Role of inhibitors in mitigating nitrogen losses from urine and fertiliser inputs in pastures

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Role of inhibitors in mitigating nitrogen losses from urine and fertiliser inputs in pastures

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Summary Final Report | Climate Change Plan of Action
Agricultural Inventory

Project Code: MAF-POL/CP02 AG-INVENT-15

Business/Institution: Landcare Research

Programme Leader: Dr Jagrati Singh

Programme Title: Role of inhibitors in mitigating nitrogen losses from urine and fertiliser inputs in pastures

Goal: To make available, through publication, results of research on the mitigation potential of nitrification (DCD – Dicyandiamide) and urease (Agrotain) inhibitors to reduce N losses from cattle urine and urea application to pasture soil.

Context of the project:

Nitrification inhibitors are one of the promising mitigation options to reduce N losses through gaseous emissions and leaching from grazed pastures. In NZ, a number of studies have been conducted using DCD. However, little work has been done on the application of urease inhibitors to cattle urine, and research trials using DCD were conducted under lysimeters in cool South Island conditions. Only recently have a few trials been conducted under field conditions to assess nitrous oxide (N_2O) emissions and nitrate (NO_3^-) leaching.

A research project over the past four years investigated the effect of the inhibitors DCD and Agrotain on nitrogen dynamics in soil. DCD impacts on N_2O emissions, NH_3 volatilisation and nitrate (NO_3^-) leaching were examined in a study that used a novel technique to measure NH_3 volatilisation and N_2O emissions simultaneously to determine the influence of inhibitors on the emissions of these gases. A glasshouse study was undertaken to elaborate the changes in N dynamics with the urease inhibitor Agrotain applied to urine and urea fertiliser.

These studies have provided data that will help understand and strengthen research on the effectiveness of inhibitors in New Zealand pastures. Publication of results will make this information available to a wide audience and will add to scientific literature, valuable data on which NZ can build strategies for reducing greenhouse gas emissions.

Approach:

Complete analysis, and prepare for publication, data collected from recent research in:
1. A glasshouse study of the impact of DCD on N losses (both gaseous and leaching), and
2. An experiment to elaborate the changes in N dynamics with Agrotain applied to urine and urea fertiliser, which included measurements of mineral N and changes in N gases, also conducted under glasshouse conditions.

Outcomes:

Submission, for publication in international peer-reviewed journals, two papers that increase understanding of the inhibitors**,** DCD and Agrotain on nitrogen dynamics in pasture soil, and thus their potential role in mitigating greenhouse gas (nitrous oxide) emissions.

Recommendations

Not applicable

Summary/Publications:

Two manuscripts have been submitted for publication:

1.

Submitted to:	*Nutrient Cycling in Agroecosystems*
Title:	Influence of dicyandiamide on nitrogen transformation and losses in pasture soil cores.
Authors:	Jagrati Singh, Surinder Saggar and Nanthi Bolan

2.

Submitted to:	*Australian Journal of Soil Research*
Title:	Impact of urease inhibitor on nitrogen dynamics in pasture soil cores receiving urea fertiliser and cattle urine.
Authors:	Jagrati Singh, Nanthi Bolan and Surinder Saggar.

These manuscripts form the body of this report.

1. Influence of dicyandiamide on nitrogen transformation and losses in pasture soil cores

Abstract

In New Zealand, urine deposited by grazing animals represents the largest source of nitrogen (N) losses, as gaseous emissions of ammonia (NH_3) and nitrous oxide (N_2O), and leaching of nitrate (NO_3^-).We determined the effect of dicyandiamide (DCD) on gaseous emissions from pasture with increasing rates of urine-N application, mineral N transformations and potential leaching of N using undisturbed soil cores of Manawatu sandy loam at field capacity. The treatments included four levels of urine-N applied at 0 (control), 14.4, 29.0 and 57.0 g N m^{-2} with and without DCD at 2.5 g m^{-2}. Results showed a significant increase ($P<0.05$) in NH_3 and N_2O-N emissions as urine application was increased. The addition of DCD to corresponding urine treatments reduced N_2O emissions by 33%, 56%, and 80%, respectively. The addition of DCD with urine to the intact soil cores at field capacity moisture content resulted in a significant increase in the soil NH_4^+-N concentration but little change in NH_3 emissions. Addition of DCD to urine reduced potential NO_3^--N leaching by 60–65% but potential NH_4^+-N leaching increased by 2–3.5 times. There was no difference in pasture dry matter production with and without DCD treatments.

Keywords Ammonia, Nitrous oxide emissions, nitrification inhibitor, nitrate leaching

Introduction

Interest in losses of N as nitrous oxide (N_2O) and ammonia (NH_3) emissions and as nitrate (NO_3^-) leached from the soil has increased during the last decade mainly because of the environmental impacts of these losses. Nitrous oxide, a potent greenhouse gas, makes up 17% (on a CO_2 equivalent basis) of New Zealand's emissions inventory (MfE 2008), with most of these emissions (about 90%) being derived from agricultural practices. Ammonia (NH_3) can cause toxicity in vegetation, induce acidification, and contribute to eutrophication in N sensitive environments (Sheppard et al. 2005), and can act as a secondary source of NO and N_2O (Bouwman 1990). Globally, nitrate leaching is a major environmental issue as it can reduce surface and ground water quality, increase surface water eutrophication, and may harm human health when present in high concentrations. 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}, and regional authorities have developed regional resource plans that may restrict land uses to protect water resources.

The largest source of these losses in animal agriculture is from animal excreta, recycling N inputs from fertiliser and biological N fixation within the soil-plant system (Bolan et al. 2004; Saggar et al. 2004b; Clark et al. 2005). Grazing ruminants utilize little of the N in feed and can excrete 75–90% of ingested N (Whitehead 1995) as urine and dung. This added N, especially animal urine, acts as the dominant substrate for N losses due to high localized concentrations of readily available N in each patch. Nitrate leaching from agricultural land is the main source of NO_3^- in surface and ground water (Di and Cameron 2002a). The expansion of the dairy industry (MAF 2003) has contributed most to the increase in N use in New Zealand, and current management of intensively managed pastures has been unable to reduce gaseous N emissions and NO_3^- leaching significantly. Thus, nitrification inhibitors

(NIs) are being promoted in New Zealand to reduce these losses. NIs can reduce leaching of NO_3^- and emissions of N_2O directly, by reducing the fraction of NH_4^+-N oxidised to NO_3^- (Aulakh et al. 1984; Bronson et al. 1992). A commonly recommended NI in New Zealand is dicyandiamide (DCD). It is not a broad-spectrum bactericide and does not affect other heterotrophs that are responsible for much of the soil's biological activity (Amberger 1989).

DCD has been used to increase the efficiency of N in fertilisers or manures, with variable results (Amberger 1989; Wadman et al. 1993; Davies and Williams 1995; Williamson et al. 1998). New Zealand lysimeter (Di and Cameron 2002b, 2003, 2004c, 2005, 2007) and field studies (Hoogendoorn et al. 2008; Smith et al. 2008), including an Australian study (Kelly et al. 2008) of DCD on urine patches, showed N_2O emissions were reduced by 25–80% and NO_3^- leaching was reduced by 47–76%. However, limited studies have been undertaken to study the overall effect of DCD on N transformations, including gaseous emissions of both NH_3 and N_2O and leaching of NH_4^+ and NO_3^-. The objective of our glasshouse study was to understand the effect of DCD on urine-N dynamics by quantifying the effects of DCD on NH_3 and N_2O emissions, mineral N, leaching of N (NH_4^+-N and NO_3^-- N), and herbage yield. Our aim was to help improve strategies for mitigating N losses from grazed pastures.

Materials and methods

Experimental details

Collection and preparation of soil cores

Intact soil cores (100 mm diameter, 100 mm depth) were collected from a sheep-grazed permanent pasture site with a mixture of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) at Frewens Research block, Massey University Turitea campus, Palmerston North. The soil at this site is a Manawatu fine sandy loam, classified as a weathered fluvial recent soil (Hewitt 1998). Pertinent soil chemical and physical properties are given in Table 1. The soil 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. The sward on the cores was then cut and the cores weighed and maintained at field-capacity throughout the experiment. Three cores were placed in each chamber (discussed under gaseous emissions) of a glasshouse maintained at 15–20^oC during August, 2005.

Treatments

The eight treatments in triplicate included: two controls (T1– without DCD and T2 – with DCD at 2.5 g m^{-2} (25 kg ha^{-1})) plus six urine and urine plus DCD treatments, (T3 – urine at 14.4 g N m^{-2} (144 kg N ha^{-1}), T4 – urine at 14.4 g N m^{-2} + DCD, T5 – urine at 29 g N m^{-2} (290 kg N ha^{-1}), T6 – urine at 29 g N m^{-2} + DCD, T7 – urine at 57 g N m^{-2} (570 kg N ha^{-1}), T8 – urine at 57 g N m^{-2} + DCD). These treatments are hereafter referred to as T1, T2, T3, T4, T5, T6, T7, and T8. DCD was mixed with urine before application. The volumes of urine added were 17, 34, and 67 ml and they increased water-filled pore space (WFPS) of the soil cores from 60% (at field capacity moisture content) to 64%, 68%, and 78% for the 14.4 g Nm^{-2} (T3 and T4), 29 g Nm^{-2} (T5 and T6), and 57 g Nm^{-2} (T7 and T8) treatments respectively. Because the moisture content of soil cores increased with addition of urine, no water was added to the soil cores until they dried below the field capacity moisture content

Leachate collection from cores

At the end of the glasshouse experiment, one core from each replicated chambers of T5, T6, T7, and T8 treatments was leached with 2.5 pore volumes of deionized water. These

treatments were selected because the rate of urine applied was closest to the actual rate in sheep grazed pasture (250–350 kg N ha^{-1}) and in cow urine patches (600 kg N ha^{-1}). Herbage on the cores was trimmed close to the soil surface and water added using a peristaltic pump at 1 ml min^{-1}. A layer of silica sand was spread on the surface of each core and a filter paper placed on top to ensure uniform distribution of water on the core surface. Leachate was collected initially in small volumes of 5, 10, 15, and 20 (x 4) ml, then in volumes of 50 (x 4) and 100 (x 7) ml; the leachates were then analysed separately for mineral N (NH_4^+ and NO_3^- ions).

Gaseous emissions

The chambers used for NH_3 and N_2O emission were modified PVC 'sewer-hatches' attached to 250 mm diameter x 150 mm deep sections of PVC pipe with a sealed base (Saggar et al. 2004b). Each chamber had two removable lids with one or two ports for sampling N_2O or NH_3 fluxes, respectively. The lid with one port was fitted with a 10 cm length of tubing (3.2 mm diameter) with a three-way stopcock for sampling N_2O gas. The lid with two ports had one input port connected to a compressed air supply and the other (exhaust) port connected to a chemical trap to absorb NH_3. Ammonia emissions were measured for 10–12 hours daily overnight for 14 days after application of the treatments using the active flux method with a constant air supply at 1 dm^3 min^{-1} and 50 ml 0.05 M H_2SO_4 acid traps to capture NH_3 (Wulf et al. 2001; Singh et al. 2003). After measuring NH_3, the lids were removed and the chambers left open to equilibrate under ambient conditions. Ammonia collected in 0.05 M H_2SO_4 was analysed colorimetrically for total NH_4^+-N using a Technicon autoanalyser. The NH_3 flux (mg N m^{-2} hr^{-1}) was then calculated using the equation:

$$N_{(NH_3\ flux)} = \frac{C \times V}{a \times D} \qquad (1)$$

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

For N_2O measurements, chambers were closed with the single-port lid (Saggar et al. 2004b) and 3 gas samples were taken from each chamber at 0 min (t0), 30 min (t30), and 60 min (t60) using 60 ml polypropylene syringes fitted with 3-way stopcocks. These gas samples were transferred to evacuated vials and analyzed using a Shimadzu GC – 17A gas chromatograph with a ^{63}Ni-Electron capture detector (Hedley et al. 2006); N_2O (mg m^{-2} hr^{-1}) flux was estimated from the measurements made at the three times (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. Precision of the gas chromatographic data at ambient N_2O concentrations was ± 1% or better. Increases in N_2O concentrations within the chamber headspace were generally linear ($R^2 > 0.90$) over time. Therefore, N_2O flux (µg m^{-2} hr^{-1}) was calculated from equation 2 using the ideal gas law.

$$F = \rho \times \frac{V}{A} \times \frac{\Delta c}{\Delta t} \times \frac{273}{(T + 273)} \qquad (2)$$

where F = gas flux (µg m^{-2} h^{-1}); ρ = density of gas (g m^{-3}); V = volume of the chamber (m^3); A = base area of the chamber (m^2); $\Delta c/\Delta t$ = average rate of change of concentration with time (ppmv h^{-1}); and T = temperature (°C) in the chamber.

Emissions of NH_3 and N_2O were monitored for 14 and 50 days, respectively. Background concentrations of NH_3 and N_2O were measured from all 24 chambers before applying the treatments. Nitrous oxide was measured daily for the first week to capture immediate changes

in gas fluxes, then measured on alternate days for two weeks, then twice in the next week. For the remaining period, measurements were taken only once a week as the fluxes approached the background levels

Analyses

Urine analysis
Urine was collected from Friesian cows during milking at the Massey University No.1 Dairy farm. Fresh urine samples from individual cows were pooled and frozen (-18 ^{0}C) within an hour of collection. Urine was analyzed for total N (Ebina et al. 1983) and total C (Bremner and 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.

Soil analysis
At the end of the glasshouse experiment, unleached soil cores were split into upper (0–50 mm) and lower (50–100 mm) sections. A subsample of 5 g (oven dry equivalent) was taken from both the sections of each soil core to determine the concentrations of NH_4^+ and NO_3^--N, by extracting with 30 ml 2M KCl (1:6 soil:extractant ratio). The extracts were analysed colorimetrically for NH_4^+ and nitrate (NO_3^-) using a Technicon autoanalyser (Blakemore et al. 1987).

Herbage analysis
Herbage was cut at 2 cm height twice during the 50 day period, dried at 65^{0}C to constant weight, and weighed. Cumulative dry matter (DM) yield was recorded for each chamber. The dried herbage was finely ground in a cutting mill and analysed for total N by the Kjeldahl digestion method (McKenzie and Wallace 1954). Recovery of added N through plant uptake was calculated using the equation:

$$N\,recovery = \frac{[Herbage\,N(urine/urea \pm DCD)] - [HerbageN(control \pm DCD)]}{N\,added(urine/urea)} \qquad (3)$$

Leachate analysis
Leachates collected from the soil cores were analysed for NH_4^+ and NO_3^- concentrations using a Technicon autoanalyser (Blakemore et al. 1987).

Statistical Methods

An analysis of variance using SAS software (version 8) was performed on total NH_3 and N_2O emitted, mineral N, total amount of ions (NH_4^+ and NO_3^-) leached, and herbage DM yield and N uptake using the General Linear Model (GLM) procedure. Means were compared using Fishers Least Significant Difference (LSD) at 5% significance.

Results

Ammonia emissions

NH_3 emissions in all urine treatments with or without DCD peaked within 24 hours, then declined sharply. Emissions reached the background level 14 days after commencing measurements (Fig. 1). These emissions increased with increasing rates of urine both with and without DCD and were significantly greater than emissions from the control treatment (T1), which ranged from 0.08 to 2.25 mg NH_3-N m^{-2} d^{-1}, and did not change with the addition

of DCD (0.10 to 1.60 mg NH_3-N m^{-2} d^{-1}). The highest emission peak (798 mg NH_3-N m^{-2} d^{-1}) was from the treatment receiving the highest level of urine application (T7). Addition of DCD to this urine treatment (T8) caused a small reduction (698 mg NH_3-N m^{-2} d^{-1}) in the peak. Similar trends in NH_3 emissions were observed between 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, in all DCD treatments (T4, T6, and T8) NH_3 emissions after 3 days increased slightly compared to urine alone. These differences in NH_3 emissions remained until day 14 when emissions in all the treatments reached the background level. No significant difference ($P > 0.05$) was found in total amount of NH_3 emitted from treatments with DCD (T4, T6, and T8) as compared to the urine-only treatments (T3, T5, and T7) (Table 2).

Nitrous oxide emissions

Application of urine to pasture soil cores with and without DCD increased N_2O emissions significantly compared to the control (Fig. 2). N_2O emissions from the control ranged from 0.07–2.30 mg N_2O-N m^{-2} d^{-1} with DCD, and 0.07–0.66 mg N_2O-N m^{-2} d^{-1} without DCD. Total N_2O-N emitted in the control with DCD (0.02 g N m^{-2}) did not differ significantly from that without DCD (0.01 g N m^{-2}). The urine treatments T3, T5, T7, without DCD gave peaks of 57.5, 135 and 196 mg N_2O-N m^{-2} d^{-1}, respectively within 24 hours of application (Fig. 2). 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 throughout the measurement period emissions remained lower than in the corresponding urine treatments without DCD. Emissions reached background levels by day 50 (results shown only to day 36), except in the T7 treatment which maintained higher emissions than the background. Addition of DCD reduced total N_2O emissions over 50 days by 33%, 56%, and 80% in the T4, T6, and T8 treatments, respectively (Table 2).

Mineral nitrogen

At the end of the experiment, mineral N present in the soil cores receiving urine with and without DCD was still significantly higher than in control treatments. In all urine treatments without DCD, N was mainly in the NO_3^--N form, whereas in urine treatments with DCD, N was predominantly in NH_4^+-N form (Fig. 3). NH_4^+-N content increased by 2.7–12.6 times at both 0–50 and 50–100 mm depths in DCD treatments with increased urine rates (Fig. 3 a), the percent increase being greater at 50–100 mm depth. Apparently, either the applied urine N moved into the soil and was subsequently hydrolysed to NH_4^+ ions, and/or hydrolysed NH_4^+ ions moved into the soil, leading to increased NH_4^+-N leaching with DCD. However, the NO_3^--N contents of the treatments receiving DCD decreased by 36–80 % at both 0–50 and 50–100 mm depths.

Dry matter yield and N recovery

Dry matter yield increased with increasing rates of urine application except in treatments T7 and T8, and compared to the control treatments was significantly higher both with and without DCD (Table 3). DCD applied to urine treatments had no consistent and significant effect on the DM yield.

The rate of urine application had a significant negative effect on N recovered from herbage (Table 3). N recovery was highest (21.5%) in T3, followed by T5 (16.4%) and T7 (5%).

Nitrogen recovery was lower with the addition of DCD in T4 and T6 compared to the non-DCD treatments T3 and T5, respectively.

Nitrogen leaching

Ammonium leaching
Changes in mean NH_4^+-N concentration in the leachate samples with cumulative drainage volume from each core are shown in Fig. 4a. Concentrations in each breakthrough curve peaked almost immediately as the leaching started, peaks being 15.7 and 34.5 mg N l^{-1} for T5 and T7 (without DCD), respectively. Addition of DCD in the T6 and T8 treatments produced significantly higher peaks (29.3 and 68.3 mg N l^{-1}). Total NH_4^+-N leached in the cores increased significantly with the addition of DCD, resulting in increases in NH_4^+-N leached of 2–3.5 times (Fig. 5).

Nitrate leaching
Leaching breakthrough curves for NO_3^--N are presented in Fig. 4b. Peak NO_3^--N concentration in the leachate from urine treatments without DCD (T5 and T7) reached 201 and 290 mg N l^{-1} respectively (Fig. 4b). However, peak NO_3^--N concentration in the leachate from the DCD treatments (T6 and T8) was significantly reduced ($P< 0.05$) to 68 and 118 mg N l^{-1} respectively. The total amount of NO_3^--N leached from the urine-only treatments T5 and T7 (13.8 and 21.7 g N m^{-2}, respectively) was significantly higher than the DCD treatments T6 and T8 (5.48 and 7.61 g N m^{-2}). Addition of DCD resulted in a 60–65% reduction in apparent NO_3^--N leaching from the soil cores.

Cumulative amounts of NH_4^+ and NO_3^--N in the leachate samples of all the cores (Fig. 5) show clearly that most N (56–95%) leached as NO_3^--N, with NH_4^+-N representing only a small proportion of the total. Thus, although adding DCD to the urine increased 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) (Fig. 5), resulting in a reduction of about 45 % in total N leached.

Discussion

Addition of DCD to increasing rates of urine resulted in: (1) a significant increase in soil NH_4^+-N concentration, causing a marginal increase in total NH_3 emissions from urine and enhancing the potential for NH_4^+-N leaching by a factor of 2–3.5; (2) a significant decrease in N_2O emissions and NO_3^--N accumulation as well as a 60–65% decrease in potential NO_3^- leaching; and (3) no significant effect on pasture yield.

Nitrogen emitted as NH_3 from all urine treatments with and without DCD ranged from 0.95–4.38% of urine-N. This is at the lower end of the range of reported losses and may result from the lack of wind and relatively high soil moisture rates in our experiment. For example, Bronson et al (1999) concluded that 38% of urine-N was lost from a sandy soil, while Whitehead and Bristow (1990) measured 18% loss of urine-N as NH_3 from microplots receiving cattle urine. However, cattle urine applied to lysimeters lost up to 2.3% (Clough et al. 1998) and 7% (Clough et al. 2003) of its N. Our value for NH_3 loss from urine also lies within the 3.7–26.9% range of NH_3 reported by Lockyer and Whitehead (1990) from urine applied at different times of the year. Dicyandiamide inhibits or delays nitrification of NH_4^+ to NO_3^--N, thus increasing the concentration of NH_4^+-N in the soil. In our study, despite the increase of NH_4^+ concentrations in the soil when DCD was applied with urine compared to

urine alone (Fig. 3), NH_3 emission did not increase significantly. This may be attributed to rapid movement of urea-N into the soil, resulting in retention of the NH_4^+-N produced by hydrolysis of this urea. However, this might not be true for surface application of urea or where urine is voided onto dry soil/ pasture, as found in extensive lysimeter and field studies by Rogers (1983), Rao and Puttanna (1987), Davies and Williams (1995), Puttanna et al. (2001), and Singh et al. (glass house study, not published). In these studies, NH_3 emissions increased significantly when DCD was added to urea applications. Surface-applied urea and urine applied to dry soil provides a greater opportunity for hydrolysed NH_4^+ to escape as NH_3, compared to the application of urine to the soil cores at field capacity moisture content. This argument is consistent with the findings of Rao and Puttanna (1987), Puttanna et al. (2001), and Rodgers (1983) who showed that NH_3 volatilisation losses from DCD-treated urea could be substantially reduced by deep placement. The volumes of urine added in our study 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 urine-N to be distributed more evenly and to a greater soil 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 the NH_4^+ produced would be deeper in the soil in the T8 treatment. However, in a lysimeter study Di and Cameron (2004c) found no significant difference in the total amount of NH_3 emitted from cow urine with and without the application of DCD.

In our experiment 0.45–3.57% of urine-N was emitted as N_2O (Table 2), with peak N_2O emissions in all urine treatments, i.e. with and without DCD, within 24 hours of application of treatments. Oenema et al. (1997) estimated that 0.1–3.8% of urine-N from urine spots in grazed pastures was emitted to the atmosphere as N_2O and de Klein et al (2003) also reported N_2O emission factors of 0.3–2.5% for cow urine in New Zealand soils, depending on rainfall and soil drainage classes. The stimulatory effect of urine can be attributed to the increased N availability, increased soil WFPS and supply of easily available C (i.e. solubilisation of soil C caused by an increase in pH after urine or urea application) (Williams et al. 1999, Ambus et al. 2007, Kelliher et al. 2005). 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. Emissions of N_2O have often been shown to increase 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 and Doran 1984). The percentage of added N emitted as N_2O in the experiment increased exponentially with the increasing volume of urine N applied. In an incubation study, increasing volumes of added urine with a constant concentration of urine-N resulted in a significant increase in N_2O emissions (Van Groenigen et al. 2005).

Nitrous oxide can be produced either by nitrification (Bremner et al. 1980) or by denitrification (Firestone and Davidson 1989), with both processes possibly occurring simultaneously in adjacent soil pores of different aerobicity and having 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. High concentrations of NO_3^- ions in soil are a major source for N_2O emissions (Stevens and Laughlin 1998). In our study, using DCD with urine proved effective for reducing N_2O emissions, lowering them by 33–80% compared to urine treatments without DCD (Table 2). Similarly, Di and Cameron (2003, 2006) showed that N_2O flux decreased 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 urine without DCD compared to cores with DCD (Fig. 3b). The mean values of NO_3^--N for all urine treatments indicated that by the end of the experimental period the NO_3^--N concentration in the soil with DCD was reduced by 25–33% compared to that in soil without DCD. This decrease in NO_3^--N can be mainly attributed to the direct effect of DCD on nitrification; moreover, the increase in NH_4^+-N concentration in DCD-treated soil may have contributed to the lower NO_3^--N concentrations, because an increase in ammonium salts inhibits nitrification (Monaghan and Barraclough 1992). There was a substantial (7–9 times) increase in NH_4^+-N concentration in cores receiving urine treatments with DCD compared to cores without DCD. Higher accumulation of NH_4^+-N in soil with the addition of DCD to N sources such as fertiliser, slurry and urine, has been reported (Abbasi and Adams, 2000; Cookson and Cornforth, 2002; Merino et al. 2002; Puttanna et al. 2001). In the absence of added N (i.e. the control soil), application of DCD did not affect soil NH_4^+-N and NO_3^--N concentrations, probably because pasture uptake of NH_4^+-N limited net nitrification. Competition for NH_4^+-N from other microorganisms (Tietema and Wessel 1992) may also have contributed to the negligible increases in NH_4^+-N in the control treatment with added DCD.

Our results showed values for all the measured NH_4^+ and NO_3^- in the leachates peaked 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 throughout the 50-day experimental period by adding water which could have maintained a 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 leaching was present mainly as NO_3^--N in the soil cores in the T5 and T7 treatments receiving urine only, so that NO_3^- formed the major component of N output in the leachate from urine treatments (Fig. 5). This NO_3^--N concentration was significantly reduced at both soil depths with the addition of DCD to the soil cores. The NO_3^- breakthrough curves show the clear difference between urine-only (T5 and T7) and urine plus DCD (T6 and T8) treatments. The 60–65% reduction in total NO_3^- leaching loss attributed to DCD in the urine treatments at 29 and 57 g N m^{-2} is similar to the 68–76% reduction when DCD was added to autumn-applied urine-N (1000 kg N ha^{-1}) by Di and Cameron (2004c, 2006, 2008).

In contrast, the addition of DCD increased the NH_4^+ concentration in soil cores 7–9 fold. This increase was apparent in the amount of NH_4^+ found in the leachate of these cores which was 2–3.5 times higher than that found in the leachate of soil cores without DCD (Fig. 5). Cookson and Cornforth (2002) found similar results. Although NH_4^+ ions are strongly adsorbed on the soil colloids, the accumulation of NH_4^+ ions would have progressively saturated the cation-exchange capacity (10 $cmol_c$ kg^{-1}) making leaching more likely. However, because the column depth in our study was only 100 mm, less NH_4^+ leaching might be expected 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 (Fig. 5).

Addition of DCD did not affect pasture yield significantly in the urine treatments in our study; however, the effect of DCD on herbage yield varies (Cookson and Cornforth, 2002; Di and Cameron, 2002b; Gioacchini et al. 2002; Smith et al. 2005). The trend of 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} may be attributed to an increase in NH_4^+ concentration resulting in salt injury and NH_4^+ toxicity. Herbage yields in the T7 and T8 treatments (57 g urine-N m^{-2}) were lower than those in 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 and Wolton 1975), particularly where applications of highly concentrated urine occur (e.g., at concentrations 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 and Wolton, 1975), and is probably caused by a combination of high salt concentrations and NH_4^+ toxicity.

Conclusions

Our glasshouse study showed DCD application to in-situ soil cores at field-capacity moisture content can reduce N losses via N_2O emissions (33–80% reduction) and potential NO_3^- leaching from urine spots with increasing N concentrations. DCD decreased NO_3^--N accumulation, resulting in a 60–65% reduction in potential NO_3^--N leaching. However, DCD increased NH_4^+-N concentrations in the soil, causing a 2–3.5 fold increase in potential NH_4^+-N leaching, and slightly elevated NH_3 emissions. Our results also showed DCD addition did not affect herbage yields from urine treatments.

Our study was conducted under controlled conditions, so our conclusions should be applied to field conditions with caution.

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Figure Captions

Figure 1 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.

Figure 2 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.

Figure 3 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.

Figure 4 Concentrations of (a), NH_4^+-N and (b), NO_3^--N in the drainage water from soil cores receiving urine at 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 Total leaching losses of N in the leachate from soil cores receiving urine at 29 g N m^{-2} and 57 g N m^{-2}, with and without DCD.

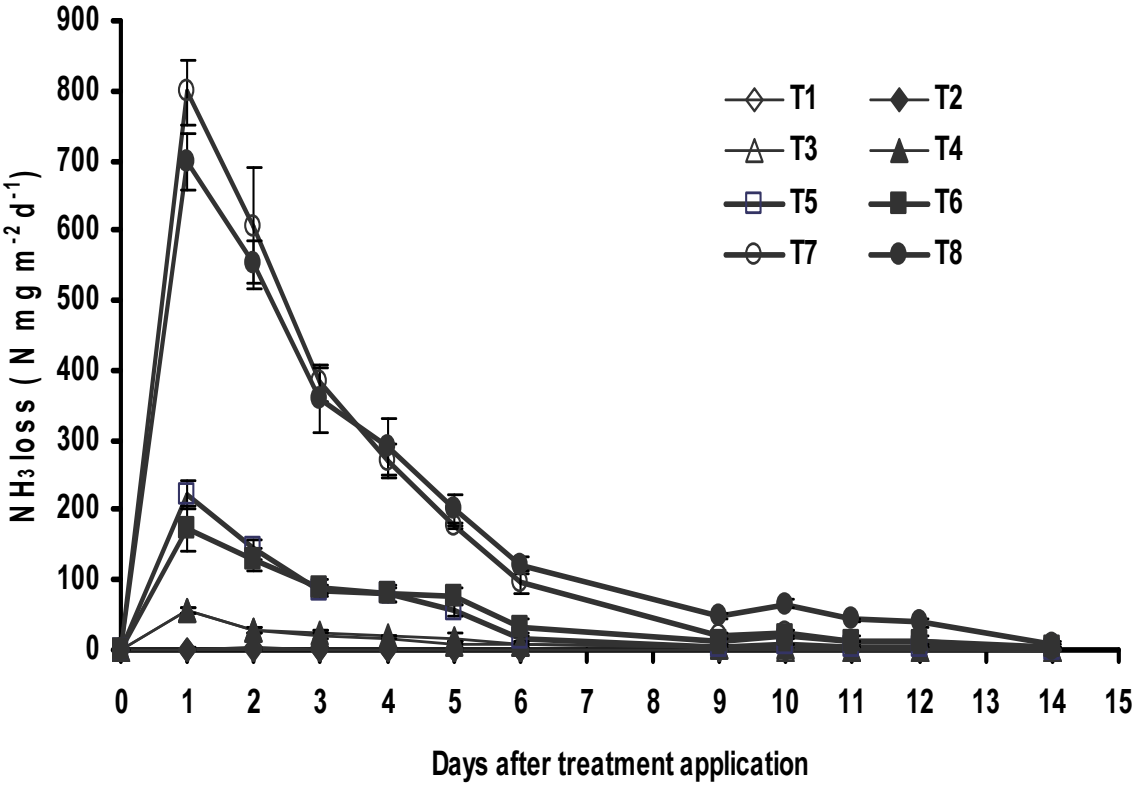

900
800
700
600
500
400
300
200
100
0
NH3 loss (N mg m-2 d-1)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days after treatment application
T1
T2
T3
T4
T5
T6
T7
T8

Fig. 1

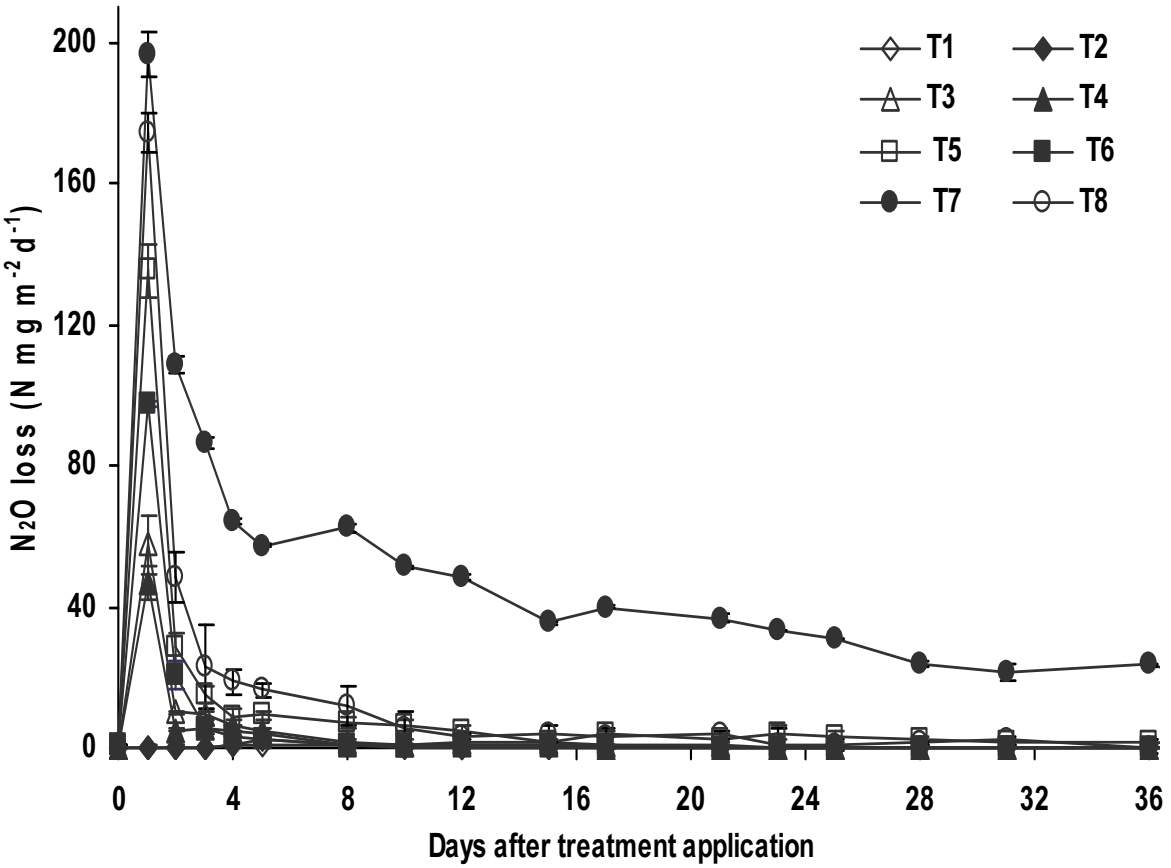
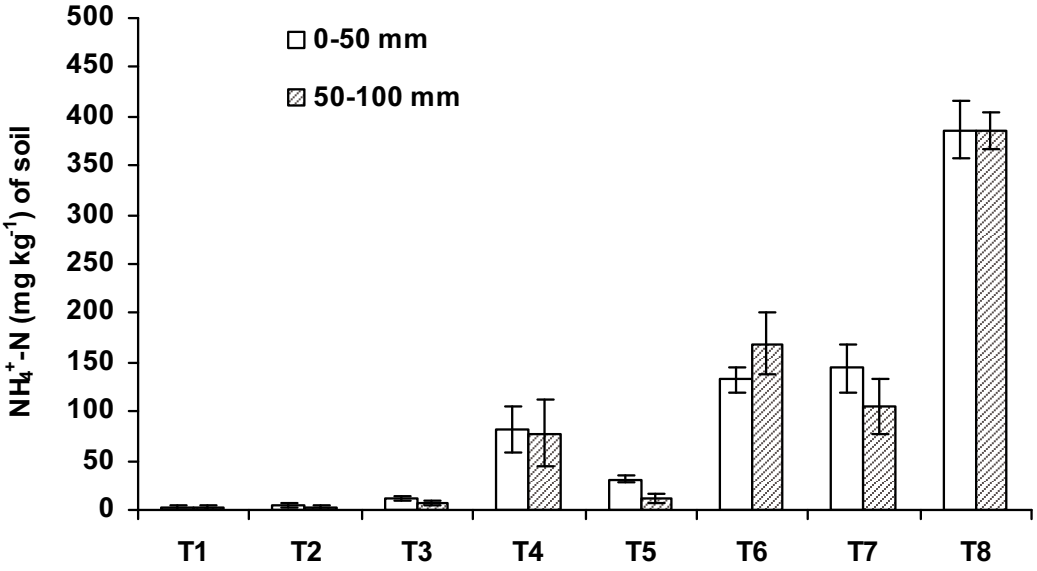

T1
T2
T3
T4
T5
T6
T7
T8
200
160
120
80
40
0
N2O loss (N m g m-2 d-1)
0
4
8
12
16
20
24
28
32
36
Days after treatment application

Fig. 2

(a)


0-50 mm
50-100 mm
NH_4^+-N (mg kg^{-1}) of soil
500
450
400
350
300
250
200
150
100
50
0
T1
T2
T3
T4
T5
T6
T7
T8

(b)

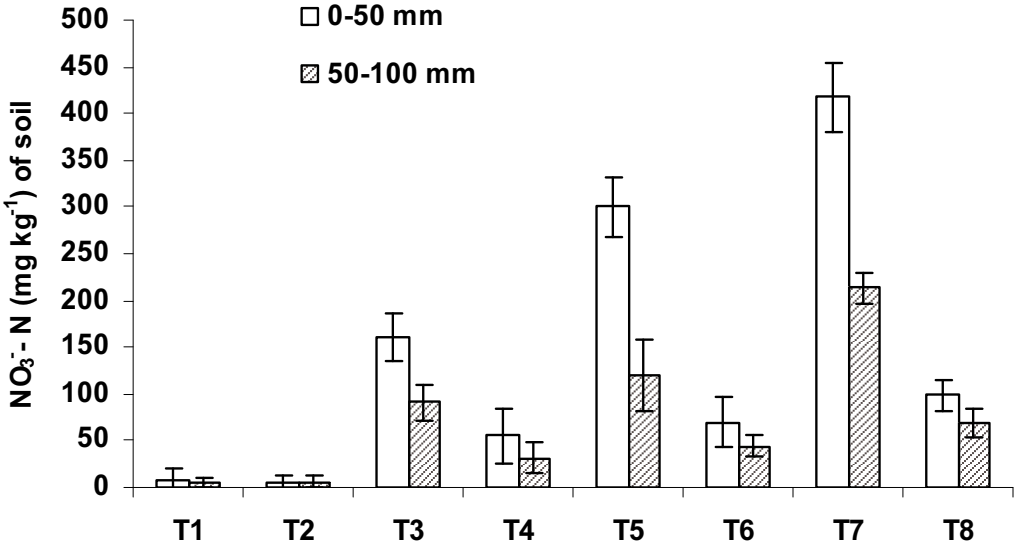
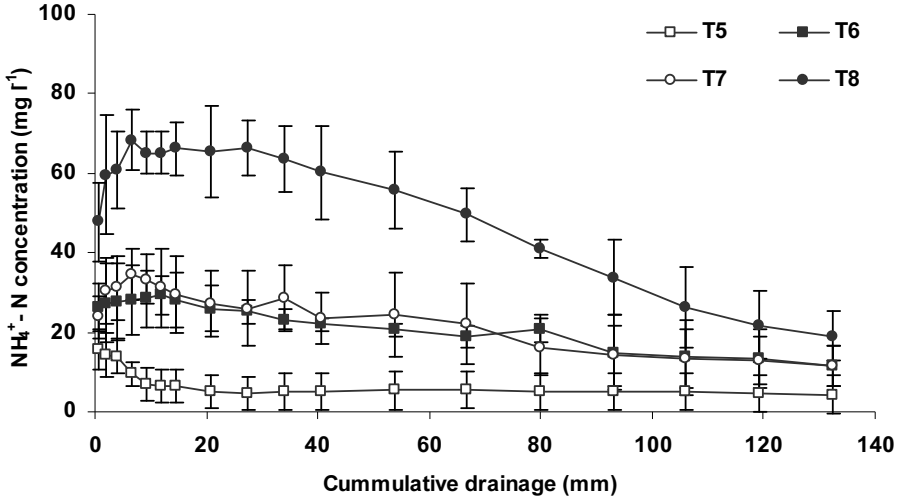

0-50 mm
50-100 mm
NO_3^-- N (mg kg^{-1}) of soil
500
450
400
350
300
250
200
150
100
50
0
T1
T2
T3
T4
T5
T6
T7
T8

Fig. 3

(a)


T5
T6
T7
T8
NH_4^+ - N concentration (mg l^{-1})
Cummulative drainage (mm)

(b)

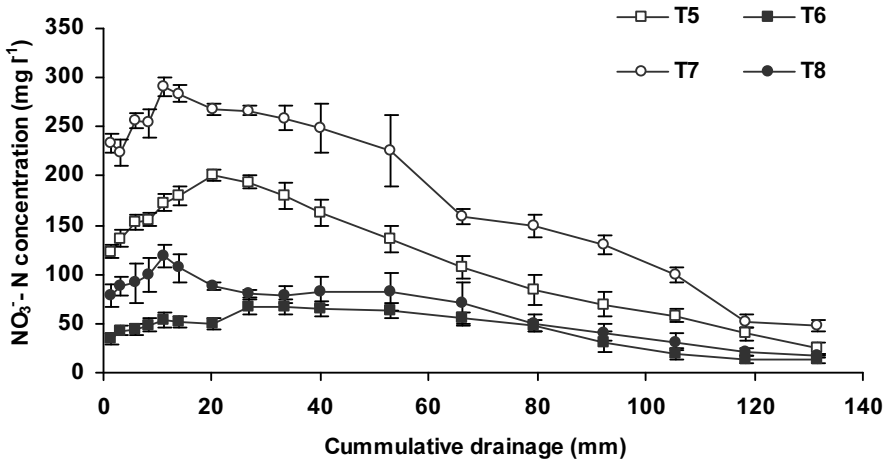

T5
T6
T7
T8
NO_3^- - N concentration (mg l^{-1})
Cummulative drainage (mm)

Fig. 4

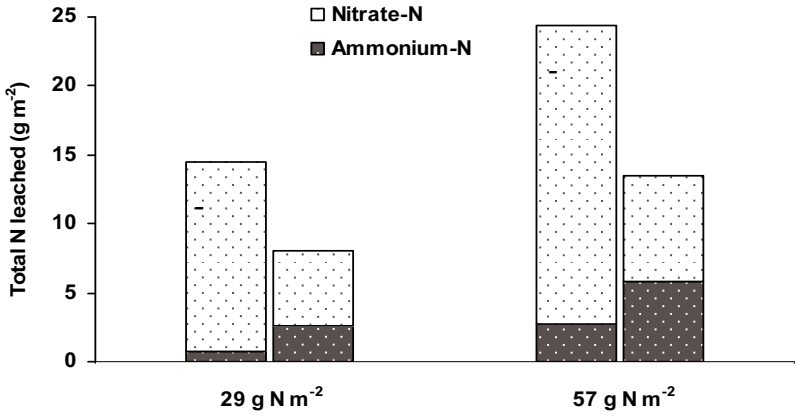

Nitrate-N
Ammonium-N
Total N leached (g m^{-2})
29 g N m^{-2}
57 g N m^{-2}

Fig. 5

Table 1 **Chemical and physical properties of Manawatu fine sandy loam 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 ($cmol_c kg^{-1}$)
0–50	1.2	12	0.30	3.5	5.70	10
50–100	1.2	12	0.15	1.5	6.20	nd

Table 2 **Total N applied and N emitted as NH_3 and N_2O (mg 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	N_2O-N (g N m^{-2})	% of added N emitted
T1	-	0.01 c		0.01 f	
T2 (+ DCD)	-	0.01 c		0.02 f	
T3	14.4	0.15 c	0.95	0.12 d	0.74
T4 (+DCD)	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 (+DCD)	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 (+DCD)	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 $P< 0.05$ level.

Table 3 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 (%)	Added N recovered (%)	DM response (g DM g^{-1} N)
T1	-	151.4 c*	2.86	-	-
T2 (+DCD)	-	143.4 c	2.89	-	-
T3	14.4	218.1 b	3.40	21.5	4.63
T4 (+DCD)	14.4	182.9 bc	3.49	15.6	2.74
T5	29.0	268.4 a	3.40	16.4	4.03
T6 +(DCD)	29.0	224.7 b	3.48	12.6	2.80
T7	57.0	224.1 b	3.22	5.0	1.27
T8 +(DCD)	57.0	225.4 ab	3.23	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 $P < 0.05$ level.

2. Impact of urease inhibitor on nitrogen dynamics in pasture soil cores receiving urea fertiliser and cattle urine

Abstract

The grazed pastures in New Zealand are the major contributor to gaseous losses of nitrogen (N) to the atmosphere; including ammonia (NH_3) and nitrous oxide (N_2O) emissions which cause environmental degradation through their effects on soil acidification, eutrophication, global warming and stratosphere ozone depletion. Ammonia is the major form of gaseous N loss and its loss results in poor efficiency of surface applied urea and voided urine - N to temperate grasslands. Glasshouse experiments were conducted to study the effect of a urease inhibitor (UI), N-(n-butyl) thiophosphoric triamide (NBPT), commercially known as Agrotain, applied to urine and urea on urea hydrolysis and NH_3 and N_2O emissions. Treatments included commercially available products Sustain Yellow (urea + agrotain + 4% sulphur coating), Sustain Green (urea + agrotain) and urea, and bovine urine (600kg N/ha) with and without Agrotain applied to intact soil cores of fine sandy loam soil. The addition of Agrotain to urine and urea reduced NH_3 emission by 22% to 48%. Agrotain was also effective in reducing N_2O emissions (48% to 62%) from both urine and urea. The reduction in N_2O emissions varied with the type and amount of N applied and plant N uptake. Pasture growth was slightly but insignificantly higher in the soil cores receiving Agrotain with urea than urea alone.

Introduction

In intensively managed pastures nitrogen (N) is the nutrient element that most strongly regulates pasture production. However, magnitude of N input determines N surplus and its potential loss. The increased use of fertiliser N and high concentration of excreted N in urine patches (up to c. 1000 kg N /ha) contribute to the surplus of N. The inability of pastures to use surplus N results in the N losses through ammonia (NH_3) volatilisation, nitrous oxide (N_2O) emission and nitrate (NO_3^-) leaching which has both economical and environmental implications. Quantitatively, NH_3 is the major form of gaseous N loss and can cause toxicity in vegetation, lead to acidification, contribute to eutrophication of N sensitive environment (Sheppard *et al*. 2005) and act as a secondary source of NO and N_2O (Bouwman 1990).

A large portion of the applied N is lost by NH_3 volatilisation, especially under tropical conditions (Vlek & Craswell 1979; Freney *et al.* 1981). Ammonia losses from 1.7 to 56% of the applied N fertilizer have been reported, which depend on soil moisture, temperature, soil pH, wind velocity, soil organic C and N, and fertliliser type (Black et al. 1985; Fenn and Hossner 1985; Freney et al. 1985; de Data et al. 1989; Gioacchini et al. 2002). Similar losses (4-41%) of the N from applied cattle urine through NH_3 volatilisation have been measured from temperate grasslands (Lockyer and Whitehead 1990; Whitehead and Raistrick 1993; Zaman and Blennerhassett 2008).

The use of urease inhibitors (UIs) is one of the mitigation options to reduce NH_3 emissions and to control N dynamics in soils. The UIs regulate the transformation 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). N-(n-butyl) thiophosphoric triamide (NBPT, known by the commercial

name of Agrotain) is an efficient UI (Carmona et al. 1990,; Watson 2000; Gioacchini et al. 2002; Zaman et al 2006). After application, Agrotain is quickly converted in soil to its more effective oxon analogue N-(n-butyl)phosphoric triamide (BNPO), which then forms a tridentate ligand with the urease enzyme (Manunza et al. 1999) slowing urea hydrolysis. There is limited information on the effect of Agrotain on changes in N dynamics and subsequent NH_3 loss in pasture soils in New Zealand. At the time of writing this paper, results of another New Zealand field study involving urea and DAP fertilisers with UI and nitrification inhibitor was published (Zaman et al. 2008). However, this study did not consider the effect of UI on urine-N transformation and N gaseous losses. In New Zealand, NBPT-amended urea products called Sustain Green (urea plus Agrotain), Sustain Yellow (urea plus Agrotain and 4% sulphur coating) have recently become commercially available (http://www.summitquinphos.co.nz). These formulations need to be evaluated under temperate conditions of New Zealand for their potential in mitigating N losses. The objective of this study was to assess the inhibitory effect of UI Agrotain on N transformations from urea and urine applications in pasture soil and to determine its subsequent effect on the changes in gaseous emissions of NH_3 and N_2O. The relative effect of Agrotain and elemental sulphur coating on the N transformations and losses from applied urea was also determined.

Materials and Methods

Experimental details

Two experiments were conducted using in-situ soil cores collected from Massey University sheep farm. The first experiment, compared N transformation and emissions of NH_3 and N_2O from urea and Sustain Yellow (urea plus Agrotain and elemental S coating), and urine and urine plus Agrotain. The second experiment, compared 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 N transformations and gaseous emissions of NH_3 and N_2O.
Intact soil cores (100 mm diameter, 100 mm depth) from three representative sites were collected from a sheep-grazed permanent legume-based pasture at Frewens Research Block, Massey University, Turitea campus. The soil at this site is Manawatu fine sandy loam, classified as a weathered fluvial recent soil (Hewitt 1998). Some pertinent soil chemical and physical properties are given in Table 1. The soil-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. The sward on the cores was then cut and the cores weighed and maintained at field-capacity moisture content throughout the experiment.

Treatments

The first experiment comprised six treatments with three replications: control without UI; control with UI; urine without UI; urine with UI; urea without UI; urea with UI (Sustain Yellow, a commercial fertiliser manufactured by Summit Quinphos Ltd. by mixing sulfur-coated (4%) urea granules with Agrotain @ 1 l t^{-1} urea). Nitrogen in the form of urine and urea was added at the rate of 47.6 g N /m^2 (476 kg N /ha) and 60.0 g N /m^2 (600 kg N /ha), 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 urea). The fertiliser-grade urea and Sustain Yellow granules (2-4 mm) were applied to the soil surface. 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) for dairy cattle, with 80-90% being urea N (Haynes & Williams 1993). The experiment was set

up in a glasshouse maintained at a temperature ranging from 15 to 20^{o}C May, 2005.

The second experiment also comprised the 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 urea); urea with UI (Sustain Yellow) and S-coated urea (32% N, 27% S). Nitrogen in the form of urea and amended forms of urea was applied @ 10 g N /m^2 (100 kg N /ha). 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) was used to overcome the urea scorch observed at the high rate of N application (600 kg N /ha) in Experiment 1. The temperature in the glass house was maintained at 25-30^0 C, which was higher than that of Experiment 1. 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 in Experiment 1 and 2, respectively) and glasshouse conditions (temperature).

Gaseous emissions

The chambers used for NH_3 and N_2O emission were modified PVC 'sewer-hatches' attached to 250 mm diameter x 150 mm deep sections of PVC pipe with a sealed base (Saggar et al. 2004b). Each chamber had two removable lids with one or two ports for sampling N_2O or NH_3 fluxes, respectively. The lid with one port was fitted with a 10 cm length of tubing (3.2 mm diameter) with a three-way stopcock for sampling N_2O gas. The lid with two ports had one input port connected to a compressed air supply and the other (exhaust) port connected to a chemical trap to absorb NH_3. Ammonia emissions were measured for 10–12 hours daily overnight for 14 days after application of the treatments using the active flux method with a constant air supply at 1 dm^3/min and 50 ml 0.05 M H_2SO_4 acid traps to capture NH_3 (Wulf et al. 2001; Singh et al. 2003). After measuring NH_3, the lids were removed and the chambers left open to equilibrate under ambient conditions. Ammonia collected in 0.05 M H_2SO_4 was analysed colorimetrically for total NH_4^+-N using a Technicon autoanalyser. The NH_3 flux (mg N/m^2/hr) was then calculated using the equation:

$$N_{(NH_3\ flux)} = \frac{C \times V}{a \times D} \qquad (1)$$

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

For N_2O measurements, chambers were closed with the single-port lid (Saggar et al. 2004b) and 3 gas samples were taken from each chamber at 0 min (t0), 30 min (t30), and 60 min (t60) using 60 ml polypropylene syringes fitted with 3-way stopcocks. These gas samples were transferred to evacuated vials and analyzed using a Shimadzu GC – 17A gas chromatograph with a ^{63}Ni-Electron capture detector (Hedley et al. 2006); N_2O (mg/m^2/hr) flux was estimated from the measurements made at the three times (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. Precision of the gas chromatographic data at ambient N_2O concentrations was ± 1% or better. Increases in N_2O concentrations within the chamber headspace were generally linear ($R^2 > 0.90$) over time. Therefore, N_2O flux (µg/m^2/ hr) was calculated from equation 2 using the ideal gas law.

$$F = \rho \times \frac{V}{A} \times \frac{\Delta c}{\Delta t} \times \frac{273}{(T + 273)} \quad (2)$$

where F = gas flux (μg/m^2/h); ρ = density of gas (g/m^3); V = volume of the chamber (m^3); A = base area of the chamber (m^2); Δc/Δt = average rate of change of concentration with time (ppmv/h); and T = temperature (°C) in the chamber.

Nitrous oxide was measured daily for the first week to capture immediate changes in gas fluxes, followed by alternate days for two weeks, 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 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.
In both the experiments the background emissions of NH_3 and N_2O were taken from all the chambers a day before applying the treatments. The gaseous emissions were monitored till these reached the background levels (2 to 5 weeks for NH_3 and 6 to 10 weeks for N_2O).

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

$$\%\ reduction\ in\ NH_3 / N_2O - N = \frac{[(A - C) + (B - D)]}{(A - C)} X100 \quad (3)$$

where, *A* = total NH_3/N_2O-N emission from N only treatment
B = total N_2O-N emission from N+DCD treatment
C = total N_2O-N emission from the control (nil N) without inhibitor treatment
D = total N_2O-N emission from the control with inhibitor treatment

Analysis

Urine analysis
Urine was collected from Friesian cows during a milking session at the Massey University No.1 Dairy farm. Fresh urine samples from individual cows 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 concentration of 7.9 g/l and 3.5 g/l, respectively.

Soil analysis :Mineral N, and Total C and N
At the end of the experiment, soil cores were split into upper (0-50 mm) and lower (50-100 mm) sections. A sub-sample of 5g (oven dry equivalent) was taken from each core and extracted with 2 *M* KCl solution by shaking for 1 hr (1:6 soil: extractant ratio). The extracts were analysed colorimetrically for nitrate (NO_3^-) and ammonium (NH_4^+) contents 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 analysis
It was not possible to measure soil pH of the incubating soil cores. Therefore, the same treatments were applied simultaneously to the sieved soils maintained at field capacity moisture content. Soil pH was measured periodically for 15 days for the both the

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

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, herbage on the cores was cut at 2 cm height from the soil surface twice during the experimental period, dried at 65°C 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).

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.

Results

Ammonia emissions

The NH_3 flux in the control treatments (both with and without UI) did not fluctuate much over the experimental period of 30 and 15 days in Experiment 1 and 2, respectively (Figure 1). The application of N in the form of urine @ 47.6 g N /m^2 and urea @ 60 (Experiment 1) and 10 g N /m^2 (Experiment 2), significantly increased NH_3 emissions within1-2 days of application as compared to control treatment (Figure 1 a & b). The NH_3 flux from urine increased within 24 hours of application and reached the peak value of 999 mg NH_3-N /m^2 /d whereas emissions in urea treatments peaked after two days of application in both the experiments. These NH_3 fluxes dropped quickly for the first five days before gradually coming to background levels. Addition of UI to urine and urea resulted in a significant reduction ($P< 0.05$) in NH_3 emissions and also delayed the time to reach the peak emissions (T_{max}). This delay was one day in urine treatment with reduction of NH_3 peak to 375 mg NH_3-N /m^2 /d, and 3-6 days in urea treatments receiving The addition of UI reduced the total amount of NH_3-N emission in urine treatment from 4.36 to 3.39 g NH_3-N /m^2 and 19.7 to 14.3 g NH_3-N /m^2 in urea treatment in Experiment 1, resulting in a 22.4% and 27.5% reduction in NH_3 losses, respectively (Table 3).

The total NH_3 emission in Experiment 2 in 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). 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).

Nitrous oxide emissions

The N_2O emission flux in the control treatments in both the experiments remained almost constant during the experimental period (Figure 2 a & b). There was variation in the effect of UI on N_2O emissions from urine and different rates of urea (60 and 10 g N /m^2) in the two experiments. Urine application resulted in peak N_2O emission within 24 hours, followed by a progressive decline with time (Figure 2a). 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 (urine+UI), and the subsequent

N_2O emissions remained lower than in the urine-only treatment throughout the experiment resulting in a 62% reduction in N_2O emissions (Table 3).

In the urea treatment in Experiment 1, the highest emission of 11.2 mg N_2O-N /m^2 /d was obtained on day 3 but then decreased markedly within a day (Figure 2a). This decline was followed by a small but steady increase in emissions, giving a peak value of 6.21 mg N_2O-N /m^2 /d on day 30 (Figure2a). Addition of Sustain Yellow resulted in a smaller initial peak (2.20 mg N_2O-N /m^2 /d) but significantly higher second peak than from the urea-only treatment. However, overall there was no significant difference in total N_2O emissions in the urea and Sustain Yellow treatments (Table 3).

The application of urea, Sustain Green, Sustain Yellow and S-coated urea, in Experiment 2 resulted in an increase in N_2O emission within a day (Figure 2b). 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 /d) was obtained 4 days after application; a subsequent smaller peak (23 mg N_2O-N /m^2 /d) was observed after 19 days. Similar trends were found in all the other treatments. The peak emissions obtained at these two days for Sustain Green and Sustain Yellow were significantly lower compared to those in the urea treatment. However, in the S-coated urea treatment, the second peak was significantly higher (45 mg N_2O-N /m^2 /d), with subsequent emissions also remaining higher than urea treatment. This accounted for 56% and 49% emissions reduction with Sustain Green and Sustain Yellow respectively, and 42% emission increase with S-coated urea (Table 4).

Mineral nitrogen

At the end of the experiment, soil mineral N content (NH_4^+ and NO_3^-) measured in both the experiments was significantly ($P < 0.05$) higher in soil cores receiving N than in the control at both the depths (Figure 3 & 4). All soil cores contained higher concentrations of NO_3^- than NH_4^+-N in all the treatments at both 0-50 and 50-100 mm depths (Figure 3 and 4). Mineral N (NH_4^+ and NO_3^-) was more evenly distributed in urine treatment as compared to the urea treatment (Figure 3). Significantly higher NH_4^+-N and NO_3^--N concentrations were found at 0-50 mm depth than at 50-100 mm depth in case of all the urea treatments (Figure 3 and 4). This difference was expected as urea was surface applied and thus NH_4^+ was confined to the top soil layer. Addition of UI to the urine caused no significant effect either on NH_4^+-N or NO_3^--N concentrations at either depth (Figure 3). In the soil cores receiving urea amended either with UI or S, the trend of NH_4^+ and NO_3^- distributions was same as in the respective urea treatments, however in Experiment 1 the concentration of NH_4^+-N at both soil depths increased significantly with addition of UI where as no such change was observed in Experiment 2.

Soil pH

The mean pH of the soil receiving N in the form of urine or urea, with and without UI in both the experiments, was significantly higher than that in the control soil (Figure 5). 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 (Figure 5a). Addition of UI to urine delayed the pH increase by a day and the maximum pH value obtained was 6.7 (Figure 5a). In the urea treatment, pH increased from 5.7 to 6.9 in Experiment 1 and 5.4 to 6.3 in Experiment 2 after 48 hours of application which then decreased rapidly after day 4 (Figure 5 a & b). Addition of UI to urea resulted in gradual increase in pH and the pH remained

significantly lower throughout the measurement period as compared to urea alone treatments in both the experiments. This may be attributed to the effect of UI on the rate of urea hydrolysis and the associated release of OH^- ions. The S-coated urea treatment in Experiment 2 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 5b).

Herbage dry matter yields

No herbage DM yield data were recorded for Experiment 1. The herbage DM yield and N uptake in the control treatment of Experiment 2, was significantly lower than those in the N treatments (Table 5). However, the DM yield differences among N treatments were not significant although the trend found was that the highest yield of 232 g /m^2 was with the Sustain Green, followed by the Sustain Yellow (223 g /m^2), urea (194 g /m^2) and S-coated urea (150 g /m^2) treatments. The N uptake was significantly ($P<0.05$) higher in Sustain Green and Sustain Yellow significantly compared to that in the urea treatment and was the least in the S-coated urea (Table 5).

Discussion

The results of our study have indicated that: (i) while NH_3 emissions were higher from urea fertiliser than urine, the opposite trend was noticed 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. 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).

When urea or urine is applied to the soil, urease enzyme hydrolyzes the urea-N ($CO(NH_2)_2$) to NH_4^+ ions (Eq. 4). 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 5), resulting in the release of NH_3 gas.

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + 2OH^- + CO_2 \quad (4)$$

$$NH_4^+ + OH^- \rightarrow NH_3 + H_2O \quad (5)$$

In this study, following the hydrolysis of urea-N in urine and urea treatments, the soil pH increased to 6.9 in Experiment 1 and 6.3 in Experiment 2. A slightly 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 amount in urea (60 g N /m^2) and urine (47.6 g N /m^2) in Experiment 1. The pH in the treatments receiving Agrotain remained significantly lower than the respective urine and urea treatments throughout the measurement periods. This may be attributed to the effect of UI on the slowing the rate of urea hydrolysis and the associated release of OH^- ions.

The NH_3 emissions from urine and urea applied in this study are comparable in magnitude to results reported by other workers (Zaman & Blennerhassett 2008, Zaman et al. 2008, Watson *et al*. 1998). 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). 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 which could explain high pH and high NH_4^+ and NH_3 concentrations within 24 hours of urine application. In this study, the amount of NH_3 emitted (19.7 g N /m^2) and the peak flux were, however, higher in the urea than in the urine treatment (4.36 g N /m^2) in Experiment 1. The plausible explanation for this can be that the urea was surface applied, thus giving more chances for the release of NH_4^+ ions as NH_3; NH_4^+ ions from urine would however, have moved into the soil core reducing the NH_4^+ concentration at the soil surface and thus decreasing NH_3 losses. 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 6); NH_3 in solution is in turn maintained by NH_4^+ - NH_3 equilibrium (Eq. 7) as follows.

$$NH_3\ (aq) \leftrightarrow NH_3\ (g) \qquad (6)$$

$$NH_4^+\ (aq) \leftrightarrow NH_3\ (aq) + H^+\ (aq) \qquad (7)$$

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 slightly 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 3-6 days in the urea treatments (Figure 1 a & b). 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 down urea hydrolysis long enough for urea to diffuse away from the zone of placement. Treating urea with UIs also reduced the rise in soil pH (Figure 5 a & b) 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 and thus reducing NH_3 emissions.

In Experiment 1, slightly 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 occur 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; Zaman et al. 2008). 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 slowly 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 difference in N_2O emission between these two experiments may be attributed to the high level of NH_3 emission in Experiment 1, thereby resulting in a decrease in NO_3^- concentration in soil. 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) for the former treatment. Sherlock & Goh (1983) measured greater losses of N_2O from simulated urine than aqueous urea and 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) and Koops *et al.* (1997). In Experiment 1, urine was added to the soil cores at field-capacity moisture content, creating 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 attributed this initial stimulation of N_2O production to either chemodenitrification or 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 but, as the effect of UI started diminishing, there was a gradual increase in N_2O emissions; the data also showed a peak after 30 days and remained higher than in the urea treatment until the termination of the experiment. However in Experiment 2, the N_2O emissions in the UI and S 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 in Experiment 2, thereby 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 concentration of NH_4^+ ions for nitrification would initially be lower in the UI and S amended-urea treatments than in the urea-alone treatment, thereby resulting in a decrease in N_2O emissions produced during nitrification. Dobbie and Smith (2003) also observed lower N_2O emissions from soil treated with urea plus Agrotain. The decrease in N_2O emissions resulted in a slightly higher NO_3^--N concentration in the soil cores (Figure 7 b) receiving UI and S amended-urea compared to the urea only treatment. This increase in NO_3^--N concentration was also found in urine and higher rate of urea treatments in Experiment 1. However, there was reduction in N_2O emissions from urine treatment with addition of Agrotain in the absence of plants. The reasons are unknown.

The data indicated that S-coated urea was effective in decreasing NH_3 losses (Table 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, thereby adversely affecting plant growth as not enough N was available for the plants. The later release of N from S-coated urea later in the experimental period when plant growth was already effective 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 contains 27% S, Sustain Yellow has only 4% S.

Conclusions

Urease inhibitor was effective in reducing NH_3 volatilisation and N_2O emissions from both urine and urea but the reduction in N_2O emission varied depending on the plant uptake and build up in nitrate concentration. Urease inhibitor increased N uptake, thereby resulting in an increase in herbage dry matter yields from urea fertiliser, though the long term effect of UI on herbage yield requires further examination. Elemental S coated urea reduced NH_3 significantly but slightly increased N_2O emissions during the experimental period of 6 weeks. The S coating of urea in the presence of UI (i.e.Sustain Yellow)did not show any additional benefit in relation to reducing N gaseous emissions compared with urea in the presence of UI alone (i.e. Sustain Green).

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Figure Captions

Figure 1 Ammonia emissions from (a) urine and urea applications, with and without UI in Experiment 1 (b) urea and amended-urea treatments in Experiment 2. 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

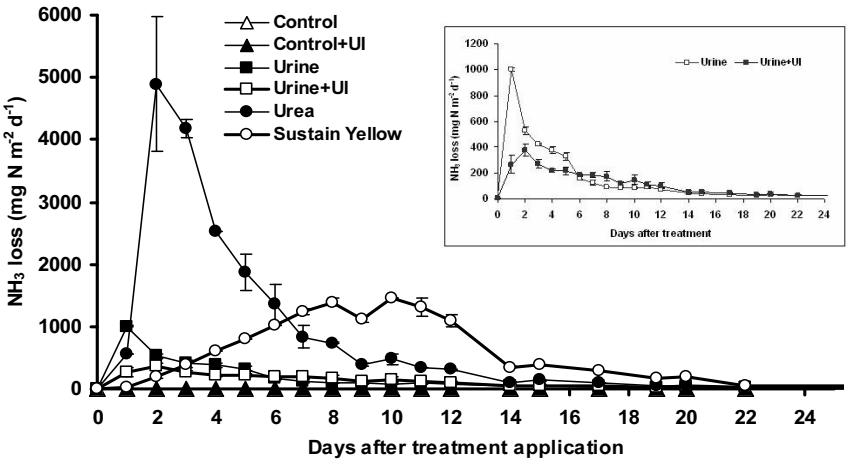
Figure 2 Nitrous oxide emissions from (a) urine and urea applications, with and without UI in Experiment 1 (b) urea and amended-urea treatments in Experiment 2. The inset gives the enlarged graph for N_2O emissions in the urea and Sustain Yellow treatments. Each value represents a mean of three replicates with standard deviation shown by vertical bars

Figure 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 in Experiment 1. 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)

Figure 4 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 in Experiment 2. 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.

Figure 5 pH distribution in Manawatu sandy loam soil receiving (a) urine and urea, with and without UI in Experiment 1 (b) urea and amended-urea treatments in Experiment 2.

(a)


Control
Control+UI
Urine
Urine+UI
Urea
Sustain Yellow
NH3 loss (mg N m-2 d-1)
Days after treatment application
Urine
Urine+UI
Days after treatment

(b)

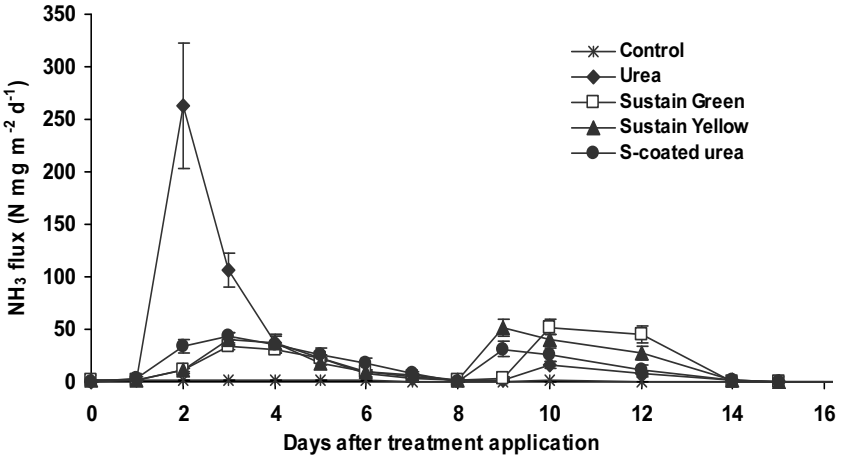
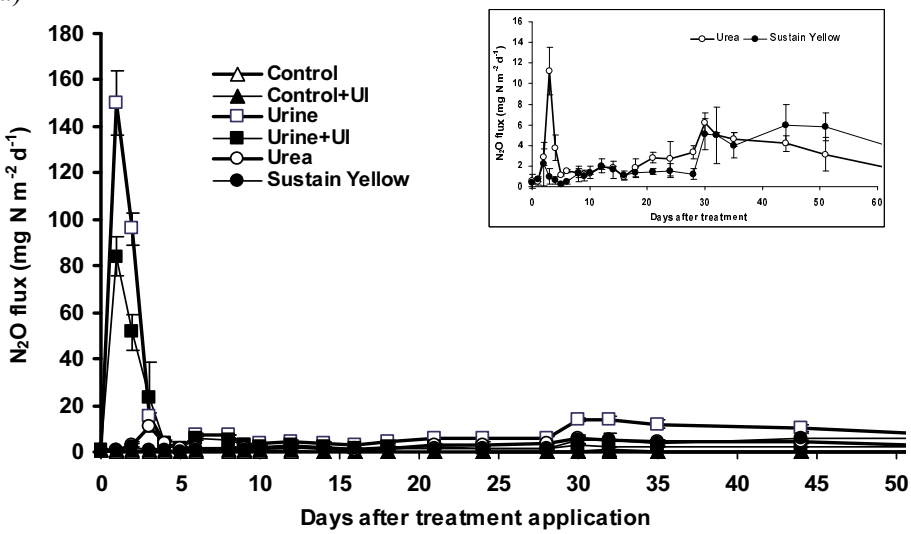

Control
Urea
Sustain Green
Sustain Yellow
S-coated urea
NH3 flux (N mg m-2 d-1)
Days after treatment application

Figure 1

(a)


Control
Control+UI
Urine
Urine+UI
Urea
Sustain Yellow
N2O flux (mg N m-2 d-1)
Days after treatment application
Urea
Sustain Yellow
N2O flux (mg N m-2 d-1)
Days after treatment

(b)

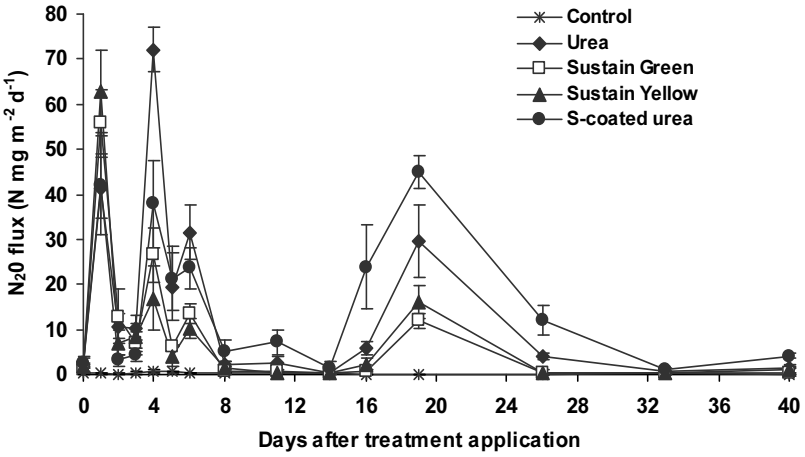
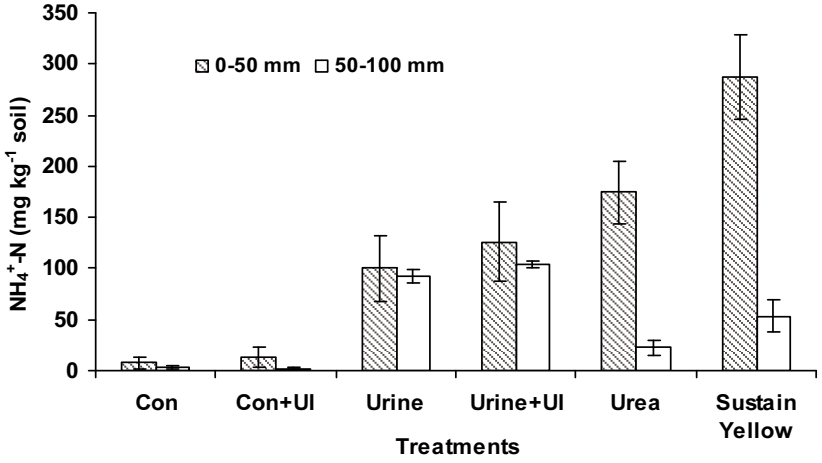

Control
Urea
Sustain Green
Sustain Yellow
S-coated urea
N20 flux (N mg m-2 d-1)
Days after treatment application

Figure 2

(a)


350
300
250
200
150
100
50
0
0-50 mm
50-100 mm
NH4+-N (mg kg-1 soil)
Con
Con+UI
Urine
Urine+UI
Urea
Sustain Yellow
Treatments

(b)

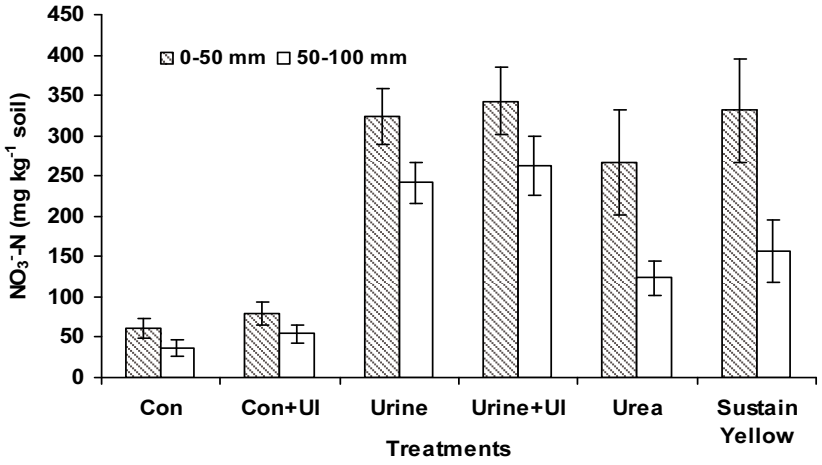
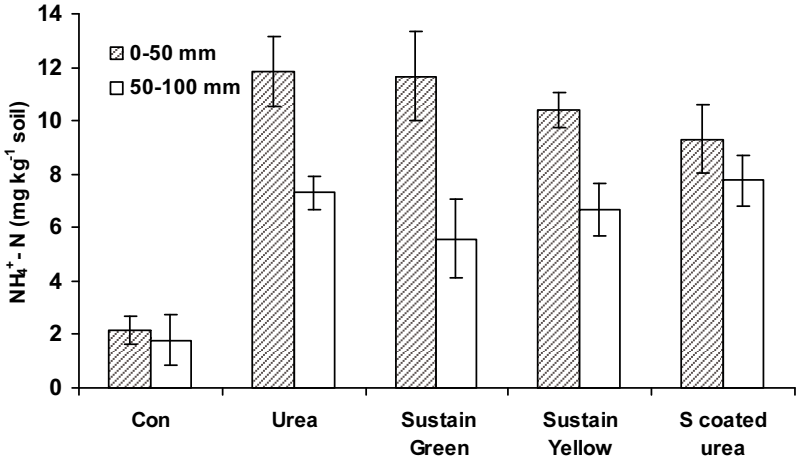

450
400
350
300
250
200
150
100
50
0
0-50 mm
50-100 mm
NO3--N (mg kg-1 soil)
Con
Con+UI
Urine
Urine+UI
Urea
Sustain Yellow
Treatments

Figure 3

(a)


0-50 mm
50-100 mm
NH_4^+ - N (mg kg^{-1} soil)
14
12
10
8
6
4
2
0
Con
Urea
Sustain Green
Sustain Yellow
S coated urea

(b)

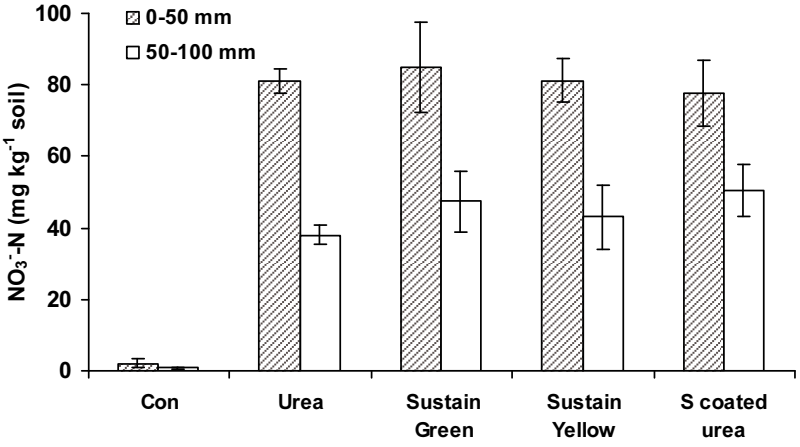
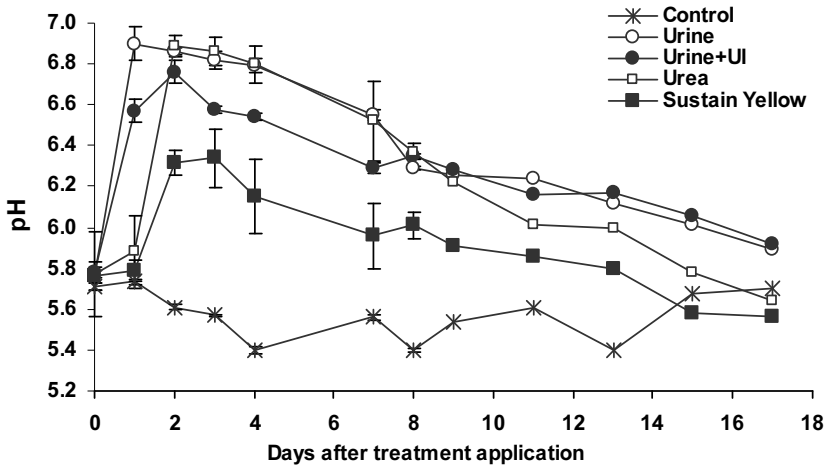

0-50 mm
50-100 mm
NO_3^--N (mg kg^{-1} soil)
100
80
60
40
20
0
Con
Urea
Sustain Green
Sustain Yellow
S coated urea

Figure 4

(a)


Control
Urine
Urine+Ul
Urea
Sustain Yellow
pH
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
0
2
4
6
8
10
12
14
16
18
Days after treatment application

(b)

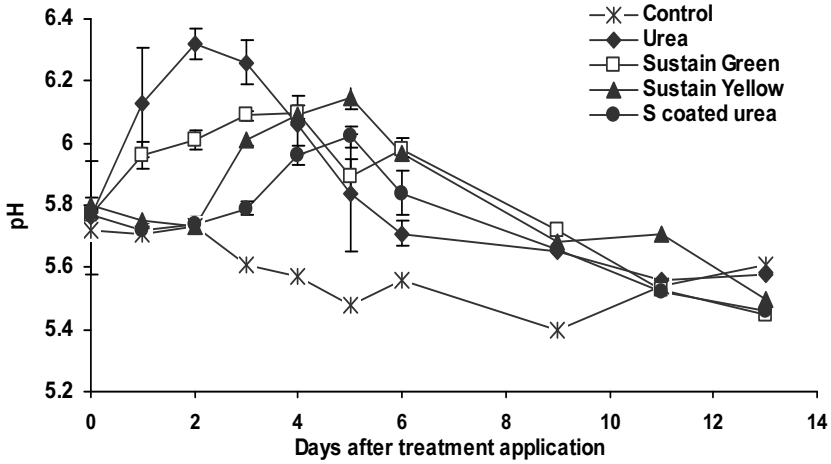

Control
Urea
Sustain Green
Sustain Yellow
S coated urea
pH
6.4
6.2
6
5.8
5.6
5.4
5.2
0
2
4
6
8
10
12
14
Days after treatment application

Figure 5

Table 1 **Chemical and physical properties of the soil from 0-50 and 50-100 mm depths**

	Depth	Bulk	Clay	Total N	Total C	pH	C.E.C
Manawatu	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

Table 2 **Characteristics of nitrogen treatments used in Experiment 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	-	4
Sustain Green	Urea+ Agrotain	-	46	-	-
S-coated Urea	Urea+S coating	-	32	14	27

Table 3 **Total N applied and N emitted as NH_3 and N_2O (g N /m^2) in Experiment 1 over the experimental period from urine and urea treatments with and without UI**

Treatments	N added (g N /m^2)	NH_3-N (g N /m^2)	% of added N emitted	N_2O-N (g N /m^2)	% of added N emitted	Total % of N emitted
Control	-	0.13 e*	-	0.014 c	-	-
Control+UI	-	0.11 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)		0.58		0.16		

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

Table 4 Total N applied and N emitted as NH_3-N and N_2O-N (g N /m^2) in Experiment 2 over the experimental period from urea and urea-amended treatments

Treatments	N added (g N /m^2)	NH_3-N (g N /m^2)	% of added N emitted	N_2O-N (g N /m^2)	% of added N emitted	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

Table 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

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4. Further Reference

Singh, J. The role of inhibitors in mitigating nitrogen losses from cattle urine and nitrogen fertiliser inputs in pastures (PhD Thesis, Massey University)