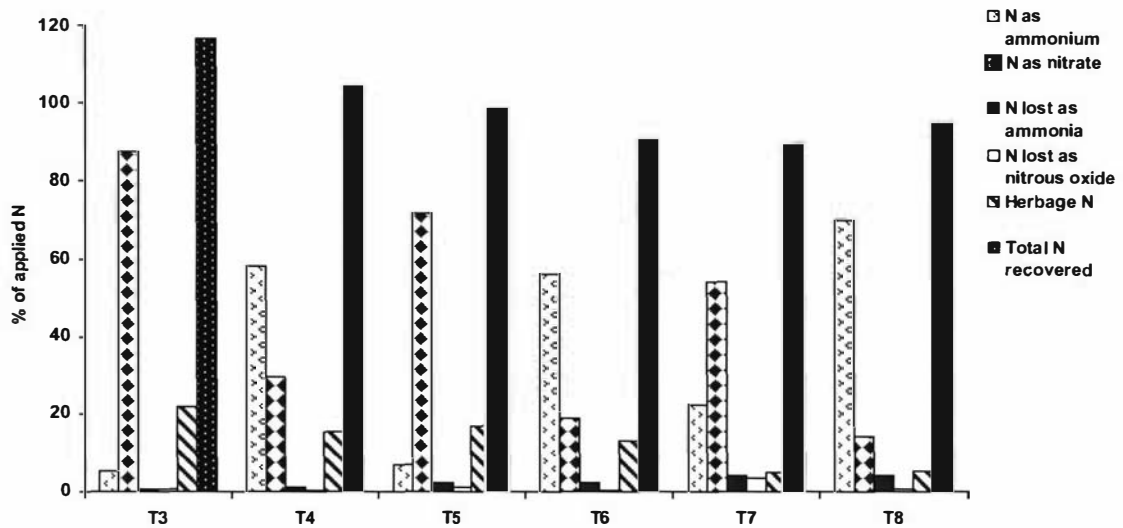


Figure 5.8 **The percent of applied N lost as NH_3 and N_2O, plant uptake and mineral N left in the soil cores receiving varying rates of urine±DCD at the end of the experiment.**

Table 5.6 **The amount of N (mg kg^{-1} soil) as NH_4^+ and NO_3^- in the soil, N lost as NH_3 and N_2O and plant N measured in intact soil cores receiving varying rates of urine±DCD, at the end of the experiment**

Treatments	NH_4^+-N	NO_3^--N	NH_3-N	N_2O-N	Herbage uptake	N added	% Recovery
T1	2.00	4.48	0.135	0.114	40.70	-	
T2	3.59	4.01	0.109	0.144	39.81	-	
T3	9.53	125	1.43	1.13	70.7	137	117
T4	83.2	44.6	1.66	0.764	60.8	137	104
T5	20.3	198	6.04	3.11	85.6	269	98.5
T6	152	55.4	6.15	1.32	75.6	269	90.4
T7	124	301	23.4	19.7	68.7	549	89
T8	386	82.6	24.2	3.86	70.2	549	95

5.3.2.6 Nitrogen leaching

Ammonium leaching

The changes in mean NH_4^+-N concentration measured in the leachate samples with cumulative drainage volume from each core are depicted in Figure 5.9 (a). The peak concentration in each breakthrough curve emerged almost immediately as the leaching started. The peak NH_4^+-N concentration in the drainage was found to be 15.7 and 34.5 mg N l^{-1} for the T5 and T7 (without DCD) treatments, respectively. These peaks increased to 29.3 and 68.3 mg N l^{-1} with the addition of DCD in the T6 and T8 treatments, respectively. The total amount of NH_4^+-N leached in the cores receiving urine significantly increased from 0.72 (T5) to 2.55 g N m^{-2} (T6) and from 2.68 (T7) to 5.84 g N m^{-2} (T8) with the addition of DCD, resulting in 2- to 3.5 - fold increases in NH_4^+-N leached (Table 5.7).

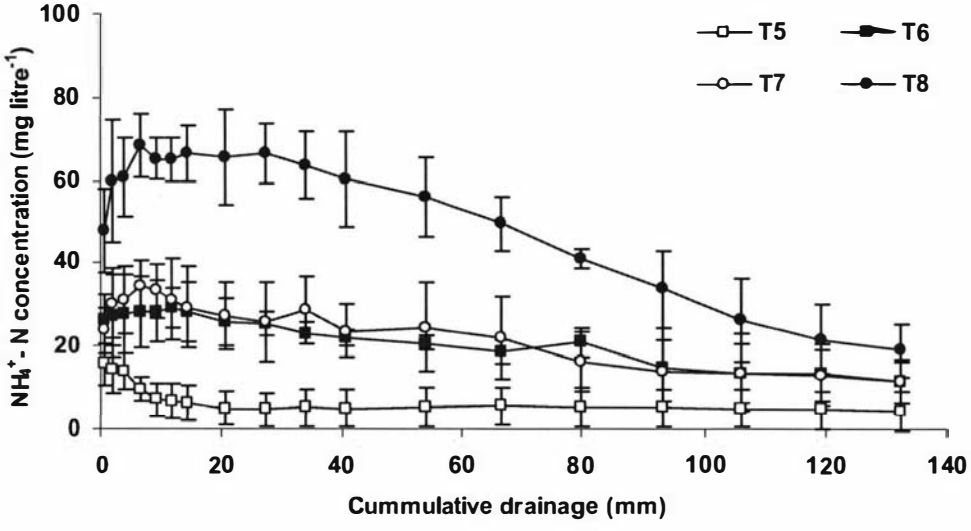
Nitrate leaching

The N leached was predominantly in the NO_3^- form, comprising 89 to 95% in the T5 and T7 (without DCD) treatments and 56 to 68% in the T6 and T8 (with DCD) treatments. The NO_3^--N leaching breakthrough curves are presented in Figure 5.9 (b). The peak NO_3^- concentration in the leachate from the T5 and T7 treatments reached 201 mg N l^{-1} and 290 mg N l^{-1}, respectively (Figure 5.9 b). However, the peak NO_3^- concentration in the leachate from the T6 and T8 treatments receiving DCD was significantly reduced ($P< 0.05$) to 68 and 118 mg N l^{-1}, respectively. The total NO_3^--N leaching losses from the T5 and T7 treatments were 13.8 and 21.7 g N m^{-2}, respectively and significantly higher than the 5.48 (T6) and 7.61 g N m^{-2} (T8) with DCD application (Table 5.7). Addition of DCD resulted in a 60% to 65% reduction in apparent NO_3^- leaching losses from the soil cores.

Figure 5.10 shows the cumulative amounts of both NH_4^+ and NO_3^--N in the leachate samples of all the cores. These results clearly show that NO_3^--N constituted the major part, and that NH_4^+-N represented only a small proportion, of the total amount of N leached. Thus, although the addition of DCD to the urine application resulted in an increase in the amount of NH_4^+-N leached, there was still a significant reduction in the

total N leached from 14.5 (T5) to 8.03 g N m^{-2} (T6) and 24.4 (T7) to 13.4 g N m^{-2} (T8) (Figure 5.11), resulting in about a 45 % reduction in total N leached.

(a)



(b)

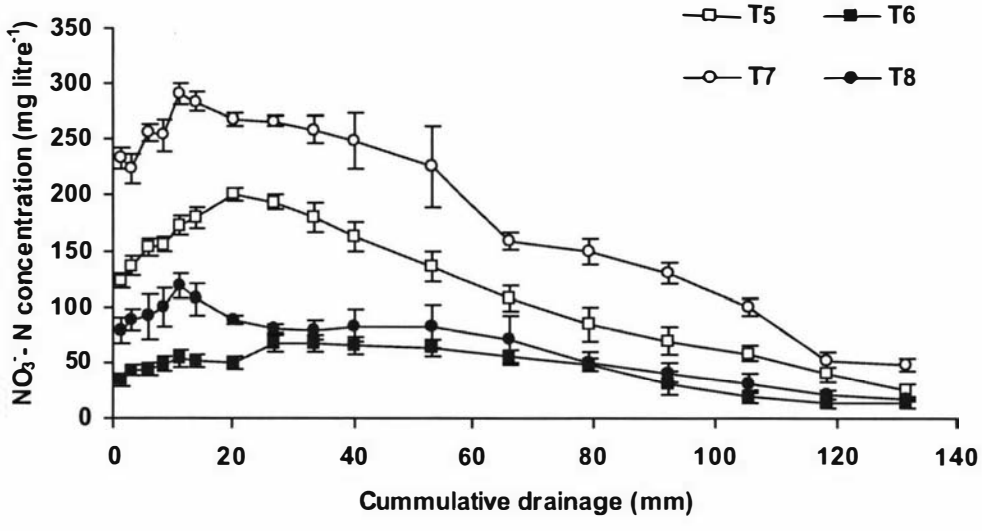


Figure 5.9 **Concentrations of (a), NH_4^+-N and (b), NO_3^--N in the drainage water from soil cores receiving urine @ 29 g N m^{-2} and 57 g N m^{-2} with and without DCD. Each value represents a mean of three replicates with standard deviation shown by vertical bars.**

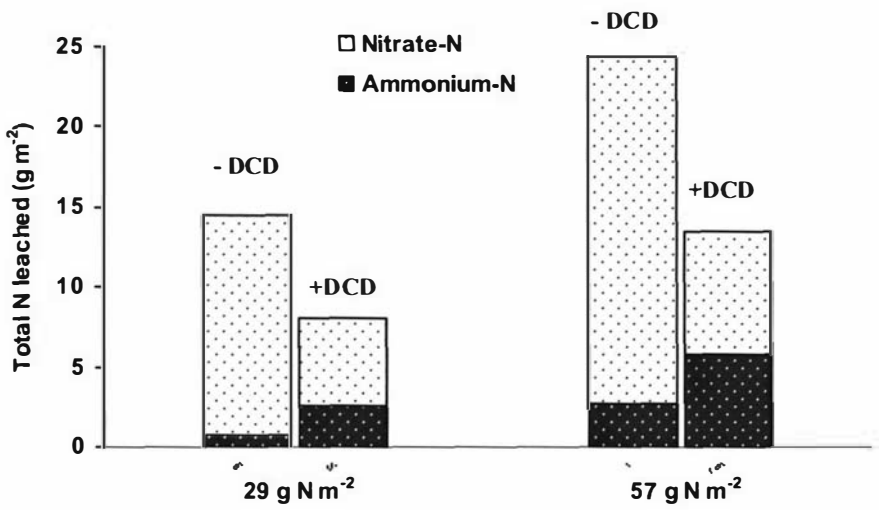


Figure 5.10 Total leaching losses of N in the leachate from soil cores receiving urine @ 29 g N m^{-2} and 57 g N m^{-2}, with and without DCD.

5.3.2.7 Cation leaching

Potassium leaching

In treatments T5 (29 g N m^{-2}) and T7 (57 g N m^{-2}) receiving urine alone without DCD, the K^+ concentration in the leachate reached a peak of 100 and 228 mg K^+ l^{-1} respectively (Figure 5.11 a). When DCD was applied along with urine in the T6 and T8 treatments, the peak K^+ concentration was significantly reduced ($P<0.05$) to below 65 and 124 mg K^+ l^{-1}, respectively. The total K^+ lost from the T5 and T7 treatments were 9.55 and 18.7 g K^+ m^{-2}, respectively as compared to the T6 (5.53 g K^+ m^{-2}) and T8 (11.9 mg K^+ m^{-2}) treatments, respectively; thus, the addition of DCD decreased K^+ leaching by 36-42% (Table 5.7).

Magnesium leaching

The concentration of Mg^{+2} in the leachate from all the treatments was significantly lower than the K^+ and Ca^{+2} concentrations. The Mg^{+2} concentration was higher in the leachate from the soil cores receiving the high dose of urine (57 g N m^{-2}; T7), with a peak value of 54.7 mg Mg^{+2} l^{-1}, than in the leachate of cores receiving the lower amount of urine (29 g N m^{-2}; T5) with a peak value of 36.7 mg Mg^{+2} l^{-1} (Figure 5.11 b). When DCD was applied, the peak Mg^{+2} concentration was significantly

($P<0.05$) reduced to around 23.3 and 22.7 mg Mg^{+2} l^{-1} in the T6 and T8 treatments, respectively. The amounts of Mg^{+2} leached from the T5 and T7 treatments were 2.63 and 3.93 g Mg^{+2} m^{-2}, respectively. Where DCD was applied, the amount of Mg^{+2} leached was decreased significantly ($P<0.05$) to 1.75 and 1.95 g Mg^{+2} m^{-2} (Table 5.7) in the T6 and T8 treatments, respectively, causing a 33% and 50% reduction, respectively. No significant difference was found in the amount of Mg^{+2} leached in the T6 and T8 treatments.

Calcium leaching

The concentration of Ca^{+2} in the leachate from the urine treatments was also reduced by the application of DCD. The peak Ca^{+2} concentrations obtained immediately after leaching started decreased from 193 mg Ca^{+2} l^{-1} and 290 mg Ca^{+2} l^{-1} in the T5 and T7 treatments, respectively, to 63.0 and 81.3 mg Ca^{+2} l^{-1} with the addition of DCD in the T6 and T8 treatments, respectively (Figure 5.11 c). The total amount of Ca^{+2} leached decreased from 14.5 and 20.8 g Ca^{+2} m^{-2} in the T5 and T7 treatments to 4.05 and 5.87 g Ca^{+2} m^{-2} in the T6 and T8 treatments, respectively, with added DCD (Table 5.7). The reduction in Ca leaching with DCD application was equivalent to 72% at both rates of urine application.

Table 5.7 Total leaching losses of K^+, Mg^{+2}, Ca^{+2}, NH_4^+-N and NO_3^--N in the cumulative drainage of 132 mm from the soil cores receiving urine at varying rates, with and without DCD.

Treatments	Potassium leached (g K^+ m^{-2})	Magnesium leached (g $Mg^{+2}m^{-2}$)	Calcium leached (g Ca^{+2} m^{-2})	Ammonium leached (g N m^{-2})	Nitrate leached (g N m^{-2})
T5	9.55	2.63	14.5	0.72	13.8
T6	5.53	1.75	4.05	2.55	5.48
T7	18.9	3.92	20.8	2.68	21.7
T8	11.9	1.95	5.87	5.84	7.61
LSD (0.05%)	2.07	0.99	1.75	1.23	1.98

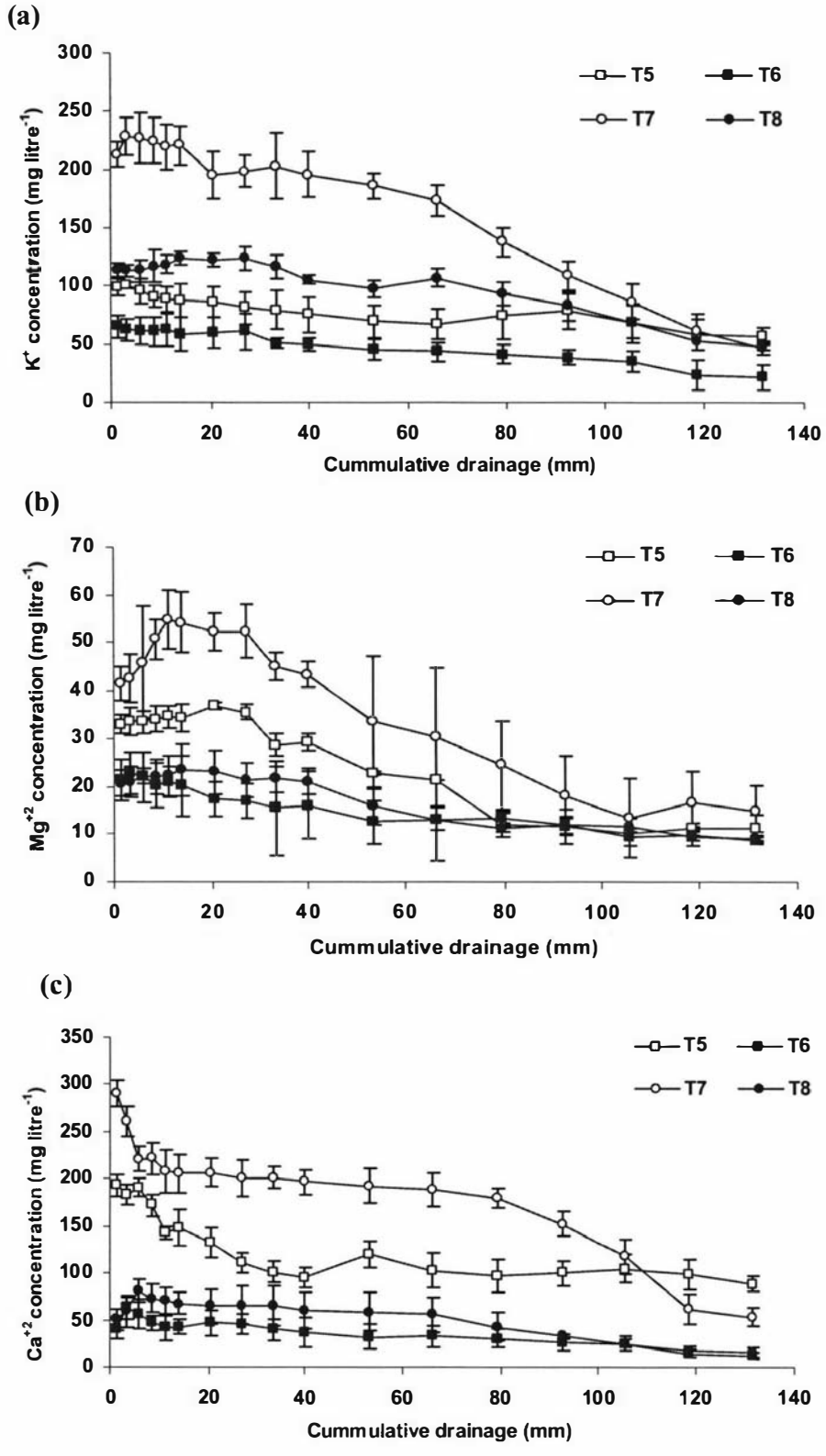


Figure 5.11 **Concentrations of (a) K^+; (b) Mg^{+2} and (c) Ca^{+2} in the drainage water from the soil cores receiving urine @ 29 g N m^{-2} and 57 g N m^{-2}, with and without DCD. Vertical bars are the standard deviations for three replicates.**

Relationship between the NO_3^- and cations leached

The total amount of cations leached was plotted against the total nitrate leached for all the treatments (Figure 5.12). The concentration of NO_3^- and the four cations (K^+, Mg^{+2}, Ca^{+2} and NH_4^+) in the leachate were converted to the same unit of $mol_c\ m^{-2}$ to compare the charge balance. The results indicated that 4–70% of the cationic charge in the leachate was balanced by NO_3^- charge. The following highly significant (Pr=0.0001) linear relationship was found between the total NO_3^- leached and sum of the cations and total NO_3^- leached ($r^2 = 0.876$)

$Y = 0.9983\,X + 38.468$

Where Y = total cationic charge ($mol_c\ m^{-2}$) and X = nitrate charge ($mol_c\ m^{-2}$).

The slope (0.998) indicated that the change in the concentration of total basic cations was balanced by the change in the concentration of nitrate in the leachate. However the high intercept value indicated that that there was a significant contribution by other anions such as Cl^- to cation leaching.

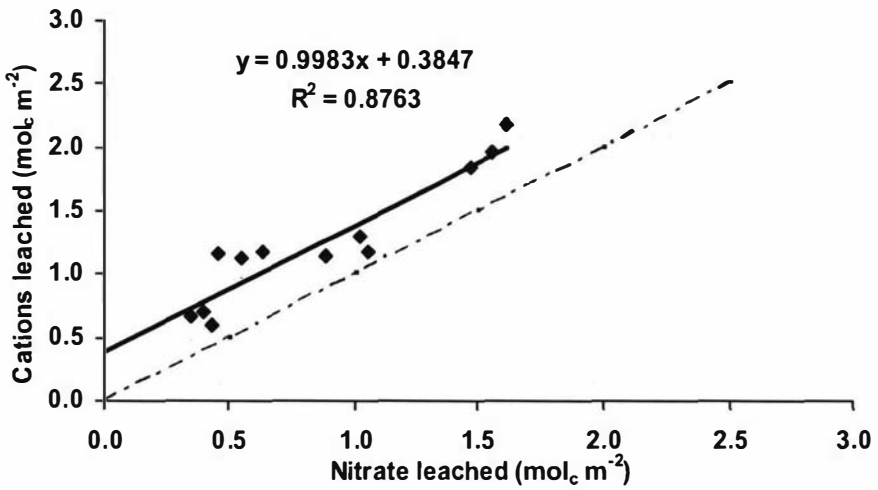


Figure 5.12 The relationship between the concentration of NO_3^- and cations (Ca^{+2}, Mg^{+2}, K^+ and NH_4^+) and NO_3^- in the leachate from the soil cores.

5.4 General Discussion

Addition of DCD resulted in: (1) a significant increase in soil NH_4^+-N concentration in both the urea and urine applications resulting in a 35-39% increase in total NH_3 losses from urea and a marginal increase from urine; (2) inhibition of both accumulation of NO_3^--N and emission of N_2O throughout the experimental period from both the urea and urine applications; (3) a 60-65% decrease in NO_3^- leaching and also a significant reduction in cation leaching from the urine application; (4) a 2 - to 3.5-fold increase in NH_4^+-N leaching; and (5) a slight positive effect on herbage yield and N uptake in the urea application but no significant effect on yield with the urine application. In this section, these results are discussed in detail in relation to those obtained in other studies and possible reasons are given for these observations.

In our study, there was a significant increase in the total amount of NH_3 emitted with the addition of DCD to the urea treatments (UrT6 and UrT8) (Table 5.1), but this increase was not significant in the urine treatments (Table 5.4). The finding of a significant DCD-related increase in NH_3 emitted from urea application is in accordance with the extensive studies of Rodgers (1983), Rao & Puttanna (1987), Davies & Williams (1995) and Puttanna *et al.* (2001). In Rodgers (1983) study, DCD applied in conjunction with urea was found to increase NH_3 volatilisation by between 20-68%, whilst, in the Rao & Puttanna (1987) study, it not only increased the total amount of NH_3 lost, but also greatly extended the period over which volatilisation occurred. DCD inhibits or delays the process of nitrification of NH_4^+ to NO_3^--N, thus increasing the concentration of NH_4^+-N in the soil. In the urine application, despite increasing NH_4^+ concentrations in the soil cores (Figure 5.7 a), NH_3 volatilisation did not increase significantly because urine rapidly permeated into the soil after application and the urea-N in urine was distributed down the soil depth; this resulted in retention of NH_4^+-N produced by hydrolysis of this urea. In Experiment 1, in contrast, urea was applied to the surface of the soil cores and provided a greater opportunity for the hydrolysed NH_4^+ to escape as NH_3. These results are consistent with the studies of Rao & Putanna (1987), Putanna *et al.,* (2001) and Rodgers (1983) which showed that the NH_3 volatilisation losses from DCD-treated urea could be substantially reduced by its deep placement. Di & Cameron (2004c) also found no significant difference in the total amount of NH_3 emitted from cow urine with and without the application of DCD.

In the urea treatments (UrT6 and UrT8), the percentage increase in NH_3 emissions with addition of DCD increased with increasing urea dose. However, in the control and UrT3 treatment (with 2.5 g added urea N m^{-2}), DCD reduced NH_3 volatilisation; this might be due to greater NH_4^+ uptake by herbage. In experiment 2, the volumes of urine added were 17, 34 and 67 ml for the 14.4 g N m^{-2} (T3 and T4), 29 g N m^{-2} (T5 and T6) and 57 g N m^{-2} (T7 and T8) treatments, respectively. The larger volume of urine in the T7 and T8 treatments would have allowed more even distribution and movement of urine N to a greater depth than in the other treatments. Thus, the addition of DCD caused a lower increase in NH_3 volatilisation in the T8 (3%) than in the T6 (5%) and T4 (15%) treatments as NH_4^+ produced would be furthest away from the soil surface in the T8 treatment.

Nitrous oxide emissions for the whole period ranged from 1.05 to 2.16% (Table 5.1) and 0.45 to 3.57% (Table 5.4) at various levels of urea and urine applications, respectively. Oenema *et al.* (1997) estimated that between 0.1 and 3.8% of urine-N from urine spots in grazed pastures was emitted to the atmosphere as N_2O. In the present experiment, the soil cores with urea showed a lag period of 4 days for N_2O emission, which was possibly due to the time required for the hydrolysis of urea. On the other hand, peak N_2O emissions were observed in all the urine treatments, both with and without DCD, within 24 hours of application of treatments. This stimulatory effect can be attributed to the increased N availability, increased soil water-filled pore space (WFPS) and supply of easily available C (i.e., solubilisation of soil carbon due to an increase in pH after urine or urea application; Williams *et al.* (1999)). The WFPS of the soil cores increased from 60% (at field capacity) to 64%, 68% and 78% with the addition of 17 ml (14 g N m^{-2}), 34 ml (29 g N m^{-2}) and 67 ml (57 g N m^{-2}) urine respectively. It has often been noticed that N_2O emission increases with increasing WFPS, with an exponential increase above 60% WFPS (Anger *et al.* 2003). These WFPS values are within the range of values previously reported to favour N_2O production by both nitrifiers and denitrifiers (Linn & Doran 1984). The percentage of added N emitted as N_2O increased with the increasing volume of urine N applied. Van Groenigen *et al.* (2005), in an incubation study, also observed that increasing volumes of added urine with a constant concentration of urine-N resulted in a significant increase in N_2O emissions.

Nitrous oxide can be produced either by nitrification (Bremner *et al.* 1980) or by denitrification (Firestone & Davidson 1989). It is possible that nitrification and denitrification can occur simultaneously in adjacent soil pores of different aerobicity and have a combined impact on the release of N_2O (Jarvis *et al.* 1994). Dicyandiamide restricts conversion of NH_4^+ to NO_3^-, thus inhibiting production of N_2O via nitrification and limiting the substrate (NO_3^-) for N_2O emission through denitrification. The use of DCD with urea and urine proved to be an effective way of reducing N_2O emissions. Application of DCD reduced N_2O emissions by 34 to 93% and 45 to 81% from the urea (Table 5.1) and urine (Table 5.4) treatments, respectively. DCD reduced N_2O emission by 40% in a dry sandy loam grassland soil from ammonium sulphate application (Skiba *et al.* 1993), and 58-78% when mixed with urea from barley fields (McTaggart *et al.*, 1997). Similarly, Di & Cameron (2003) showed that N_2O flux can be reduced by 76% for autumn urine and 78% for spring urine application.

At the end of the experiment, the concentration of NO_3^--N was higher at both 0-50 and 50-100 mm depths in soil cores receiving urea or urine without DCD compared to cores with DCD (Figure 5.3 b and 5.7 b). The high concentration of NO_3^- ions in soil provides a major source for N_2O emission, as stated by Stevens & Laughlin (1998). The mean values of NO_3^--N for all the urea and urine treatments indicated that, at the end of the experimental period, the NO_3^--N concentration in the soil with DCD was reduced by almost 50% (urea) and 25% to 33% (urine) compared to that in soil cores without DCD. This decrease in NO_3^--N can be attributed mainly to the direct effect of DCD on nitrification; the increase in NH_4^+-N concentration in DCD-treated soil may have also contributed to the lower NO_3^--N concentrations as an increase in ammonium salts has been shown to inhibit nitrification (Monaghan & Barraclough 1992). There was a substantial (3-8 times) increase in NH_4^+-N concentration in cores receiving urea (UrT6 and UrT8) and urine treatments with DCD compared to cores without DCD. Higher accumulation of NH_4^+-N in soil with the addition of DCD to various N sources, such as fertiliser, slurry and urine, has been found by various workers (Abbasi & Adams, 2000; Cookson & Cornforth, 2002; Merino *et al.*, 2002; Puttanna *et al.*, 2001). In the absence of N addition (i.e., the control soil), the application of DCD did not affect soil NH_4^+-N and NO_3^--N concentrations; this may be attributed to the pasture uptake of NH_4^+-N limiting net nitrification. Competition for NH_4^+-N from other microorganisms

may have also contributed (Tietema & Wessel 1992) to the negligible increases in NH_4^+-N in the control treatment with added DCD. Nitrification has also been shown to be dependent on readily oxidisable C supply (Tietema & Wessel, 1992) which may have been restricted in the control soil.

Results from this study showed that the peak values for all the measured properties (K^+, Mg^{+2}, Ca^{+2}, NH_4^+, NO_3^-) in the leachates occurred within the first pore volume. Urine applied to the soil cores at field-capacity moisture content was distributed uniformly. Further, soil cores were maintained at field-capacity moisture content throughout the experimental period of 50 days by adding water which could have maintained the uniform solute distribution. Thus, when soil cores were leached, the concentration of the solutes in the leachate from the first pore volume was high and then decreased with increasing pore volumes. As discussed earlier, the soil mineral-N before commencing leaching was mainly present as NO_3^- in the soil cores at 0-50 and 50-100 mm depths in the T5 and T7 treatments receiving urine only. Thus, NO_3^- formed the major component of N output in the leachate for urine treatments (Figure 5.10). This NO_3^- concentration was significantly reduced at both the depths with the addition of DCD to the soil cores. The NO_3^- breakthrough curves show the obvious difference between the urine (T5 and T7) and urine+DCD (T6 and T8) treatments. The 60-65% reduction in total NO_3^- leaching loss due to the application of DCD to the urine treatments @ 29 and 57 g N m^{-2} is similar to the 74-76% reduction with DCD added to autumn-applied urine-N (1000 kg N ha^{-1}) by Di & Cameron (2004c).

In contrast, the addition of DCD increased the NH_4^+ concentration in soil cores by 7- to 9- fold. This increase was apparent in the amount of NH_4^+ found in the leachate of these cores which was 2 to 3.5 times higher than that found in the leachate of soil cores without DCD (Figure 5.11). A similar result was also found by Cookson & Cornforth (2002). Although, NH_4^+ ions are strongly adsorbed on the soil colloids, the accumulation of NH_4^+ ions would have caused the saturation of the cation-exchange capacity (10 $cmol_c\ kg^{-1}$) making them susceptible to leach. But, as the column depth in our study was only 10 cm, one would expect less NH_4^+ leaching under field conditions with a deeper soil profile. Although there was an increase in the NH_4^+ concentration of the leachate from the T6 and T8 treatments, the addition of DCD reduced total N leaching by 45% for these treatments, mainly by reducing NO_3^- leaching (Figure 5.10).

In addition to the reduction in NO_3^- leaching, the treatment of soil cores with DCD also resulted in decreased leaching losses of the major cations K^+, Mg^{+2} and Ca^{+2}. According to Figure 5.13, the relationship between the cations and NO_3^- leached for all the treatments was linear (r^2 = 0.876, F<0.0001), indicating that the charge ratio between the amount of cations and NO_3^- leached was the same. As the electrical charge of the soil solution is always balanced or neutral, and charge balancing is a fundamental requisite in all systems – soil, plant and animal, leaching of anionic constituents in soil needs to be balanced by an equivalent loss of cationic constituents. Nitrate is a weakly held anion (non-specific adsorption), in contrast to other anions like phosphate which undergoes specific adsoption; when nitrate is subject to leaching it takes the cations along as charge-balancing counter ions. Thus, the reduction in NO_3^- leaching also caused a decrease in the leaching of counter ions (cations). From Figure 5.12 it is clear that, even when the leaching loss of NO_3^- became close to zero, there was considerable loss of cations. These losses probably represent the background leaching loss of cations as counter ions with other native anions such as Cl^- and SO_4^- from the grazed grassland soil. This also explains the increase in leaching of NH_4^+, even though there was a reduction in NO_3^- leaching with the addition of DCD. Brye & Norman (2004) have observed that anionic dissolved organic carbon can also play an important role in maintaining the soil-solution charge balance. Thus dissolved organic carbon can be one of the factors, along with other anions, that can explain the difference in cation leaching not accounted for nitrate leaching. The leaching reductions of 72% for Ca^{+2}, 33 - 50% for Mg^{+2} and 36 - 42% for K^+ obtained with added DCD in this study are similar to those found by Di & Cameron (2004b) i.e., a reduction of 50%, 52% and 65% for Ca^{+2}, Mg^{+2} and K^+, respectively, with the addition of DCD to urine spots in a free draining silt loam soil under field conditions.

The dominant cations in the leachate in the present study were Ca^{+2} and K^+ with the concentrations of Mg^{+2} and NH_4^+ being low. This dominance of Ca^{+2} as a counter ion was attributed to the large amount of exchangeable Ca^{+2} in the soil (Table 4.1). The high concentration of K^+ in the leachate was likely to be because of its high concentration in cattle urine (6 to 11 g K l^{-1}; Ledgard *et al.* (1982); Williams *et al.* (1989)), and can also be attributed to the absence of any 2:1 type clay minerals for

absorbing K^+ ions in the Manawatu sandy loam soil (Williams *et al.* 1990). Moreover, it is likely that urine-derived NH_4^+ would have replaced adsorbed K^+ from soil colloids.

The amount of cations leached increased with increasing rate of urine treatment (Figure 5.11). The concentration of all cations, including K^+, Mg^{+2}, Ca^{+2} and NH_4^+, increased significantly in the T7 treatment as compared to the T5 treatment. The increase in NH_4^+ and K^+ leaching can be attributed to the higher input of these ions in T7 than T5. The increase in the leaching of Ca^{+2} and Mg^{+2} with increasing rate of urine application can be explained by the ratio law which indicates that, in dilute solutions, divalent cations mostly stay on the exchange sites and it is mainly the monovalent ions that remain in solution with the anions. But, as the soil solution concentrations and NO_3^- ions increases at the higher urine rate more divalent cations are found in solution. Thus, with increasing nitrate input it is likely that increasing levels of Ca^{+2} and Mg^{+2} will be leached, firstly because of the presence of more associated soluble anions and, secondly, because of the ratio law described above.

Addition of DCD did not affect pasture yield significantly in either urea or the urine treatments, but did increase the N concentration in herbage in the urea treatment. However, the trend was opposite in the urine treatments where the addition of N to soil cores was higher than in the urea treatments. The slight decrease in DM yield with the addition of DCD in the urine treatments at the lower rates of 14.4 and 29 g N m^{-2}, and also in the control, may be attributed to the increase in NH_4^+ concentration resulting in salt injury and NH_4^+ toxicity. The effect of DCD on herbage yield is found to be quite variable (Cookson & Cornforth, 2002; Di & Cameron, 2002b; Gioacchini *et al.*, 2002; Smith *et al.*, 2005). Herbage yields in both the T7 and T8 treatments (57 g urine-N m^{-2}) were reduced as compared to treatments receiving urine at the lower rates. A few days after the application of urine in the T7 and T8 treatments, the herbage turned brown around the leaf margins and some of the plants died. This effect has been referred to as urine scorch or urine burn and has been observed in the field (Doak 1952; Holmes 1968; Richards & Wolton 1975), particularly where applications of highly concentrated urine occur (e.g., concentration of urine N >1%; (Quin 1977). The main effect of urine burn is thought to be on the root system of the plants rather than on the leaves (Richards & Wolton, 1975), and is probably caused by a combination of high salt and NH_4^+ toxicity.

5.5 Conclusions

In intensively grazed pasture, urine excretions act as the hot spots for N losses. The N added via urine is well in excess of that which can be taken up by the pasture in a growing season and thus liable to losses through gaseous emissions and/or leaching. Addition of a nitrification inhibitor (DCD) has been shown to be effective in reducing some of these losses considerably.

The main conclusions that can be drawn from this study in relation to DCD-induced changes in N-related dynamics in soils are as follows:

- Ammonia emissions were significantly increased by the addition of DCD to urea but not to urine application.
- DCD reduced N_2O emissions by 34 to 93% and 45 to 81% in the urea and urine treatments, respectively, depending on their application rate.
- DCD affected mineral N transformations of urea and urine N in soils. DCD increased NH_4^+-N concentrations in soil by inhibiting nitrification, with a corresponding decrease in NO_3^--N concentrations. The NH_4^+-N concentrations decreased more gradually in DCD-treated soil than in the untreated soil, which also contributed to lower NO_3^--N concentration in both the DCD treated urea and urine treatments.
- The accumulation of NH_4^+-N in the soil with added DCD was reflected by a 2.5- to 3- fold increase in NH_4^+-N leaching, whereas the reduction in NO_3^--N concentration resulted in NO_3^--N leaching being reduced by 60-65% in the urine treatments. The overall effect of DCD was reduced mineral-N leaching.
- Addition of DCD also reduced anion-induced cation (K^+, Mg^{+2}, Ca^{+2}) leaching.
- There was no significant effect of DCD addition on herbage yields either in the urea or urine treatments.

Our study has shown that, under controlled conditions, DCD can reduce N losses from soil via reducing N_2O emissions and NO_3^- leaching and cations leaching from urine spots. There was some trend of slightly elevated NH_3 volatilisation with DCD but the increases in the urine treatments were not significant. The actual benefits of DCD under field conditions would depend on whether the inhibitor could remain active over protracted periods in different soils and under different

environmental conditions. Therefore, the effectiveness of DCD under field conditions, and the effect of soil type on DCD degradation, forms the focus of our next chapters.

Chapter 6

Combined effect of urease and nitrification inhibitors on N dynamics in pasture soils

6.1 Introduction

Strategies for regulating the fate and behaviour of N in the pasture system focus mainly on improving the use efficiency of fertiliser N and excretal N, and reducing environmental N pollution. Included among these strategies is the use of inhibitors with N fertilisers or added to the urine spots in grazed pastures. Urease inhibitors (UIs) retard the hydrolysis of soil-applied urea or urine and reduce the accumulation and volatilisation of NH_3 (Tomar *et al.* 1985; Wang *et al.* 1991). Nitrification inhibitors (NIs) reduce N_2O flux and nitrate leaching from NH_4^+ and urea based fertilisers (Bronson *et al.*, 1992; Mosier *et al.*, 1998; Skiba *et al.*, 1993), organic manures and urine spots (Di & Cameron, 2002b, 2003; Hatch *et al.*, 2005), but they can sometimes enhance NH_3 volatilisation losses (Rao & Puttanna, 1987). This increased NH_3 volatilisation may negate the beneficial effects of NIs on the reduction in N_2O emission and nitrate leaching. It is hypothesized that a combination of urease and nitrification inhibitors is conducive to retaining more urea-N in the soil-plant system.

To test this hypothesis, glasshouse and field-plot studies were conducted involving urease inhibitor i.e., Agrotain (NBPT) and nitrification inhibitor i.e., DCD, with urea and urine. The research aim was to improve the N-use efficiency of applied urea and urine and reduce loss of N to the environment.

The main objective of this study was to investigate the individual and combined effects of urease inhibitor (Agrotain) and nitrification inhibitor (DCD) on the fate and behaviour of applied urea and urine–N. The specific objectives were as follows:

- To examine the combined effect of inhibitors on NH_3 and N_2O losses from applied urea and cattle urine in a pasture soil.
- To examine the effect of these inhibitors on changes in mineral N in the soil.
- To study the effect of these inhibitors on pasture yield and N uptake.

6.2 Materials and Methods

6.2.1 Experimental set-up

6.2.1.1 Glasshouse experiment

The study used intact soil cores (100 mm diameter, 100 mm depth), that were similar to those described in Chapter 3 section 3.2.4.1, collected from the same site and prepared similarly for the application of treatments. The soil at this site was Manawatu fine sandy loam and its characteristics have been described in Chapter 4 of this thesis. The experiment with four treatments viz., Control, Urea+NI, Urea+UI and Urea+UI+NI in triplicate was set up in the glasshouse maintained at a near constant temperature ranging from 15-20°C. The urease and nitrification inhibitors used in this study were Agrotain (Sustain Yellow) and DCD (@ 25 kg ha^{-1}), and urea was applied @ 100 kg N ha^{-1}. The treatment details are given in Table 6.1. Background measurements for NH_3 and N_2O emissions were taken before applying treatments.

6.2.1.2 Field-plot Experiment

Experimental site and Soil Characteristics

This experiment was a part of a TechNZ-funded project entilted 'Development of technology to minimise N losses from grazed dairy pastures' that included establishing the efficacy of nitrification and urease inhibitors on gaseous losses of N_2O, drainage losses of NO_3^- from pasture and evaluating the efficacy and practical usability

of a 'real time' inhibitor delivery system called a Taurine device under normal dairy-grazing conditions. Details of the experiment are given in Gillingham *et al.* (2006). This experiment was intiated on 10th May 2005 at the Massey University's Dairy farm pasture with predominantly perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) on Tokomaru silt loam soil. The chemical and physical properties of the soil at the site are presented in Table 6.2. The rainfall distribution for the experimental period (10th May to 29th June 2005) is shown in Figure 6.1. The average air temperature during this period was 12.2^0 C. The soil temperature varied from 8.5 to 15^0 C with an average of 12^0 C (Figure 6.1).

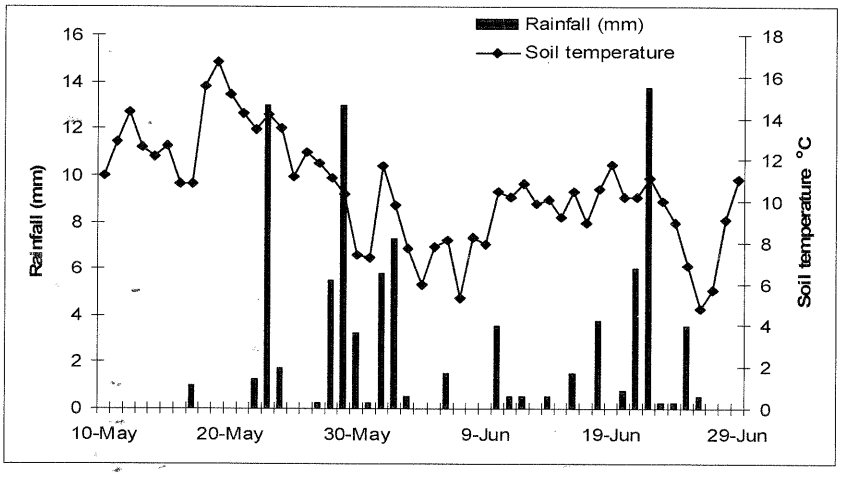


Figure 6.1 Daily rainfall and soil temperature (0-50 mm) distribution for May to July 2005 for the field-plot study.

Table 6.1 Treatments applied in the glasshouse study and Field-plot experiment

Treatment No.	Treatment	Referred as in text	Inhibitor rate
	Glasshouse study		
1	Urea*	Ur	-
2	Urea + DCD***	Ur+NI	25 kg ha^{-1}
3	Sustain Yellow**	Ur+UI	0.1% (w/w)
4	Sustain Yellow+DCD	Ur+UI+NI	0.1% (w/w), 25 kg ha^{-1}
	Field-plot study		
1	Water only (Control)	Control	-
2	Urine*	Urine	-
3	Urine + DCD	Urine + NI	7 kg ha^{-1}
4	Urine + Agrotain	Urine + UI	3 l ha^{-1}
5	Urine+DCD+Agrotain	Urine+NI+UI (Urine+UI+NI)	3 l ha^{-1}, 7 kg ha^{-1}

* Urea was surface applied @100 kg N ha^{-1} and urine was applied @600 kg N ha^{-1}
**commercial product manufactured by Summit Quinphos Ltd. Containing urea granules coated with Agrotain (0.1% w/w). It contains 44% N and 4% S.
** DCD was added in solution form after broadcasting of Sustain Yellow.

Table 6.2 Chemical and physical properties of Tokomaru silt loam soil at the experimental site

	Depth (cm)	Total N (%)	Total C (%)	pH (soil:water1:2.5)	Bulk Density (Mg/m^3)	C.E.C ($c\ mol_c\ kg^{-1}$)
Tokomaru	**0-5**	0.35	3.64	5.47	1.01	22.3
silt loam	**5-10**	0.22	2.18	5.00	1.01	nd

Experimental design

A completely randomised block design with three replications was used. Fifteen plots, each plot with a 1 m x 1.5 m area and separated by a 0.5 m buffer zone were established. The area was fenced off two months before the start of the experiment to avoid excretal N deposition from grazing animals. A pre-conditioning harvest (2 cm height) was taken before applying the treatments described in Table 6.1. Cattle urine at the rate of 600 kg N ha^{-1} was applied. Inhibitors were mixed with the urine before application at the appropriate rate and sprayed on to the plots with a watering can. Urine was collected from Friesian cows during the milking session at the Massey Dairy farms. After collection, the urine was stored for three days in tight sealed plastic

containers below 4°C to avoid urine hydrolysis. Before the urine application to the plots, a urine sample was bulked from each container and analyzed for total N (Ebina *et al.*, 1983) and total C (Bremner & Tabatabai, 1971). The urine applied had an average total N and C concentration of 6.5 g l^{-1} and 2.8 g l^{-1}, respectively.

6.2.2 Ammonia measurements

For the glasshouse experiment, NH_3 emission was measured for 20 days after application of the treatments using the active flux method discussed in Chapter 3 Section 3.2.4.2. For the field-plot experiment, NH_3 emission was estimated for 15 days using passive samplers containing an oxalic acid ($C_2H_2O_4$) coating, as described in Chapter 3 Section 3.2.5.1. Four passive samplers of 21 mm (diameter) × 90 mm (long) were placed diagonally in each plot (Plate 3.3). These samplers were removed regularly after 6 days of exposure, for the measurement of trapped NH_3 and were recapped until analysis. The NH_3-saturated oxalic acid was dissolved in water and the concentration of NH_4^+-N in the solution was measured using an auto-analyser. The volatilisation loss of NH_3-N from each plot was estimated as the sum of NH_4^+-N in the four vials of each plot.

6.2.3 Nitrous oxide measurements

In both the experiments, N_2O emissions were determined using the closed chamber technique described in Chapter 3 Section 3.2.4.3. The emissions were measured for 36 days in the glasshouse experiment and for 50 days in the field plot study. In the field-plot study, one open base chamber one per plot was inserted 100 mm into the soil one day before measurements. During the course of measurements over 50 days, the chambers were covered with baskets, which were insulated with aluminium foil to minimise fluctuations in temperature. Basal N_2O emission was measured one day before the application of treatments in both the experiments. Measurements of N_2O emissions were taken daily for the first week to capture immediate changes in N_2O fluxes. Subsequent measurements were made on alternate days for the next two weeks,

and then twice a week in the fourth week. For the remaining period of two weeks, measurements were taken once a week. The N_2O flux was calculated as discussed in Section 3.2.4.3 of Chapter 3. Cumulative fluxes were calculated using the mean values from two consecutive daily fluxes.

6.2.4 Soil Sampling and analysis

At the end of the glasshouse experiment, soil cores were cut at 0-50 mm and 50-100 mm depths. A sub-sample of 5 g (oven dry equivalent) was taken from each soil core to determine the concentration of NH_4^+ and NO_3^- -N, as discussed in Section 4.2.4.2. In the field-plot study, three soil samples at two depths (0-50 and 50-100 mm) were collected, three from each replicate plot within each block outside the chamber area on 1, 4, 5, 7, 12, 17, 21, 28, 37 and 48 days after the treatments were applied. Composite samples were obtained by bulking together the three samples from each replicate plot to determine soil water content, pH (0-50mm), mineral N and DCD (0-50 mm) concentrations. Field-moist samples were weighed (Mt) and oven-dried (105^0C) to a constant mass (Ms). Gravimetric soil water content (SWC) was calculated

$$SWC = \frac{M_t - M_s}{M_s} \times 100$$

The volumetric soil water (θv) content was then calculated by multiplying the gravimetric SWC with the soil bulk density (ρ_b). The water filled pore space (WFPS) was calculated as follows

$$WFPS = \frac{\theta_v}{Total\ soil\ porosity}$$

where, total soil porosity was calculated by the formula

$$Total\ soil\ porosity = 1 - \frac{\rho_b}{\rho_s}$$

The particle density of the soil (ρ_s) was assumed to be 2.65 g cm^{-3}.

Field-moist samples were stored overnight at $<4^0$C before sieving (2mm). A sub-sample 5 g (oven dry equivalent) was extracted with 30 ml 2M KCl for NH_4^+ and NO_3^- as described in Section 4.2.4.2.

Another sub-sample (10 g oven dry equivalent) was extracted with deionised water (1:2, soil:water ratio). The solution was shaken on an orbital shaker for 30 minutes, centrifuged and filtered (Whatman No. 40). The soil extract was analysed for DCD concentration by a manual colorimetric method at 540 nm (Vilsmeier 1982).

Soil pH was measured at a 1:2.5 soil:water ratio using a combined electrode pH meter (Blakemore *et al.*, 1987). Total C and N in the soil were measured by combustion in a Leco FP-2000 CNS (LECO Corp., St Joseph, MI, USA).

6.2.5 Herbage analysis

Herbage from the glasshouse in-situ cores was cut twice to 2 cm height and from the field-plots mown three times on days 25, 50 and 65 after the treatment application. The harvested material was weighed, with a sub sample dried at 100°C for 24 hours to determine dry matter (DM) content, and the cumulative DM yield was recorded. In addition, hand-cut samples were collected, dried at 65°C for 24 hours and analysed for total N content as discussed in Section 4.2.4.3. The dry matter response to N input was calculated by the formula given in Section 5.2.3.3.

6.2.6 DCD Degradation

The rate of degradation of DCD as measured by its half-life ($t_{1/2}$) was calculated by fitting an exponential curve relating the change in DCD concentration in the soil with time.

$$N = N_0 \exp(-kt) \qquad \text{Eq (6.1)}$$

where, N is the amount of DCD remaining in the soil at time (t), N_0 is the amount of DCD recovered at the beginning of the experiment in the soil and k is the decay constant. The DCD half-life (time taken to reduce DCD concentration to half of the initial values) was calculated as:

$$t_{½} = 0.693/k \qquad \text{Eq (6.3)}$$

6.2.7 Statistical Analysis

An analysis of variance using SAS software (version 8) was performed on the results of total NH_3 and N_2O emitted, mineral N, total herbage DM and herbage N uptake using the General Linear Model (GLM) procedure. Mean comparisons were done using Fishers Least Significant Difference (LSD) at 5% significance. The correlation analyses between ammonium, nitrate and DCD were conducted using the SAS package.

6.3 Results

6.3.1 Ammonia emissions

In the glasshouse experiment, the addition of urea (Ur) to in-situ soil cores resulted in an NH_3 peak (176 mg N m^{-2} d^{-1}) 48 hours after application (Figure 6.2). When NI was added with urea (Ur+NI), a significant increase in the NH_3 peak (264 mg N m^{-2} d^{-1}) resulted. The amount of N emitted as NH_3 increased from 0.61 (Ur) to 0.88 g N m^{-2} in the Ur+NI treatment, resulting in a 44% increase in NH_3-N emission (Table 6.3). The application of UI to urea (Ur+UI; Sustain Yellow) decreased the NH_3 peak (27.4 mg N m^{-2} d^{-1}) significantly, and the peak was delayed by 2 days as compared to urea alone (Figure 6.2). The emissions remained low with the UI application throughout the 15 day measurement period. The total amount of NH_3-N emitted was reduced from 0.61 to 0.17 g N m^{-2} with UI, resulting in a 72% decrease in NH_3-N loss (Table 6.3). The emission of NH_3-N with the addition of DCD to Sustain Yellow (Ur+UI+NI) followed a similar trend to Sustain Yellow (Ur+UI), with a slight but insignificant increase in emissions from 0.17 (Ur+UI) to 0.18 g N m^{-2} (Ur+UI+NI). The use of the combined inhibitors (Ur+UI+NI) resulted in a 70% decrease in NH_3-N emissions as compared to the Ur treatment (Table 6.3). In all the treatments, NH_3 emissions reached the background levels by day 15.

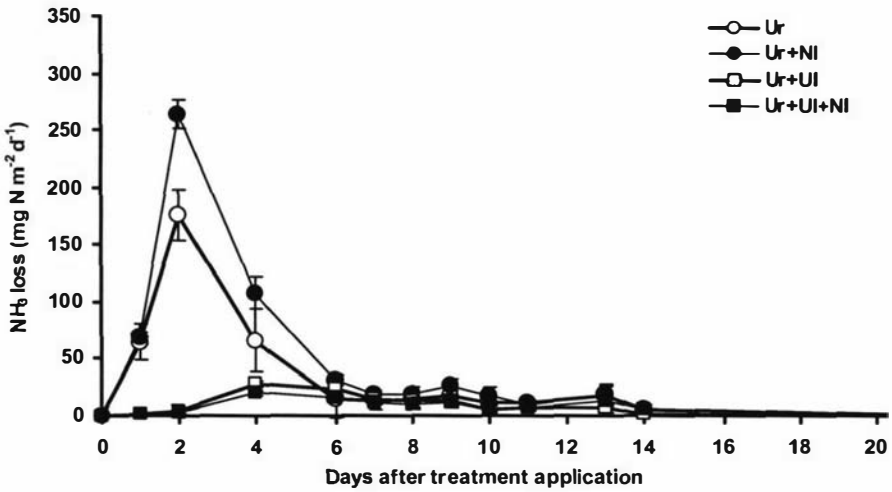


Figure 6.2 **Ammonia volatilisation losses from urea applied, with and without urease and nitrification inhibitors. Each value represents a mean of three replicates with standard deviation shown by vertical bars.**

In the field-plot study, NH_3-N trapped by the passive samplers for all the urine treatments was about 21 to 33 times greater than the control (water only) and was indicative of the trend of NH_3 loss from each treatment (Figure 6.3). The data obtained from passive samplers indicate that the addition of DCD to urine (Urine+NI) increased the NH_3 loss by 50%, whereas the addition of Agrotain either alone (Urine+UI) or with DCD (Urine+NI+UI) reduced the NH_3 loss by 6% and 4%, respectively (Figure 6.3).

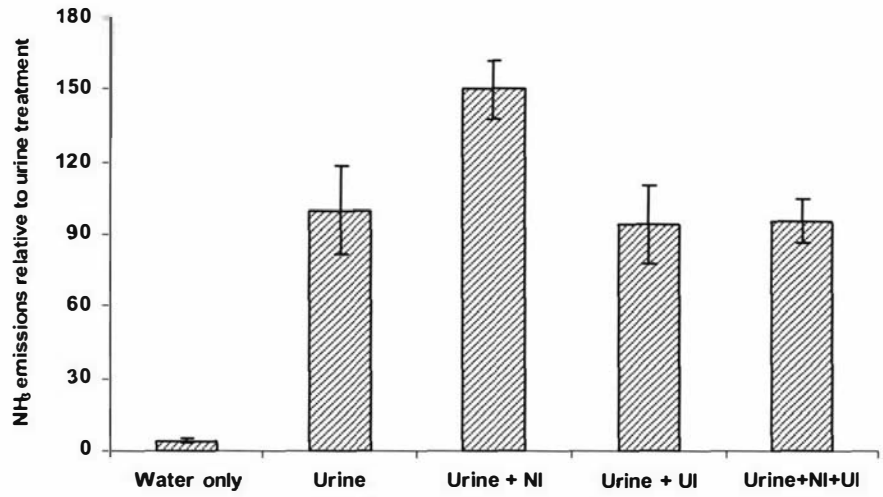


Figure 6.3 **The amount of NH_3-N released following the urine application with urease and nitrification inhibitors, relative to urine alone. Vertical bars denote the standard deviation between the replicate plots.**

6.3.2 Nitrous oxide emissions

In the glasshouse experiment, N_2O emission in the urea (Ur) treatment peaked to 2.94 mg N m^{-2} d^{-1}, 7 days after the application of the treatment before gradually declining to the background levels (Figure 6.4). Where DCD was applied to urea (Ur+NI), the N_2O flux was reduced to 0.7 mg N m^{-2} d^{-1} and remained significantly lower ($P<0.05$) than from urea alone throughout the experimental period of 37 days. This resulted in a 75% reduction in N_2O emissions. However, in the presence of urease inhibitor (Ur+UI), N_2O emissions were lower than from urea for 11 days and then started to increase, peaking to 4.73 mg N m^{-2} d^{-1} on day 26. The total increase in N_2O-N emissions with the urease inhibitor from 0.04 (Ur) to 0.07 g N m^{-2} (Ur+UI) was, however, not significant (Table 6.3). No peak was obtained when both the inhibitors were added (Ur+UI+NI) and the N_2O flux always remained below 1.03 mg N m^{-2} d^{-1} throughout the experimental period. The total N_2O-N emission decreased from 0.04 g N m^{-2} (Ur) to 0.01 and 0.02 g N m^{-2} with DCD alone (Ur+NI) or in combination with UI (Ur+UI+NI), resulting in 75% and 50% emission reductions, respectively (Table 6.3).

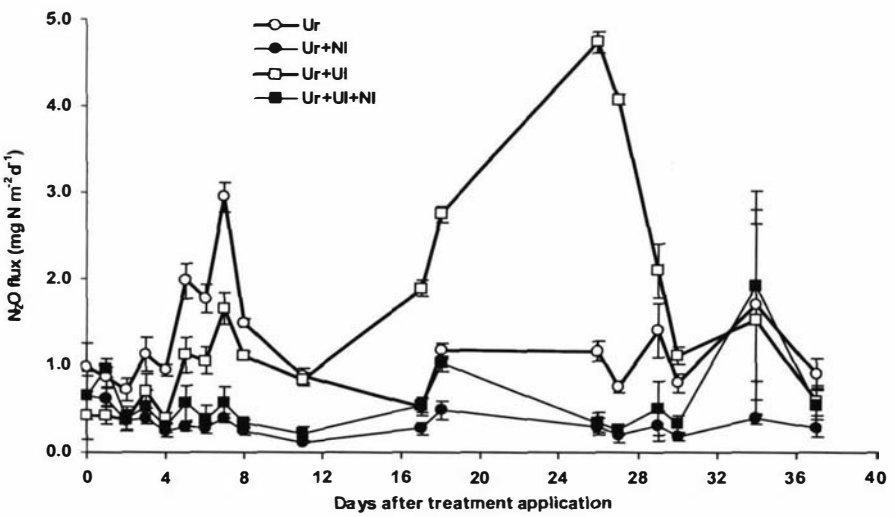


Figure 6.4 Nitrous oxide losses from urea applied with and without urease and nitrification inhibitors. Each value represents a mean of three replicates with standard deviation shown by vertical bars.

Table 6.3 Total N applied and N emitted as NH_3 and N_2O (g N m^{-2}) over the experimental period from soil cores receiving urea with and without urease and nitrification inhibitors

Treatment	N added (g N m^{-2})	NH_3-N (g N m^{-2})	% of added N emitted as NH_3	N_2O-N (g N m^{-2})	% of added N emitted as N_2O
Ur	10	0.61	6	0.044	0.4
Ur + NI	10	0.88	8.8	0.011	0.1
Ur + UI	10	0.17	1.7	0.070	0.7
Ur+UI+NI	10	0.18	1.8	0.023	0.2
L.S.D (0.05%)		0.22		0.05	

In the field-plot study, N_2O emissions in all treatments showed two peaks (Figure 6.5). The first small peak was observed within 24 hours of urine application. The emissions were 0.054, 0.045, 0.077, 0.042 and 0.031 kg N ha^{-1} d^{-1} for the control, Urine, Urine+NI, Urine+UI and Urine+NI+UI treatments, respectively (Figure 6.5); in the urine treatment, emissions were 2- to 8-fold greater than the background emission rates. A second peak occurred in all treatments, except the control (water only), at day 12 and corresponded with a high rainfall event (Figure 6.1). The peak was highest in the Urine+UI treatment (0.29 kg N ha^{-1} d^{-1}), followed by the Urine treatment (0.21 kg N ha^{-1} d^{-1}). The peaks for the Urine+NI and Urine+NI+UI treatments were significantly lower (0.08 and 0.13 kg N ha^{-1} d^{-1}) than the peak for the Urine treatment, and remained low throughout the experimental period of 50 days. The total N_2O emissions in the Urine+UI treatment (3.76 kg N ha^{-1}) did not differ significantly from those in the urine treatment (3.37 kg N ha^{-1}). However, total emissions were reduced to 1.62 and 2.05 kg N_2O-N ha^{-1}, respectively, in the Urine+NI and Urine+NI+UI treatments. The application of DCD to the Urine and Urine+UI treatments consequently resulted in 68% and 51% reductions, respectively (Table 6.4). The total amount of N emitted as N_2O from the control treatment was 0.81 kg N ha^{-1}.

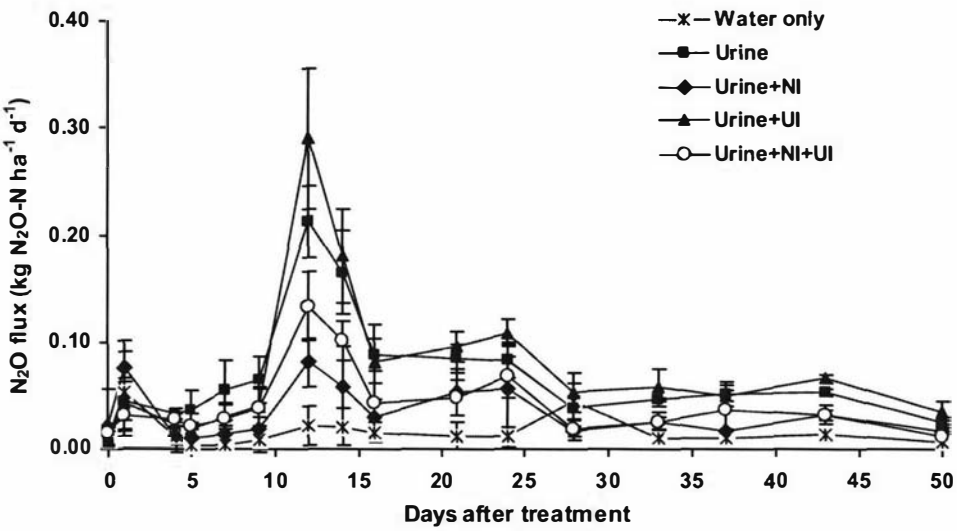


Figure 6.5 **Nitrous oxide fluxes (kg N $ha^{-1}d^{-1}$) following the application of urine, with and without urease and nitrification inhibitors, in autumn to pasture on Tokomaru silt loam. Each value represents a mean of three replicates with standard deviation shown by vertical bars.**

Table 6.4 **Total N applied and N emitted as N_2O (kg N ha^{-1}) over the experimental period from plots receiving various treatments**

Treatment	N added (kg N ha^{-1})	N_2O-N (g N m^{-2})	% of added N emitted as N_2O	% reduction
Water only (control)	-	0.81	-	-
Urine	600	3.37	0.43	-
Urine+NI	600	1.62	0.13	68
Urine+UI	600	3.76	0.49	-
Urine+NI+UI	600	2.05	0.21	51
L.S.D (0.05)		1.01		

6.3.3 DCD degradation analysis

The amount of DCD recovered on day 1 from 0-50 mm depth soil in the field-plots treated @ 7 kg ha^{-1} was 4.3 mg kg^{-1} (2.17 kg ha^{-1}) and 4.9 mg kg^{-1} (2.47 kg ha^{-1}) (Figure 6.6) for Urine+NI and Urine+NI+UI, respectively. The DCD concentrations reduced with time. The calculated half-life of DCD in this soil under the prevailing

climatic conditions was 24.5 and 27.5 days for these two treatments, respectively (Table 6.5). Soil NH_4^+-N showed a significant positive correlation, (r^2 = 0.81; P < 0.0001) with DCD concentration while soil NO_3^--N had a non-significant negative correlation (r^2 = -0.38).

Table 6.5 Half-life ($t_{½}$) of DCD in plots receiving urine with DCD alone and combined with Agrotain

Treatment	Degradation rate constant (day^{-1})	Half-life (days)	r^2
Urine	-	-	-
Urine + NI	0.029 ± 0.003	24.5 ± 2.5	0.81
Urine+NI+UI	0.025 ± 0.002	27.5 ± 2.5	0.90
P = 0.2590			

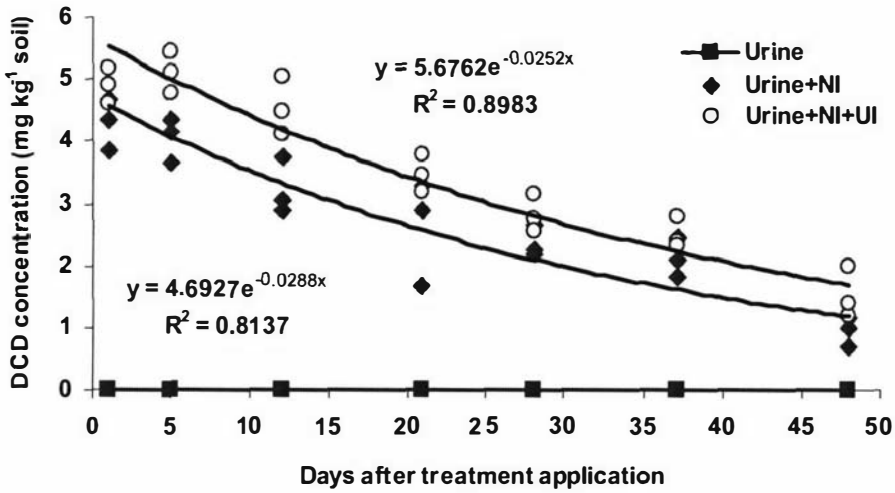
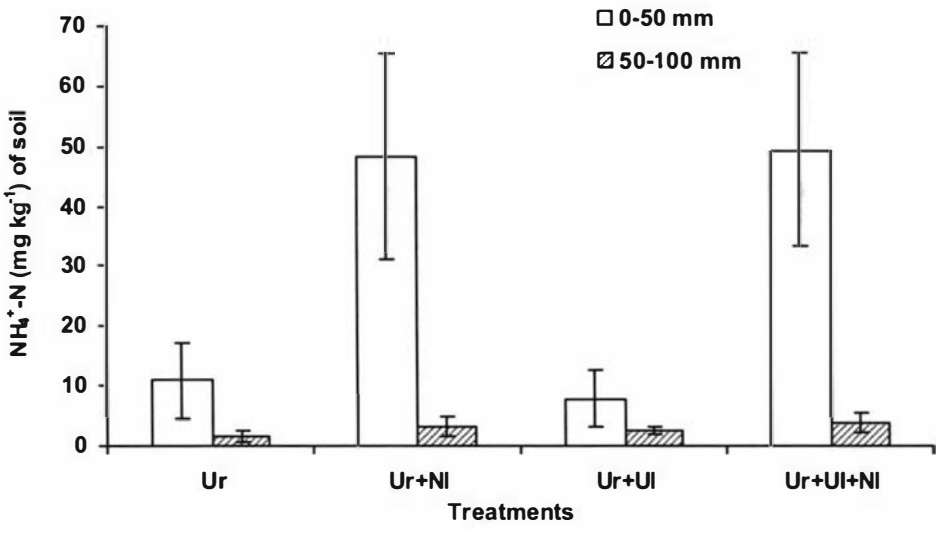


Figure 6.6 Mean DCD concentrations in Tokomaru silt loam following the application of urine treatments with DCD alone and combined with Agrotain. Error bars represent the standard deviation of the three replicates

6.3.4 Nitrogen transformation

At the end of the glasshouse experiment, mineral N in the soil at 0-50 and 50-100 mm depths was mainly present as NO_3^- in both the urea (Ur) and Ur+UI treatments (Figure 6.7). However, in the treatments where DCD was added to urea i.e., Ur+NI and Ur+UI+NI, mineral N was mainly present as NH_4^+ at 0-50 mm depth. The addition of DCD to the Ur and Ur+UI treatments increased the NH_4^+-N concentration at 0-50 mm depth significantly, from 10.9 mg N kg^{-1} (Ur) to 48.3 (Ur+NI) and 49.3 mg N kg^{-1} (Ur+UI+NI), respectively (Figure 6.7 a). There was also a significant ($P<0.05$) increase in the NH_4^+-N concentration at 50-100 mm depth from 1.69 in Ur to 3.28 in Ur+NI and 3.75 mg N kg^{-1} in Ur+UI+NI. The addition of DCD to urea (Ur+NI) decreased the NO_3^--N concentration at both 0-50 and 50-100 mm depths, from 53.5 and 24.5 mg kg^{-1} soil (Ur) to 18.8 and 10.5 mg kg^{-1} soil (Ur+NI), respectively (Figure 6.7 b). When DCD was added along with UI (Ur+UI+NI), the amount of NO_3^--N decreased from 41.0 and 21.5 mg kg^{-1} (Ur+UI) to 21.5 and 12.0 mg kg^{-1} at 0-50 and 50-100 mm depth, respectively. The NH_4^+-N and NO_3^--N concentrations at either 0-50 or 50-100 mm depths did not differ significantly in the Ur+UI and Ur treatments (Figure 6.7 a & b).

(a)



(b)

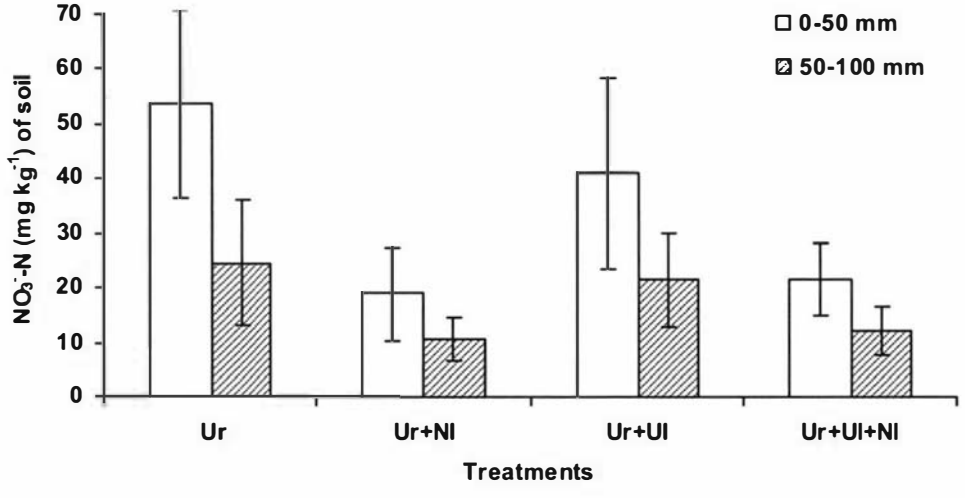
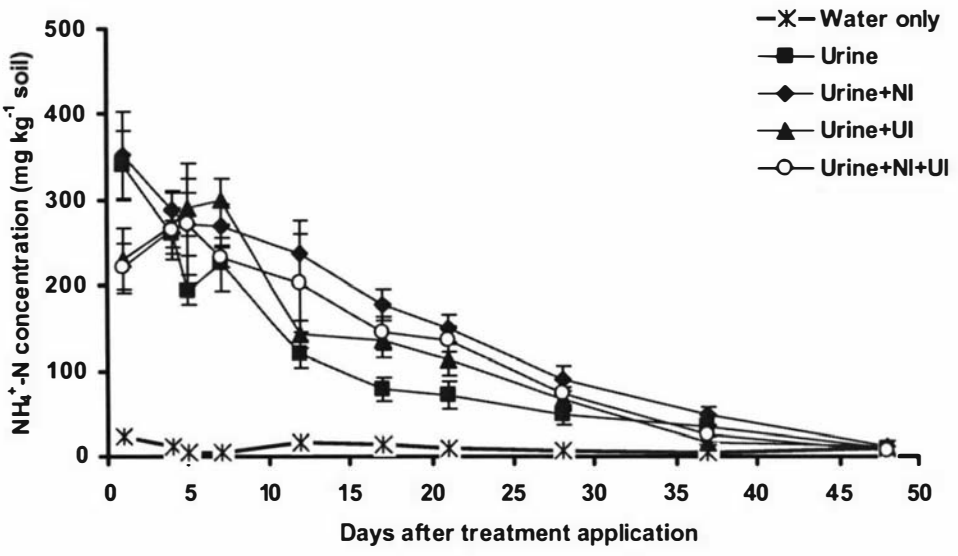


Figure 6.7 **Distribution of (a) NH_4^+ and (b) NO_3^- concentration at 0-50 mm and 50-100 mm depths in soil cores receiving urea, with both urease and nitrification inhibitors. Vertical bars denote standard deviation for the nine replicates**

In the field-plot study, NH_4^+-N and NO_3^-–N concentrations in the soil receiving water only were 23.4 and 29 mg N kg^{-1} soil at 0-50 mm depth and 19.6 and 25 mg N kg^{-1} soil at 50-100 mm depth, respectively, a few hours after treatment application (Figure 6.8, 6.9). The addition of urine (with and without inhibitors) increased the mineral N concentration of the soil at both depths significantly compared with the control treatment. The NH_4^+-N concentrations reached peak values within 24 hours of application of the urine (341 mg N kg^{-1} soil) and Urine+NI (352 mg N kg^{-1} soil) treatments at 0-50 mm depth and then decreased with time, reaching the background levels by the end of the experiment (Figure 6.8 a). In the Urine treatment a significant reduction ($P< 0.05$) in NH_4^+-N concentration occurred at 0-50 mm depth after the peak, where ca. 65% of the NH_4^+-N present on the first day of application disappeared rapidly during the first 12 days. The concentration then further decreased, and at the end of 50 days very little NH_4^+-N (10.0 mg kg^{-1} soil) was present at this depth. However, addition of DCD to urine (Urine+NI) was effective in inhibiting the transformation of NH_4^+ to NO_3^--N in the soil (Figure 6.9), thus keeping the NH_4^+-N concentration significantly higher than that from urine throughout the experiment (Figure 6.8). The net rate of change in NH_4^+-N concentration for the first 16 days was found to be higher for the Urine treatment (16.4 mg kg^{-1} soil d^{-1}) than that for the Urine+NI treatment (10.9 mg kg^{-1} soil d^{-1}). There was a similar treatment difference in the 50-100 mm layer, although NH_4^+-N levels were much lower than those at 0-50 mm depth (Figure 6.8 b). Application of Agrotain did not prevent the build up of NH_4^+-N in soil, although it did delay the time of peak concentration by 5 and 7 days in the Urine+UI and Urine+NI+UI treatments, respectively (Figure 6.8 a). The peak values obtained in these Urine+UI and Urine+NI+UI treatments were 299 and 273 mg NH_4^+-N kg^{-1} soil, respectively; values decreased with time and were at background levels by end of the experiment. The net rate of change of NH_4^+-N concentrations for the first 10-12 days was 16.2 and 10.7 mg kg^{-1} soil d^{-1}, respectively in the Urine+UI and Urine+NI+UI treatments.

(a)



(b)

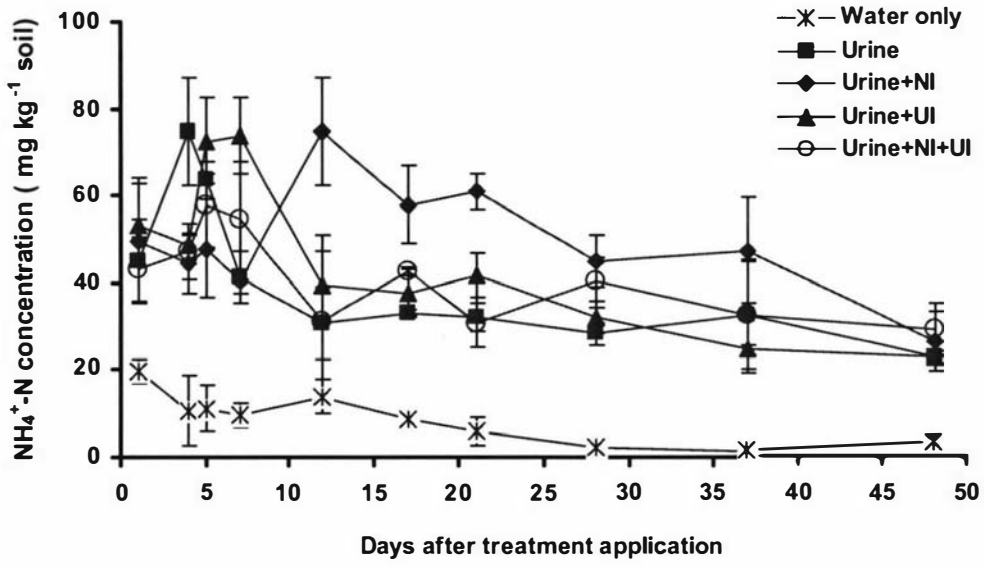
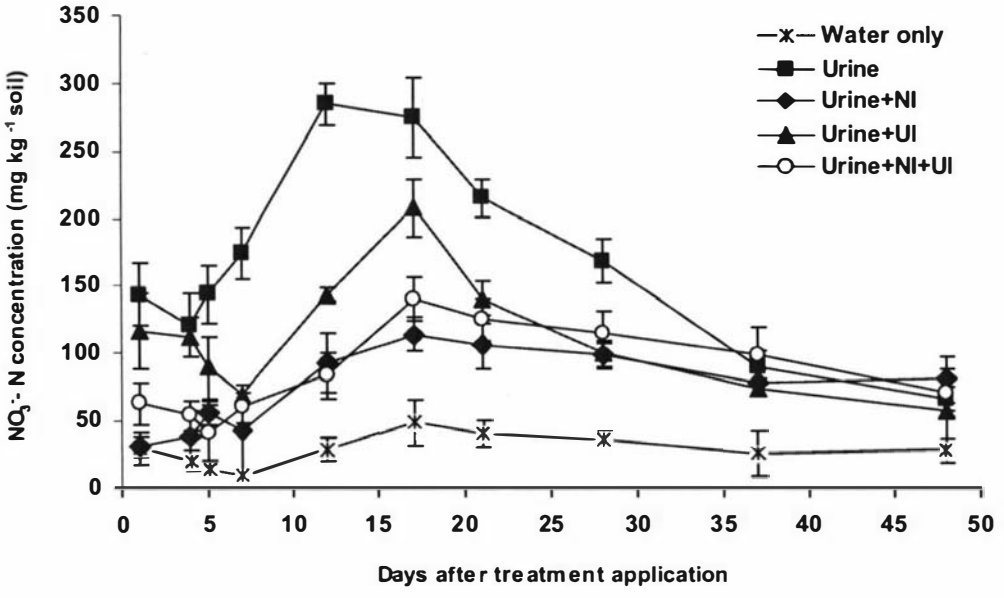


Figure 6.8 **Distribution of soil NH_4^+-N concentration at (a) 0-50 mm and (b) 50-100 mm depth following the application of urine, with and without urease and nitrification inhibitors, to pasture on Tokomaru silt loam. Each value represents a mean of three replicates with standard deviation shown by vertical bars.**

In all the treatments along with the control, a slow build-up of NO_3^--N was observed at both 0-50 and 50-100 mm depths, and then a decrease after reaching a peak value (Figure 6.9). The concentration of NO_3^--N in soil amended with urine alone increased progressively and a maximum of 294 mg N kg^{-1} soil was obtained after 12 days at 0-50 mm depth, whereas a peak of 92.3 mg N kg^{-1} soil was observed after 17 days at 50-100 mm depth. The concentration of NO_3^--N then decreased in both the layers, despite the continuing decrease in NH_4^+-N (Figure 6.8). The application of inhibitors with urine significantly affected the NO_3^--N concentration. The rate of nitrification, as measured from the increase in NO_3^--N, was found to be higher for the urine treatments without DCD than with DCD. Values at 0-50 mm soil depth for Urine alone and Urine+UI were 22.1 and 12.4 mg kg^{-1} d^{-1}, respectively and for Urine+NI and Urine+NI+UI were 5.02 and 6.54 mg kg^{-1} d^{-1}, respectively (Table 6.6). Similarly, at 50-100 mm soil depth, the rate of nitrification was highest for Urine (3.97 mg $kg^{-1}d^{-1}$) treatment. The addition of UI and NIs, both alone and in combination decreased the nitrification rate and the maximum reduction was seen in the Urine+NI (0.57 mg kg^{-1} d^{-1}) (Table 6.6). With the addition of Agrotain to urine (Urine+UI), the maximum NO_3^- concentration (207 mg N kg^{-1} soil) at 0-50 mm depth was reached only on the 17th day (Figure 6.9), and was significantly lower than that found in the Urine treatment; NO_3^--N then decreased progressively until the end of the experiment. Like NH_4^+-N, NO_3^- -N concentrations at both 0-50 and 50-100 mm soil depths in the Urine+UI treatment remained significantly higher than those in the control throughout the experiment. Nitrate concentrations at 50-100 mm soil depth in all the treatments showed similar trends to those at 0-50 mm depth, with the urine treatment having higher NO_3^--N concentrations than the treatments with inhibitors (Figure 6.9).

(a)



(b)

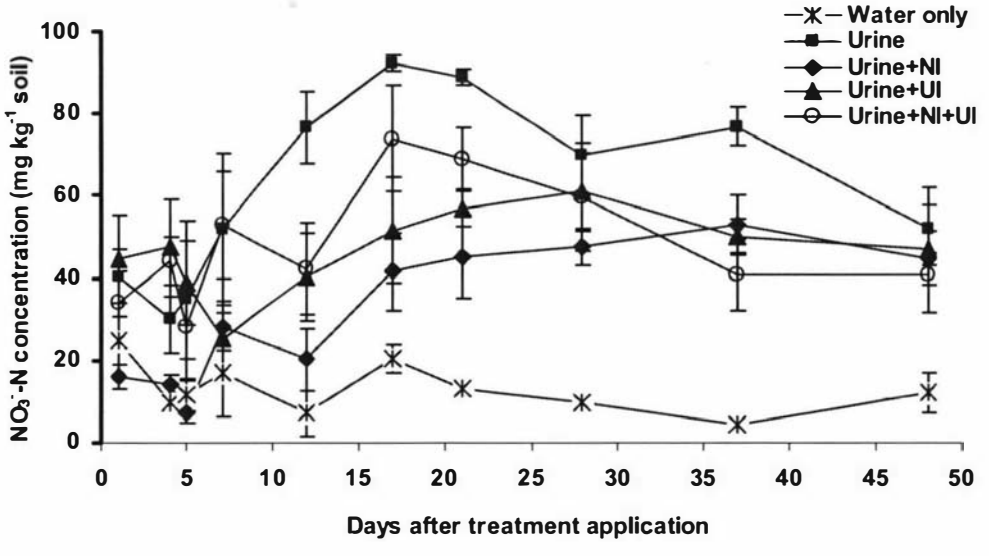


Figure 6.9 **Distribution of soil NO_3^--N concentration at (a) 0-50 mm and (b) 50-100 mm depth following the autumn application of urine, with and without urease and nitrification inhibitors to the pasture on Tokomaru silt loam. Each value represents a mean of three replicates with standard deviation shown by vertical bars.**

Table 6.6 Nitrification rate in various treatments in the Tokomaru silt loam soil in the field-plot study.

Treatments	0-50 mm			50-100 mm		
	Peak value of NO^-_3-N (mg kg^{-1})	Day for peak value	Nitrification rate (mg $kg^{-1}d^{-1}$)	Peak value of NO^-_3-N (mg kg^{-1})	Day for peak value	Nitrification rate (mg $kg^{-1}d^{-1}$)
Water only (Control)	48.5	17	1.15	20.5	17	-0.25
Urine	284	12	22.1	92.3	17	3.97
Urine+NI	114	17	5.02	53.0	37	0.76
Urine+UI	207	17	12.4	61.0	28	1.30
Urine+NI+UI	141	17	6.54	74.0	17	2.90

6.3.5 Dry matter yield and nitrogen uptake

The dry matter (DM) yield from the cores receiving urea with and without inhibitors in the glasshouse experiment is given in Table 6.7. The DM yield from the cores receiving urea alone (180 g m^{-2}) was the lowest among all the four treatments. Application of inhibitors individually (Ur+NI and Ur+UI) increased the grass dry matter production non-significantly to 181 and 202 g m^{-2}, respectively. The DM yield with the addition of the combined inhibitors (Ur+UI+NI) was 205 g m^{-2} and the increase of 13% over the Ur treatment was significant. The amount of N taken up by herbage followed the trend: Ur+UI+NI (5.54g N m^2) > Ur+UI (5.43 g N m^{-2}) > Ur (4.8 g N m^{-2}) > Ur+NI (4.73 g N m^{-2}); however, the treatment differences were not significant (Table 6.7).

Table 6.7 Total DM yield, percent N in DM and N uptake by the herbage from soil cores receiving urea with and without inhibitors

Treatment	N added ($g\ m^{-2}$)	Total DM ($g\ m^{-2}$)	N in DM (%)	N uptake ($g\ m^{-2}$)
Ur	10	180	2.66	4.80
Ur + NI	10	181	2.67	4.73
Ur + UI	10	202	2.68	5.43
Ur +UI+ NI	10	205	2.70	5.54
L.S.D. (0.05%)		23.5		0.90

In the field experiment, pasture was cut on three occasions on days 25, 50 and 75 after the application of the treatments. Over the three pasture growth periods, the combined (3 cuts) pasture dry matter yields were significantly higher in the plots receiving N as urine both with and without inhibitors, than in the control (3.89 t ha^{-1}) (Table 6.8). The highest herbage yield was obtained in the treatment with the combined inhibitors (6.52 t ha^{-1}) followed by the Urine+UI (6.11 t ha^{-1}), Urine+NI (5.97 t ha^{-1}) and Urine only (5.79 t ha^{-1}); however, the differences in yields were not significant. The dry matter yield responses per unit N added also followed a similar trend to those of DM with 3.17, 3.48, 3.71 and 4.41 kg DM yield kg^{-1} added N for the Urine, Urine+NI, Urine+UI and Urine+NI+UI treatments, respectively (Table 6.8). The N uptake by the herbage pasture was significantly increased by the urine application when compared to the control (124 kg N ha^{-1}). The highest N (309 kg N ha^{-1}) was taken up by pasture receiving urine with combined inhibitors (Urine+NI+UI), followed by 278, 277 and 268 kg N ha^{-1} in the Urine+NI, Urine+UI and Urine treatments, respectively.

Table 6.8 Total dry matter (DM) yield, percent of added N in DM and DM response to the added urine-N in autumn with and without urease and nitrification inhibitors

Treatment	Total DM (t ha^{-1})	N added (kg ha^{-1})	N in DM (%)	DM response (kg DM kg^{-1} N)
Water only (Control)	3.89	-	3.20	-
Urine	5.79	600	4.63	3.17
Urine + NI	5.97	600	4.66	3.48
Urine +UI	6.11	600	4.54	3.71
Urine+NI+UI	6.52	600	4.74	4.41
L.S.D (0.05%)	0.79			

6.3.6 Water-filled pore space

At the beginning of the field-plot study, conditions were very dry and the soil in all the plots was significantly below field capacity. This is reflected in the WFPS distribution at day 0 (Figure 6.10). With the application of the treatments (water for the control and urine), there was a small increase in WFPS in all the treatments. Water-filled pore space subsequently decreased until the next rainfall event 12 days after treatment applications (Figure 6.10). A total of 89.5 mm of precipitation was received during the experimental period of 50 days, with 56% of it in the last 30 days. The WFPS corresponded well with the rainfall events during the experiment and remained higher than that at field-capacity moisture content (WFPS=0.53) in all the treatments for the last 30 days of the experiment. There was no significant difference in the WFPS between the treatments (Figure 6.10).

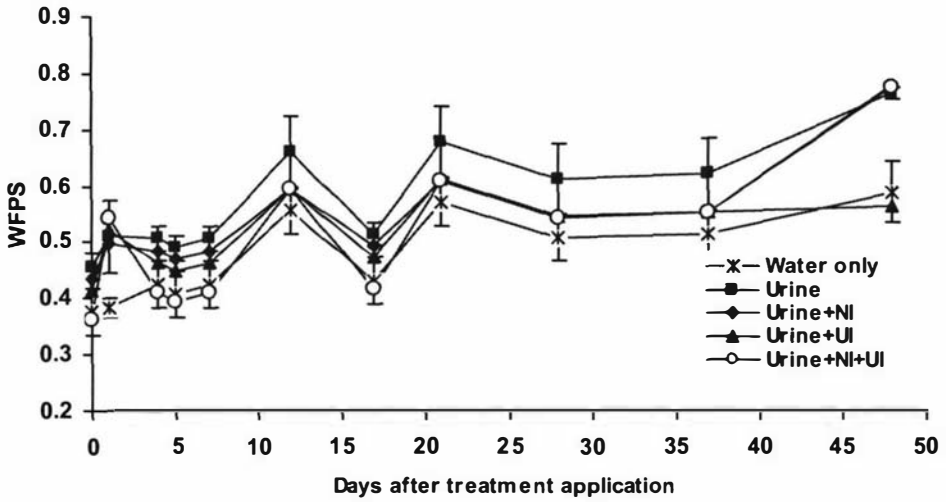


Figure 6.10 WFPS distribution (0-50 mm) for all the treatments in the field-plot study on Tokomaru silt loam .Each value represents a mean of three replicates with standard deviation shown by vertical bars

6.4 General discussion

The results obtained in both the glasshouse and field-plot study indicated that urease (Agrotain) and nitrification (DCD) inhibitors when applied together had a positive effect in reducing N losses from urine and urea fertiliser. The results suggested that: (1) application of Agrotain alone was an effective means of reducing NH_3 emissions; however, it proved to be ineffective in reducing N_2O emissions from both urea and urine; (2) the addition of DCD alone was effective in reducing N_2O emissions, but it increased NH_3 emissions from both urea and urine and increased NH_4^+-N concentrations in the soil; (3) the combined application of Agrotain and DCD was successful in reducing both NH_3 and N_2O emissions; (4) the combined application also caused more N uptake by the herbage, and thus an increase in herbage yield, as compared to application of these inhibitors alone. These results are discussed in detail in this section.

As indicated in earlier chapters, the urea N in urine and urea-based fertilisers undergoes hydrolysis by urease enzyme activity within a short period after application

to soils (from a few hours to few days duration); the NH_4^+ ions formed resulted in an increase in soil pH close to the zone of hydrolysis. This increase in pH with a corresponding increase in NH_4^+ ions is likely to have induced NH_3 volatilisation. In the field experiment, after a few hours from the urine application, there was an increase in soil pH from 5.50 to 6.30 (Figure 6.11) and NH_4^+-N showed a peak concentration at 0-50 mm depth (Figure 6.8 a). Vallis *et al.* (1982) similarly found that 80% of urea in urine voided onto pasture was hydrolysed in 2 hrs as a result of the urease enzyme in soil or plant residues. This increase in pH and high NH_4^+ concentration are prerequisites for NH_3 volatilisation and resulted in high losses of NH_3 both from urea in the glasshouse experiment and urine in the field experiment. The peak NH_4^+ concentration decreased rapidly after day 1 when urine was applied in the field-plot study. However, with the addition of NI to urine, the rate of nitrification of soil NH_4^+-N was reduced, and thus comparatively high NH_4^+-N concentrations were maintained for a longer period (in excess of 50 days); an increase in NH_3 volatilisation loss thereby resulted. Added DCD also kept the soil pH higher for a longer time than in the Urine-only treatment (Figure 6.11) and resulted in increased NH_3 volatilisation. This result differs from the results reported in Chapter 5 which showed no significant increase in NH_3 volatilisation when DCD was added to different levels of urine. This might be due to the fact the soil cores in glasshouse study (Chapter 5) were maintained at field-capacity moisture content. The urine was consequently more deeply distributed down the core than in the soil in the current field experiment, in which the soil was quite dry at the time of urine application.

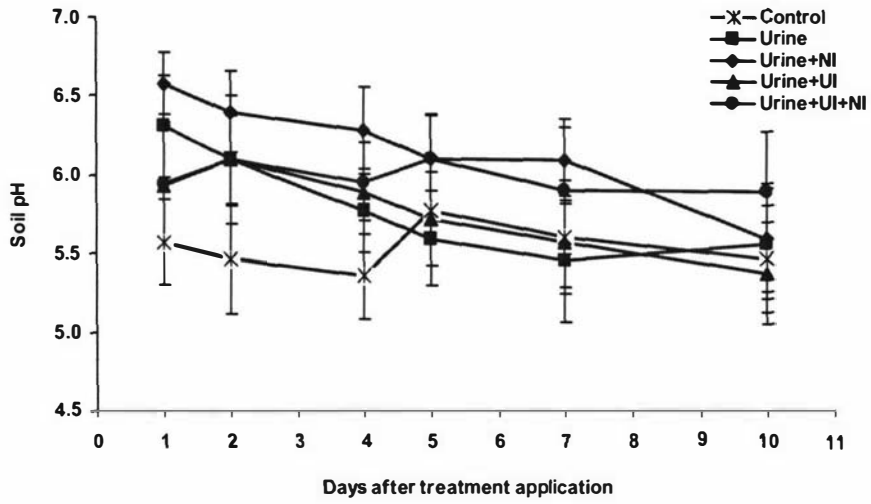


Figure 6.11 pH distribution (0-50 mm) in Tokomaru silt loam with application of water and urine, with and without N inhibitors. Each value represents a mean of four replicates with standard deviation shown by vertical bars.

The addition of UI inhibited urea hydrolysis and thus delayed the peak NH_4^+-N concentration in soil by 2 days; this helped urea to diffuse away from the surface, thereby reducing NH_3 volatilisation. The reduction in NH_3 volatilisation was greater in the glasshouse experiment than in the field-plot study, in spite of the fact that urea based N sources were surface applied in the former experiment. This could be attributed to the coating of urea granules with Agrotain, enabling the inhibitor to remain close to the zone of high N concentration. Furthermore, in the field-plot experiment, no rainfall occurred for the first five days (Figure 6.1). In the glasshouse experiment, water was added every alternate day to the soil cores to maintain field-capacity moisture content, and would have assisted movement of the urea down the soil depth. The efficiency of NBPT has been found to be negatively correlated with organic C and clay content, and positively correlated with the sand content of the soil (Bremner & Chai 1986; Watson *et al.* 1994a). This may explain the lower effectiveness of NBPT in the field-plot study in which soil was the silt loam compared to the sandy loam in the glasshouse experiment. The lighter texture of the sandy loam soil probably allowed a greater diffusion of the unhydrolysed urea within the soil (Christianson *et al.* 1993; Zhengping *et al.* 1996), and hydrolysis would have taken place below the soil surface, resulting in very low NH_3 losses. However, UI was effective in the field in inhibiting urea hydrolysis, as shown

by the soil NH_4^+-N concentration being lower than in the Urine only treatment within 24 hours of application. Nitrification inhibitor in the Urine+NI+UI treatment kept N in the NH_4^+ form for a longer period than in the Urine+UI treatment, but as the NH_4^+ ions produced were probably below the soil surface this increase in NH_4^+-N did not cause a significant increase in NH_3 volatilisation.

The nitrification of NH_4^+ to NO_3^--N following the excretion of urine in pasture soils usually occurs within 14-60 days (Field *et al.* 1985; Shand *et al.* 2000; Whitehead 1995; Williams *et al.* 1999; Williams & Haynes 1994), depending on soil temperature and moisture content. A similar time frame was also observed in our study where soil NO_3^--N levels in the urine treatment peaked on day 12, coinciding with a decline in soil NH_4^+-N concentrations (Figure 6.9). The addition of DCD slowed down the nitrification process. These results are similar to those found previously for liquid DCD applied to a urine patch (Di & Cameron, 2002b, 2004a; Smith *et al.*, 2005) and effluent (Williamson *et al.*, 1996). In these studies, the DCD was effective in delaying nitrification of NH_4^+ to NO_3^--N for upto 99 days when applied to effluent under field-plot study (Williamson *et al.,* 1996) and for 60-110 days after application to cattle urine (Cookson & Cornforth 2002 and Smith *et al.,* 2005). As the rate of DCD is known to influence both its longevity and effectiveness in inhibiting nitrification in soil (Williamson *et al.* 1996; Puttanna *et al.* 1999), the DCD application rate needs to be taken into account when comparing the results of this study with others. The rate of DCD application was lower in the present study (7 kg DCD ha^{-1}) than in other studies reported in the literature (12 - 15 kg DCD ha^{-1} - Williamson *et al.* 1996; Williamson *et al.* 1998; Di & Cameron 2002b, 2004c); 10 - 30 kg DCD ha^{-1} - Cookson & Cornforth, 2002; Smith *et al.,* 2005). Even at this low DCD application rate, there was significantly ($P<0.05$) more NH_4^+-N, and consequently less NO_3^--N, in the plots receiving DCD than those not receiving DCD (Urine and Urine+UI). Thus, DCD limited the substrate pool for N_2O production, directly by reducing nitrification and indirectly by reducing the availability of NO_3^--N for denitrification. The N_2O fluxes in both the urea and urine applications with the addition of Agrotain were not substantially different from those in the urea and urine-alone treatments, although there was a small increase in N_2O emissions. Urease inhibitor proved ineffective as a means of reducing N_2O emissions. The rationale for its use to reduce N_2O emission was that slower

hydrolysis of urea to NH_4^+, together with the uptake of NH_4^+ by grass, would result in a generally reduced concentration of NH_4^+ in the soil. Thus, lower NH_4^+ ions would undergo potential nitrification and denitrification. However, this effect on NO_3^--N was not translated effectively to a reduction in N_2O emissions.

The combined application of UI and NI was effective in reducing N_2O emissions by 50% in both the urea and urine applications. Although DCD enhanced soil NH_4^+ concentrations when added with a N source, its combination with Agrotain effectively regulated the concentration of NH_4^+ and NO_3^- in the soil, reducing both nitrification-denitrification losses and thus causing a reduction in N_2O emissions. This is in agreement with the results of Xu *et al.* (2000) who found that hydroquinone (a urease inhibitor) and DCD decreased N_2O emissions by 25% in plots fertilized with urea. These reductions in N_2O emission were much higher than the reductions caused by DCD alone.

Under well-drained conditions in both the experiments, the influence of UI on the hydrolysis of the applied urea was short-lived (Zhao *et al.* 1992) and for this reason there was not any difference in soil NH_4^+-N concentrations in the Ur and Ur+UI treatments after 36 days. However, the combination of UI with NI gave the highest NH_4^+-N concentration in the soil at the end of the glasshouse experiment. Similarly, the rate of decrease in NH_4^+-N concentration was lowest in the Urine+NI+UI treatments in the field-plot study. Thus, under these experimental conditions, there was a synergistic effect of Agrotain and DCD on the retention of exchangeable NH_4^+-N in soil after an application of N source. In comparison with the urea and urine-only treatments, the soil samples treated with inhibitors in both experiments had a lower concentration of NO_3^--N, with the lowest values in the treatments with NI (Ur+NI, Urine+NI) and combined UI and NI (Ur+UI+NI and Urine+NI+UI). Xu *et al.* (2001) found a synergistic effect of DCD and hydroquinone (UI) on urea-^{15}N transformations and recovery of fertiliser ^{15}N in the soil after the application of urea.

Application of UI+NI together to urine induced an increase in N uptake by plants and even increased the pasture yield, as compared to NI and UI added singly to urine. An increase in the fertiliser-derived N taken up by the plants in the presence of both urease and nitrification inhibitors has also been found by Soliman & Monem (1996) in a rice crop and Xu *et al.* (2001) in a wheat crop.

The DCD half life of 28 days at 12°C obtained in the present study is almost similar to that found in other studies e.g., a half-life of 22 days at 20°C with an application of 33μg DCD-N /g soil (Rajbanshi *et al.* 1992) and 39 days at 22°C for 60 μg DCD-N /g soil (Williamson *et al.,* 1996). Di & Cameron (2004a) reported the half-lives of DCD at 8°C and 20° C to be 111-116 days and 18-25 days respectively when DCD was applied @ 7.5 and 15 kg ha^{-1}. Clearly, soil temperature and soil type are important factors in determining the rate of degradation of DCD in soil. No lag phase in the degradation of DCD at a temperature of ca. 12°C was observed in the present study. According to Williamson *et al.,* (1996), the threshold temperature above which a lag phase was not detected occurred between 13 and 16° C. In the field-plot study, it was possible that a short lag phase had occurred at the temperature found in field soil, but was undetected because of the long time span between the addition of DCD and soil sampling, and also because of the low recovery of added DCD in the soil. DCD has mostly been applied at rates of 10-30 kg ha^{-1} (Amberger & Germannbauer, 1990; Rodgers *et al.*, 1985; Williamson *et al.*, 1998). However, the present study has shown that the application rate of 7.5 kg ha^{-1} is also effective in reducing nitrification when N concentrations of urine-N are high (600 kg N ha^{-1}) under soil temperatures ranging from 8 to 15°C.

6.5 Conclusions

- The combination of urease and nitrification inhibitors retarded NH_3 emissions from pasture soil following the application of urea or urine, as compared to the application of NI (DCD) alone, which resulted in an increase in NH_3 emissions.
- There was a considerable decrease in N_2O emissions from applied urea and urine when combined inhibitors were used. The use of UI (Agrotain) alone was ineffective in reducing N_2O emissions.
- There was an increase in herbage yield and N uptake using combined inhibitors as compared to separate applications of individual inhibitors.

These studies indicate that there is scope for reducing NH_3 and N_2O gas emissions from urea and urine applied to pasture by using a combination of UI and NI.

Chapter 7

Degradation kinetics of dicyandiamide in four soils and its effect on nitrous oxide emission – an incubation study

7.1 Introduction

In recent decades, DCD has received increasing attention and gradually has become the most extensively used nitrification inhibitor (NI) because it is highly effective in inhibiting nitrification, non-volatile and completely decomposed into CO_2 and NH_4^+ (Trenkel 1997). However, the effectiveness of DCD in reducing N losses as nitrous oxide (N_2O) emission and nitrate (NO_3^-) leaching is quite variable (Davies & Williams, 1995; Merino *et al.* 2001; Prasad & Power, 1995) and depends on several environmental and soil conditions. Most studies involving DCD have focussed on its value in enhancing fertilizer and urine N-use efficiency (Di & Cameron 2002b, 2003, 2004c; Zhu 1992), but little research has been conducted on its fate in different soil types. As DCD is degraded by micro-organisms (e.g., *Rhodococcus* sp., *Pseudomonas* sp.) (Hallinger *et al.* 1990), factors affecting microbial activity are likely to influence DCD degradation.

The bioactivity and persistence of DCD as measured by its half-life decreases with an increase in soil temperature (Guiraud *et al.* 1989); Di & Cameron 2004a) and organic matter content of the soil (Amberger 1986; Sahrawat *et al.* 1987), and increases with an increase in the concentration of DCD applied (Puttanna *et al.* 1999). The effect of temperature on the persistence of DCD in soils is well established. For example, Williamson *et al.* (1996) observed that the rate of degradation of DCD was very low at a temperature less than 15°C. Similarly, studies in New Zealand have shown the half-live of DCD to be 111-116 days at 8°C and 18-25 days at 20°C in a silt loam soil when DCD

was applied @ 7.5-15 kg DCD ha^{-1} (Di & Cameron 2004a). Degradation studies of DCD in a loamy sand found half lives of 14.7 days at 22°C and 52.2 days at 8°C when DCD was applied @ 30 mg DCD kg^{-1} soil (Bronson *et al.* 1989). Clearly, soil type and the amount of DCD applied are important factors controlling the degradation of DCD. However, the optimum application rate of DCD required for the inhibition of nitrification under different soil and climatic conditions is not clear. A review of the literature indicates that various levels of DCD have been tested to achieve inhibition of nitrification. For example, Reddy (1964) and Rao & Puttanna (1987) found that inhibition of nitrification was effective at a DCD application rate of 25 and 15-20 mg kg^{-1} soil, respectively. McCarty & Bremner (1989) obtained nitrification inhibition in three soils for 21 days using 10 mg DCD kg^{-1} soil, and Di & Cameron (2004c) found that DCD applied even at the low rate of 7.5 mg kg^{-1} (7.5 kg ha^{-1}) was effective in nitrification inhibition. The work done in New Zealand on DCD degradation by Di & Cameron (2002b, 2003, 2004c) is limited to only one soil type. Moreover, it is also not clear that what effect DCD application with urine will have on the general microbial population in the soil, apart from reducing the activity of nitrifier bacteria. Thus the present study was conducted using four soil types with different concentrations in organic matter content, texture and mineralogy, two rates of DCD (10 and 20 kg DCD ha^{-1}), and one rate of urine (600 mg N kg^{-1}) application at a constant temperature; specific objectives were:

1. To quantify the rate of degradation of DCD in four contrasting soils.
2. To study the influence of soil type on DCD effectiveness in inhibiting nitrification, and thus affecting N_2O emissions.
3. To assess the effect of DCD application on microbial activity as measured by microbial biomass and respiration in four contrasting soils.

7.2 Materials and Methods

7.2.1 Soil sampling and preparation

Four pasture soils under intensive dairy farming from North Island (Tokomaru silt loam, Manawatu sandy loam, Egmont brown loam and Horotiu silt loam) which varied in organic matter (C and N concentration), texture and mineralogy were selected (Table 7.1). Bulk samples (0-10 cm depth) were collected with a shovel from three locations on each site. Field moist soils were sieved through a 2-mm sieve. Soil moisture contents were adjusted to -10 kPa pressure (taken as field capacity moisture content) 4 kg soil were pre-incubated for 4 weeks at 25^0C.

Table 7.1 Selected properties of the soils sampled at 0-10 cm depth

	Tokomaru silt loam	Manawatu sandy loam	Egmont brown loam	Horotiu silt loam
	Non-allophanic		Allophanic	
Soil type	Argillic fragic Perch-gley Pallic	Dystric Fluventic Eutrochrept	Typic Dystrandept	Typic Orthic Allophanic Soil
pH	5.62	5.25	5.64	5.95
CEC ($cmol_c\ kg^{-1}$)	22.3	10.0	27.1	35.6
Total C (%)	3.07	3.91	8.47	6.59
Total N (%)	0.29	0.40	0.81	0.57
Sand (%)	8.5	62.5	55.0	30.5
Silt (%)	68.4	25.3	22.8	52
Clay (%)	23.0	12	22.0	17

7.2.2 Experimental set-up

Four treatments with three replications each were used in this experiment. These included: (i) water only (control), (ii) 600 mg N kg^{-1} as dairy cow urine, (iii) 600 mg N kg^{-1} as dairy cow urine plus 10 mg DCD kg^{-1} soil and (iv) 600 mg N kg^{-1} as dairy cow urine plus 20 mg DCD kg^{-1}. These treatments are, hereafter, referred to as control, urine only, urine+DCD1 and urine+DCD2, respectively. To calculate the application rate of treatments, the bulk density of all the soils was assumed to be 1 Mg m^{-3}. Fresh

urine was collected from lactating dairy cows at Massey University No. 4 dairy farm and the DCD was dissolved in water before applying to the soils.

The urine-N rate of 600 mg N kg^{-1} was used to simulate the N loading rate under a typical dairy cow urine patch in a grazed pasture soil. The two DCD rates were designed to test whether these are sufficient to inhibit nitrification in all the soils at 25°C.

Samples of each soil (1kg oven-dry equivalent) were weighed into polythene bags and the treated soils were incubated at 25°C. The soil moisture content was maintained near 80% field capacity moisture content by monitoring the weight changes of each soil sample and adding water to it. Changes in DCD, NH_4^+-N, NO_3^--N concentrations over time in the soil were used to measure the effectiveness of DCD in inhibiting nitrification.

Influence of DCD on N_2O and CO_2 emissions from different soils

A parallel incubation study was conducted for 58 days to measure the N_2O and CO_2 emissions from these soils receiving the same treatments, where 80 g of soil (oven-dry equivalent) was weighed into a urine cup (125 ml), treated and placed in Agee jars of 1.8 l volume. The jars were sealed with air-tight screw-cap lids having a rubber septum in them. The gas samples for both N_2O and CO_2 were taken after closing the jars for 24 hours. After every gas measurement, jars were left open to equilibrate with the ambient atmosphere and water was added, if required, to maintain the soil moisture constant at 80% field capacity. The gas samples were taken daily for the first week, followed by every alternate day for the second week and then twice a week for the third week. Subsequent measurements were taken once a week for four more weeks. The gas samples were analysed using a Shimadzu GC – 17A gas chromatograph with a 63Ni-Electron capture detector, and N_2O and CO_2 fluxes (mg kg^{-1} soil d^{-1}) were then calculated using the equation of Mosier & Mack (1980) given in Chapter 3. Cumulative fluxes were calculated from the area under the curve relating daily fluxes to the measurement period.

7.2.3 Soil analysis

7.2.3.1 DCD analysis

Sub samples of 10g (oven dry equivalent) soil were taken from each bag on 0, 1, 3, 6, 16, 23, 31 and 40 days of incubation and were extracted with deionised water (1:2 soil to water ratio) with 1 hr shaking, followed by centrifugation and filtration (Toyo 5C filter paper). The extracts were analysed for DCD concentration using a colorimetric method at 540 nm (Vilsmeier, 1982).

7.2.3.2 Mineral nitrogen, soil pH and soil texture

Sub samples of 5 g (oven dry equivalent) were taken on 1, 3, 6, 16, 31 and 40 days of incubation from each replicate to determine the concentration of NH_4^+ and NO_3^- -N, as discussed in Section 4.2.4.2. Soil pH was measured on 1, 3, 5, 12, 19, and 40 days after incubation at a 1:2.5 soil: water ratio using a combined electrode pH meter (Blakemore *et al.* 1987).

Determination of particle size-fractions (sand, silt and clay) was carried out following the hydrometer method (Bouyoucos 1962).

Total C and N in the soil were measured by combustion in a Leco FP-2000 CNS (LECO Corp., St Joseph, MI, USA).

7.2.3.3 Microbial biomass carbon

Soil microbial biomass C (Vance *et al.* 1987) was determined on 1, 3, 6, 16, 31 and 40 days of incubation by using duplicate sub-samples of 5 g (oven dry equivalent) from each replicate. This method is discussed in detail by (Ross 1992). In brief, one-half of the above mentioned soil samples was chloroform fumigated. Both the fumigated and unfumigated soil samples were extracted with 0.05 M K_2SO_4. Carbon in the subsequent filtrates was determined with a high TOC II (total organic carbon) analyser. Microbial-C flush was calculated as the difference between the amount of C extracted from the fumigated and unfumigated samples and converted to microbial biomass C using the relationship: microbial C = C flush/0.41 (Sparling & West 1988).

7.2.4 Estimation of nitrification inhibition index

A nitrification inhibition index was calculated at sampling days 1, 3, 6, 16, 31 and 40 to indicate the effectiveness of DCD, using the following formula (McCarty & Bremner 1989):

$$(C-T)/C \times 100 \quad \textbf{(7.1)}$$

where T and C are the concentrations of NO^-_3-N (urine treatments) in the presence and absence of DCD (NO_3^--N in the control treatment was subtracted from both T and C), respectively.

7.2.5 Dicyandiamide half-life

The following first-order equation was used to calculate the degradation rate of DCD from the data obtained over an incubation period of (40 days):

$$N = N_0 \exp(-kt) \quad \textbf{(7.2)}$$

where N *is* the amount of DCD remaining in the soil at time (t), N_0 is the amount of DCD recovered at the beginning of the experiment in the soil and k is the decay constant. The DCD half-life ($t_{½}$) was calculated as discussed in Section 6.2.6 of Chapter 6.

7.2.6 Statistical analysis

The mean values of DCD, NH_4^+ and NO_3^--N, soil pH and soil microbial biomass C were calculated from three replicates of each treatment, and least significant difference (l.s.d.) values were calculated following analysis of variance using SAS version 8. The 5% confidence level is regarded as statistically different. An analysis of variance for N_2O and CO_2 emissions and regression analysis for the degradation rate of DCD was also carried out using SAS package.

7.3 Results

7.3.1 Degradation of DCD

Recovery of DCD at day 0 in the four soils varied between 80 and 100%. The Tokomaru and Manawatu soils showed a higher recovery of 98% and 100%, as compared to the 92% in the Egmont soils and 80% in the Horotiu soil. The soil-extract colour was observed to be darker in the high organic-C allophanic soils (Egmont and Horotiu soils) as compared to the other two soils. The concentration of DCD in all the soils, except Horotiu, decreased rapidly with time and the amount remaining in the soil became negligible by 40 days of incubation at both application rates (Figure 7.1). The DCD applied at the higher rate maintained a higher DCD concentration in all the four soils throughout the study period. The $t_{½}$ (time taken by DCD to reduce to half of initial values) varied among the soils (Table 7.2). The $t_{½}$ was higher ($P< 0.05$) with the application of 20 mg DCD kg^{-1} soil than with 10 mg DCD kg^{-1} soil in all the soils except Horotiu soil. The highest DCD $t_{½}$ of 31.9 (DCD2) and 38.7 (DCD1) days were obtained in the Horotiu soil and the lowest (6.56 and 10.7 days) in the Egmont soil. The change in the concentration of DCD with time in soils varied with both the soil type and rate of DCD applied.

Table 7.2 Half-life ($t_{½}$) of DCD in four soils following the application of urine (600 mg N kg^{-1} soil) and DCD at the rates of 10 and 20 mg DCD kg^{-1} soil

Soil	DCD application rate (mg kg^{-1} soil)	Degradation rate constant (day^{-1})	Half-life (days)	R^2
Tokomaru soil	10	0.0691 ± 0.008	10.0 ± 1.18	0.87
	20	0.0446 ± 0.003	15.5 ± 1.05	0.97
Manawatu soil	10	0.0707 ± 0.006	9.80 ± 0.84	0.93
	20	0.0526 ± 0.003	13.2 ± 0.75	0.99
Egmont soil	10	0.1056 ± 0.011	6.56 ± 0.69	0.91
	20	0.0646 ± 0.003	10.7 ± 0.50	0.99
Horotiu soil	10	0.0179 ± 0.002	38.7 ± 4.38	0.88
	20	0.0217 ± 0.001	31.9 ± 1.47	0.96

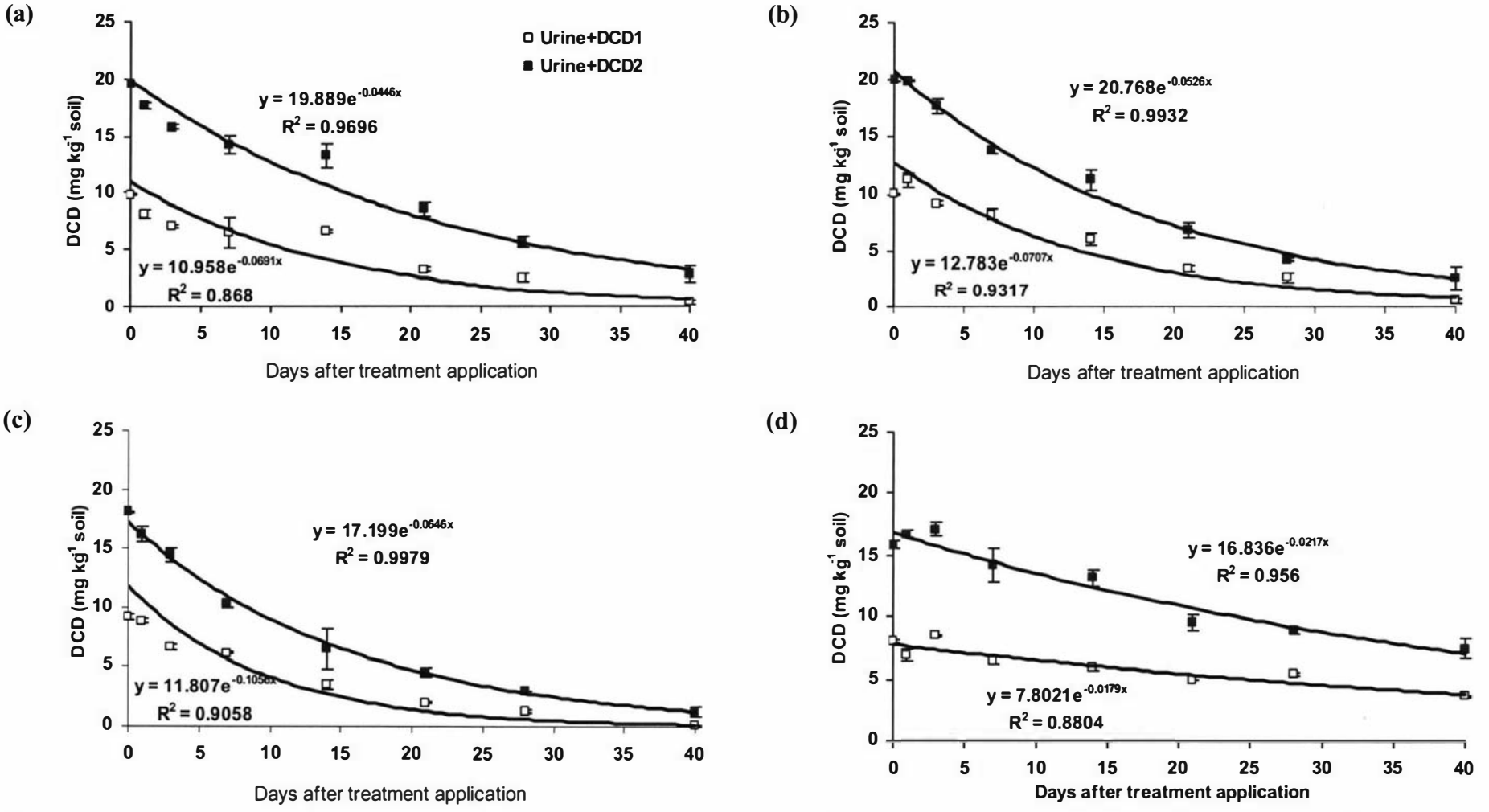


Figure 7.1 **Mean DCD concentration in (a) Tokomaru silt loam (b) Manawatu sandy loam (c) Egmont brown loam (d) Horotiu silt loam following the application of urine with DCD applied at 10 and 20 kg ha^{-1}. Error bars represent the standard deviation of the three replicates.**

7.3.2 Effect of DCD

7.3.2.1 Nitrous oxide emission

Application of urine resulted in sharp increases in N_2O emission in all the four soils with the peak emissions attained within a day. The emissions decreased sharply on the next day (Figure 7.2). The highest N_2O emission peak was measured in the Manawatu soil (2.52 mg N $kg^{-1}d^{-1}$ soil), followed by the Tokomaru soil (2.11 mg N $kg^{-1}d^{-1}$ soil), Egmont soil (0.728 mg N $kg^{-1}d^{-1}$ soil) and Horotiu soil (0.211 mg N $kg^{-1}d^{-1}$ soil). However, another peak of 0.471 mg N $kg^{-1}d^{-1}$ soil was observed in the Horotiu soil on day 12. In the Tokomaru soil, a second smaller peak (0.39 mg N kg^{-1} soil) was observed on day 17 (Figure 7.2). The total N_2O emitted over the incubation period of 58 days after urine application was highest in Tokomaru soil (11.2 mg N kg^{-1} soil), followed by Egmont (10.3 mg N kg^{-1} soil), Manawatu (8.13 mg N kg^{-1} soil) and Horotiu (7.04 mg N kg^{-1} soil) soils. Addition of DCD at both rates significantly reduced peak emissions in all the soils, and also maintained lower N_2O emissions than those from the urine-alone treatment throughout the incubation period. Maximum emissions reduction (85 and 90%) were obtained in Tokomaru soil, with cumulative N_2O emissions reduced from 11.2 to 1.93 and 1.34 mg N kg^{-1} soil with the addition of DCD1 and DCD2, respectively (Table 7.3). In Horotiu soil, emission reductions were 60 and 63% and in Manawatu soil 56 and 57%, with DCD1 and DCD2, respectively. The lowest reductions of 42 and 45% were observed in Egmont soil. Although, a higher reduction of N_2O emission was obtained when DCD was added at the higher rate (DCD2), to all four soils, the difference in N_2O reduction between the DCD2 and DCD1 rates was not significant. The N_2O emission flux in the control treatments of all the soils remained almost constant throughout the incubation period, with cumulative N_2O emissions ranging from 0.14 to 0.39 mg N kg^{-1} soil (Table 7.3).

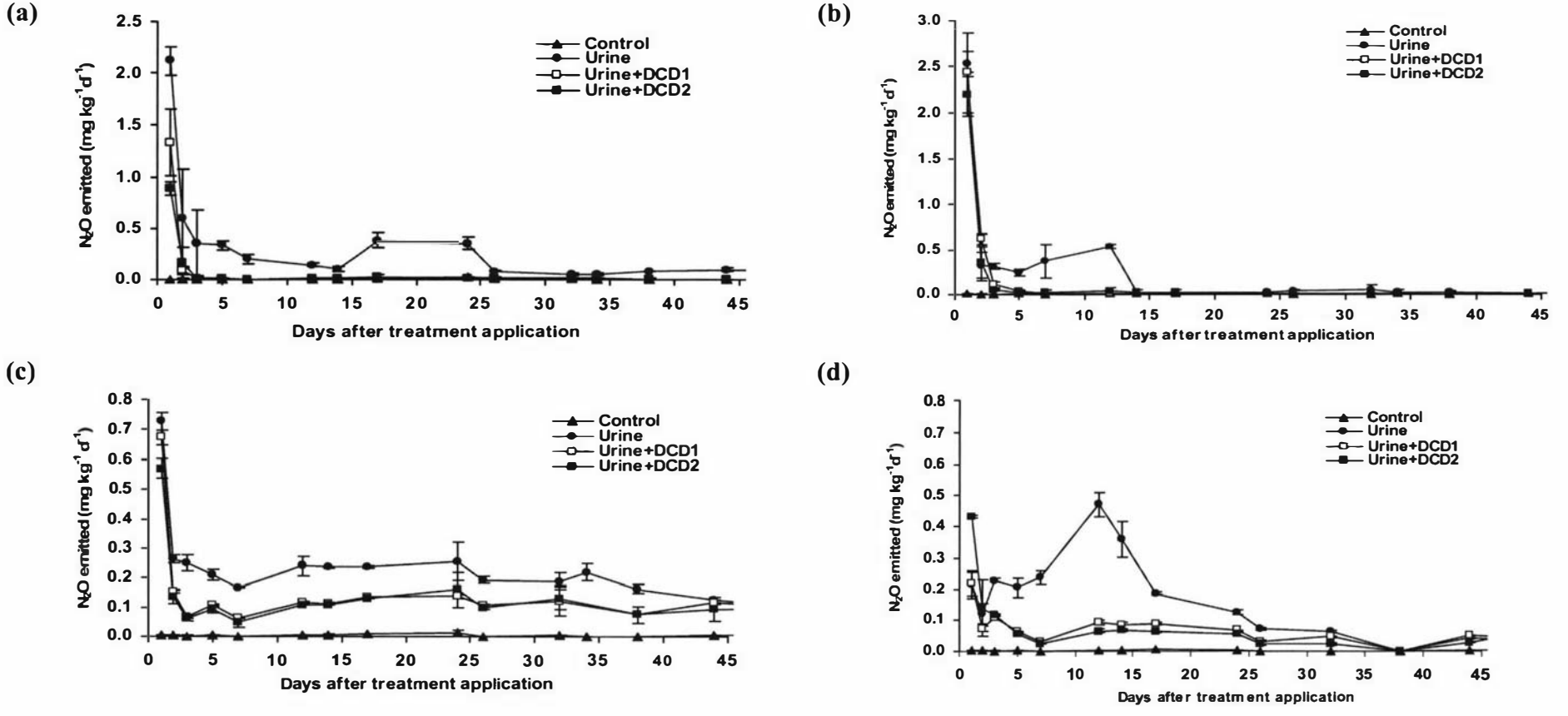


Figure 7.2 **Nitrous oxide losses for various treatments from (a) Tokomaru silt loam (b) Manawatu sandy loam (c) Egmont brown loam (d) Horotiu silt loam during the incubation period (note the scales for the y-axis). Each value represents a mean of three replicates with standard deviation shown by vertical bars.**

Table 7.3 Total N emitted as N_2O-N (mg N kg^{-1} soil) for the four soils over the incubation period of 50 days

Treatment	Total N_2O-N (mg kg^{-1} soil)	% reduction in N_2O-N with DCD
Tokomaru silt loam		
Control	0.27 c	-
Urine only	11.2 a	-
Urine+DCD1	1.93 b	85 %
Urine+DCD2	1.34 bc	90 %
L.S.D. (0.05%)	1.14	
Manawatu sandy loam		
Control	0.39 c*	-
Urine only	8.13a	-
Urine+DCD1	3.75 b	56 %
Urine+DCD2	3.71 b	57 %
L.S.D. (0.05%)	1.26	
Egmont brown loam		
Control	0.22 c	-
Urine only	10.3 a	-
Urine+DCD1	6.10 b	42 %
Urine+DCD2	5.80 b	45 %
L.S.D. (0.05%)	1.64	
Horotiu silt loam		
Control	0.14 c	-
Urine only	7.04 a	-
Urine+DCD1	2.87 b	60 %
Urine+DCD2	2.67 b	63 %
L.S.D. (0.05%)	0.54	

* Values followed by the same letter under one soil type do not differ significantly at the 0.05 level

7.3.2.2 Carbon-dioxide emission

In all the four soils, the amount of CO_2 respired was least in the control treatment, and highest in the urine treatments (Figure 7.3). Addition of DCD to urine resulted in a slight reduction of total CO_2-C respired in all the soils, although this reduction was not significant. The basal CO_2 respired in the control treatment during the 58 day incubation was highest in Manawatu soil (900 mg C kg^{-1}soil), followed by Egmont soil (750 mg C kg^{-1}soil), Tokomaru soil (622 mg C kg^{-1}soil) and Horotiu soil (567 mg C kg^{-1}soil). The CO_2 respired from urine only, urine+DCD1 and urine+DCD2 was, respectively, 1604, 1261 and 1209 mg kg^{-1} in Tokomaru soil, 1592,1428 and 1340 mg kg^{-1} in Manawatu soil, 1365, 1271 and 1174 mg kg^{-1} in Egmont soil and 1022, 995 and 747 mg kg^{-1} in Horotiu soil (Figure 7.3). The CO_2 evolution peaked within a day of application of the urine and urine plus DCD treatments in all the four soils and emissions were significantly higher than those in the control (water only) soils (Appendix 2). The highest peak was 454 mg CO_2-C kg^{-1} soil d^{-1} in both the Tokomaru and Manawatu soils, followed by 328 mg CO_2-C kg^{-1} soil d^{-1} in Egmont soil and 307 mg CO_2-C kg^{-1} soil d^{-1} in Horotiu soil (Appendix 2). These emissions declined sharply in all the urine-treated soils and were similar to those in the control soils after 5 to 7 days incubation. The highest increase in CO_2 emissions with urine application was observed in the Tokomaru soil, with a 2-fold increase over the control (Figure 7.3).

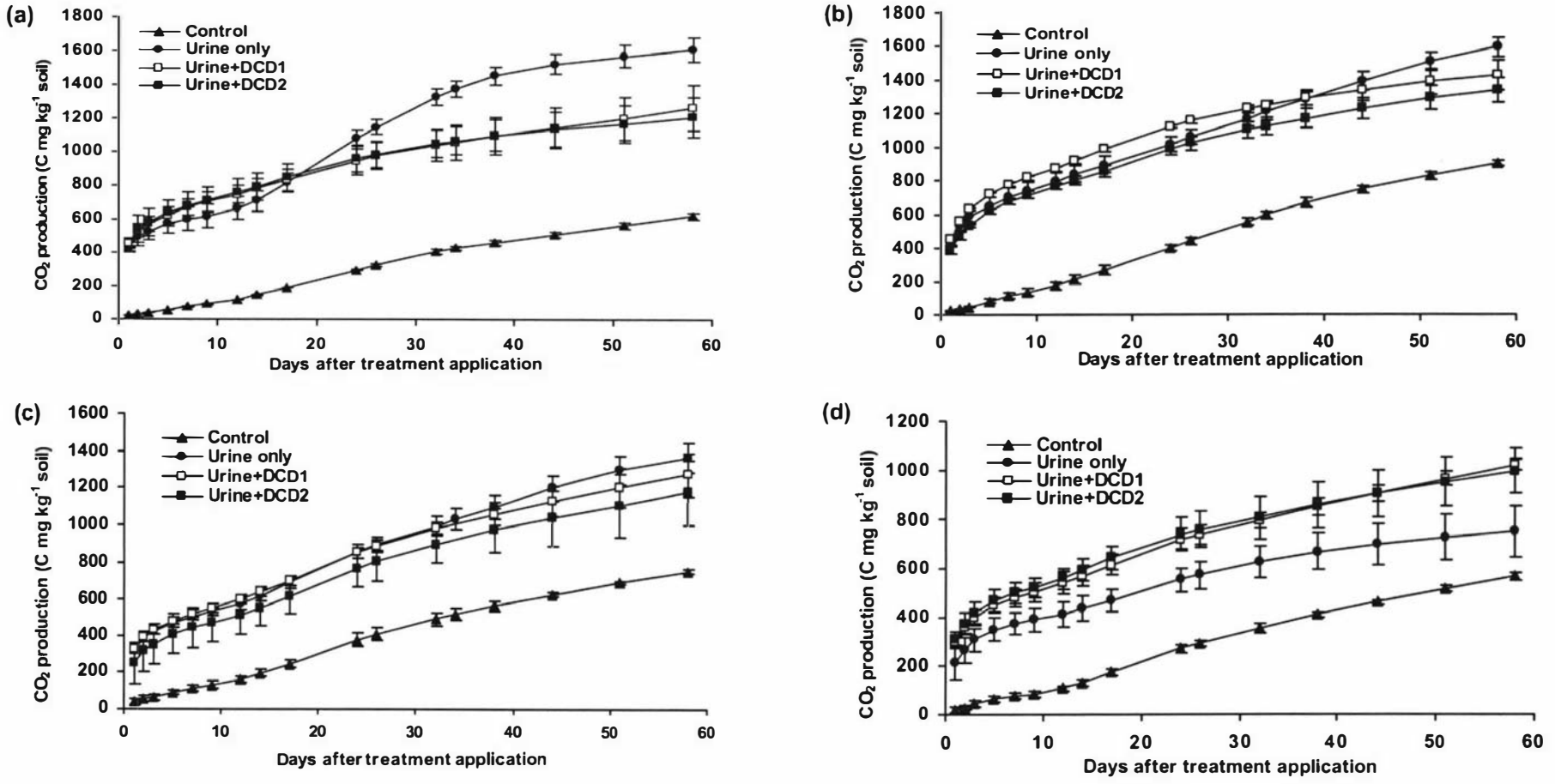


Figure 7.3 **Cumulative amount of CO_2 released in various treatments during the incubation of (a) Tokomaru silt loam (b) Manawatu sandy loam (c) Egmont brown loam (d) Horotiu silt loam (note the different scales for the y-axis). Each value represents a mean of three replicates with standard deviation shown by vertical bars.**

7.3.2.3 Mineral nitrogen

The effectiveness of DCD in inhibiting nitrification can be estimated from its ability in maintaining N in the NH_4^+-N form for a sufficient period of time before DCD degrades and thereby allows nitrification to proceed. The NH_4^+-N concentrations increased within a day in all the soils receiving urine and urine plus DCD treatments (Figure 7.4). Peak concentrations were observed 1-3 days after incubation, reaching 506, 476, 432 and 440 mg NH_4^+-N kg^{-1} soil in the Tokomaru, Manawatu, Egmont and Horotiu soils, respectively, in the urine-only treatment, and then subsequently declined (Figure 7.4). The rate of production of NH_4^+-N following urine application varied with both the soil type and rate of DCD applied. There was some DCD rate x soil type interaction (Table 7.4). In all soils, the effect of DCD application on NH_4^+-N was observed from day 6, with significantly higher ($P < 0.05$) NH_4^+-N concentration in soils with DCD as compared to those in soils with urine only. The NH_4^+ concentration was generally higher with 20 mg DCD kg^{-1} soil than with 10 mg DCD kg^{-1} soil, although the difference was only significant on days 16 and 33 (Figure 7.4). The ratio of NH_4^+-N concentrations found in urine-treated soil with DCD to those in soil without DCD were highest in Horotiu soil, ranging from 3.3 (DCD1) to 11 (DCD2) after day 16 until the end of the incubation period. This ratio ranged from 1.7 to 2.5 for Tokomaru soil, from 1.2 to 1.8 in Manawatu soil, and from 1.05 to 2.5 in Egmont soil (Figure 7.4) after day 16 until the end of the incubation period. The NH_4^+ concentration in the control of all four soils remained almost constant, with only slight fluctuations, and ranged from 0.85 to 4.54, 4.48 to 8.64, 0.59 to 2.85 and 0.08 to 2.37 mg N kg^{-1} soil in the Tokomaru, Manawatu, Egmont and Horotiu soils, respectively, during the incubation period.

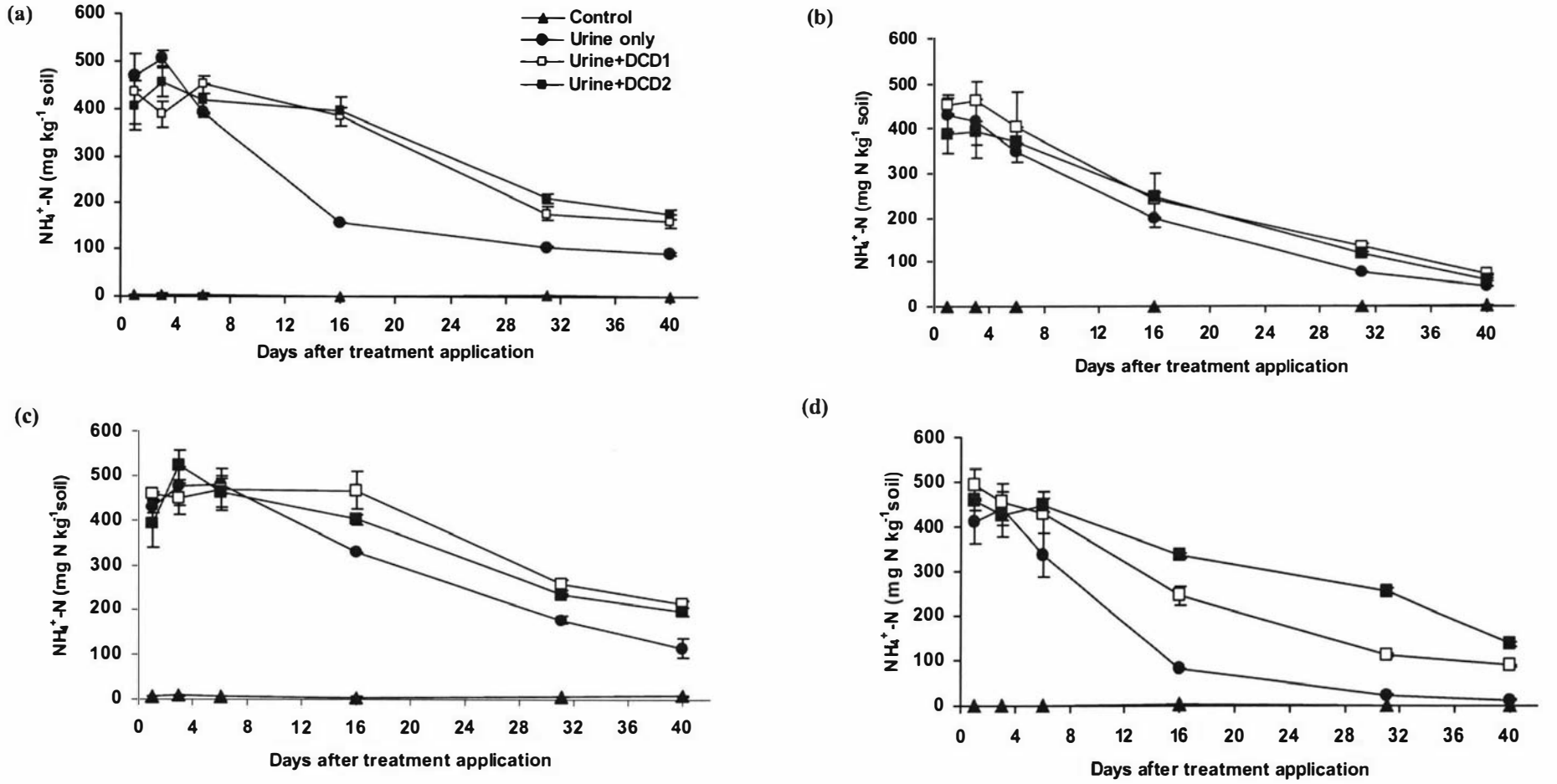


Figure 7.4 **Mean NH_4^+-N concentrations for various treatments in (a) Tokomaru silt loam (b) Manawatu sandy loam (c) Egmont brown loam (d) Horotiu silt loam. Error bars represent the standard deviation of the three replicates.**

Nitrate concentrations increased with time after the application of urine-N in all the soils with and without DCD, and were significantly higher than those of the control treatment throughout the incubation (Figure 7.5). Peak NO_3^--N concentrations of 412, 393, 546 and 521 mg N kg^{-1} soil were observed on day 16 with the urine application to Tokomaru, Manawatu, Egmont and Horotiu soils, respectively. This coincided with the sharp decrease in NH_4^+-N on day 16 in these soils (Figure 7.4). Generally, the addition of DCD at both rates decreased ($P<0.05$) NO_3^- concentrations in all four soils and no such peaks were observed. For the first 3 days, NO_3^--N concentrations in the urine treatments in all the soils were variable and showed no effect of added DCD on NO_3^--N concentration. The decrease in NO_3^--N concentration with added DCD in the urine treatment increased with the rate of DCD application; however, this difference in reduction was not significant ($P > 0.05$) on a few sampling days. The concentration of NO_3^--N in the soil was affected by both the soil type and rate of DCD application, and some rate x soil type interactions were also observed (Table 7.4). The NO_3^--N concentrations in the control treatment of all four soils did not change much over time and ranged from 5.70 to 9.85, 14.8 to 18.2, 14.4 to 19.5 and 13.9 to 15.7 mg N kg^{-1} soil in the Tokomaru, Manawatu, Egmont and Horotiu soils, respectively (Figure 7.5).

An attempt was made to relate the rate of change in soil NH_4^+-N concentrations with time to that of NO_3^--N concentrations. For example, in the urine-only treatment, the rate of change of NH_4^+-N concentrations, calculated for the period between peak concentrations and the day they reached the background level (day 31), was -14.3, -10.6, -12.2 and -14.9 mg kg^{-1} soil d^{-1} for the Tokomaru, Manawatu, Egmont and Horotiu soils, respectively. The rate of change of NO_3^--N in the urine-only treatments for the same period was 10.4, 7.3, 9.8 and 11.9 mg kg^{-1} soil d^{-1} respectively, for the Tokomaru, Manawatu, Egmont and Horotiu soils. The rate of increase in NO_3^--N was less than the rate of decrease in NH_4^+, and the differences could be attributed to NH_3 volatilisation, immobilisation of NH_4^+ and NO_3^--N, and denitrification; however, under the present experimental conditions, the potential for denitrification was low and there was no NO_3^- leaching. The rate of change in NH_4^+ and NO_3^--N concentrations in the soils with time was affected by the addition of DCD, but the differences between the rates were insignificant for all soils in the presence of inhibitors.

Table 7.4 Levels of significant differences between soil type and DCD rates on NH_4^+-N, NO_3^--N and DCD-N concentrations in the soil at different times during the experiment.

Time (Days)	Source of Variation	NH_4^+-N	NO^-_3-N
1	Soil Type	n.s.	**
	DCD rate	*	n.s.
	Soil X DCD rate	n.s.	*
3	Soil Type	*	**
	DCD rate	n.s.	**
	Soil X DCD rate	*	*
6	Soil Type	**	**
	DCD rate	*	**
	Soil X DCD rate	**	*
16	Soil Type	**	**
	DCD rate	**	**
	Soil X DCD rate	**	**
31	Soil Type	**	**
	DCD rate	**	**
	Soil X DCD rate	**	**
40	Soil Type	**	**
	DCD rate	**	**
	Soil X DCD rate	**	*

Analysis of variance performed only on soils receiving DCD
n.s. not significant, * significant ($P<0.05$), ** highly significant ($P<0.01$).

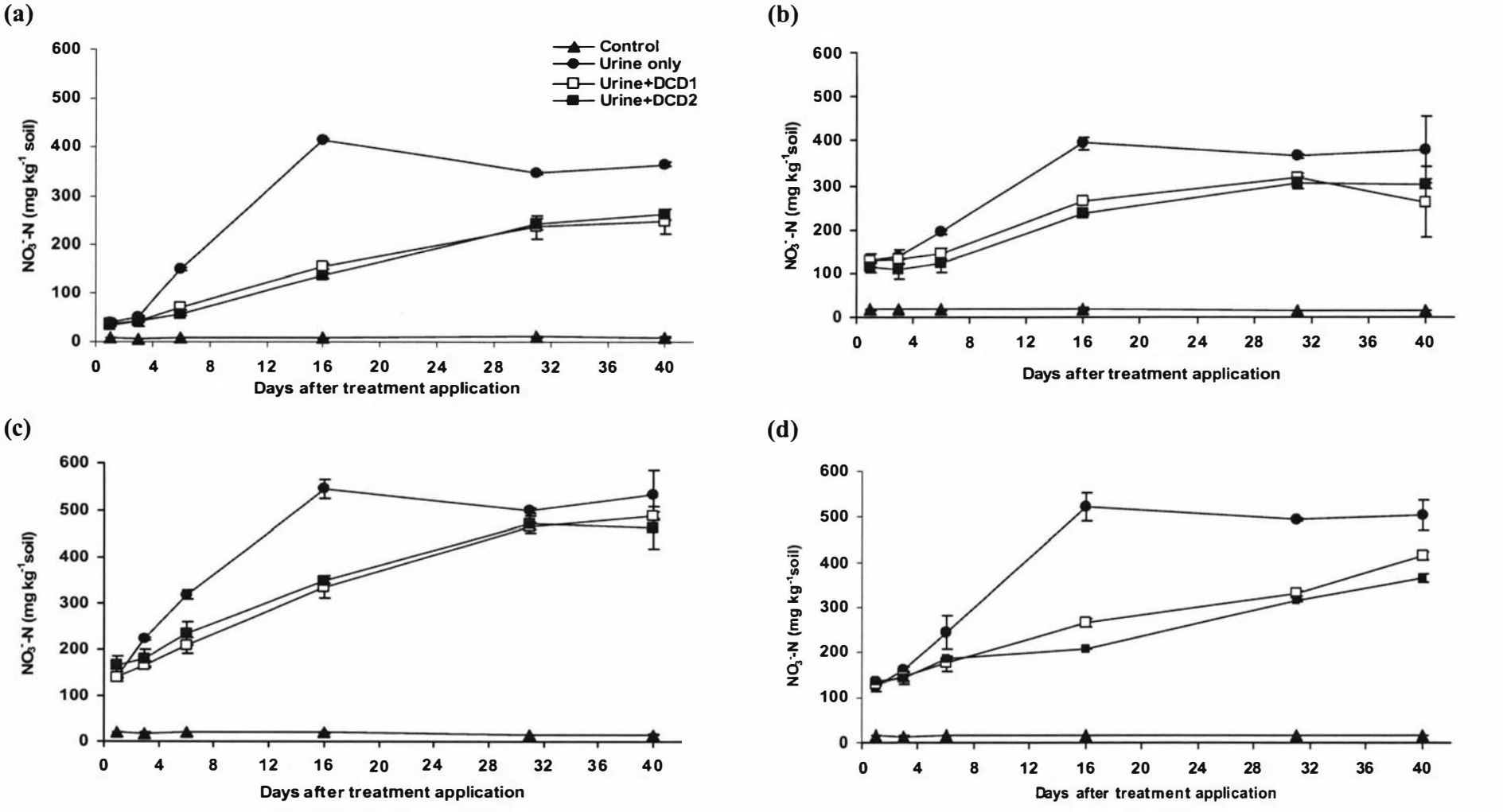


Figure 7.5 **Mean NO_3^--N concentrations for various treatments in (a) Tokomaru silt loam (b) Manawatu sandy loam (c) Egmont brown loam and (d) Horotiu silt loam. Error bars represent the standard deviation of the three replicates.**

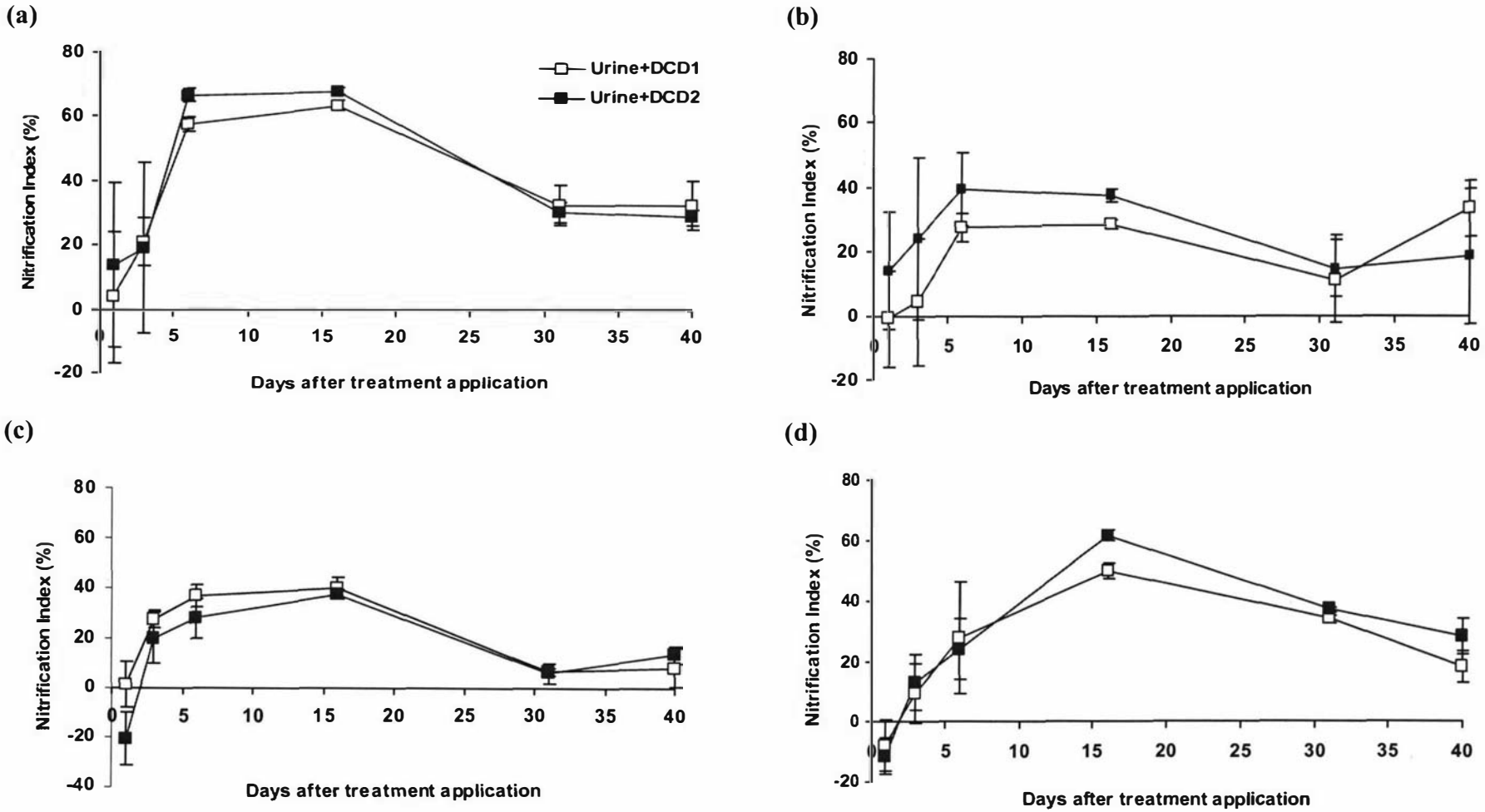


Figure 7.6 **Mean Nitrification inhibition index (NII) at various periods after urine application with two rates of DCD in the (a) Tokomaru silt loam (b) Manawatu sandy loam (c) Egmont brown loam and (d) Horotiu silt loam. Error bars represent the standard deviation of the three replicates.**

7.3.2.4 Nitrification inhibition

The nitrification inhibition index (NII) varied among the four soils but increased with time in all of them until it attained a peak value between days 6-16 (Figure 7.6). Maximum inhibition ($P<0.05$) in the DCD2 treatment was found in Tokomaru soil (66.7 %) followed by Horotiu soil (61.8%), Manawatu soil (39.2%) and Egmont soil (37.4%). The peak period of NII generally coincided with the peak period for the nitrification of urine N (Figure 7.5). The nitrification inhibition index differed between the two rates of DCD only on a few days during the incubation, but, in general, was higher at the higher DCD application rate (Figure 7.6); an exception was in Egmont soil. Very small or negative NII values were observed at the beginning of the incubation; these coincided with low NO_3^- concentration in the soils (Figure 7.5).

7.3.2.5 Microbial biomass carbon

The changes in microbial biomass were monitored only on days 1, 3 and 7, after the application of the treatments to the soils. The microbial biomass C fluctuated with both time and urine application (with and without DCD) but, generally, the differences were not significant (Table 7.3). The mean microbial biomass concentration over the first 7 days was higher in Egmont (1067 mg C kg^{-1}soil) and Tokomaru (1003 mg C kg^{-1}soil) soils than in Horotiu (977 mg kg^{-1}soil) and Manawatu (683 mg kg^{-1}soil) soils. Application of urine, with or without DCD, resulted in a slight increase in microbial biomass C over the control treatment in all the soils except Horotiu soil (Table 7.5). There was no consistent effect of the DCD application rate on microbial biomass C in any of the soils.

Table 7.5 Total microbial biomass C (mg kg^{-1}soil) for various treatments in four different soils at various periods during the incubation.

Treatment	Microbial biomass C (mg kg^{-1} soil)		
	Day 1	Day 3	Day 7
Tokomaru silt loam			
Control	1131 b	770 b	1107 b
Urine only	1105 b	957 ab	1325 a
Urine+DCD1	1182 b	1023 a	1274 ab
Urine+DCD2	1384 a	923 ab	1023 b
L.S.D. (0.05%)	134	228	319
Manawatu sandy loam			
Control	805 a	618 a	626 b
Urine only	1038 a	760 a	890 a
Urine+DCD1	975 a	944 a	984 a
Urine+DCD2	891 a	645 a	1004 a
L.S.D. (0.05%)	349	388	161
Egmont brown loam			
Control	1063 a	1150 a	989 b
Urine only	1197 a	1145 a	1201 ab
Urine+DCD1	1158 a	963 a	1199 ab
Urine+DCD2	1158 a	936 a	1245 a
L.S.D. (0.05%)	222	373	230
Horotiu silt loam			
Control	958 a	985 a	988 ab
Urine only	828 a	765 a	921 b
Urine+DCD1	887 a	724 a	1190 a
Urine+DCD2	920 a	867 a	1050 ab
L.S.D. (0.05%)	196	292	246

* Values followed by the same letter under each soil type and day do not differ significantly at the 0.05 level

7.3.2.6 Soil pH

The pH of the four soils ranged from 5.25 to 5.95. During the incubation, the pH in the control treatment showed an initial slight fluctuation in all four soils but then remained almost constant for the entire incubation period.

Addition of urine, both with and without DCD, increased the pH within a day in all the soils (Figure 7.7). In the urine-only treatment, the pH decreased sharply after day 5, followed by a gradual decrease, and was lower than the pH of the control treatment by the end of the experiment. The addition of DCD to urine resulted in only a gradual decrease in pH after the peak value, compared to the urine-only treatment, and the pH remained significantly higher in the DCD treatments than urine-only treatments till the end of the experiment. Hydrolysis of urea-N contained in urine to NH_4^+ may have produced enough OH^- ions to temporarily raise the pH of the soil. These NH_4^+ ions get nitrified quickly and a sharp decrease in pH was observed in urine treatments. As nitrification generates 2 protons per ion of NH_4^+ nitrified, the increase in soil acidity was expected. The addition of DCD inhibited the nitrification process, which resulted in the gradual decrease in soil pH compared to its urine-only treatment. No significant difference was observed in soil pH with the different DCD rates.

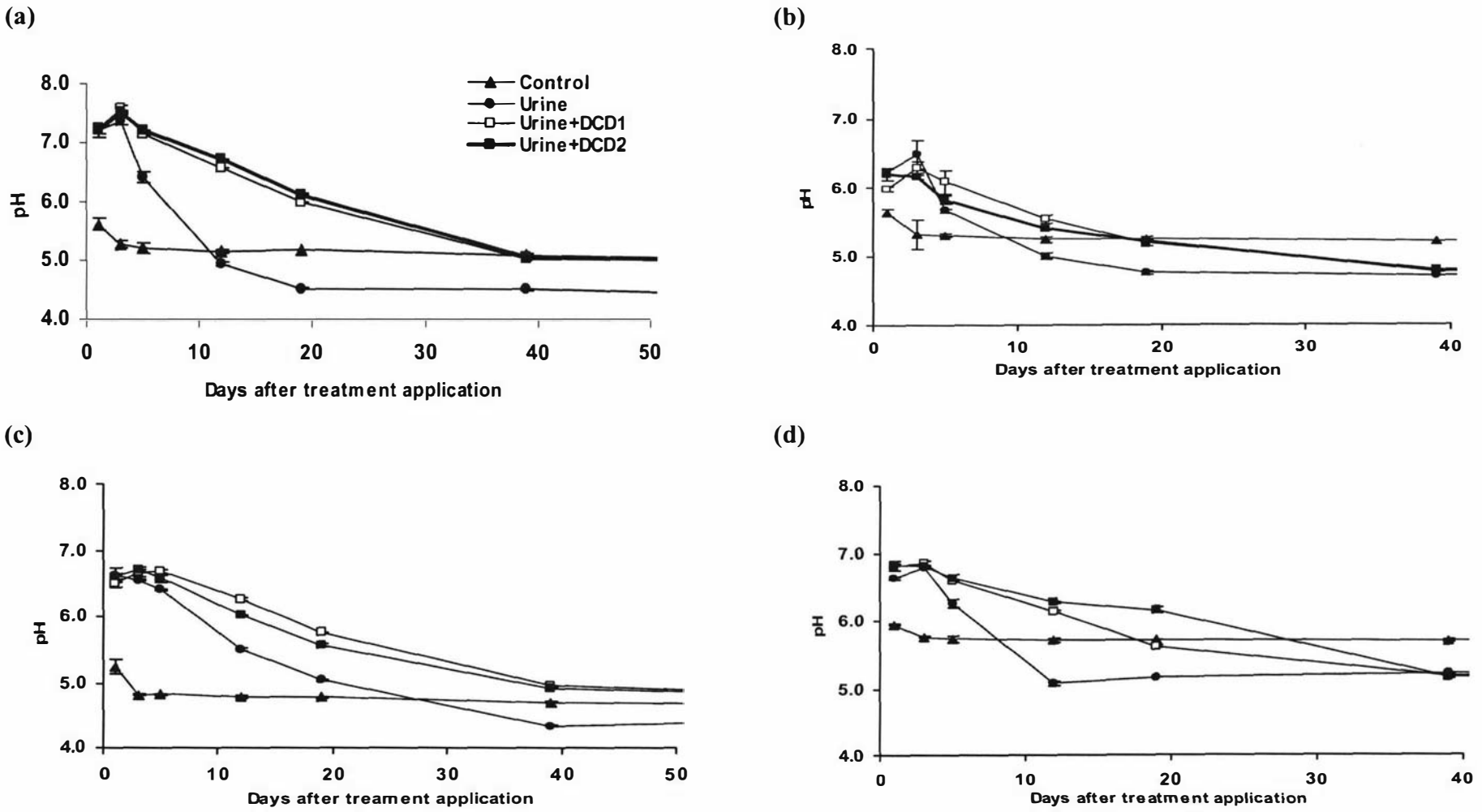


Figure 7.7 Geometric mean of pH levels at different periods following the application of various treatments .in (a) Tokomaru silt loam (b) Manawatu sandy loam (c) Egmont brown loam and (d) Horotiu silt loam

7.4 General Discussion

The results in this chapter indicate that: (i) the rate of DCD degradation was affected by both the level of DCD application and soil type; (ii) DCD caused a significant inhibition of nitrification of urine N, as measured by its nitrification inhibition index; (iii) DCD application caused a significant decrease in N_2O emissions from urine nitrogen; (iv) DCD application did not cause any significant impact on microbial activity as measured by microbial biomass and respiration. These observations are discussed below.

The recovery of DCD using water as an extractant immediately after addition to soils (i.e at day 0) varied with the soil type, ranging from 80 to 100%. The difference in recovery between the soils may be attributed to the interference resulting from the colour of the soil extract and/or the sorption of DCD by soil colloids. The interference from soil colour was adjusted by using appropriate control samples during the colorimetric measurement of DCD. Dicyandiamide sorption takes place mainly on organic matter surface sites as the DCD molecule containing two active functional groups (-NH_2 and =NH), bound to the carboxyl (COOH) functional groups of organic matter through hydrogen bonds (Bowatte 2003; Zhang *et al.* 2004). This may be the main reason for the low recovery of DCD in the Horotiu (80%) and Egmont (92%) soils. Both these soils are high in organic matter (Table 7.1), but differed in their CEC and the nature of the allophane, which resulted in differences in DCD sorption between the two soils. These observations are supported by the results obtained by Zhang *et al.*, (2004), which showed that a mollisol with comparatively high organic matter and CEC sorbed more DCD, as compared to an alfisol with lower organic matter and CEC.

The degradation of DCD, as shown by the DCD concentrations in the soil and the estimated DCD half-life ($t_{½}$) value, varied with the level of DCD application and the soil type (Table 7.2). These observations are supported by the studies of Rajbanshi *et al.,* (1992), Puttanna *et al.,* (1999), and Williamson *et al.,* (1996) who found that, in arable and pasture soils that were not pre-exposed to DCD, the rate of degradation of DCD decreased with increasing level of DCD application. In soils that received repeated applications of DCD, the rate of degradation of DCD was rapid and was less affected by the level of DCD application. The effect of level of DCD application on

degradation, especially in freshly treated soils, may be attributed to the lag period required for the buildup in microbial communities involved in the degradation of DCD.

There was no significant difference in the rate of degradation of DCD in the two non-allophanic soils. However, in the allophanic soils, the rate of degradation was faster in Egmont soil ($t_{1/2}$ = 6.67 to 10.7) than in Horotiu soil ($t_{1/2}$ = 31.9 to 38.7), which may be again attributed to the difference in the organic matter and allophane content in the two soils. DCD is known to decompose more rapidly when the soil organic matter content is high (Amberger & Vilsmeier 1979), as in the Egmont soil.

The results from the incubation studies reported in this chapter showed DCD was effective in inhibiting nitrification at both application rates and the effectiveness varied among the four soils. The concentration of NH_4^+-N was higher in the presence than absence of DCD in the urine treatments, and the difference was pronounced in the Horotiu silt loam soil which had 18 to 26% NH_4^+ of the initial quantity in DCD-treated soils, as compared to 2.9% in the urine-only treated soil at the end of the incubation period (40 days). Tokomaru silt loam soil then had 36-38% NH_4^+ in the DCD treatments as compared to 17% of the initial quantity in the urine-only treatment; relative percentages of NH_4^+ were lower in the Manawatu and Egmont soils (Figure 7.4). Another index used to measure the inhibition by DCD was the nitrification inhibition index (NII) which gave highest values in Tokomaru silt loam, followed by Horotiu silt loam and then Manawatu and Egmont soils (Figure 7.6). McCarty & Bremner (1989) also found that the NII values for DCD applied @ 5 mg kg^{-1} soil varied from 8 to 41% in three different soil types at 25° C. The variation in the effectiveness of DCD in inhibiting nitrification in the soils could be attributed to a number of factors that include different organic matter content, aggregate size and pH of the soil (Reddy 1964; Zhang *et al.* 2004). Maximum reduction in N_2O emissions with DCD addition to urine was observed in Tokomaru silt loam, followed by Horotiu silt loam soil> Manawatu soil> Egmont soil; this agreed with the NII.

The total N_2O emitted over the incubation period of 58 days after urine application was highest in Tokomaru soil (11.2 mg N kg^{-1} soil) followed by Egmont (10.3 mg N kg^{-1} soil), Manawatu (8.13 mg N kg^{-1} soil) and Horotiu (7.04 mg N kg^{-1} soil) soils. Clough *et al.* (1996, 1998) found that the levels of N_2O emissions from an organic soil receiving urine N at 500-1000 kg N ha^{-1} were not significantly different to

those from mineral soil. The results in this study showed that N_2O emissions resulting from urine application were slightly higher (although non-significant) in non-allophanic (Tokomaru and Manawatu soil) than allophanic (Egmont and Horotiu soil) soils. However, the two non-allophanic soils in this study contain less organic matter (Table 7.1) than the two allophanic soils. The difference in urine-born N_2O emission between these two groups of soils could be attributed to the differences in the nature and amount of clay and the structural properties of the soils (Clough *et al.* 1998; Khalil *et al.* 2006).

Egmont and Horotiu soils had comparatively high total C concentrations (Table 7.1). However, microbial biomass C concentrations were higher in the Tokomaru and Egmont soils than in the Horotiu and Manawatu soils (Table 7.5); this may be attributed to the difference in clay content in these soils (Table 7.1). It has often been shown that clay particles protect microbial metabolities produced during the decomposition of organic substrates (Ladd *et al.* 1981; McGill *et al.* 1981).

The results from this study clearly show that DCD applied at either rate did not have a significant impact on the microbial biomass. Application of urine is likely to increase the soluble carbon content of soil through urine-derived carbon input and pH-induced solubilization of soil carbon (Kelliher *et al.* 2005). However, in this study, the addition of urine, both with and without DCD, did not cause any consistent change in microbial biomass C levels. Di & Cameron (2004a) also did not find any obvious effect of urine application, in the presence and absence of DCD, on microbial biomass C concentrations of soil under pasture.

Microbial respiration, as measured by CO_2 release, increased within a day of the application of urine, both with and without DCD, in all the soils suggesting an immediate and significant rise in microbial activity. Urine contains a very small (0.01%) amount of soluble C, so this increase in respiration could be explained by the solubilization of soil organic C with the urine application (Monaghan & Barraclough 1993) providing substrate for increased metabolism. Moreover, the hydrolysis of urea produces CO_2, so the measured levels of CO_2 from the treated soils would have been derived from both urea hydrolysis and increased microbial respiration.

The CO_2 production was higher in Manawatu (1314 mg CO_2-C kg^{-1} soil) and Tokomaru (1174 mg CO_2-C kg^{-1} soil) soils (non-allophanic) than in Egmont (1140 mg CO_2-C kg^{-1} soil) and Horotiu (833 mg CO_2-C kg^{-1} soil) soils (allophanic). It has been

found that allophane protects the microbial metabolites produced during decomposition of organic substrate or stabilizes organic residues and microbial biomass (Saggar *et al.* 1994). Thus, in soils with high allophane content, micro-organisms could have diverted more C metabolites to new biomass, resulting in reduced CO_2 emission. Similarly, Martin *et al.* (1982), who compared the release of C as CO_2 from a number of allophanic and non-allophanic soils of Chile, found that allophanic soils with comparatively high organic matter (8.8-16.1%) lost from 920-1910 mg C kg^{-1} soil compared to 1430 – 2430 mg C kg^{-1} soil in non-allophanic soils with a lower organic matter concentration (1.6-2.6 %).

Addition of DCD at both rates did not show any significant impact on CO_2 emitted from the urine-treated soils, as compared to the urine-alone treatments, though a small decrease was observed. Results found by Weiske *et al.* (2001) from a three-year field study however, showed that NIs (DCD and DMPP) decreased CO_2 emission from fertilized plots by 7-10% and 28% respectively as compared to plots without NIs. No other reports on the side-effects of DCD on C mineralization could be found in the literature and thus results need to be further tested under field conditions.

7.5 Conclusions

From the results obtained in this study, the following conclusions can be drawn:

- The rate of degradation of DCD varied between the soils, with the differences being greater in the allophanic soils with high organic matter.
- Dicyandiamide was effective in reducing N_2O emissions from all the soil types at both application rates of 10 and 20 mg DCD kg^{-1} soil.
- The effectiveness of DCD in inhibiting nitrification varied considerably between soils; this was attributed to the difference in the nature and amount of soil organic matter and clay content. Maximum inhibition was observed in the soil with low organic matter and a high clay content
- Although DCD was effective in inhibiting nitrification, there was no consistent relationship between nitrification inhibition and rate of DCD degradation.

- Application of DCD did not result in any change in microbial biomass and respiration rate in the soils.

Chapter 8

Modelling the effect of nitrification inhibitor (DCD) on nitrous oxide emissions from urine application

8.1 Introduction

Currently there is an increasing interest and research activity in New Zealand in the use of nitrification inhibitors (Nis) as one of the mitigation options for reducing N_2O emissions from grazed pastures. New Zealand government draft policy (http://www.maf.govt.nz/climatechange/discussion-document/11-pillars.htm) recommends farmers to use NIs with N fertilisers and, in grazed pastures, to reduce environmental effects of N pollution through leaching and gaseous emission, thereby avoiding penalties. Government-funded incentives are proposed to encourage farmers to use NIs. Information is needed, therefore, to obtain realistic emission-reduction estimates from NIs at multiple scales. But the current IPCC approach for calculating N_2O emissions from agricultural and managed pastoral soils used in New Zealand (Ministry of Environment 2005) is not sufficiently flexible to allow the assessment of mitigation options.

The emissions rates from excretal input and from various soil types in New Zealand are very different and uncertain, as also shown in Chapter 7 of this thesis. This makes extrapolation to regional scales and beyond very challenging. Saggar *et al.* (2002) initiated a more robust, process-based approach to estimate field-scale N_2O emission estimates for grazed pasture systems, that is internationally acceptable and quantifies N_2O emissions at the field level more accurately, by adapting the denitrification-decomposition (DNDC) model developed by Li *et al.* (1992a, b) to

New Zealand conditions. The major modifications are related to pasture growth, N input from animals, evapo-transpiration and soil moisture regimes (Giltrap *et al.* 2004; Saggar *et al.* 2004a; Saggar *et al.* 2007a). The adapted NZ-DNDC model has been validated for N_2O emissions against field measurements from two dairy pastures with contrasting soil types (Saggar *et al.* 2004a) and from a sheep pasture (Saggar *et al.* 2007b). The model simulated effectively the soil water-filled pore-space (WFPS) which is considered to be one of the important soil properties controlling N_2O emissions, and general pulses and trends in N_2O emissions from both sheep and dairy-grazed pastures, and also captured the observed effects of excretal and fertiliser N inputs on N_2O emissions. A series of sensitivity tests conducted on NZ-DNDC showed that the model predicted well the changes in pasture production and N_2O emissions with changes in climate, soil properties, fertiliser management and grazing regimes (Saggar *et al.* 2007a). The results of these model simulations demonstrate the value and flexibility of the process-based modelling approach for assessing the efficacy of potential N_2O mitigation options.

Most of the New Zealand research on NIs in pastoral agriculture is concentrated on the use of dicyandiamide (DCD) (Francis *et al.*, 1995, Williamson *et al.*, 1998; Di & Cameron 2002b, 2003, 2004b,c) which shows that DCD reduces NO_3^- leaching and N_2O emissions and has the potential to reduce environmental impacts of N use. Results from my laboratory incubation study discussed in Chapter 7, on the effectiveness of DCD applied to cattle urine, show emissions reductions vary with soil organic C content, mineralogy and soil texture. Therefore, a single emission reduction factor cannot be applied across a range of soil types and climate. Furthermore, it was not possible to develop a regression model incorporating soil properties to predict DCD-induced reductions in N_2O emission in the present study.

As the NZ-DNDC model adequately accounts for the effect of soil types and climatic conditions on N_2O emissions, an attempt is made here to extend this model in simulating N_2O emissions reductions from the use of DCD in different soil types. The specific objective of this chapter is:

- To adapt and then test the NZ-DNDC model for predicting changes in N_2O emissions from urine application as influenced by DCD addition.

8.1.1 Model Description

The DNDC model of Li *et al.*, (1992 a, b) is a process-based model that forms a bridge between global C and N biogeochemical cycles and the basic ecological drivers of the C and N cycles in the soil (Figure 8.1). The model consists of four sub-models that include: thermal-hydraulic, crop growth, decomposition and denitrification. The thermal-hydraulic sub-model uses basic climate data to simulate soil moisture conditions and to capture anaerobic microsite formation and sequential substrate reduction. The crop growth sub model simulates growth of various crops from sowing to harvest. Above-ground biomass is accumulated based on daily N and water uptake. The decomposition sub-model has four soil carbon pools: litter, microbial, labile and passive. Each pool has a fixed C:N ratio and decomposition rate is influenced by soil texture (clay content), soil moisture and temperature. The decomposition sub model provides initial NO_3^- and soluble C pools for the initiation of denitrification. The denitrification sub-model is responsive to available NO_3^- and soluble C and is also activated by rain events, and changes in soil moisture and temperatures. An increase in WFPS caused by rain or irrigation events decreases soil oxygen availability. The WFPS, soluble C, soil temperature, soil pH, available N and denitrifier biomass control the rate of denitrification (Frolking *et al.* 1998). DNDC has been designed so that soil moisture has a large influence on N_2O fluxes through its impact on the volume of soil in which denitrification occurs and the duration of denitrifying conditions. Among these sub models, crop growth and decomposition operate on a daily time-step while the denitrification sub-model operates on an hourly time-step.

DNDC has been successfully used to produce estimates of N_2O emission for US, Canada, China, Germany and UK. The model has been modified for New Zealand's grazed pasture systems and named NZ-DNDC model (Saggar *et al.* 2004a). In brief, the modifications incorporated into the NZ-DNDC model include: addition of a modifier for perennial pasture growth with adjusted N–fixation rates, quantification of N inputs from animals, and modification of potential evapo-transpiration, soil water flows and soil moisture regimes to capture long term saturated conditions that are very typical of New Zealand winters (Giltrap *et al.*, 2004; (Saggar *et al.* 2002, 2004a).

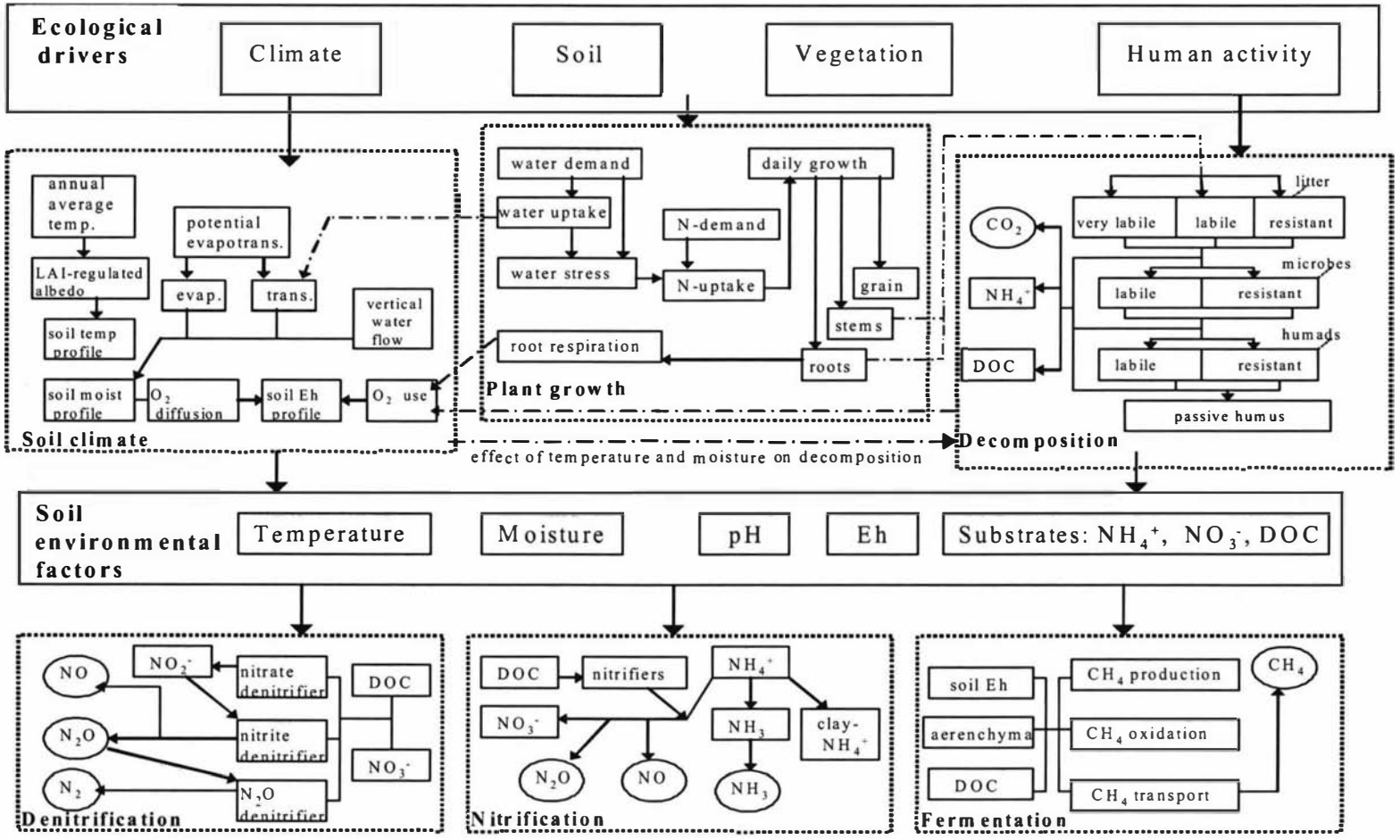


Figure 8.1 Structure of the DNDC model (Li *et al.*1992a)

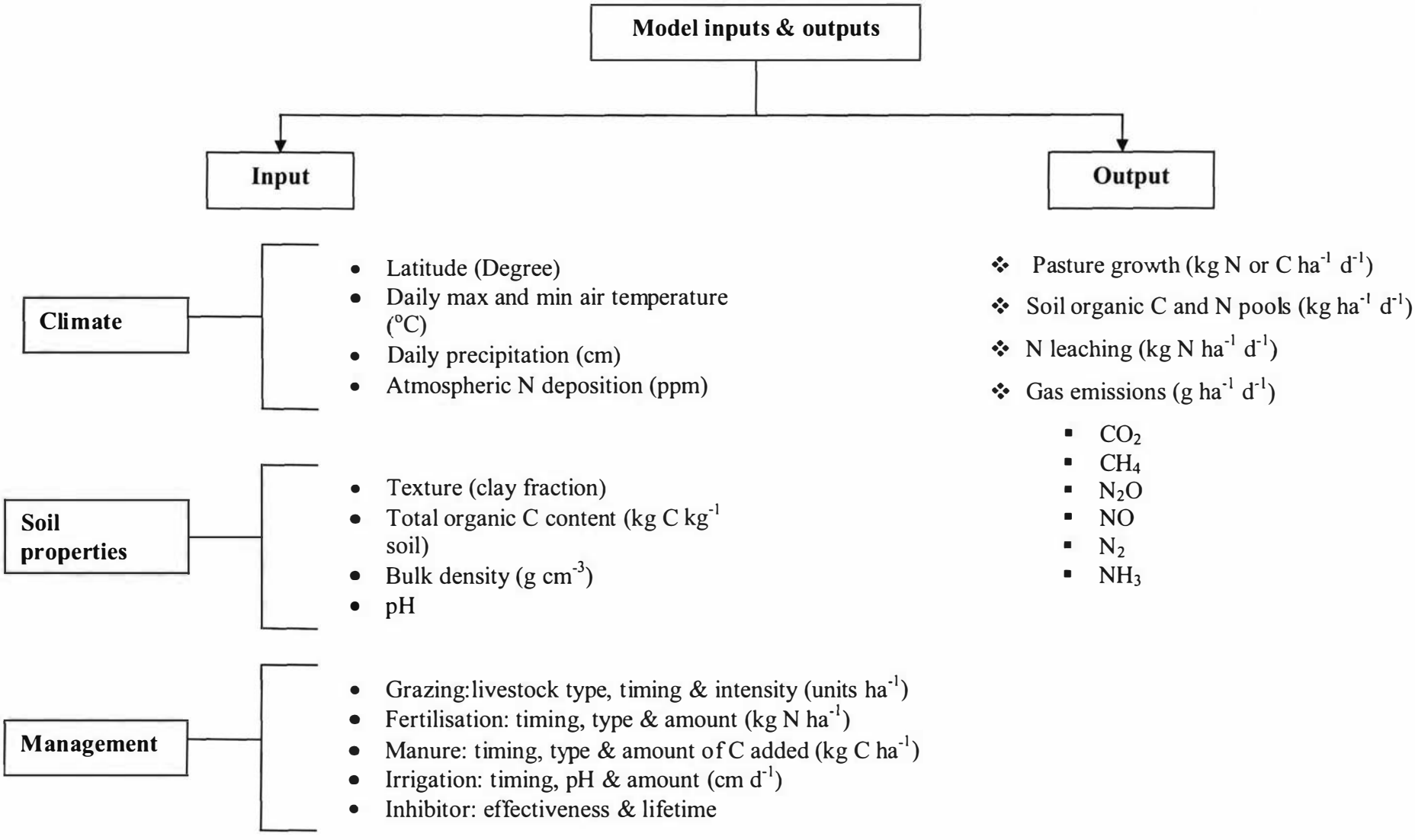


Figure 8.2 Input parameters required by the NZ-DNDC model and the model outputs

8.1.2 Input parameters

An attempt was made to simulate the effect of DCD on N_2O emissions from urine application under the field conditions reported in Chapter 6. The model input parameters are given in Figure 8.2. The grazing parameter takes into account the amount of excretal input by grazing animals and has a built-in model value which is estimated from animal type, animal numbers and grazing period. Animal grazing also results in the removal of sufficient above-ground plant biomass to meet the animals' food requirements. As urine was applied manually in the field-plot study reported in Chapter 6, the grazing excretal-N input parameter is therefore taken as zero. Instead, the urine-N input (600 kg N ha^{-1}) is simulated as a simultaneous application of urea fertiliser and rainfall, as cow urine has almost all N in the urea form. The changes in WFPS are calculated from rainfall and the infiltration values. For climate inputs, the text files with data arranged in Julian days, mean air temperature (^{o} C) and rainfall (mm) are used. The mean air temperature and rainfall data were obtained from the weather station located at the site of the experiment. Other input parameters describing the soil are silt loam soil texture; 1.01 mg cm^3 for bulk density; 5.62 for soil pH and 0.53 for water-filled pore space at field capacity.

8.1.3 Model adaptation

The NZ-DNDC model was further modified by introducing a factor to account for the effect of NI on N transformation (i.e. nitrification rate), thereby controlling the supply of nitrate substrate for N_2O emission (Giltrap et al., 2006). The factor introduced to scale the inhibition of nitrification from the application of NI is:

$(1-N_{eff})$

where, N_{eff} = inhibitor efficiency index (0-1).

The parameter used is to mimic the effect of NIs such as DCD, by scaling down the process of nitrification, resulting in decreased NO_3^--N production and eventually reducing the overall amount of NO_3^--N available for denitrification. For the model adaptation, it was assumed that the NI remains equally effective for a specific period

(N), which in the present study was ≥50 days. The original nitrification rate for urine treatment is then multiplied by (1-N_{eff}) to obtain the modified nitrification rate in the model for urine+DCD treatment.

Another minor modification was made to the model such that the amount of ammonia volatilisation occurring immediately following the "urea" application (here 600 kg N ha^{-1}) was the same as the ammonia volatilisation that the model uses when urine is an input as part of a grazing event.

8.2 Materials and Methods

The NZ-DNDC model was adapted to simulate N_2O emission from urine application with and without DCD following the modifications discussed above and briefly reported by (Giltrap *et al.* 2006). The input data files along with climate files are presented in Appendix 1. The model was run to account for nitrification inhibition ranging from 0 to 100%.

Data on N_2O emission, WFPS and mineral N obtained from the field-plot study reported in Chapter 6 were used to compare with model simulations. This experiment (Chapter 6) has five treatments: Control (water only), Urine only, Urine+DCD, Urine+Agrotain, Urine+DCD+Agrotain. The three treatments viz., Control, Urine only and Urine+DCD were used to compare the model simulations of N_2O emissions.

Data on N_2O emissions and related soil (NH_4^+, NO_3^-, WFPS) and environmental (rainfall, air temperature, soil temperature) parameters are described in detail in Chapter 6 of this thesis.

The percentage reduction in total N_2O-N emitted with addition of DCD to urine was calculated using the following equation:

$$\%\ reduction\ in\ N_2O-N = \frac{(A-B)}{(A-C)} X100 \qquad \textbf{(8.1)}$$

where, A = total N_2O-N emitted from urine only treatment

B = total N_2O-N emitted from urine+DCD treatment

C = total N_2O-N emitted from control (nil N) treatment

8.3 Results and Discussion

8.3.1 Model parameterisation

The model was parameterised to account for NI efficacy in inhibiting nitrification from 0 to 100%. For this, N_{eff} values were varied from 0 to 1 where 0 accounted for no inhibition and 1 accounted for 100% inhibition. The total N_2O-N simulated emissions for the 50 day study period, with N_{eff} values of 0, 0.2, 0.4, 0.6, 0.8 and 1 were 4.56, 4.03, 3.37, 2.55, 1.51 and 0.23 kg N ha^{-1}, respectively (Figure 8.3), resulting in simulated reductions in total N_2O-N emission of 14%, 32%, 55%, 83% and 117%, with 20%, 40%, 60%, 80% and 100% nitrification inhibition, respectively.

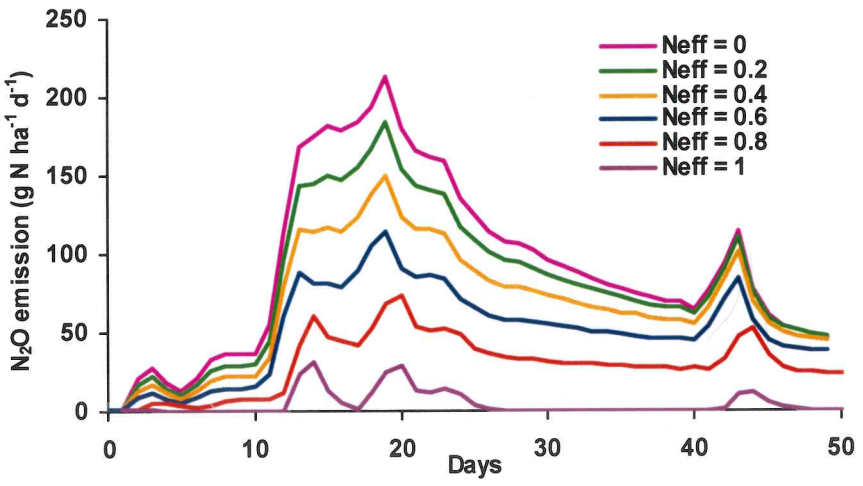


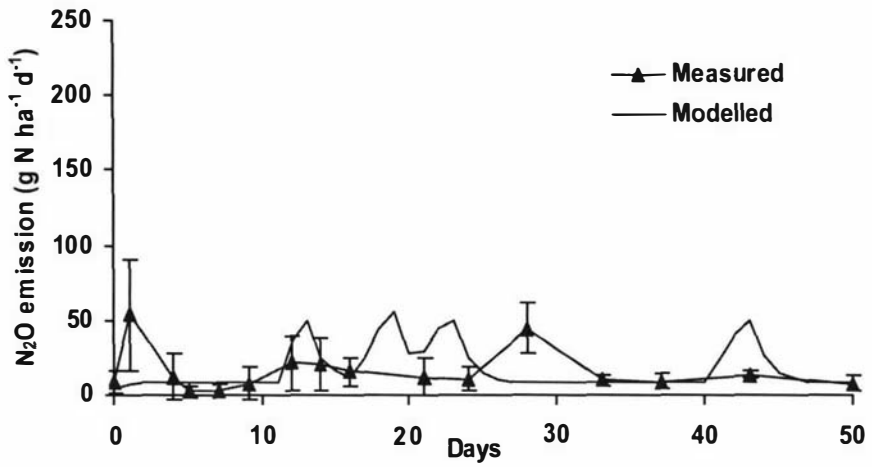
Figure 8.3 NZ-DNDC modelled N_2O emissions for the urine+DCD plots using N_{eff} factor 0 to 1.

8.3.2 Model Simulated N_2O emission, WFPS and mineral N

The NZ-DNDC simulated emissions for the control (water only) and urine application are shown in Figure 8.4 a and 8.4 b. Emissions from the control treatment

were low (0.87 kg N ha^{-1}) and varied slightly during the 50 day period (Figure 8.4 a). The simulated N_2O emissions for the urine treatment varied from 1.8 to 213 g N ha^{-1} d^{-1} with the total model estimate of 4.56 kg N ha^{-1} (4560 g N ha^{-1}) over the 50 day period (Figure 8.4 b). The model-simulated peak emission was 18 days after urine application, followed by a subsequent decline and fluctuations until the end of the experimental period.

(a)



(b)

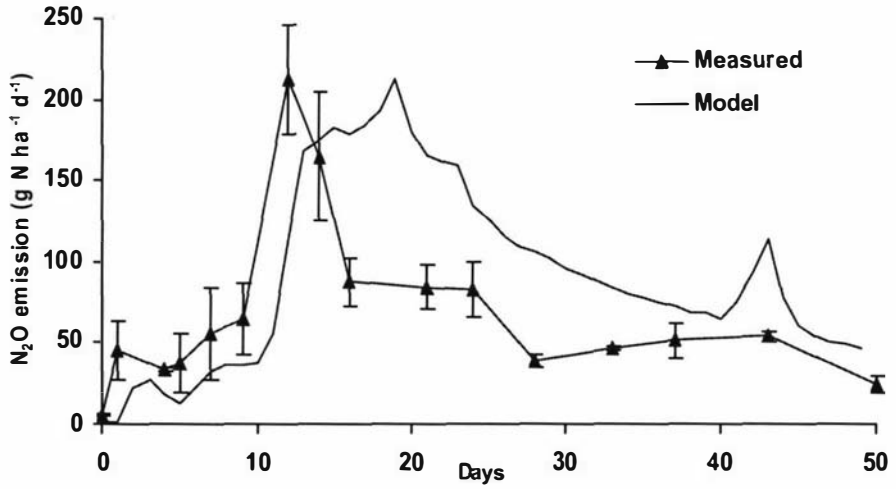


Figure 8.4 **Measured and NZ-DNDC simulated N_2O emissions for (a) the control treatment (b) the urine-only treatment. The measured values are the mean of three replicates. Error bars represent the standard deviation values.**

The application of urine simulated a small increase in the WFPS with no significant subsequent changes during the 50 day period (Figure 8.5). The model simulated an average WFPS of 0.53 which was close to the field-capacity moisture content for the entire experimental period. The measured values represent the mean of the five treatments, including the water control. Since there was no absolute control (no water/urine application) treatment in the field-plot study, it was not possible to examine the effect of the urine application on WFPS and to compare the simulated versus measured values.

The range in the measured and simulated mineral N concentrations (NH_4^+ and NO_3^- -N) in soil for the control, urine and urine+DCD treatments are shown in Table 8.1. The simulated NH_4^+-N concentrations for the control treatment was low and fluctuated slightly from 4.33 to 7.39 mg N kg^{-1} soil. Simulated NO_3^--N was also low in the control treatment and fluctuated between 3.44 to 13.7 mg N kg^{-1} soil. The simulated NH_4^+-N concentrations for the urine treatment reached a peak after 12 days; this subsequently declined until the end of 50 days. The modelled NH_4^+-N in 0-100 mm depth soil varied from 1.36 to 238 mg N kg^{-1} (Table 8.1). A constant build-up was observed in simulated NO_3^--N in the urine treatment until the end of the 50 day period, with concentrations ranging from 9.29 to 147 mg N kg^{-1} soil. The mineral N concentrations simulated using the N_{eff} factor of 0.8 showed an increase in the NH_4^+-N concentration in the urine+DCD treated plots as compared to the urine plots; the trend was reversed for NO_3^--N (Table 8.1). This trend was consistent with the effect of DCD addition, which inhibits the conversion of NH_4^+ to NO_3^- and results in an increase in NH_4^+ concentration.

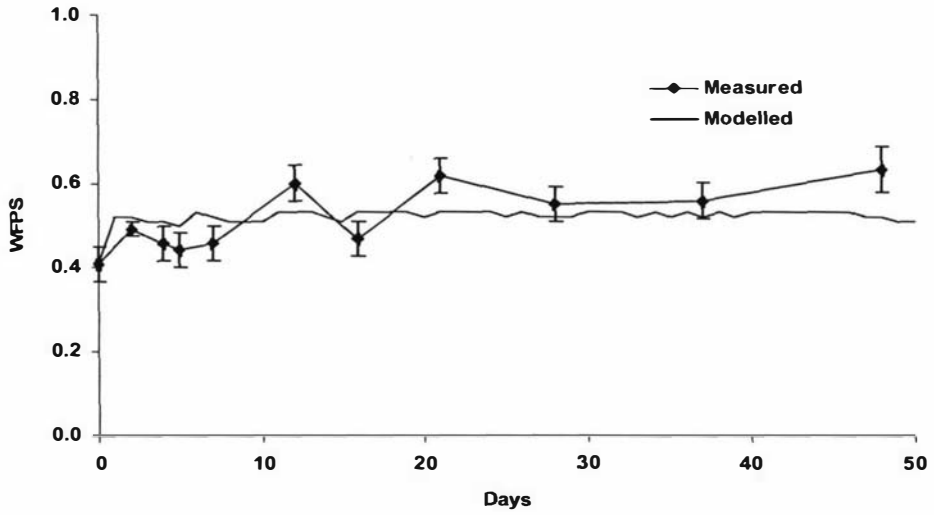


Figure 8.5 **Mean measured and simulated WFPS for all the treatments in the field-plot study. The measured values are the mean of all the five treatments. Error bars represent the standard deviation values for the measured data.**

Table 8.1 **Range and means of simulated and measured NH_4^+-N and NO_3^--N in the pasture soil for the control, urine and urine+DCD treatments. Mean values are given in brackets.**

Treatment	NH_4^+-N (mg kg^{-1} soil)		NO_3^--N (mg kg^{-1} soil)	
	Measured	**Modelled**	**Measured**	**Modelled**
Control	5.19-10.9 (8.04)	4.31-7.39 (5.85)	7.17-20.5 (13.8)	3.44-13.7 (8.57)
Urine	21.5-193 (89.4)	1.36-238 (123)	23.2-185 (115)	9.29-147 (82.9)
Urine+DCD	21.5-201 (119)	1.36-282 (189)	23.2-78.0 (53.4)	9.24-44.5 (25.8)

8.3.3 Comparison of the measured and modelled N_2O emission, WFPS and mineral N data

Overall, the NZ-DNDC simulated well the average daily N_2O fluxes from the control, urine and urine+DCD treatments compared with the measured daily N_2O fluxes from these treatments for the corresponding periods (Figure 8.4 a & b and 8.6). For the control treatment, the total modelled emission of 0.87 kg N ha^{-1} was close to the measured value in the field plot study (0.81± 0.24 kg N ha^{-1}) (Figure 8.7). In the urine-only treatment, the model slightly overestimated the total emission. The modelled emission was 4.56 kg N_2O-N ha^{-1} compared with the urine-only treatment of 3.37 ± 0.28 kg N_2O-N ha^{-1} (Figure 8.7). The measured N_2O emission peak in the urine-only treatment was observed 12 days after treatment application, whereas the model simulated N_2O emission peak occurred 18 days after treatment application (Figure 8.4 a). The total measured N_2O-N emission from the urine+DCD treatment was 1.62 ± 0.34 kg N_2O-N ha^{-1}, resulting in a 68% reduction in emissions with the addition of DCD to urine. This emission reduction was in the range of those simulated using N_{eff} values of 0.6 (2.55 kg N_2O-N ha^{-1}) and 0.8 (1.51 kg N_2O-N ha^{-1}) which gave reductions of 55% and 83%, respectively (Figure 8.6).

As mentioned above, with 20%, 40%, 60%, 80% and 100% nitrification inhibition (based on the N_{eff} values used in the model), the model-simulated reduction in total N_2O-N emissions was 14%, 32%, 55%, 83% and 117%, respectively.

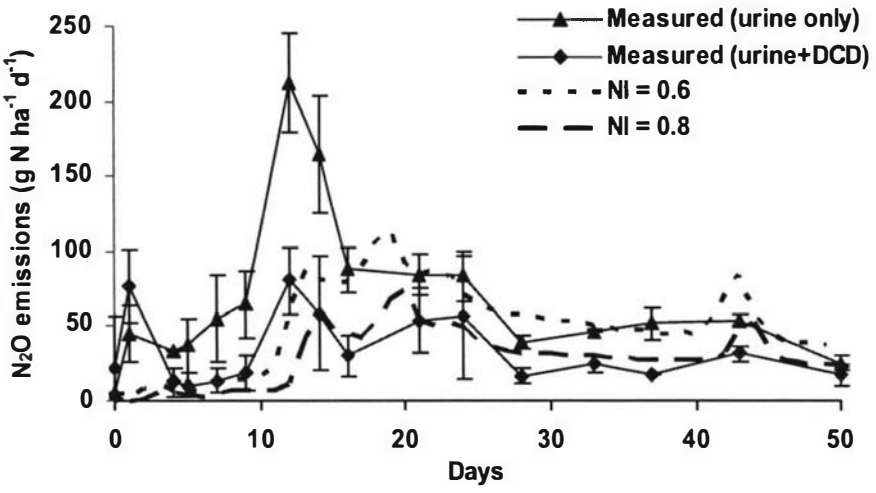


Figure 8.6 **Measured and simulated N_2O emissions for the urine+DCD treatment using Neff values of 0.6 and 0.8. The measured values are the mean of three replicates. Error bars represent the standard deviation values for the measured data.**

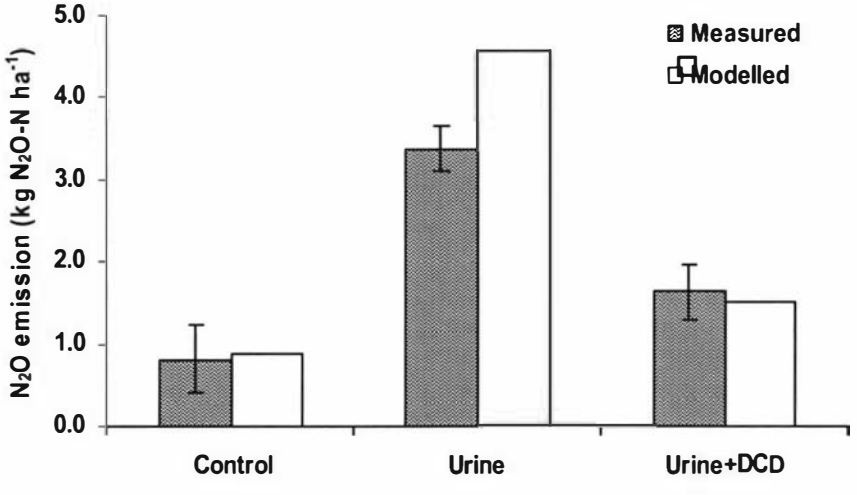


Figure 8.7 **Total measured and simulated N_2O emission for the control, urine and urine+DCD treatments. Error bars represent the standard error; for the urine+DCD treatment the simulation was obtained for a N_{eff} value of 0.8.**

The percent reduction in N_2O-N emissions from urine with the application of DCD increased with the increasing assumed percent DCD-induced nitrification inhibition (Figure 8.6). However, there was a slight discrepancy between these two values. At

the lower rates of nitrification inhibition (20-60%), the simulated reduction in N_2O emissions was slightly less than the corresponding nitrification inhibition indicating that, although denitrification is the main source of N_2O emission, some of N_2O may have been derived during the oxidation of NH_4^+ to NO_3^-. However, at the higher rates of nitrification inhibition (80-100%), the trend was the opposite. This may be attributed to the indirect effect of DCD (i.e., other than the inhibition of nitrification inhibition) on N_2O emission. For example, DCD-treated soil maintained a higher pH value than the untreated soil, thereby affecting the release of N_2O. It also has to be pointed out that nitrification inhibition caused by the addition of DCD also affects complete denitrification, thereby altering the ratio between N_2O and N_2 emission. Therefore, it may not be appropriate to relate the DCD-induced inhibition of nitrification solely to the reduction of N_2O emission.

The trend in N_2O emissions was similar between the measured and the modelled flux as for the control, urine and urine+DCD treatments, and the model was able to predict the peaks and the subsequent decline in emissions with time. However, the predicted peak in the model for the urine treatment was delayed by 7 days when compared to the measured peak.

The results of the simulations obtained with the 0.6 and 0.8 N_{eff} values suggest that, by using the appropriate inhibition efficiency factor, the model has the ability to account for NI efficacy in reducing N_2O emission. Such emission reductions cannot be generalised across all soil types and climate conditions as N_{eff} of DCD will vary with soil type, as demonstrated by monitoring the effect of DCD on different soils in Chapter 7. However, the results show that NZ-DNDC has the potential to account for changes in N_2O emissions with the addition of NI when the N_{eff} of a soil type is known. For this to be accomplished, more field data are required on the effect of DCD in a range of New Zealand soils.

The modelled WFPS values were within the measured values (Figure 8.5). The measured WFPS of the soil before the treatment application (0.41) showed a slight increase to 0.49 with the application of the treatments but then remained lower than the field-capacity moisture content (WFPS=0.53) until day 12. Although there was less fluctuation in the modelled WFPS than the measured values, the model simulated changes in WFPS agreed quite well with the measured changes in the top 5

cm depth for the plots treated with urine (Figure 8.5), with the difference between the simulated and measured WFPS values being generally less than 15%. These changes in WFPS covered all the major rainfall events.

The simulated mineral N values followed the same trend as the measured values in the urine+DCD treated plots, showing an increase in NH_4^+-N concentration as compared to the urine plots. The simulated NO_3^--N concentration in the urine+DCD plots was found to be lower than in urine-only plots. The simulated values of the mineral N data were observed to lie almost within the range of the measured values (Table 8.1), but on certain days the model tended to over- or under-estimate the mean concentration. In order to achieve more reliable field-scale estimates of mineral N, further model refinement is needed.

8.4 Conclusions

Application of DCD appears to have a short-term potential to reduce N_2O emissions and its effect may vary with soil type and climatic conditions. The NZ-DNDC model has the potential to account for the effect of DCD in reducing these emissions in a range of soil types and climatic conditions. More field data are required to further verify the ability of this model to predict emission-reductions with DCD application in different soil types.

The main conclusions that can be drawn from this study are as follows:

- NZ-DNDC effectively simulated the effect of DCD on N_2O emissions reductions.
- This modified NZ-DNDC model could be applied to soils using the estimated N_{eff} of DCD.
- The trend of N_2O emissions simulated by the NZ-DNDC model was similar to that observed under field measurements with both urine and urine+DCD application.
- The NZ-DNDC model needs to be further improved for predicting changes in NH_4^+ and NO_3^--N concentrations in soil.

Chapter 9

Summary and Conclusions

This chapter provides a summary of the major research findings obtained in the various laboratory incubation, glasshouse and field experiments conducted to examine the effect of urease (UI - Agrotain) and nitrification (NI - DCD) inhibitors in controlling N transformations and mitigating N losses through gaseous emissions of nitrous oxide (N_2O) and ammonia (NH_3) and nitrate (NO_3^-) leaching from urine and urea fertilisers. Furthermore, the degradation of NI (DCD) in four soil types, and the application of a process-based model to predict the effectiveness of the NI in reducing N_2O emissions, are also discussed. Finally, given the current knowledge of effectiveness of inhibitors, future research areas in relation to quantification and modelling of inhibitors-induced reductions in N losses, and the impact of inhibitors on various soil and plant processes associated with N transformation, are proposed.

9.1 Nitrogen in grazed pastures

The major land use in New Zealand is pastoral farming of sheep and cattle to produce wool, meat and milk for domestic consumption and the export market. In these legume-based pastures, nitrogen (N) is derived from biological N fixation by clover, fertiliser and N recycled through farm effluent application and the uneven deposition of animal excret. The major source of N loss in intensively managed grazed pastures is from cattle urine patches. The N deposited through animal excreta is far in excess of immediate plant requirements and hence is susceptible to losses as NH_3 and N_2O emissions and removal to drainage water through nitrate (NO_3^-) leaching. The quantities of N lost through these processes from grazed pastures depend on a number of factors including animal type, grazing intensity, grazing management, soil type, topography, effluent management and climatic conditions (Haynes & Williams 1993; Bolan *et al.* 2004).

9.2 Nitrogen loss and effect of inhibitors

Loss of N, occurring mainly through NH_3 volatilisation, biological denitrification and NO_3^- leaching, generally increase with farming intensity (Ledgard 2001) and so, unless effective controls can be found to minimise these losses, they could cause environmental degradation, thereby limiting the productivity of New Zealand pastoral soils and resulting in decreased N-use efficiency and an economical loss to farmers. There has been increasing interest in the use of inhibitors to mitigate environmental impacts of N losses from animal excreta and effluent application through leaching and gaseous emissions. This research was conducted to examine the potential role of urease and nitrification inhibitors as tools to manage these losses.

The UIs are used to control urea hydrolysis and the subsequent ammonification process through their effect on urease enzyme. The NIs are used to control the oxidation of NH_4^+ ions to NO_3^- ions (i.e. nitrification). These inhibitors are also likely to affect the leaching of basic cations which accompany NO_3^- to maintain charge balance in the soil solution (Figure 9.1).

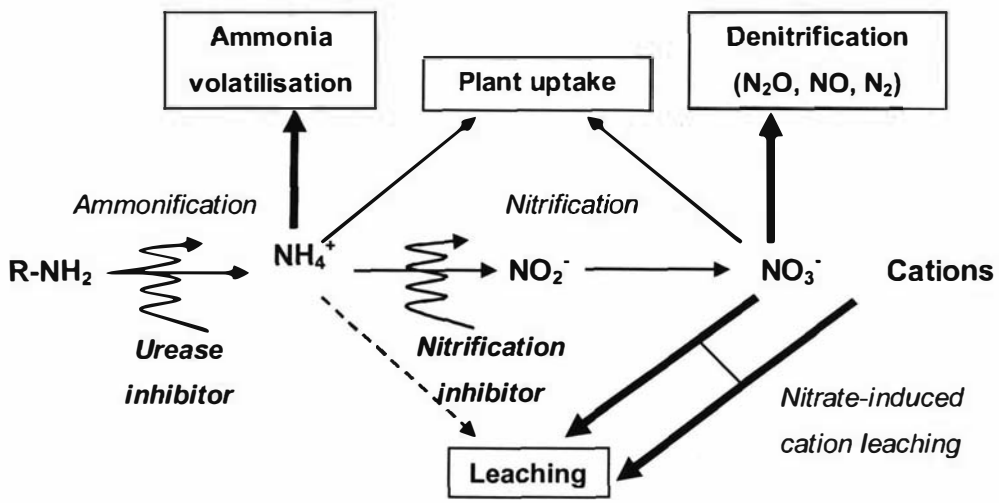


Figure 9.1 **Mechanisms of ammonification and nitrification, and the role of inhibitors in controlling the transformations of nitrogen and losses of nitrogen and cations.**

9.2.1 Ammonification and nitrification reactions

Urease inhibitors retard the hydrolysis of urea molecules derived from urine and urea fertiliser input, thereby preventing the immediate increases in the concentration of NH_4^+ ions and pH of the soil zone close to urea molecules (Figure 4.5, 4.9 and 6.10). In the absence of the UI, hydrolysis of urea results in the accumulation of exchangeable NH_4^+ in the soil within 1-2 days after urine deposition or urea application, whereas the presence of UI slows down the formation of exchangeable NH_4^+, due to the inhibition of urea hydrolysis (Chapter 6). Although the application of UI does not prevent the build-up of NH_4^+-N in the soil, it delays the peak concentration by 5-7 days in the urine treatment. For example, the results in Chapter 6 indicated that urine application @ 600 kg N ha^{-1} without UI, resulted in a peak NH_4^+ concentration of 341 mg N kg^{-1} soil within a day after application, whereas in the presence of UI, the NH_4^+ concentration reached a peak value of 299 mg N kg^{-1} soil, 5 days after application. Similarly, the pH value reached a maximum of 6.9 and 6.7 after a few hours and one day of urine application in the absence and presence of UI, respectively (Chapter 4). Urease inhibitor however had little or no effect on the conversion of NH_4^+ to NO_3^-, though the complete nitrification had been delayed by 5 days (Chapter 6).

Addition of NI to both urea and urine was effective in inhibiting the transformation of NH_4^+ to NO_3^--N in the soil, and maintaining the NH_4^+-N concentration significantly higher than that without NI. High concentrations of NH_4^+-N were observed in the soil cores receiving DCD with urea (36.2 mg N kg^{-1} soil) or urine (386 mg N kg^{-1} soil), as compared to those without DCD (7.48 and 144 mg N kg^{-1} soil, respectively) (Chapter 5). The rate of conversion of NH_4^+ to NO_3^- (nitrification rate) was almost 4 times lower in the soil receiving urine+DCD (5.02 mg NO_3^--N kg^{-1} d^{-1}) as compared to that in urine-alone treatment (21.2 mg NO_3^--N kg^{-1} d^{-1}), as seen in Chapter 6.

The application of both urease and nitrification inhibitors together following the application of urea and/ urine did not affect the highest concentration of NH_4^+-N produced after urea hydrolysis, although it delayed the time of hydrolysis by 7 days, as also observed in the application of UI alone. The peak NH_4^+ concentration obtained after the urine application to the pasture soil (Chapter 6) was 341 mg N kg^{-1} soil and the addition of the combined inhibitors to urine resulted in a peak of 273 mg NH_4^+-N kg^{-1}

soil after 7 days. The NH_4^+-N produced in the combined inhibitors treatment remained in the soil for a longer time compared to that in the Urine-alone or Urine+UI treatments. The rate of change of NH_4^+-N after attaining the peak concentration was 10.7 mg kg^{-1} d^{-1} in the Urine+NI+UI treatment as compared to 16.4 and 16.2 mg kg^{-1} d^{-1} in the Urine-only and Urine+UI treatments, respectively. The NO_3^- produced with the combined application of inhibitors to urea was significantly lower (21.5 mg N kg^{-1} soil), than that in the urea alone treatment (53.5 mg kg^{-1} soil), and was close to that in the urea+NI treatment (18.8 mg N kg^{-1} soil). The rate of nitrification was reduced significantly to 6.54 mg $kg^{-1}d^{-1}$ with the combined application of inhibitors to urine, compared to the 22.1 and 12.4 mg $kg^{-1}d^{-1}$ in the Urine-only and Urine+UI treatments, respectively.

9.2.2 Ammonia volatilisation

The incorporation of UI (Agrotain) with urine and urea (Sustain Yellow – urea+UI+elemental S; Sustain Green - urea+UI) was found to be highly effective in reducing NH_3 volatilisation and delaying the time for peak NH_3 loss (T_{max}), both in the urine treatment and urea treatment at low (100 kg N ha^{-1}) and high (600 kg N ha^{-1}) levels of N input to the pasture soils (Chapter 4). One of the important factors that control the UI-induced reduction in NH_3 volatilisation is the diffusion of NH_4^+ ions away from the zone of high soil pH associated with urea hydrolysis. The results have indicated that urea fertiliser N and urine N in the presence of UI, remain as urea N for a longer period, and that these neutral urea molecules diffuse to a greater depth than the NH_4^+ ions derived from the hydrolysis of urea fertiliser and urine in the absence of UI. Thus, the diffusion of urea molecules to lower soil depths reduces subsequent NH_3 volatilisation. The total reduction in NH_3 losses resulting from UI application varied from 23% to 46% in the urine and urea treatments. The application of UI caused a greater reduction in NH_3 emission from urea than from urine as Agrotain is coated on to urea granules, thereby remaining close to the site of high N concentration (Chapter 4 and 6). No difference was found in gaseous N emissions and N transformations between the Sustain Yellow (urea+UI+elemental S) and Sustain Green (urea+UI) treatments, indicating that the S coating did not influence the effect of Agrotain on N transformations of urea.

The application of DCD showed an increase in the total amount of NH_3 emitted in the urea and urine treatments (Chapter 5). DCD inhibits or delays the process of nitrification of NH_4^+ ions to NO_3^- ions, thereby increasing the concentration of NH_4^+ ions in the soil. The increased NH_4^+-N resulted in an increase in NH_3 emissions (35-44%) in the surface-applied urea treatments (Chapter 5 and 6). However, the increase in NH_3 volatilisation was not significant when DCD was applied to urine; this was attributed to the rapid permeation of urine into the soil, thereby providing less chance for the release of NH_3 gas. The application of UI and NI together with either urea or urine reduced NH_3 losses to a similar extent as when UI applied alone (Chapter 6).

9.2.3 Nitrous oxide emissions

The application of UI to urine and urea (Sustain Yellow and Sustain Green) showed varied effects on N_2O emissions under different conditions. In the glasshouse experiment under the high temperature of 25-30^{0}C (Chapter 4), UI decreased N_2O emissions when applied with urea at the lower rate (100 kg N ha^{-1}). The reason for this can be that the slower hydrolysis of urea to NH_4^+ caused by the UI, together with uptake of NH_4^+ by the grass (Table 4.4), would have resulted in a generally reduced concentration of NH_4^+ in the soil. Thus, less NH_4^+ was available to undergo potential nitrification and denitrification. However, UI was not effective in reducing N_2O emissions when applied with urine under field conditions, and with urea under glasshouse conditions at lower temperatures (Chapter 6). The effect of UI on N_2O emissions might also depend on the nitrification rate in soil, which is temperature dependant.

On the other hand, NI (DCD) was very effective in reducing N_2O emissions from urea and urine applied to pasture soil under both glasshouse and field conditions. As the application of DCD delays the microbial transformation of NH_4^+ to NO_3^--N, the concentration of NO_3^--N remains low in the soil, resulting in a lower amount of substrate for the release of N_2O by denitrification. This was evident from the concentration of NO_3^--N present in the soil during the experimental period, (Figure 6.9) and at the end of the experiment in soil cores (Chapter 5 & 6). For example, the results in Chapter 6 indicated that the NO_3^- concentration in the soil at 0-50 mm depth reached

a peak value of 294 mg N kg^{-1} soil 12 days after the application of urine (600 kg N ha^{-1}), whereas the NO_3^- concentration in the presence of NI reached a peak value of only 114 mg N kg^{-1} 17 days after application. The total reduction in N_2O emissions from urea and urine under different application conditions varied from 33 to 93%. When both the inhibitors were applied together, there was a considerable reduction of N_2O emissions (50%) from urea or urine applied alone.

9.2.4 Nitrogen leaching

DCD inhibits or delays the process of nitrification of NH_4^+-N to NO_3^--N, thus increasing the concentration of NH_4^+-N in the soil. The addition of DCD increased the NH_4^+ concentration in soil cores by 7- to 9- fold, thereby increasing the amount of NH_4^+ in the leachate by 2- to 3.5- times more than that found in the leachate without DCD (Figure 5.10). Although NH_4^+ ions are expected to be retained by cation-exchange sites, the accumulation of excess NH_4^+ ions would have caused the saturation of CEC, thereby making the NH_4^+ ions liable to leaching. However, it has to be pointed out that in urine- and urea-treated soils, NO_3^- ions act as an important charge balancing anion, thereby controlling the leaching of co-cations, including NH_4^+ (i.e. anion-induced cation leaching). Therefore, any reduction in NO_3^- leaching due to the application of NI to urine- or urea-treated soils is likely to result in a decrease in the leaching of co-cations, including NH_4^+ ions.

The reduction in NO_3^--N concentrations in soil due to the application of DCD to the urine treatments resulted in a 60-65% decrease in NO_3^- leaching loss (Chapter 5). Although there was an increase in the NH_4^+ concentration of the leachate with the addition of DCD, the decrease in NO_3^- leaching with DCD application resulted in an overall reduction of 45% in total N leached as NO_3^- was the main N component in the leachate, (Figure 5.10). Thus, DCD reduces N leaching through its direct effect on NO_3^- leaching and indirect effect on anion-induced NH_4^+ leaching.

9.2.5 Nitrogen-induced cation leaching

The dominant cations in the leachate in the study conducted with urine applied to pasture soil were Ca^{+2} and K^{+}, with low concentrations of Mg^{+2} and NH_4^{+} (Chapter 5). The reduction in NO_3^{-} leaching resulting from DCD application caused a corresponding decrease in the leaching of counter ions (cations). The 60-65% decrease in NO_3^{-} leaching with the DCD application caused leaching reductions of 72% for Ca^{+2}, 33 to 50% for Mg^{+2} and 36 to 42% for K^{+}. Nitrate is a weakly held anion (non-specific adsorption), in contrast to other anions like phosphate which undergoes specific adsoption; when nitrate is subject to leaching it is associated with cations as charge-balancing counter ions. Thus, the reduction in NO_3^{-} leaching also caused a decrease in the leaching of counter ions (cations). However, urine also contains other non-specifically adsorbed anions such as Cl^{-} and SO_4^{-}, which may also contribute to anion-induced cation leaching.

9.2.6 Nitrogen-use efficiency

The incorporation of Agrotain along with urea as Sustain Yellow and Sustain Green (Chapter 4) and with urine (Chapter 6) showed a slight increase in dry matter yield, by 15-20% and 6%, as compared to that in the urea and urine-alone treatments, respectively. Application of UI with urea/urine has been very effective in inhibiting and delaying urea hydrolysis, resulted in better diffusion of urea molecules in the soil and, consequently, reduced volatilisation losses. Thus, this delay in the release of NH_4^{+} ions, and the associated reduction in NH_3 losses, resulted in more N being taken up by the plants and, thereby, greater dry matter yields.

Addition of DCD did not affect pasture yields and pasture N concentrations significantly compared with those in the urine and urea-alone treatments (Chapter 5) in the glasshouse studies; however in the field-plot study it increased dry matter yields slightly (3%). As DCD maintained soil N in the NH_4^{+} form for a longer time (Chapter 6), the differences in pasture yields in the different experiments might have been a consequence of the change from NO_3^{-} to NH_4^{+} nutrition, to which the plants were subjected. Moreover, DCD has been reported to produce phytotoxic effects in clover and lettuce (Macadam *et al.* 2003). Thus, the differences in the dry matter yields in

treatments with and without DCD can be attributed to the above two factors and their interaction (induced ammonia nutrition or DCD toxicity per se).

The application of the two inhibitors (UI+NI) together in the urea and/or urine treatments resulted in an increase in dry matter yield by 13% (Chapter 6) and also in a higher dry matter response to applied N, compared with those found when these inhibitors were applied individually.

9.3 Modelling the effect of inhibitors

Although the study has demonstrated that the combined application of UI and NI is more effective in mitigating N losses when compared to their individual applications, urease enzyme is produced by all microbial and plant species in soils and it is difficult to control activity of this enzyme using UI. Therefore, the modelling exercise focussed on the value of NI in mitigating N_2O emissions.

The effectiveness of NI (DCD) in controlling nitrification has been found to be influenced by numerous environmental and soil factors, such as organic matter, pH, and temperature. The varying effectiveness of DCD makes it difficult to predict the sustainability of using DCD for N management in grazed pastures in different regions and land-management regimes. The laboratory incubation experiment was therefore conducted to see the effectiveness of DCD at different concentrations in four different soils of New Zealand (Chapter 7). The effectiveness of DCD in inhibiting N_2O emissions varied among the soils, which may be attributed to differences in organic matter and in clay type (allophane) and content in the different soils. Although DCD was effective in inhibiting nitrification, there was no consistent relationship between nitrification inhibition and rate of DCD degradation.

As the NZ-DNDC model accounts for the effect of soil type and climatic conditions on N_2O emissions, this model was further modified to simulate the effect of DCD in reducing N_2O emissions. The model was able to simulate adequately the N_2O emissions from urine application for Tokomaru soil under field conditions with an NI efficiency index (N_{eff}) of 0.6-0.8. This modified NZ-DNDC model could thus be applied to different soil types using the estimated/measured N_{eff} of DCD. The model was, however, not able to effectively simulate the NH_4^+ and NO_3^- concentrations in the soil

and, therefore, needs to be further tested and modified to be able to accurately predict mineral N in the presence of applied inhibitor.

9.4 Future research

The research work described in this thesis suggests a number of areas that require further investigation. Some of the major research areas relating to urease and nitrification inhibitors in managing N dynamics in grazed pastures include:

- More long-term field studies are needed to examine the effect of urease and nitrification inhibitors on various pathways of N losses, especially under intensively managed grazed pastures. For example, in the present study there was clear evidence that urease inhibitors tend to decrease NH_3 volatilisation, whereas the nitrification inhibitors show the opposite effect. Quantitatively, NH_3 volatilisation results in a greater N loss than from N_2O emissions, especially at high levels of N input through urea-based fertilisers and urine deposition in dairy pastures. Similarly, while nitrification inhibitors have been shown to have a positive effect in reducing N_2O emissions and NO_3^- leaching, the urease inhibitors have shown no effect on these N losses. It is therefore important to examine the relative effects of these two groups of inhibitors in controlling N losses at various levels of N input.
- A range of natural and synthetic N transformation inhibitors are available in the market. Of the commercially developed nitrification inhibitors, the most widely used are Nitrapyrin and Dicyandiamide (DCD), with a newer product (3,4-dimethylpyrazole phosphate; DMPP) being investigated mostly in Europe. Other products include Etridiazole (a fungicide), 2-ethynylpyridine (2-EP) and various coated acetylene products. Some of natural products include essential oils from certain plant species such as *Mentha spicata* and *Artemisia annua* (Usha & Patra 2003), neem cake (*Azadirachta indica*) and karanj (*Pongamia glabra* Vent) (Sahrawat 2003). There is also a variety of UIs available, such as phenylphosphorodiamidate (PPD), hydroquinone and the extensively used N–(n-butyl)thiophosphric triamide (NBPT). The effects of these products on the

transformation and loss of N need to be examined in relation to their persistence and impact on the microbial community.

- In addition to reducing N losses, inhibitors have been shown to be beneficial in controlling the nitrate-induced leaching losses of basic cations such as potassium, calcium and magnesium. The relative role of readily leachable anions, such as nitrate and chloride, in controlling the leaching of basic cations as charge balancing co-ions needs to be examined in grazed pastures. The value of inhibitors in reducing the leaching losses of these basic cations by controlling nitrate leaching needs to be examined in conjunction with the uptake of these cations by plants and the subsequent implications on the cation-anion balance in plants.
- Nitrogen transformation inhibitors affect soil pH through their effects on N transformations and leaching, and plant uptake of associate cations. It has been consistently shown that the pH of urea- and urine-treated soil remained high for a longer period in the presence than absence of nitrification inhibitors; this is attributed mainly to the decrease in proton release due to the inhibition of nitrification. The plant uptake of excessive NH_4^+ ions in the presence of nitrification inhibitors is likely to affect the rhizosphere pH due to the cation/anion balance in plants. The long term-effects of these processes in controlling the pH of soil resulting from the application of inhibitors need to be examined in detail.
- Although most inhibitors are specific in controlling the transformation of urea and ammonium N, they can also affect other beneficial and detrimental microorganisms in soils. The long-term effect of commercial inhibitors on microbial communities needs to be examined.
- Certain plant species, such as *Brachiaria humidicola,* have been shown to inhibit the nitrification process by suppressing the growth of ammonia-oxidising bacteria, accumulating NO_3^- in the soil, and enhancing N absorption. However, there is little information in the literature on the type of N taken up by plants after the application of inhibitors, or on their effects on the biochemical processes of N assimilation. For example, in the presence of nitrification inhibitors pasture plants tend to take up less nitrate-N, thereby reducing nitrate toxicity to grazing animals. The relative uptake of ammonium and nitrate nitrogen in the presence of inhibitors needs to be examined.

- Modification of the NZ-DNDC model to parameterize it for simulating N losses through gaseous emissions and nitrate leaching in the presence of inhibitors requires urgent attention. The accuracy of the model to predict these losses from urine and urea-based fertilisers could be further improved by examining the soil and climatic factors affecting the degradation and persistence of inhibitors, and the efficiency of inhibitors in controlling the transformation of various forms on N input.

References

Abbasi M.K., Shah Z. & Adams W.A. (2003). Effect of the nitrification inhibitor nitrapyrin on the fate of nitrogen applied to a soil incubated under laboratory conditions. *Journal of Plant Nutrition and Soil Science*, 166, 513-518.

Agriculture and Agri-Food Canada (2003). The Health of our water: Towards a sustainable agriculture in Canada. Ch. 7d. http://www.res2.agr.gc.ca/publications/hw/07d_e.htm

Amberger A. (1986). Potentials of nitrification inhibitors in modern N- fertilizer management. *Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, 149, 469-484.

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

Amberger A. and Germannbauer M.P (1990) Effect of the nitrification inhibitors 1-Amidino-2-thiourea and dicyandiamide in combination with urea and ammonium-sulfate. *Fertilizer Research,* 21, 179-183

Amberger A. & Vilsmeier K. (1979). Inhibition of the nitrification of liquid manure nitrogen by dicyandiamide. *Zeitschrift fur Acker und Pflanzenbau-Journal of Agronomy and Crop Science*, 148, 239-246.

Amtul Z., Atta-ur-Rahman, Siddiqui R.A. & Choudhary M.I. (2002). Chemistry and mechanism of urease inhibition. *Current Medicinal Chemistry*, 9, 1323-1348.

Anger M., Hoffmann C. & Kuhbauch W. (2003). Nitrous oxide emissions from artificial urine patches applied to different N-fertilized swards and estimated annual N_2O emissions for differently fertilized pastures in an upland location in Germany. *Soil Use and Management*, 19, 104-111.

Aulakh M.S., Rennie D.A. & Paul E.A. (1984). Acetylene and N-Serve effects upon N_2O emissions from NH_4^+ and NO_3^- treated soils under aerobic and anaerobic conditions. *Soil Biology & Biochemistry*, 16, 351-356.

Ball P.Roger. (1979). Nitrogen relationships in grazed and cut grass-clover systems. PhD Thesis, Massey University, Palmerston North, New Zealand. 217pp.

Beauchamp E.G. (1997). Nitrous oxide emissions from agricultural soils. *Canadian Journal of Soil Science*, 77, 113-123.

Belastegui Macadam X.M., del Prado A., Merino P., Estavillo J.M., Pinto M. & Gonzalez-Murua C. (2003). Dicyandiamide and 3,4-dimethyl pyrazole phosphate decrease N_2O emissions from grassland but dicyandiamide produces deleterious effects in clover. *Journal of Plant Physiology*, 160, 1517-1523.

Black A.S., Sherlock R.R., Cameron K.C., Smith N.P. & Goh K.M. (1985). Comparison of three field methods for measuring ammonia volatilization from urea granules broadcast on to pasture. *Journal of Soil Science*, 36, 271-280.

Blakemore L.C., Searle P.L. & Daly B.K. (1987). Methods for chemical analysis of soils. In: *New Zealand Soil Bureau Science Report No. 80* Lower Hutt.

Bock E., Koops H.-P., Harms H. & Ahlers B. (1991). The biochemistry of nitrifying organisms. In: *Variations in autotrophic life* (eds. Shively JM & Barton LL). Acadamic Press London, pp. 171-200.

Bolan N.S., Saggar S., Luo J.F., Bhandral R. & Singh J. (2004). Gaseous emissions of nitrogen from grazed pastures: Processes, measurements and modelling, environmental implications and mitigation. *Advances in Agronomy*, 84, 37-120.

Bouwman A.F. (1990). Exchange of greenhouse gases between terrestrial ecosystems and the atmosphere. In: *Soils and the greenhouse effect* (ed. Bouwman AF). John Wiley & Sons Ltd Chichester, pp. 61-127.

Bouyoucos G.J. (1962). Hydrometer method improved for making particle size analysis of soils. *Agronomy Journal*, 54, 464-465.

Bowatte W.M.S.D. (2003). Urine nitrogen in hill country pasture soils. PhD thesis, Massey University Palmerston North, New Zealand. pp. 274.

Bremner J.M. (1995). Recent research on problems in the use of urea as a nitrogen fertilizer. *Fertilizer Research*, 42, 321-329.

Bremner J.M., Blackmer A.M. & Waring S.A. (1980). Formation of nitrous-oxide and dinitrogen by chemical decomposition of hydroxylamine in soils. *Soil Biology & Biochemistry*, 12, 263-269.

Bremner J.M. & Chai H.S. (1986). Evaluation of N-butyl phosphorothioic triamide for retardation of urea hydrolysis in soil. *Communications in Soil Science and Plant Analysis*, 17, 337-351.

Bremner J.M. & Chai H.S. (1989). Effects of phosphoroamides on ammonia volatilization and nitrite accumulation in soils treated with urea. *Biology and Fertility of Soils*, 8, 227-230.

Bremner J.M., McCarty G.W. & Higuchi T. (1991). Persistence of the inhibitory effects of phosphoroamides on urea hydrolysis in soils. *Communications in Soil Science and Plant Analysis*, 22, 1519-1526.

Bremner J.M., McCarty G.W., Yeomans J.C. & Chai H.S. (1986). Effects of phosphoroamides on nitrification, denitrification, and mineralization of organic nitrogen in soil. *Communications in Soil Science and Plant Analysis*, 17, 369-384.

Bremner J.M. & Mulvaney R.L. (1978). Urease activity in soils. In: *Soil Enzymes* (ed. Burns RG). Academic Press London, pp. 149-196.

Bremner J.M. & Tabatabai M.A. (1971). Use of automated combustion techniques for total carbon, total nitrogen, and total sulphur analysis of soils. In: *Instrumental methods for analysis of soils and plant tissue* (ed. Walsh LM). Soil Science Society of America, Madison.

Broadbent F.E., Nakashima T. & Chang G.Y. (1985). Performance of some urease inhibitors in field trials with corn. *Soil Science Society of America Journal*, 49, 348-351.

Bronson K.F., Mosier A.R. & Bishnoi S.R. (1992). Nitrous-oxide emissions in irrigated corn as affected by nitrification inhibitors. *Soil Science Society of America Journal*, 56, 161-165.

Bronson K.F., Touchton J.T., Hauck R.D. & Kelley K.R. (1991). N-15 recovery in winter-wheat as affected by application timing and dicyandiamide. *Soil Science Society of America Journal*, 55, 130-135.

Bronson K.F., Touchton J.T. & Hauck R.S. (1989). Decomposition rate of dicyandiamide and nitrification inhibition. *Communications in Soil Science and Plant Analysis*, 20, 2067-2078.

Brye K.R. & Norman J.M. (2004). Land-use effects on anion-associated cation leaching in response to above-normal precipitation. *Acta Hydrochimica et Hydrobiologica*, 32, 235-248.

Bundy L.G. & Bremner J.M. (1973). Inhibition of nitrification in soils. *Soil Science Society of America Journs*, 37.

Buresh R.J., Dedatta S.K., Padilla J.L. & Samson M.I. (1988). Field-evaluation of 2 urease inhibitors with transplanted lowland rice. *Agronomy Journal*, 80, 763-768.

Byrnes B.H. & Freney J.R. (1995). Recent developments in the use of urease inhibitors in the tropics. *Fertilizer Research*, 42, 251-259.

Byrnes B.H., Savant N.K. & Craswell E.T. (1983). Effect of a urease inhibitor phenyl phosphorodiamidate on the efficiency of urea applied to rice. *Soil Science Society of America Journal*, 47, 270-274.

Cameron K.C., Di H.J., Moir J., Roberts A., Pellow R. & Christie R. (2005). Treating grazed pasture soil with a nitrification inhibitor "ECO-N" to decrease nitrate leaching. In: *Developments in Fertliliser Application Technologies and Nutrient Management* (eds. Currie LD & Hanly JA) Fertiliser and Lime Research Centre, Massey University, Palmerston North, pp. 93-103.

Carmona G., Christianson C.B. & Byrnes B.H. (1990). Temperature and low concentration effects of the urease inhibitor N-(n-butyl) thiophosphoric triamide (Nbtpt) on ammonia volatilization from urea. *Soil Biology & Biochemistry*, 22, 933-937.

Carran R.A., Ball P.R., Theobald P.W. & Collins M.E.G. (1982). Soil nitrogen balances in urine-affected areas under two moisture regimes in Southland. *New Zealand Journal of Experimental Agriculture*, 10, 377-381.

Carran R.A., Theobald P.W. & Brock J.L. (2000). Novel passive samplers for assessing NH_3 volatilisation from difficult sites. In: *Soil 2000: New Horizons for a New Century. Australian and New Zealand Second Joint Soils Conference.* (eds. Adams JA & Metherell AK) Lincoln University, New Zealand Society of Soil Science, pp. 47-48.

Christianson C.B., Baethgen W.E., Carmona G. & Howard R.G. (1993). Microsite reactions of urea-nBTPT fertilizer on the soil surface. *Soil Biology & Biochemistry*, 25, 1107-1117.

Christianson C.B., Byrnes B.H. & Carmona G. (1990). A comparison of the sulfur and oxygen analogs of phosphoric triamide urease inhibitors in reducing urea hydrolysis and ammonia volatilization. *Fertilizer Research*, 26, 21-27.

Clay D.E., Malzer G.L. & Anderson J.L. (1990). Ammonia volatilization from urea as influenced by soil-temperature, soil-water content, and nitrification and hydrolysis inhibitors. *Soil Science Society of America Journal*, 54, 263-266.

Clayton H., McTaggart I.P., Parker J., Swan L. & Smith K.A. (1997). Nitrous oxide emissions from fertilised grassland- A 2-year study of the effects of N fertiliser form and environmental conditions. *Biology and Fertility of Soils*, 25, 252-260.

Clough T.J., Ledgard S.F., Sprosen M.S. & Kear M.J. (1998). Fate of N labelled urine on four soil types. *Plant and Soil*, 199, 195-203.

Clough T.J., Sherlock R.R., Cameron K.C. & Ledgard S.F. (1996). Fate of urine nitrogen on mineral and peat soils in New Zealand. *Plant and Soil*, 178, 141-152.

Cookson W.R. & Cornforth I.S. (2002). Dicyandiamide slows nitrification in dairy cattle urine patches: effects on soil solution composition, soil pH and pasture yield. *Soil Biology & Biochemistry*, 34, 1461-1465.

Crush J.R. (1987). Nitrogen fixation. In: *White clover* (eds. Baker MJ & Williams WM). CAB International Wallingford, UK, pp. 185-202.

Davies D.M. & Williams P.J. (1995). The effect of the nitrification inhibitor dicyandiamide on nitrate leaching and ammonia volatilization - a UK nitrate sensitive areas perspective. *Journal of Environmental Management*, 45, 263-272.

De Boer W. & Kowalchuck G.A. (2001). Nitrification in acid soils: microorganisms and mechanisms. *Soil Biology & Biochemistry*, 33, 853-866.

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, 77-85.

de Klein C.A.M. & Monaghan R.M. (2005). The impact of potential nitrous oxide mitigation strategies on the environmental and economic performance of dairy systems in 4 New Zealand catchments. In: *Non-CO_2 Greenhouse Gases (NCGG-4)*. Millipress Rotterdam The Netherlands, pp. 593–600. .

de Klein C.A.M. & van Logtestijn R.S.P. (1994). Denitrification and N_2O emission from urine-affected grassland soil. *Plant and Soil*, 163, 235-241.

Denmead O.T. (1983). Micrometeorological methods for measuring gaseous losses of nitrogen in the field. In: *Gaseous loss of nitrogen from plant-soil systems* (eds. Freney JR & Simpson JR). Martinus Nijhoff/Dr W Junk Publishers The Hague, pp. 133-157.

Di H.J. & Cameron K.C. (2000). Calculating nitrogen leaching losses and critical nitrogen application rates in dairy pasture systems using a semi-empirical model. *New Zealand Journal of Agricultural Research*, 43, 139-147.

Di H.J. & Cameron K.C. (2002a). Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutrient Cycling in Agroecosystems*, 64, 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, 395-403.

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

Di H.J. & Cameron K.C. (2004a). 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, 927-932.

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

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

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 pastures. *Agriculture Ecosystems & Environment*, 109, 202-212.

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

Duxbury J.M., Bouldin D.R., Terry R.E. & Tate R.L. (1982). Emissions of nitrous-oxide from soils. *Nature*, 298, 462-464.

Ebina J., Tsutsui T. & Shirai T. (1983). Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation. *Water Research*, 17, 1721-6.

Fenn L.B. & Hossener L.R. (1985). Ammonia volatilization from ammonium or ammonia-forming nitrogen fertilizers. *Advances in Soil Science*, 1, 123-169.

Field T.R.O., Theobald P.W., Ball P.R. & Clothier B.E. (1985) Leaching losses of nitrate from cattle urine applied to a lysimeter. In *Proceedings of Agronomy Society of New Zealand*, 15, 137-141.

Firestone M.K. & Davidson E.A. (1989). Microbiological basis of NO and N_2O production and consumption in soil. In: *Exchange of trace gasses between terrestrial ecosystems and the atmosphere* (eds. Andreae MO & Schimel DS). John Wiley New York, pp. 7-21.

Fox R.H., Piekielek W.P. & Macneal K.E. (1996). Estimating ammonia volatilization losses from urea fertilizer using a simplified micro-meteorological sampler. *Soil Science Society of America Journal*, 60, 139-146.

Francis G.S., Haynes R.J. & Speit P.H. (1995) The effects of a nitrification inhibitor on leaching losses and recovery of mineralized nitrogen by a wheat crop ploughing-in temporary leguminous pastures. *Fertilizer Research*, 41, 33-39.

Frankenberger W.T. & Tabatabai M.A. (1982). Amidase and urease activities in plants. *Plant and Soil*, 64, 153-166.

Freney J.R. (1997). Strategies to reduce gaseous emissions of nitrogen from irrigated agriculture. *Nutrient Cycling in Agroecosystems*, 48, 155-160.

Freney J.R. & Black A.S. (1988). Importance of ammonia volatilization as a loss process. In: *Advances in Nitrogen Cycling in Agricultural Ecosystems.* (ed. Wilson JR). CAB International Wallingford, pp. 156-173.

Freney J.R., Denmead O.T., Watanabe I. & Craswell E.T. (1981). Ammonia and Nitrous-Oxide losses following applications of ammonium-sulfate to flooded rice. *Australian Journal of Agricultural Research*, 32, 37-45.

Freney J.R., Simpson J.R. & Denmead O.T. (1983). Volatilization of ammonia. In: *Gaseous Loss of Nitrogen from Plant-Soil Systems* (eds. Freney JR & Simpson JR). Martinus Nijhoff/Dr W Junk Publishers The Hague, pp. 1-32.

Frolking S.E., Mosier A.R., Ojima D.S., Li C., Parton W.J., Potter C.S., Priesack E., Stenger R., Haberbosch C., Dorsch P., Flessa H. & Smith K.A. (1998). Comparison of N_2O emissions from soils at three temperate agricultural sites: simulations of year round measurements by four models. *Nutrient Cycling in Agroecosystems*, 52, 77-105.

Gale G.C. & Atkins I.M. (1969). Inhibition of urease by hydroxamic acids. *Archives Internationales de Pharmacodynamie et de Therapie*, 180, 289-298.

Gillingham A.G., Saggar S., Clothier B.E., Zaman M., Green S., Ross D., Singh J. & Francis G.S. (2006). Development of technology to minimise N losses from grazed pastures. In: *Confidential Summit-Quinphos funded research contract to Agresearch, Progress Report 2*, p. 21.

Giltrap D.L., Saggar S., Singh J. & Li C. (2006). Modelling the effects of nitrification inhibitors on nitrous oxide emissions from grazed pastures. In: *'Soil and Society' New Zealand Soil Science Society Conference* Rotorua, New Zealand.

Giltrap D.L., Saggar S., Tate K.R. & Li C. (2004). Using the "NZ-DNDC" model to simulate the effects of changing land management on nitrous oxide and carbon dioxide emissions from New Zealand grazed pastures. In: *3rd Australian New Zealand Soils Confernce* (ed. Singh B) University of Sydney, Australia.

Gioacchini P., Nastri A., Marzadori C., Giovannini C., Antisari L.V. & Gessa C. (2002). Influence of urease and nitrification inhibitors on N losses from soils fertilized with urea. *Biology and Fertility of Soils*, 36, 129-135.

Goh K.M. & Williams P.H. (1999). Comparative nutrient budgets of temperate grazed pastures. In: *Nutrient disequilibria in global agroecosystems: concepts and case studies.* (eds. Fresco LO, Smailing EM & Oenema O). CAB International Wallingford, UK, pp. 173-191.

Grundmann G.L., Renault P., Rosso L. & Bardin R. (1995). Differential effects of soil water content and temperature on nitrification and aeration. *Soil Science Society of America Journal*, 59, 1342-1349.

Guiraud G. & Marol C. (1992). Influence of temperature on mineralization kinetics with a nitrification inhibitor (mixture of dicyandiamide and ammonium thiosulphate). *Biology and Fertility of Soils*, 13, 1-5.

Guiraud G., Marol C. & Thibaud M.C. (1989). Mineralization of nitrogen in the presence of a nitrification inhibitor. *Soil Biology & Biochemistry*, 21, 29-34.

Hallinger S., Wallnofer P.R., Goldbach H. & Amberger A. (1990). Several aspects of bacterial dicyandiamide degradation. *Naturwissenschaften*, 77, 332-334.

Hargrove W.L., Kissel D.E. & Fenn L.B. (1977). Field measurement of ammonia volatilization from surface application of ammonia salts to a calcareous soil. *Agronomy Journal*, 69, 473-476.

Hatch, D., Trindade, H., Cardenas, L., Carneiro, J., Hawkins, J., Scholefield, D., & Chadwick, D. (2005). Laboratory study of the effects of two nitrification inhibitors on greenhouse gas emissions from a slurry-treated arable soil: impact of diurnal temperature cycle. *Biology and Fertility of Soils, 41*, 225-232.

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

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

Hendrickson L.L., Omholt T.E. & O'Connor M.J. (1987). Effect of phenylphosphorodiamidate on immobilzation and ammonia volatilization. *Soil Science Society of America Journal*, 51, 1067-1071.

Hesse P.R. (1971). *A textbook of soil chemical analysis*, London.

Holmes W. (1968). The use of nitrogen in the management of pasture for cattle. *Herbage Abstracts*, 38, 265-277.

Hooper A.B., Vannelli T., Bergmann D.J. & Acciero D. (1997). Enzymology of oxidation of ammonia to nitrite by bacteria. *Antonie van Leeuwenhoek*, 71, 59-67.

Irigoyen I., Muro J., Azpilikueta M., Aparicio-Tejo P. & Lamsfus C. (2003). Ammonium oxidation kinetics in the presence of nitrification inhibitors DCD and DMPP at various temperatures. *Australian Journal of Soil Research*, 41, 1177-1183.

Jacinthe P.A. & Pichtel J.R. (1992). Interaction of Nitrapyrin and Dicyandiamide with soil humic compounds. *Soil Science Society of America Journal*, 56, 465-470.

Jarvis S.C., Hatch D.J., Pain B.F. & Klarenbeek J.V. (1994). Denitrification and the evolution of nitrous oxide after the application of cattle slurry to a peat soil. *Plant and Soil*, 166, 231-241.

Joo Y.K., Christians N.E. & Bremner J.M. (1987). Effect of N-(normal-butyl) thiophosphoric triamide (Nbpt) on growth-response and ammonia volatilization following fertilization of kentucky bluegrass (Poapratensis L) with urea. *Journal of Fertilizer Issues*, 4, 98-102.

Joo Y.K., Christians N.E., Spear G.T. & Bremner J.M. (1992). Evaluation of Urease Inhibitors as urea amendments for use on Kentucky bluegrass turf. *Crop Science*, 32, 1397-1401.

Keeney D.R. (1980). Factors affecting the persistence and bioactivity of nitrification inhibitors. In: *Nitrification inhibitors: potentials and limitations* (ed. Stelly M). American Society of Agronomy, Madison Wisconsin, pp. 33-46.

Kelliher F.M., Sedcole J.R., Minchin R.F., Wan Y., Condron L.M., Clough T.J. & Bol R. (2005). Soil microbial respiration responses to repeated urea applications in three grasslands. *Australian Journal of Soil Research*, 43, 905-913.

Khalil M.I., Schmidhalter U. & Gutser R. (2006). N_2O, NH_3 and NO_X emissions as a function of urea granule size and soil type under aerobic conditions. *Water Air and Soil Pollution*, 175, 127-148.

Killham K. (1986). Heterotrophic nitrification. In: *Nitrification* (ed. Prosseer JI). IRL Press Oxford, UK, pp. 117-126.

Kissel D.E., Brewer H.L. & Arkin G.F. (1977). Design and test of a field sampler for ammonia volatilization. *Soil Science Society of America Journal*, 41, 1133-1138.

Kissel D.E. & Cabrera M.L. (1988). Factors affecting urea hydrolysis. In: *Ammonia volatilization from urea fertilizers* (eds. Bock BR & Kissel DE). National Fertilizer Development Center, Tennessee Valley Authority, Muscle Shoals, Alabama, pp. 53-66.

Kobashi K., Hase J.I. & Uehara K. (1962). Specific inhibition of urease by hydroxamic acids. *Biochimica et Biophysica Acta*, 62, 380-383.

Kobashi K., Munakata K.I., Takebe S. & Hase J.I. (1980). Therapy for urolithiasis by hydroxamic acids. II. Urease inhibitory potency and urinary excretion rate of hippurohydroxamic acid derivatives. *Journal of Pharmacobiodynamics.*, 3, 444-450.

Kobashi K., Takebe S. & Numata A. (1985). Specific-inhibition of urease by n-acylphosphoric triamides. *Journal of Biochemistry*, 98, 1681-1688.

Koops J.G., vanBeusichem M.L. & Oenema O. (1997). Nitrous oxide production, its source and distribution in urine patches on grassland on peat soil. *Plant and Soil*, 191, 57-65.

Kowalchuck G.A. & Stephen J.R. (2001). Ammonia-oxidising bacteria: a model for molecular ecology. *Annual Reviews Microbiology*, 55, 485-529.

Kuenen J.G. & Robertson L.A. (1994). Combined nitrification-denitrification processes. *FEMS Microbiology Reviews*, 15, 109-117.

Ladd J.N., Oades J.M. & Amato M. (1981). Microbial biomass formed from ^{14}C, ^{15}N-labelled plant material decomposing in soils in the field. *Soil Biology & Biochemistry*, 13, 119-126.

Lea P. (1993). Nitrogen metabolism. In: *Plant Biochemistry and Moleculer Biology* (eds. Lea PJ & Leegood RC). John Wiley and Sons New York, pp. 155-180.

Ledgard S.F. (1995). Leaching rife with high nitrogen application. In: *New Zealand Dairy Exporter*, pp. 20-21.

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

Ledgard S.F., Brier G.J. & Upsdell M.P. (1990). Effect of clover cultivar on production and nitrogen-fixation in clover-ryegrass swards under dairy-cow grazing. *New Zealand Journal of Agricultural Research*, 33, 243-249.

Ledgard S.F., Sprosen M.S., Penno J.W. & Rajendram G.S. (2001). Nitrogen fixation by white clover in pastures grazed by dairy cows: temporal variation and effects of nitrogen fertilization. *Plant and Soil*, 229, 177-187.

Ledgard S.F., Sprosen M.S. & Steele K.W. (1996). Nitrogen fixation by nine white clover cultivars in grazed pasture, as affected by nitrogen fertilization. *Plant and Soil*, 178, 193-203.

Ledgard S.F. & Steele K.W. (1992). Biological nitrogen-fixation in mixed legume grass pastures. *Plant and Soil*, 141, 137-153.

Ledgard S.F., Steele K.W. & Saunders W.H. (1982). Effects of cow urine and its major constituents on pasture properties. *New Zealand Journal of Agricultural Research*, 25, 661-682.

Li C., Frolking S. & Frolking T.A. (1992a). A model of nitrous oxide evolution from soil driven by rainfall events: 1. Model structure and sensitivity. *Journal of Geophysical Research*, 97, 9759-9776.

Li C., Frolking S. & Frolking T.A. (1992b). A model of nitrous oxide evolution from soil driven by rainfall events: 2. Model applications. *Journal of Geophysical Research*, 97, 9777-9783.

Liao C.F.H. & Raines S.G. (1985). Inhibition of soil urease activity by amido derivatives of phosphoric and thiophosphoric acids. *Plant and Soil*, 85, 149-152.

Lightner J.W., Mengel D.B. & Rhykerd C.L. (1990). Ammonia volatilization from nitrogen fertilized surface to orchardgrass sod. *Soil Science Society of America Journal*, 54, 1478-1482.

Linn D.M. & Doran J.W. (1984). Effect of water filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Science Society of America Journal*, 48, 1267-1272.

Liss P.S. & Slater P.G. (1974). Flux of gases across the air-sea interface. *Nature*, 247, 181-184.

Lloyd A.B. & Sheaffe M.J. (1973). Urease activity in soils. *Plant and Soil*, 39, 71-80.

Lockyer D.R. & Whitehead D.C. (1990). Volatilization of ammonia from cattle urine applied to grassland. *Soil Biology & Biochemistry*, 22, 1137-1142.

Macadam X.M.B., Prado A.d., Merino P., Estavillo J.M., Pinto M. & Gonzalez-Murua C. (2003). Dicyandiamide and 3,4-dimethyl pyrazole phosphate decrease N_2O emissions from grassland but dicyandiamide produces deleterious effects in clover. *Journal of Plant Physiology*, 160, 1517-1523.

MAF (2003). A short-term financial and physical forecast reflecting farmer, farm consultant and industry perceptions of farming trends and issues, production and financial figures. In: *Dairy monitoring report*. Ministry of Agriculture and Forestry Wellington, New Zealand, p. 58.

Manunza B., Deiana S., Pintore M. & Gessa C. (1999). The binding mechanism of urea, hydroxamic acid and N-(n-butyl)-phosphoric triamide to the urease active site. A comparative molecular dynamics study. *Soil Biology & Biochemistry*, 31, 789-796.

Marshall V.G. & DeBell D.S. (1980). Comparison of four methods of measuring volatilization of nitrogen following urea fertilisation of forest soils. *Canadian Journal of Soil Science*, 60.

Martens D.A. & Bremner J.M. (1984). Effectiveness of phosphoroamides for retardation of urea hydrolysis in soils. *Soil Science Society of America Journal*, 48, 302-305.

Martens D.A. & Bremner J.M. (1993). Influence of herbicides on transformations of urea nitrogen in soil. *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes*, 28, 377-395.

Martin J.P., Zunino H., Peirano P. & Caiozzi M. (1982). Decomposition of ^{14}C-labelled lignins, model humic acid polymers, and fungal melanins in allophanic soils. *Soil Biology & Biochemistry*, 14, 289-293.

Matson P.A. & Harriss R.C. (1995). Closed-based measurement of trace gas exchange: application and sources of error. In: *Methods in Ecology- Biogenic Trace Gases: Measuring Emissions from Soil and Water* (eds. Matson PA & Harriss RC) Oxford, United Kingdom, pp. 14-51.

McBride M.B. (1994). In: *Environmental Soil Chemistry*. Oxford University Press New York.

McCarty G.W. & Bremner J.M. (1989). Laboratory evaluation of dicyandiamide as a soil nitrification inhibitor. *Communications in Soil Science and Plant Analysis*, 20, 2049-2065.

McCarty G.W. & Bremner J.M. (1990). Persistence of effects of nitrification inhibitors added to soils. *Communications in Soil Science and Plant Analysis*, 21, 639-648.

McGill W.B., Hunt H.W., Woodmansee R.G. & Reuss J.O. (1981). PHOENIX, a model of the dynamics of carbon and nitrogen in grassland soils. *Ecological Bulletin (Stockholm)*, 33, 49-115.

McKenzie H.A. & Wallace H.S. (1954). The kjelhahl determination of nitrogen. A critical study of digestion conditions-temperature, catalyst and oxidising agent. *Australian Journal of Chemistry*, 7, 55-70.

McTaggart I.P., Clayton H., Parker J., Swan L. & Smith K.A. (1997). Nitrous oxide emissions from grassland and spring barley, following nitrogen fertiliser application with and without nitrification inhibitors. *Biology and Fertility of Soils*, 25, 261-268.

Merino P., Estavillo J.M., Graciolli L.A., Pinto M., Lacuesta M., Munoz-Rueda A. & Gonzalez-Murua C. (2002). Mitigation of N_2O emissions from grassland by nitrification inhibitor and Actilith F2 applied with fertilizer and cattle slurry. *Soil Use and Management*, 18, 135-141.

Merino P., Menendez S., Pinto M., Gonzalez-Murua C. & Estavillo J.M. (2005). 3,4-dimethylpyrazole phosphate reduces nitrous oxide emissions from grassland after slurry application. *Soil Use and Management*, 21, 53-57.

Misselbrook T.H., Nicholson F.A., Chambers B.J. & R.A. J. (2005). Measuring ammonia emissions from land applied manure: an intercomparison of commonly used samplers and techniques. *Environmental Pollution*, 135, 389-397.

Moir J.W.B., Crossman L.C., Spiro S. & Richardson D.J. (1996). The purification of ammonia-oxygenase from *Paracoccus denitrificans*. *FEBS Letters*, 387, 71-74.

Monaghan R.M. & Barraclough D. (1992). Some chemical and physical factors affecting the rate and dynamics of nitrification in urine-affected soil. *Plant and Soil*, 143, 11-18.

Monaghan R.M. & Barraclough D. (1993). Nitrous oxide and dinitrogen emissions from urine-affected soil under controlled conditions. *Plant and Soil*, 151, 127-138.

Montemurro F., Capotorti G., Lacertosa G. & Palazzo D. (1998). Effects of urease and nitrification inhibitors application on urea fate in soil and nitrate accumulation in lettuce. *Journal of Plant Nutrition*, 21, 245-252.

Mosier A.R., Bleken M.A., Chaiwanakupt P., Ellis E.C., Freney J.R., Howarth R.B., Matson P.A., Minami K., Naylor R., Weeks K.N. & Zhu Z.L. (2001). Policy implications of human-accelerated nitrogen cycling. *Biochemistry*, 52, 281-320.

Mosier A.R., Duxbury J.M., Freney J.R., Heinemeyer O. & Minami K. (1998). Assessing and mitigating N_2O emissions from agricultural soils. *Climatic Change*, 40, 7-38.

Mosier A.R. & Kroeze C. (2000). Potential impact of global atmospheric N_2O budget of the increased nitrogen input required to meet future global food demands. *Chemosphere-Global Change Science*, 2, 465-473.

Mosier A.R. & Mack L. (1980). Gas chromatographic system for precise, rapid analysis of nitrous oxide. *Soil Science Society of America Journal*, 44, 1121-1123.

Mosier A.R., Schimel S.G., Valentine D., Bronson K. & Parton W. (1991). Methane and nitrous-oxide fluxes in native, fertilized and cultivated grasslands. *Nature*, 350, 330-332.

Mulvaney R.L. & Bremner J.M. (1981). Control of urea transformations in soils. In: *Soil Biochemistry* (eds. Paul EA & Ladd JN). Marcel Dekker New York, pp. 153-196.

Nastri A., Toderi G., Bernati E. & Govi G. (2000). Ammonia volatilization and yield response from urea applied to wheat with urease (NBPT) and nitrification (DCD) inhibitors. *Agrochimica*, 44, 231-239.

Nervig R.M. & Kadis S. (1976). Effect of hydroxamic acids on growth and urease activity in Corynebacterium renale. *Canadian Journal of Microbiology*, 22, 544-551.

Nommik H. & Vathras G.L. (1982). Retention and fixation of ammonium and ammonia in soils. In: *Nitrogen in Agricultural Soils* (ed. Stevenson FJ). American Society of Agronomy Madison, WI, pp. 123-171.

O' Connor M.J. & Hendrickson L.L. (1987). Effect of phenylphosphorodiamidate on ammonia volatilization as affected by soil temperature and rate and distribution of urea. *Soil Science Society of America Journal*, 51, 1062-1066.

O' Toole P., Morgan M.A. & McGarry S.J. (1985). A comparative study of urease activities in pasture and tillage soils. *Communications in Soil Science and Plant Analysis*, 16, 759-773.

O'Toole P., Morgan M.A. & McAleese D.M. (1982). Effects of soil properties, temperature and urea concentration on patterns and rates of urea hydrolysis in some Irish soils. *Irish Journal of Agricultural Research*, 21, 185-197.

Oenema O., Velthof G.L., Yamulki S. & Jarvis S.C. (1997). Nitrous oxide emissions from grazed grassland. *Soil Use and Management*, 13, 288-295.

Parliamentary Commissioner for the Environment (2005). Growing for good – intensive farming, sustainability and New Zealand's environment. Parliamentary Commissioner for the Environment, Wellington. 236 p. http://www.pce.govt.nz

Peterson S.O., Stamatiadis S. & Christofides C. (2004). Short-term nitrous oxide emissions from pasture soil as influenced by urea level and soil nitrate. *Plant and Soil*, 267, 117-127.

Prasad R. & Power J.F. (1995). Nitrification inhibitors for agriculture, health, and the environment. *Advances in Agronomy*, 54, 233-281.

Prasertsak P., Freney J.R., Denmead O.T., Saffigna P.G. & Prove B.G. (2001). Significance of gaseous nitrogen loss from a tropical dairy pasture fertilised with urea. *Australian Journal of Experimental Agriculture*, 41, 625-632.

Prinn R., Cunnold D., Rasmussen R., Simmonds P., Alyea F., Crawford A., Fraser P. & Rosen R. (1990). Atmospheric emissions and trends of nitrous-oxide deduced from 10 years of Ale-Gauge data. *Journal of Geophysical Research-Atmospheres*, 95, 18369-18385.

Pugh K.B. & Waid J.S. (1969). The influence of hydroxamates on ammonia loss from an acid loamy sand treated with urea. *Soil Biology and Biochemistry*, 1, 195--206.

Puttanna K., Gowda N.M.N. & Rao E. (1999). Effect of concentration, temperature, moisture, liming and organic matter on the efficacy of the nitrification inhibitors benzotriazole, o-nitrophenol, m-nitroaniline and dicyandiamide. *Nutrient Cycling in Agroecosystems*, 54, 251-257.

Puttanna K., Nanje Gowda N.M. & Prakash Rao V.S. (2001). Regulation of nitrification by benzotriazole, o-Nitrophenol, m-Nitroaniline and dicyandiamide and pattern of NH_3 emissions from citronella field fertilized with urea. *Water Air and Soil Pollution*, 131, 11-17.

Quin B.F. (1977). The fate of sheep urine - nitrogen on surface irrigated pasture in Canterbury. In: *New Zealand Soil News*, p. 25.

Rajbanshi S.S., Benckier G. & Ottow J.C.G. (1992). Effects of concentration, incubation temperature, and repeated applications on degradation kinetics of dicyandiamide (DCD) in model experiments with a silt loam soil. *Biology and Fertility of Soils*, 13, 61-64.

Rao D.L.N. & Ghai S.K. (1986). Effect of phenylphosphorodiamidate on urea hydrolysis, ammonia volatilization and rice growth in an alkali soil. *Plant and Soil*, 94, 313-320.

Rao E.V.S.P. & Puttanna K. (1987). Nitrification and ammonia volatalization losses from urea and dicyandiamide-treated urea in a sandy loam soil. *Plant and Soil*, 97, 201-206.

Rao S.C. & Popham T.W. (1999). Urea placement and nitrification inhibitor effects on growth and nitrogen accumulation by no-till winter wheat. *Crop Science*, 39, 1115-1119.

Rasmussen R.A. & Khalil M.A.K. (1986). Atmospheric trace gases - trends and distributions over the last decade. *Science*, 232, 1623-1624.

Rawluk C.D.L., Grant C.A. & Racz G.J. (2001). Ammonia volatilization from soils fertilized with urea and varying rates of urease inhibitor NBPT. *Canadian Journal of Soil Science*, 81, 239-246.

Reddy G.R. (1964). Effect of mixing varying quantities of dicyandiamide with ammonia fertilizers on nitrification of ammonia in soils. *Canadian Journal of Soil Science*, 44, 254-259.

Reynolds C.M., Wolf D.C. & Armbruster J.A. (1985). Factors related to urea hydrolysis in soils. *Soil Science Society of America Journal*, 49, 104-108.

Richards I.R. & Wolton K.M. (1975). A note on urine scorch caused by grazing animals. *Journal of the British Grassland Society*, 30, 187-188.

Rodgers G.A. (1983). Effect of dicyandiamide on ammonia volatilisation from urea in soil. *Fertilizer Research*, 4, 361-367.

Rodgers G.A. & Ashworth J. (1982). Use of nitrification inhibitors to improve recovery of mineralized nitrogen by winter wheat. *Journal of Science Food and Agriculture*, 33, 1229-1226.

Rodgers G.A., Wickramasinghe K.N. & Jenkinson D.S. (1985). Mineralization of dicyandiamide, labelled with N^{15}, in acid soils. *Soil Biology & Biochemistry*, 17, 253-254.

Rosenstein I.J., Hamiltonmiller J.M. & Brumfitt W. (1981). Role of urease in the formation of infection stones - comparison of ureases from different sources. *Infection and Immunity*, 32, 32-37.

Ross D.J. (1992). Influence of sieve mesh size on estimates of microbial carbon and nitrogen by fumigation-extraction procedures in soils under pasture. *Soil Biology & Biochemistry*, 24, 343-350.

Ryden J.C. (1984). Nitrate leaching from grassland. *Nature*, 311,50-53.

Ryden J.C. (1986). Gaseous losses of nitrogen from grassland. In: *Nitrogen fluxes in intensive grassland systems.* (eds. van Meer HG, Ryden JC & Ennik GC). Martinus Nijhoff Publishers Dordrecht.

Saggar S. (2004). Changes in nitrogen dynamics of legume-based pastures with increased nitrogen fertiliser use: Impacts on New Zealand's nitrous oxide emissions inventory. *New Zealand Soil News.*, 52, 110-117.

Saggar S., Andrew R.M., Tate K.R., Hedley C.B., Rodda N.J. & Townsend J.A. (2004a). Modelling nitrous oxide emissions from dairy-grazed pastures. *Nutrient Cycling in Agroecosystems*, 68, 234-255.

Saggar S., Andrew R.M., Tate K.R., Hedley C.B. & Townsend J.A. (2003). Simulation of nitrous oxide emissions from New Zealand dairy-grazed pastures and its mitigation strategies. In: *Proceedings 3rd International Methane and Nitrous Oxide Mitigation Conference* Beijing, China.

Saggar S., Andrew R.M., Tate K.R., Rodda N.J., Hedley C.B. & Townsend J.A. (2002). Measurements and modelling of nitrous oxide emissions from dairy pastures. In: *Proceedings of the Workshop on Dairy Farm Soil Management* (eds. Currie LD & Loganathan P) Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 201-214.

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

Saggar S., Giltrap D.J., Li C., Hedley C.B., Tate K.R. & Lambie S.M. (2005a). Modelling nitrous oxide emissions from grazed grasslands in New Zealand. In: *XX international Grasslands Congress* (eds. O' Mara FP, Wilkins RJ, Mannetje Lt, Lovett DK, Rogers PAM & Boland TM). Wageningen Academic Publishers The Netherlands, p. 566.

Saggar S., Giltrap D.J., Li C., Hedley C.B., Tate K.R. & Lambie S.M. (2005b). Nitrous oxide emissions from grazed perennial pastures in New Zealand. In: *Non-CO2 Greenhouse Gases (NCGG-4)*. Millipress Rotterdam The Netherlands, pp. 113-121.

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

Saggar S., Hedley C.B., Giltrap D.L. & Lambie S.J. (2007b). Measured and modelled estimates of nitrous oxide emission and methane consumption from a sheep-grazed pasture. *Agricultural Ecosystems and Environment* (Submitted).

Saggar S., Hedley M.J., Gillingham A.G., Rowarth J.S., Richardson S., Bolan N.S. & Gregg P.E.H. (1990a). Predicting the fate of fertilizer sulfur in grazed hill country pastures by modeling the transfer and accumulation of soil-phosphorus. *New Zealand Journal of Agricultural Research*, 33, 129-138.

Saggar S., Mackay A.D., Hedley M.J., Lambert M.G. & Clark D.A. (1990b). A nutrient-transfer model to explain the fate of phosphorus and sulfur in a grazed hill-country pasture. *Agriculture Ecosystems & Environment*, 30, 295-315.

Saggar S., Tate K.R., Feltham C.W., Childs C.W. & Parshotam A. (1994). Carbon turnover in a range of allophanic soils amended with C-labelled glucose. *Soil Biology & Biochemistry*, 26, 1263-1271.

Sahrawat K.L. (2003). A systematic approach to research on the development of nitrification inhibitors from indigenous resources. *Current Science*, 84, 10-10.

Sahrawat K.L., Keeney D.R. & Adams S.S. (1987). Ability of nitrapyrin, dicyandiamide and acetylene to retard nitrification in a mineral and an organic Soil. *Plant and Soil*, 101, 179-182.

Schjoerring J.K., Sommer S.G. & Ferm M. (1992). A simple passive sampler for measuring ammonia emission in the field. *Water Air and Soil Pollution*, 62, 13-24.

Schlegel A.J., Nelson D.W. & Sommers L.E. (1986). Field-evaluation of urease inhibitors for corn production. *Agronomy Journal*, 78, 1007-1012.

Schulze E.D., Vries W., Hauhs M., Rosen K., Rasmussen L., Tamm C.O. & Nilsson J. (1989). Critical loads for nitrogen adsorption on forest ecosystems. *Water Air and Soil Pollution*, 48, 451-456.

Sears P.D., Goodall V.C., Jackman R.H. & Robinson G.S. (1965). Pasture growth and soil fertility. VIII. The influence of grasses, white clover, fertilizers and the return of herbage clippings on pasture production of an impoverished soil. *New Zealand Journal of Agricultural Research*, 8, 270-283.

Shand, C. A., Williams, B. L., Smith, S., & Young, M. E. (2000). Temporal changes in C, P and N concentrations in soil solution following applications of synthetic sheep urine to a soil under grass. *Plant and Soil, 222*, 1-13.

Sherlock R.R., Freney J.R., Bacon P.E. & van Der Weerden T.J. (1995). Estimating ammonia volatilization from unsaturated urea fertilized and urine affected soils by an indirect method. *Fertilizer Research*, 40, 197-205.

Sherlock R.R. & Goh K.M. (1983). Initial emissions of nitrous oxide from sheep urine applied to pasture soil. *Soil Biology & Biochemistry*, 15, 615-617.

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

Silva R.G., Cameron K.C., Di H.J. & Hendry T. (1999). A lysimeter study of the impact of cow urine, dairy shed effluent and nitrogen fertilizer on drainage water quality. *Australian Journal of Soil Research*, 37, 357-369.

Singh J., Bolan N. & Saggar S. (2003). A method for simultaneous measurement of ammonia volatilization and nitrous oxide emissions from intact soil cores. . In: *Tools for nutrient and pollutant management: Applications to agriculture and environmental quality* (eds. Currie LD & Hanly JA) Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 224-231.

Singh J., Saggar S. & Bolan N. (2004). Mitigating gaseous losses of N from pasture soil with urease and nitrification inhibitors. In: *Proceedings of the Australia New Zealand SuperSoil 2004 Conference* Sydney, Australia http://www.regional.org.au/au/asssi

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

Slangen J.H.G. & Keerkhoff P. (1984). Nitrification inhibitors in agriculture and horticulture: a literature review. *Fertilizer Research*, 5, 1-76.

Smith K.A., Crichton I.J., McTaggart I.P. & Lang R.W. (1989). Inhibition of nitrification by dicyandiamide in cool temperate conditions. In: *Nitrogen in organic wastes applied in soils.* (eds. Hansen JA & Henriksen K). Academic Press London, pp. 289-303.

Smith, L. C., Monaghan, R. M., Ledgard, S. F., & Catto, W. D. (2005). The effectiveness of different nitrification inhibitor formulations in limiting accumulation in a Southland pastoral soil. *New Zealand Journal of Agricultural Research, 48*, 517-529.

Soliman S.M. & Monem M.A.S.A. (1996). Effect of method of N- application and modified urea on N-15 recovery by rice. *Fertilizer Research*, 43, 143-148.

Sommer S.G., Olesen J.E. & Christensen B.T. (1991). Effects of temperature, wind speed and air humidity on ammonia volatilization from surface applied cattle slurry. *Jouranal of Agriculture Science*, 117, 91-100.

Sparling G.P. & West A.W. (1988). A direct extraction method to estimate soil microbial C: calibration *in situ* using microbial respiration and ^{14}C labelled soils. *Soil Biology & Biochemistry*, 20, 337-343.

Stelly M. (1980). Nitrification inhibitors - Potentials and Limitations. . In: *ASA Special Publication No. 38. American Society of Agronomy, Soil Science Society of America* Madison, Wisconsin.

Stevens R.J. & Laughlin R.J. (1998). Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils. *Nutrient Cycling in Agroecosystems*, 52, 131-139.

Stevenson F.J. (1982). *Nitrogen in agricultural soils.* American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI.

Stewart G.P., Mann A.F. & Fentem P.A. (1980). Enzymes of glutamate formation: gluatamate dehydrogenase, glutamine synthetase, glutamate synthase. In: *The Biochemistry of Plants* (ed. Miflin BF). Academic Press New York, pp. 271-327.

Subbarao G.V., Ito O., Sahrawat K.L., Berry W.L., Nakahara K., Ishikawa T., Watanabe T., Suenaga K., Rondon M. & Rao I.M. (2006). Scope and strategies for regulation of nitrification in agricultural systems- challenges and opportunities. *Critical Reviews in Plant Sciences*, 25, 303-335.

Terman G.L. (1979). Volatilization loss of nitrogen as ammonia from surface-applied fertilizers, organic amendments and crop residues. *Advances in Agronomy*, 31, 189-223.

Thompson R.B., Pain B.F. & Rees Y.J. (1990). Ammonia volatilization from cattle slurry following surface application to grassland. 2. Influence of application rate, wind speed and applying slurry in narrow bands. *Plant and Soil*, 125, 119-128.

Tietema A. & Wessel W.W. (1992). Gross nitrogen transformations in the organic layer of acid forest ecosystems subjected to increased atmospheric nitrogen input. *Soil Biology & Biochemistry*, 24, 943-950.

Tomar J.S., Kirby P.C. & Mackenzie A.F. (1985). Field-evaluation of the effects of a urease inhibitor and crop residues on urea hydrolysis, ammonia volatilization and yield of corn. *Canadian Journal of Soil Science*, 65, 777-787.

Trenkel M.E. (1997). Controlled-release and stabilized fertilizer in agriculture. In: *International Fertilizer Industry Association* Paris, pp. 29-40.

Usha K. & Patra D.D. (2003). Influence of natural essential oils and their by-products as nitrification retarders in regulating nitrogen utilization for Japanese mint in sandy loam soils of subtropical central India. *Agriculture Ecosystems & Environment*, 94, 237-245.

Vallis I., Harper L.A., Catchpoole V.R. & Weier K.L. (1982). Volatilization of ammonia from urine patches in a sub-tropical pasture. *Australian Journal of Agricultural Research*, 33, 97-107.

van Der Weerden T.J., Moal J.F., Martinez J., Pain B.F. & Guiziou F. (1996). Evaluation of the wind-tunnel method for measurement of ammonia volatilization from land. *Journal of Agricultural Engineering Research, 64, 11-13.*.

van Groenigen J.W., Kuikman P.J., De Groot W.J.M. & Velthof G.L. (2005). Nitrous oxide emission from urine-treated soil as influenced by urine composition and soil physical conditions. *Soil Biology & Biochemistry*, 37, 463-473.

Vance E.D., Brookes P.C. & Jenkinson D.S. (1987). An extraction method for measuring soil microbial C. *Soil Biology & Biochemistry*, 19, 703-707.

Vilsmeier K. (1980). Effect of temperature on the breakdown of dicyandiamide in the soil. *Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, 143, 113-118.

Vilsmeier K. (1982). Kurzmitteilung verbesserte kolorimetrische bestimmung von dicyandiamid in bodenextrakten. *Zeitschrift Planzenernaehr Bodenk*, 19, 703-707.

Vittori-Antisari L., Marzadori C., Gioacchini P., Ricci S. & Gessa C. (1996). Effects of the urease inhibitor N-(n-butyl) thiophosphoric triamide in low concentrations on ammonia volatilization and evolution of mineral nitrogen. *Biology and Fertility of Soils*, 22, 196-201.

Vlek P.L.G., Byrnes B.H. & Craswell E.T. (1980). Effect of urea placement on leaching losses of nitrogen from flooded rice soils. *Plant and Soil*, 54, 441-449.

Vlek P.L.G. & Carter M.F. (1983). The effect of soil environment and fertilizer modifications on the rate of urea hydrolysis. *Soil Science*, 136, 56-63.

Vlek P.L.G. & Craswell E.T. (1979). Effect of nitrogen source and management on ammonia volatilization losses from flooded rice-soil systems. *Soil Science Society of America Journal*, 43, 352-358.

Wadman W.P., Neeteson J.J. & Wijnen G.J. (1993). Field experiments with slurry and dicyandiamide - response of potatoes and effects on soil mineral nitrogen. *Netherlands Journal of Agricultural Science*, 41, 95-109.

Wang Z.-H., Liu X.-J., Ju X.-T., Zhang F.-S. & Malhi S.S. (2004). Ammonia volatilization loss from surface-broadcast urea: comparison of vented- and closed-chamber methods and loss in winter wheat-summer maize rotation in north China plain. *Communications in Soil Science and Plant Analysis*, 35, 2917-2939.

Wang Z., Van Cleemput O., Liantie L. & Baert L. (1991). Effect of organic matter and urease inhibitors on urea hydrolysis and immobilization of urea nitrogen in an alkaline soil. *Biology and Fertility of Soils*, 11, 101-104.

Watson C.J. (2000). Urease activity and inhibition - principles and practice. In: *The International Fertiliser Society Proceedings No. 454.*

Watson C.J., Miller H., Poland P., Kilpatrick D.J., Allen M.D.B., Garrett M.K. & Christianson C.B. (1994a). Soil properties and the ability of the urease inhibitor N-(n-Butyl) thiophosphoric triamide (NBTPT) to reduce ammonia volatilization from surface-applied urea. *Soil Biology & Biochemistry*, 26, 1165-1171.

Watson C.J., Poland P. & Allen M.B.D. (1998). The efficacy of repeated applications of the urease inhibitor N-(n-butyl) thiophosphoric triamide for improving the efficiency of urea fertilizer utilization on temperate grassland. *Grass and Forage Science*, 53, 137-145.

Watson C.J., Poland P., Miller H., Allen M.B.D., Garrett M.K. & Christianson C.B. (1994b). Agronomic assessment and N-15 recovery of urea amended with the urease inhibitor Nbtpt (N-(n-Butyl) thiophosphoric triamide) for temperate grassland. *Plant and Soil*, 161, 167-177.

Watson R.T., Meiro Filho L.C., Sanhueza E. & Janetos A. (1992). Sources and sinks. In: *Climate Change 1992, the supplementary report to the IPCC scientific assessment.* (eds. Houghton JT, Callander BA & Varney SK). Cambridge University Press Cambridge, pp. 25-46.

Watson S.W., Bock E., Harms H., Koops H.-P. & Hooper A.B. (1989). Nitrifying bacteria. In: *Bergeys manual of systematic bacteriology* (eds. Staley JT, Bryant MT, Pfennig N & Holt JD). Williams and Wilkins Baltimore, pp. 1808-1834.

Weiske A., Benckiser G., Herbert T. & Ottow J.C.G. (2001). Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparision to dicyandiamide (DCD on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. *Biology and Fertility of Soils*, 34, 109-117.

White R.E. (1989). Nitrogen transformations, fixation and losses - a brief introduction and overview. In: *Proceedings Nitrogen in New Zealand Agriculture and Horticulture.* (eds. White RE & Currie LD) Fertiliser and Lime Research Centre, Massey University, Palmerston North, pp. 83-87.

Whitehead D.C. (1995). *Grassland Nitrogen.* CAB International, Wallingford, UK.

Whitehead D.C., Lockyer D.R. & Raistrick N. (1989). Volatilization of ammonia from urea applied to soil: Influence of hippuric acid and other constituents of livestock urine. *Soil Biology & Biochemistry*, 21, 803-808.

Whitehead D.C. & Raistrick N. (1990). Ammonia volatilization from 5 nitrogen-compounds used as fertilizers following surface application to soils. *Journal of Soil Science*, 41, 387-394.

Whitehead D.C. & Raistrick N. (1993). The volatilization of ammonia from cattle urine applied to soils as influenced by soil properties. *Plant and Soil*, 148, 43-51.

Williams D.L., Ineson P. & Coward P.A. (1999). Temporal variations in nitrous oxide fluxes from urine-affected grassland. *Soil Biology & Biochemistry*, 31, 779-788.

Williams P.H., Gregg P.E.H. & Hedley M.J. (1990). Fate of potassium in dairy cow urine applied to intact soil cores. *New Zealand Journal of Agricultural Research*, 33, 151-158.

Williams, P. H., & Haynes, R. J. (1994). Comparison of initial wetting pattern, nutrient concentration in soil solution and the fate of ^{15}N-labelled urine in sheep and cattle urine patch areas of pasture soil. *Plant and Soil, 162*, 49-59.

Williams P.H., Hedley M.J. & Gregg P.E.H. (1989). Uptake of potassium and nitrogen by pasture from urine-affected soil. *New Zealand Journal of Agricultural Research*, 32, 415-421.

Williamson J.C. & Jarvis S.C. (1997). Effect of dicyandiamide on nitrous oxide flux following return of animal excreta to grassland. *Soil Biology & Biochemistry*, 29, 1575-1578.

Williamson J.C., Menneer J.C. & Torrens R.S. (1996). Impact of dicyandiamide on the internal nitrogen cycle of a volcanic, silt loam soil receiving effluent. *Applied Soil Ecology*, 4, 39-48.

Williamson J.C., Taylor M.D., Torrens R.S. & Vojvodic-Vukovic M. (1998). Reducing nitrogen leaching from dairy farm effluent-irrigated pasture using dicyandiamide: a lysimeter study. *Agriculture Ecosystems & Environment*, 69, 81-88.

Wood C.W., Marshall S.B. & Cabrera M.L. (2000). Improved method for field-scale measurement of ammonia volatilization. *Communications in Soil Science and Plant Analysis*, 31, 581-590.

Wood P.M. (1986). Nitrification as a bacterial energy source. In: *Nitrification, Special publication of the Society for General Microbiology* (ed. Prosser JI). IRL Press Oxford, UK, pp. 39-64.

World Resources Institute (2000). World Resources 2000-2001. World Resources Institute, Washington DC, pp. 389.

Wulf S., Maeting M., Bergmann S. & Clemens J. (2001). Simultaneous measurement of NH_3, N_2O and CH_4 to assess efficiency of trace gas emission abatement after slurry application. *Phyton*, 41, 131-142.

Xu X.K., Boeckx P., Van Cleemput O. & Zhou L.K. (2002a). Urease and nitrification inhibitors to reduce emissions of CH_4 and N_2O in rice production. *Nutrient Cycling in Agroecosystems*, 64, 203-211.

Xu X.K., Boeckx P., Wang Y.S., Huang Y., Zheng X.H., Hu F. & Van Cleemput O. (2002b). Nitrous oxide and methane emissions during rice growth and through rice plants: effect of dicyandiamide and hydroquinone. *Biology and Fertility of Soils*, 36, 53-58.

Xu X.K., Huang Y., Zhou L.K., Huang G.H. & Van Cleemput O. (2001). Effect of dicyandiamide and hydroquinone on the transformation of urea- ^{15}N in soil cropped to wheat. *Biology and Fertility of Soils*, 34, 286-290.

Xu X.K., Zhou L., Cleemput O.V. & Wang Z. (2000). Fate of urea- N in a soil-wheat system as influenced by urease inhibitor hydroquinone and nitrification inhibitor dicyandiamide. *Plant and Soil*, 220, 260-271.

Yadvinder Singh & Beauchamp E.G. (1989). Nitrogen transformations near urea in soil - effects of nitrification inhibition, nitrifier activity and liming. *Fertilizer Research*, 18, 201-212.

Zacherl B. & Amberger A. (1990). Effect of the nitrification inhibitors dicyandiamide, nitrapyrin and thiourea on *Nitrosomonas europaea*. *Fertilizer Research*, 22, 37-44.

Zaman M., Nguyen L., Blennerhassett J.D. & Quin B.F. (2005). Increasing the utilisation of urea fertilisers by pasture. In: *Developments in Fertiliser Application Technologies and Nutrient Management* (eds. Currie LD & Hanly JA) Fertiliser and Lime Research Centre, Massey University, Palmerston North, pp. 276-284.

Zerulla W., Barth T., Dressel J., Erhardt K., von Locquenghien K.H., Pasda G., Radle M. & Wissemeier A.H. (2001). 3,4-Dimethylpyrazole phosphate (DMPP) - a new nitrification inhibitor for agriculture and horticulture - An introduction. *Biology and Fertility of Soils*, 34, 79-84.

Zhang H.J., Wu Z.J. & Zhou Q.X. (2004). Dicyandiamide sorption-desorption behaviour on soils and peat humus. *Pedosphere*, 14, 395-399.

Zhao X.Y., Zhou L.K. & Wu G.Y. (1992). Urea hydrolysis in a brown soil: effect of hydroquinone. *Soil Biology & Biochemistry*, 24, 165-170.

Zhengping W., Van Cleemput O. & Baert L. (1996). Movement of urea and its hydrolysis products as influenced by moisture content and urease inhibitors. *Biology and Fertility of Soils*, 22, 101-108.

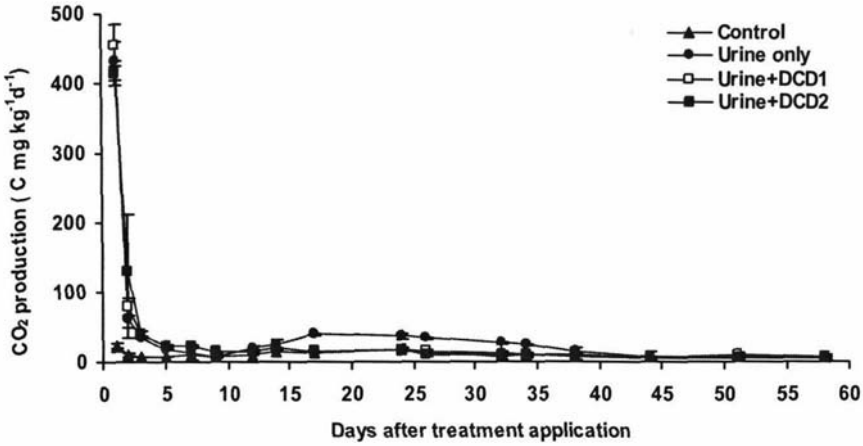
Zhu, Z. L. (1992). Efficient management of nitrogen fertilizers for flooded rice in relation to nitrogen transformations in flooded soils. *Pedosphere, 2*, 97-114.

Zia M.S., Aslam M., Rahmatullah, Arshad M. & Ahmed T. (1999). Ammonia volatilization from nitrogen fertilizers with and without gypsum. *Soil Use and Management*, 15, 133-135.

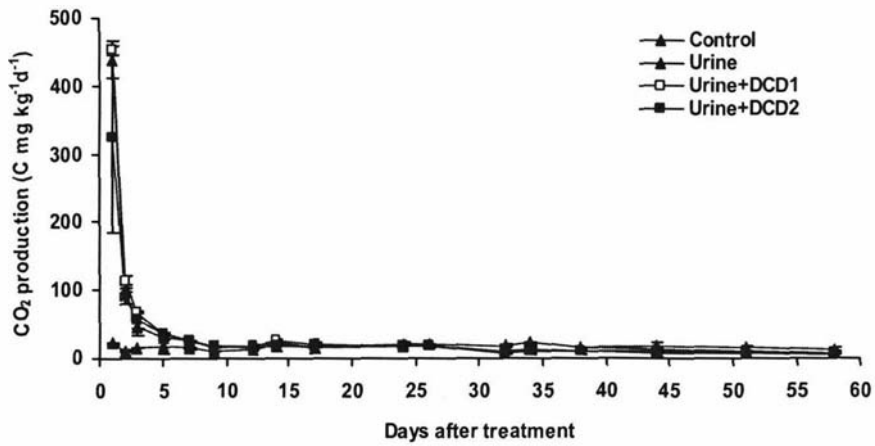
Zourarakis D. & Killorn R. (1990). The efficacy of 2 nitrification inhibitors at high-temperature in 2 Iowa soils. *Soil Science*, 149, 185-190.

Appendix 1

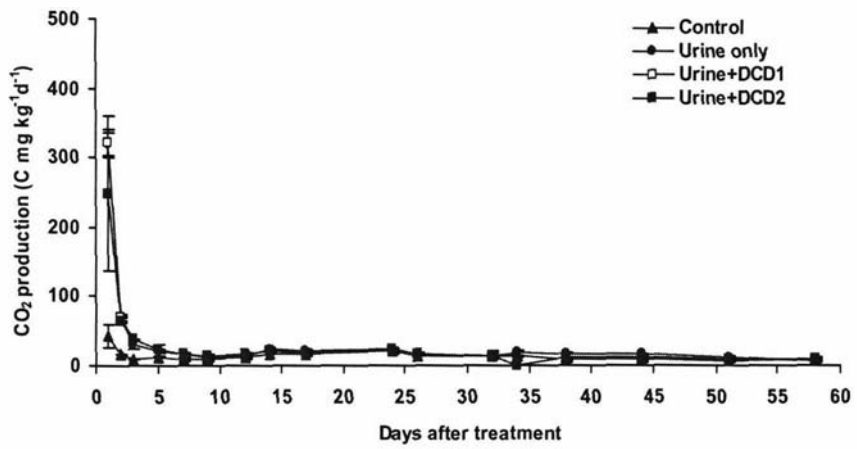
(a)



(b)



(c)



(d)

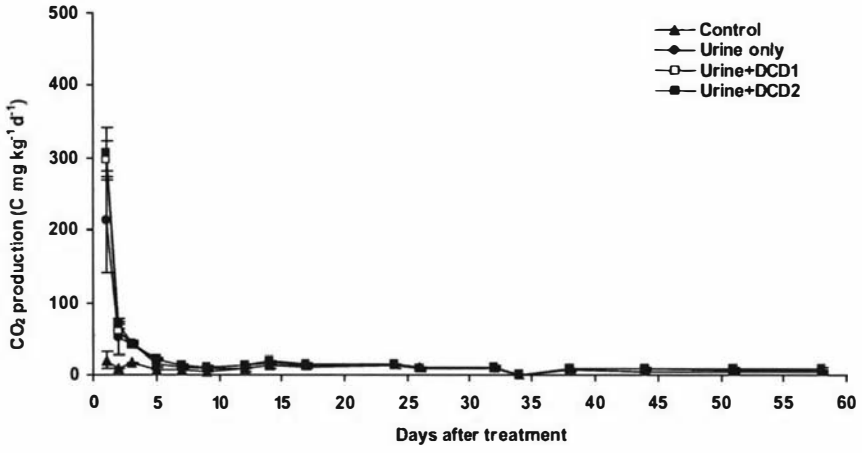


Figure A **Carbon-di-oxide losses with and without DCD from urine applied to (a) Tokomaru silt loam (b) Manawatu sandy loam (c) Egmont brown loam (d) Horotiu silt loam during the incubation period (note the difference in y-axis). Each value represents a mean of three replicates with standard deviation shown by vertical bars.**

Appendix 2

Input Parameters for NZ-DNDC

This appendix provides the list of the input parameters that were used while running the NZ-DNDC model for the field-plot study

Site data	
Site_name	Field-plot study
Simulated_Year	1
Latitude	-40.4
Daily_Record	1

Climate Data	
Climate_Data_Type	2
NO_3NH_4_in_rainfall (mg N l^{-1})	0.50
NO_3_of_atmosphere (μg N m^{-3})	0.06
Base CO_2_of_atmosphere (ppm)	350.0
Climate_file_count	1 C:\DNDC\Database\Nitrification inhibitor\D3Climate05_extrawater.txt

Soil data	
Soil texture	4 (Silt Loam)
Land use type	3 (moist grassland/pasture)
Density (g cm^{-3})	1.01
Soil pH	5.62
SOC at surface (kg C kg^{-1})	0.036
Clay fraction	0.14
Bypass Flow	0
Litter SOC (kg C kg^{-1})	0.10
Humads SOC (kg C kg^{-1})	0.15
Humus SOC (kg C kg^{-1})	0.75
Soil NO_3^- (mg N kg^{-1})	12.20

Soil NH_4^+ (mg N kg^{-1})	3.30
Moisture	0.40
Temperature (^0C)	12.2

Crop data	
Rotation number	1
Rotation ID	1
Total year	1
Years of a cycle	1
Crop total number	1
Crop ID	1
Crop Type	12 (perennial grassland)
Plant time (month d^{-1})	1 1
Harvest time (month d^{-1})	12 31
Year of harvest	1
Ground harvest	1.0
Yield (kg DM ha^{-1})	1250.0
Development rate (reproductive)	0.01
Development rate (vegetative)	0.03
Photosynthesis efficiency	0.40
Maximum photosynthesis	60.0
Intial biomass (kg DM ha^{-1})	12.50
Tllage number	0
Fertilisation number	1
Fertilisation ID	1
Month/Day/method	5 12 0 (Surface)
Depth (cm)	0.20
Nitrate	0
AmmBic	0
Urea	600.0
Anhydrous	0
NH_4NO_3	0

$(NH_4)_2SO_4$	0
NH_4HPO_4	0
Release rate	1.0
Inhibitor efficiency	0.0 (This number is 0 for Urine only or up to 1 for DCD)
Inhibitor duration	Inhibitor_duration= 0.0 (Zero if no inhibitor, otherwise >50 days)
Manure number	0
Weed number	0
Weed problem	0
Flood number	0
Leak type	1
Water control	0
Leak rate	0
Irrigation number	0
Irrigation type	0
Irrigation index	0
Climate file mode	0
Soil microbial index	0
Crop model approach	
Depth WRL cm	10.00
Slope	0
Filed capacity	0.53
Wilting point	0.28
CO_2 increase rate	0
SOC profile A	0.08
SOC profile B	1.40