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Effect of dicyandiamide (DCD) on white clover growth and nitrogen fixation in ryegrass/ white clover pasture

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Summary

The nitrification inhibitor dicyandiamide (DCD) is used in New Zealand dairy farming to reduce nitrogen (N) losses from leaching and nitrous oxide emissions. Overseas studies have shown that, in certain conditions, DCD can potentially have an adverse effect on a range of crops. White clover provides New Zealand pastoral farming with a significant competitive advantage primarily through input of N into the soil by the fixation of atmospheric N_2. However, there have been few New Zealand studies on the effect of DCD on white clover. Nitrification inhibitors work by retaining ammonium-N in the soil longer so there is more opportunity for plant uptake. In mixed pasture, white clover competes with ryegrass when soil inorganic N levels are low, but increased concentrations of inorganic N in soil favour the ryegrass. Therefore, use of DCD could indirectly reduce white clover growth in mixed pasture by reducing clover's competitiveness with ryegrass.

This study examined the effect of DCD on white clover in a mowing trial (Waikato) and two grazing trials (Waikato and Southland). Changes in soil inorganic N were measured in the mowing trial and pasture production, white clover content and the amount of atmospheric N_2 fixed by white clover were measured in all the trials.

The mowing trial consisted of urine (700 kg N/ha) and nil urine treatments, with and without DCD (10 kg/ha). Application of DCD to the urine plots resulted in an additional 32 kg N/ha of inorganic N in the top 40 cm of soil in August and September, and an additional 79 kg N/ha of inorganic N in the top 10 cm in July, compared to the urine-only plots. DCD significantly reduced the proportion of white clover in the pasture at four of the harvests in the control treatment, although there was no significant effect on total clover yield.

The long-term DCD grazing trial (eight years) in Southland consisted of two treatments with six replicate plots. One treatment had no DCD while the other received three applications of 15 kg DCD/ha in autumn/winter. The Waikato grazing trial had three treatments of nil DCD, two applications of 10 kg DCD/ha, and four applications of 10 kg DCD/ha in autumn/winter each with nine replicate plots. There was no significant difference in the proportion of white clover in pasture or total clover growth between the DCD and nil DCD treatments at either grazing trial site.

The ^{15}N isotope dilution method was used to measure N_2 fixation by clover in all three trials. ^{15}N analyses from both of the grazing trials and from the non-urine treatments of the mowing trial showed no indication of any effect of DCD on N_2 fixation. Due to uneven distribution of the ^{15}N label through the soil, no measurements of N_2 fixation were possible on the urine treatments for several months after the urine was applied. However, while later measurements showed a reduction in N_2 fixation due to urine application there was no evidence of any further adverse effect due to DCD application.

These trials set out to evaluate the hypothesis that by increasing the amount of inorganic N retained in the soil, DCD would increase the competitiveness of ryegrass in mixed ryegrass/white clover pasture and indirectly reduce clover growth. The mowing trial showed an initial trend toward less clover in the DCD treatments. However, in the urine-treated plots, clover content was reduced to very low levels and the only statistically significant reductions in clover content due to DCD occurred in the control plots. This suggests that DCD had a short-term direct rather than indirect effect. Over the full course of the mowing trial there was no significant reduction in the total amount of clover production due to DCD. There was no evidence of any adverse effect of DCD on clover in either grazing trial.

The conclusion from this limited series of mowing and grazing trials is that the application of DCD may have an initial effect in reducing clover content and clover production, but that this does not significantly affect annual clover growth compared with non DCD-treated areas of

pasture. Similarly, DCD has no effect on clover N_2 fixation in non urine-affected pasture. There was no medium-long term effect of DCD on N_2 fixation in urine patches but short-term effects were unclear and further research is required.

Introduction

Nitrification inhibitors can be an effective mitigation strategy to control nitrogen (N) losses from animal excreta. These inhibitors work by delaying nitrification and retaining N in the soil in the more immobile ammonium (NH_4) form. The most commonly used nitrification inhibitor in New Zealand is dicyandiamide (DCD). Studies have shown potential reductions in both nitrate (NO_3) leaching in lysimeters, and nitrous oxide (N_2O) emissions from pasture urine patches of up to 70% following application of DCD (e.g. Di and Cameron. 2004). While research is continuing to quantify the effects of DCD on reducing N losses and increasing pasture production, there is little or no knowledge of the effect of DCD use on some other N processes. The most important one of these is N_2 fixation by white clover. In New Zealand, a critical element of the efficiency of our pastoral systems is the “free input of N” via biological N_2 fixation by white clover and this drives the long-term sustainability of production by balancing N losses (Ledgard 2001).

DCD could affect clover either directly (e.g. through phytotoxicity), or indirectly by altering its competitiveness with other pasture species. Various studies (Maftoun and Sheibany 1979, Reeves and Touchton 1986) have shown that DCD can be phytotoxic to crops and Macadam *et al*. (2003) found that DCD applied at a rate of 25 kg/ha to a two year old grass/white clover pasture in conjunction with either N fertiliser or cattle slurry, caused chlorosis and necrosis at the clover leaf edges. Macadam *et al*. (2003) followed this up with a growth chamber trial involving clover grown in a nutrient solution, which indicated that the effects were due directly to DCD rather than indirectly due to changes in the soil eco-system. [See Appendix 1 for a review of previous research on the effects of DCD on plant growth].

In New Zealand, DCD application rates to pasture are typically much lower than 25 kg/ha and there have been no reported cases of phytotoxicity. In mixed ryegrass/white clover pastures, ryegrass is more competitive at higher soil N concentrations while clover, which can utilise atmospheric N_2, has an advantage at lower soil N concentrations (Brock and Hay 2001). It is possible that DCD could indirectly effect clover growth by retaining inorganic nitrogen in the soil and thus allow ryegrass to compete better with clover in the pasture sward. Also, when soil inorganic N content increases clover substitutes soil N for atmospheric N resulting in a reduction in N_2 fixation (Ledgard and Steele 1993).

Only a few New Zealand studies have looked at the effect of DCD on pasture composition. Moir *et al*. (2007) examined the effect of DCD on both the urine-patch and inter urine-patch areas of a grazed pasture and found that DCD had no effect on pasture clover content. Sprosen *et al*. (2009) applied DCD at various rates in both liquid and granular forms to mixed ryegrass/clover plots that had received 600 kg N/ha as artificial urine. Clover content in pasture declined initially on all the urine treated plots but seven months after treatment application the clover content on the urine-only plots was over 20% while the average of all the DCD treatments was significantly lower at 10%. Monaghan *et al*. (2009) applied DCD to a grazed South Island dairy pasture over four years and found no consistent effect of DCD on clover growth over the full duration of the trial. However, in year two of the trial the DCD treatment produced significantly less clover than the control treatment (904 versus 1326 kg DM/ha).

The current study was designed to test the hypothesis that, by increasing the amount of inorganic N retained in grazed pasture, DCD could reduce white clover production and N_2 fixation.

Materials and Methods

There were three field trials involved in this study. All the trials were on established dairy pastures. In the mowing trial, an area was fenced to exclude cattle and the plots were harvested at intervals with the clippings discarded. In the two grazing trials the plots were grazed at intervals by dairy cattle and there was no restriction on the movement of cattle between the treatments.

WAIKATO MOWING TRIAL

In May 2011, an area of well established (over ten years) ryegrass/white clover pasture on what was formerly Ruakura No.1 dairy unit (now used for calf raising) near Hamilton New Zealand was fenced off and mown. The soil type was a Te Kowhai silt loam (Typic Ochraqualf soil) and the area had been out of grazing since mid-April 2011. Soil samples were collected to determine the nutrient status of the site (Table 1). Sulphate concentration was low but all other nutrients (except for N) were within optimum ranges.

Table 1. Soil test results from site (0–75 mm). Cation concentrations are in MAF quicktest units.

pH	P Olsen ug/ml	K MAF QT	Ca MAF QT	Mg MAF QT	$S(SO_4)$ ppm	Organic matter %	TKN Kjeldahl N %	NH_4-N ppm	NO_3-N ppm
5.6	47	12	7	27	5	7.4	0.48	3	2

On the 17th June, 24 plots (1.25 x 2.5 m) were marked out and a covariate pasture cut carried out. A randomised block design of four treatments with 6 replicates of each treatment was used (Table 2).

Table 2. Mowing trial treatments.

Treatment		
Number	**Name**	**Description**
1	control	No urine or DCD
2	control+DCD	No urine + DCD @ 10 kg/ha
3	urine	Artificial urine @ 700 kgN/ha
4	urine+DCD	Artificial urine @ 700 kgN/ha + DCD @ 10 kg/ha

On the 20th June, artificial urine was applied to treatments 3 and 4 at a rate of 10 L/m^2. The composition of the urine was based on that used by de Klein *et al.* (2003) and consisted of 13.65 g/L urea, 3.4 g/L glycine, 16.31 g/L potassium bicarbonate, 1.61 g/L potassium sulphate and 5.68 g/L potassium chloride. The following day a solution of DCD (10 kg/ha) was sprayed on to treatments 2 and 4 at a rate of 800 L/ha.

Clover N_2 fixation was measured using the ^{15}N isotope dilution technique (Chalk 1985). A 0.5 m^2 microplot was marked out on each plot and on 22nd June, ^{15}N labelled ammonium sulphate was applied. Rate of application was 1 kg N/ha and ^{15}N enrichment was 99 atoms % on the urine plots and 40 atoms % on the non-urine plots. ^{15}N-labelled ammonium sulphate was reapplied to the microplots on the 16th of November 2011 and on the 23rd February 2012 at an enrichment of 40 atoms % ^{15}N on all treatments.

Dry matter production was measured by harvesting two mower strips to a height of 55 mm and drying the samples at 65 °C. Harvest intervals (3-8 weeks) were based on the normal grazing rotation periods for the time of year but were shortened when necessary to avoid pasture senescence in the urine plots. Prior to mowing, a herbage sample was collected from each microplot, separated into grass and white clover before being dried at 65 °C and analysed for nitrogen concentration and ^{15}N isotope enrichment using a Dumas elemental analyser (Europa Scientific ANCA-SL) interfaced to an isotope mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser).

A separate herbage sample was collected from the area on each plot between the two mower strips. This was dissected into grasses, white clover and weeds, and dried at 65 °C to measure the proportion by weight of clover, grass and weeds in the pasture. The proportion of clover in the pasture was multiplied by the dry matter yield at each harvest to derive clover growth. This was multiplied by the concentration of N in the clover and by the proportion of legume N derived from N_2 fixation to calculate the amount of N fixed in the harvested clover. The percentage of legume N fixed from atmospheric N_2 was calculated using the following formula from Ledgard *et al*. (1985):

%N fixed = (atoms% ^{15}N grass – atoms%^{15}N clover)/(atoms% ^{15}N grass – atoms% ^{15}N unlabelled grass) x 100

Data on the proportion of clover N derived from N_2 fixation was multiplied by the amount of clover dry matter production and the N concentration in clover to get the amount of fixed N in harvested clover.

GRAZING TRIALS

Waikato

In 2009, a grazing trial was established on an Otorohanga silt loam (Typic Orthic Allophanic soil) at AgResearch’s Tokanui dairy farm near Hamilton, New Zealand to examine the effect of DCD on pasture production. This was part of the Nitrous Oxide Mitigation Research national trial series (Gillingham *et al*. 2012). The area was resown with Halo perennial ryegrass in 2008. Soil samples were collected in March 2011 to determine the nutrient status of the site (Table 3). Due to a low potassium (K) concentration in the soil, 45 kg/ha of K was applied as potassium chloride in late-March.

Table 3. Soil test results from site (0–75 mm). Cation concentrations are in MAF quicktest units.

pH	P Olsen ug/ml	K MAF QT	Ca MAF QT	Mg MAF QT	$S(SO_4)$ ppm	Organic matter %	TKN Kjeldahl N %	NO_3-N ppm
5.5	27	3	5	13	150	14.3	0.84	9

From this trial, eighteen 10 m x 5 m plots were selected for establishment of ^{15}N isotope labelled microplots using a randomised block design consisting of three treatments by six replicates. Treatment one received no DCD, treatment two received two applications of DCD and treatment three received four applications (Table 4 has full treatment details).

Table 4. Tokanui grazing trial treatments.

Treatment Number	Name	Description
1	control	Control (No DCD)
2	DCDx2	DCD @ 10 kg/ha applied in April and June
3	DCDx4	DCD @ 10 kg/ha applied in April, May, June and August

DCD was sprayed on to treatments 2 and 3 within two days of the trial being grazed. A total of 152 kg N/ha of fertiliser-N was applied to all treatments with an additional 13 and 26 kg N/ha applied in the form of DCD to treatments 2 and 3, respectively. Treatments 2 and 3 received totals of 20 and 40 kg DCD/ha, respectively. Details of the rate and timing of DCD and N fertiliser applications are provided in Table 5.

Table 5. Timing of N fertiliser and DCD application on Tokanui grazing trial.

Date	N fertiliser and DCD application
15 April	25 kg N/ha urea. 10 kg/ha DCD on treatments 2 & 3
31 May 2011	35 kg N/ha urea. 10 kg/ha DCD on treatment 3
30 June 2011	10 kg/ha DCD on treatments 2 & 3
19 August 2011	25 kg N/ha urea. 10 kg/ha DCD on treatment 3
25 September 2011	27 kg N/ha DAP
31 March 2012	40 kg N/ha urea

^{15}N-labelled ammonium sulphate was applied to 1 m^2 microplots at the same N rate as used in the mowing trial. The ^{15}N was applied on the 1st of July and 25th of October 2011 and on the 14th of February 2012 at an enrichment of 25 atoms % ^{15}N.

Grazing management aimed to graze when pasture reached approximately 2800 kg DM/ha and leave a residual of about 1450 kg DM/ha at the end of grazing. Pasture dry matter was measured before and after each grazing using a rising plate meter. Forty measurements were taken from each plot and the average was converted into a dry matter (DM) figure using:

RPM height reading x 158 + 200 = kg DM/ha (Farmworks 2008)

Use of this equation was based on pasture calibration cuts taken in the same paddock in 2009. Pasture production for each grazing interval was calculated by subtracting the pasture cover measured after one grazing from the pasture cover measured just prior to the next grazing. Prior to each grazing a herbage sample was collected from the microplot and this sample was separated into grass and white clover (any weeds present were discarded) to be analysed for nitrogen concentration and ^{15}N isotope enrichment as described in the mowing trial. A separate herbage sample was collected from a transect across the entire plot and dissected into grasses, white clover and weeds to measure the proportion of white clover, grass and weeds in the pasture.

Southland

In 2004, a grazing trial was established on a dairy farm at Tussock Creek, 20 km north of Invercargill, New Zealand on a Pukemutu Argillic-mottled Fragic Pallic soil to investigate the effectiveness of DCD in reducing N leaching (Monaghan *et al*. 2009). N fertiliser application rate was increased in 2011 from 100-150 kg N/ha/year to 370 kg N/ha/year to evaluate the effectiveness of DCD under high N fertiliser conditions. In August 2011, microplots (1 m^2)

were established on six paired plots with each pair consisting of a DCD and nil-DCD treatment. Each DCD plot received three applications of DCD during the autumn/winter of 2011 (a total of 40 kg DCD/ha). A total of 371 kg N/ha of fertiliser-N was applied to all the plots from March 2011 to February 2012, with the DCD plots receiving an additional 26 kg N/ha in the form of DCD. Details of DCD and urea fertiliser application are provided in Table 6. The DCD was sprayed on to the plots within five days of the completion of grazing, except for the August DCD application which was not associated with a previous grazing.

^{15}N-labelled ammonium sulphate was applied to all microplots on the 23rd of August and 16th of November 2011 and on the 9th of March 2012 at a rate of 1 kg N/ha and an enrichment of 25 atoms % ^{15}N. Pasture production, proportion of white clover in pasture, and ^{15}N enrichment of clover and grass were measured in the same manner as at Tokanui. Rising plate meter readings were converted into standing pasture (kg DM/ha) based on the formulae used by L'Hullier (1988). Grazing management was determined by the farm manager but was based on standard Southland dairy practice.

Table 6. Timing and rate of N fertiliser and DCD application at Tussock Creek.

Date	N fertiliser and DCD application
11 March 2011	50 kg N/ha as urea. 15 kg/ha DCD
11 April 2011	50 kg N/ha as urea. 15 kg/ha DCD
1 August 2011	10 kg/ha DCD
12 August 2011	39 kg N/ha as urea
27 September 2011	39 kg N/ha as urea
19 October 2011	39 kg N/ha as urea
14 November 2011	39 kg N/ha as urea
12 December 2011	39 kg N/ha as urea
1 February 2012	39 kg N/ha as urea
17 February 2012	37kg N/ha as urea
5 March 2012	10 kg/ha DCD
13 March 2012	39 kg N/ha as urea
26 April 2012	39 kg N/ha as urea. 10 kg/ha DCD

Statistical analysis and modelling

Analysis of variance was carried out on all data using the GenStat (version 11.1.0.1575) statistical program (Genstat 2008). The least significant differences (LSD) presented are for individual treatment comparisons at the $p<0.05$ level.

The possible effects of DCD on clover from retaining additional NH_4 in the soil were examined using the Agricultural Production Systems sIMulator (APSIM version 7.3) software (Keating *et al*. 2003). Soil settings were based on actual data from the Ruakura mowing trial site and information from the New Zealand soil database for the Te Kowhai soil. Initial clover content in pasture was based on the data from the June covariate harvest. For non urine-affected areas, the additional soil N obtained from the breakdown of DCD was simulated by applying varying amounts of urea fertiliser in July. For modelling of urine-affected areas, 700 kg N/ha was applied as urea in June and then varying amounts of urea fertiliser in August to simulate the additional inorganic N retained in the soil by the DCD. The model was set for a cut and carry system to match the harvests taken on the mowing trial.

Results

CLIMATE DATA

Soil temperature and cumulative rainfall data for 2011/2012 for Ruakura, Tokanui and Tussock Creek are given in Figures 1, 2 and 3, respectively. The Waikato had a warmer and wetter winter than usual. At Ruakura the average June soil temperature at 10 cm depth was 2 °C above the long term average of 9 °C and in July 1 °C above the long term average of 7.7 °C. Cumulative rainfall at Ruakura over June and July was 71 mm above the long term average of 246 mm.

At Tussock Creek, low rainfall in December and January (Figure 3) severely restricted clover growth. No long term climate data was available for the Tokanui and Tussock Creek sites.

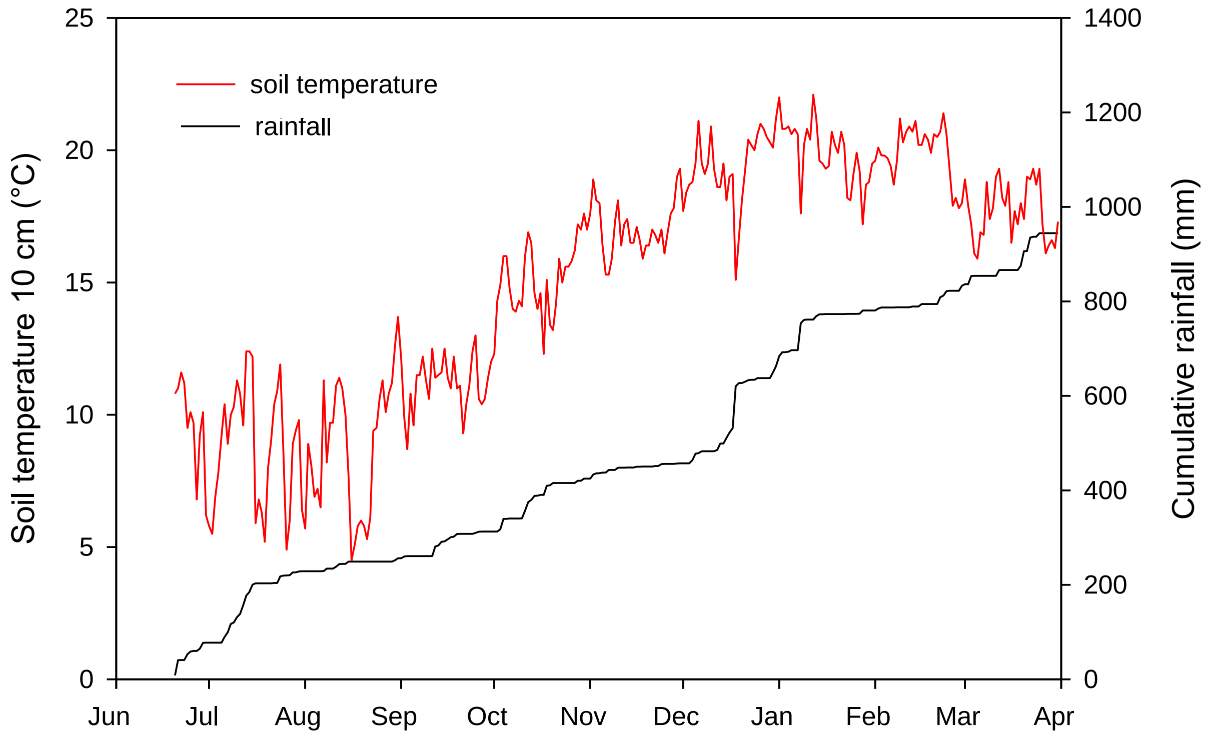

Figure 1. Ruakura soil temperature (10 cm depth, at 9 a.m.) and cumulative rainfall for the nine months following DCD application. Source: Ruakura meteorological station (NIWA agent number 26117).

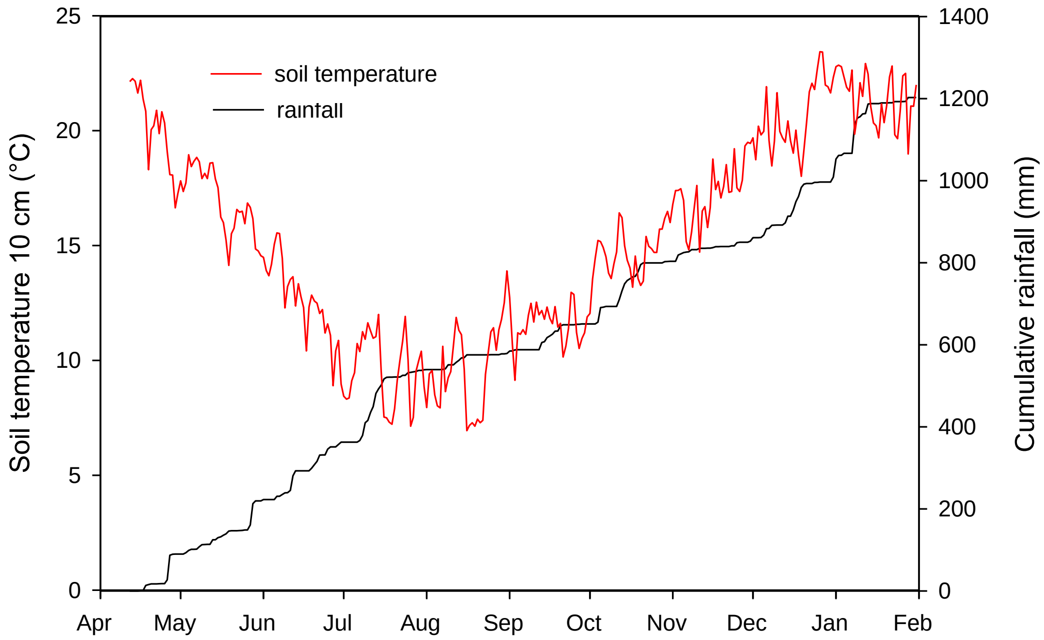

Figure 2. Tokanui soil temperature (10 cm depth, at 9 a.m.) and cumulative rainfall for the nine months following initial DCD application. Source: Tokanui farm meteorological station.

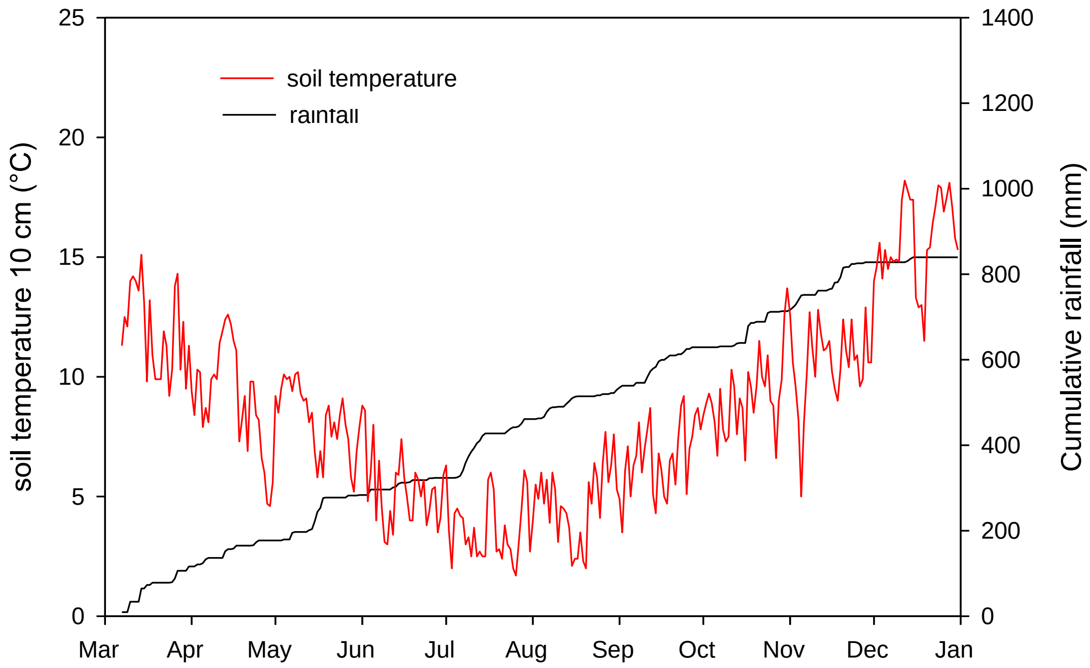

Figure 3. Tussock Creek soil temperature (10 cm depth, at 9 a.m.) and cumulative rainfall for the nine months following initial DCD application. Source: Tussock Creek farm meteorological station.

WAIKATO MOWING TRIAL

Soils

The urine+DCD treatment retained significantly more ($p<0.05$) NH_4-N in the soil than the urine-only treatment, from July through to late-September, in both the 0-10 cm (Figure 4) and 0-40 cm (Figure 5) depths. By December, the amount of NH_4-N in the urine treatment had declined to the levels found in the non-urine treatment. From June through to December, the amount of NH_4-N present in the top 40 cm of soil in the non-urine treatments averaged 7 kg N/ha and there were no significant differences between the control and control+DCD treatments at any of the samplings (detailed results not shown).

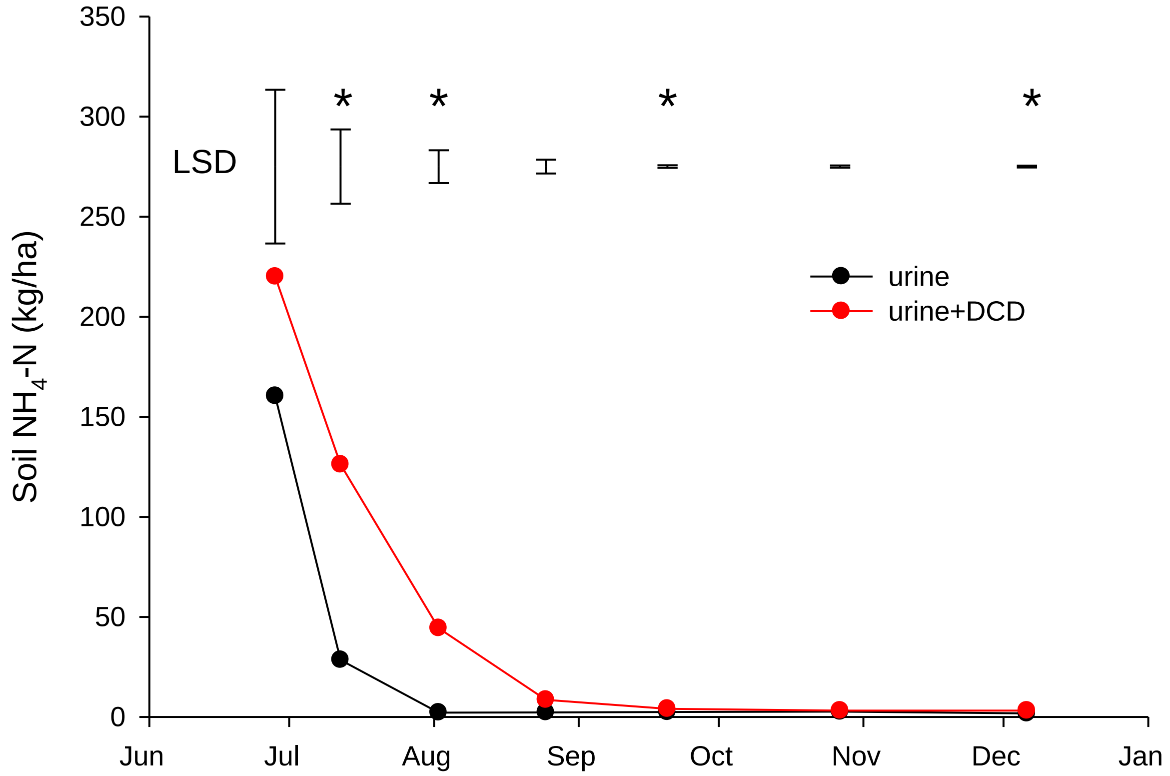

Figure 4. Soil NH_4-N in the urine and urine+DCD treatments (0-10 cm). The error bars represent the least significant difference ($LSD_{p<0.05}$) between treatments. * Difference significant at $p<0.05$.

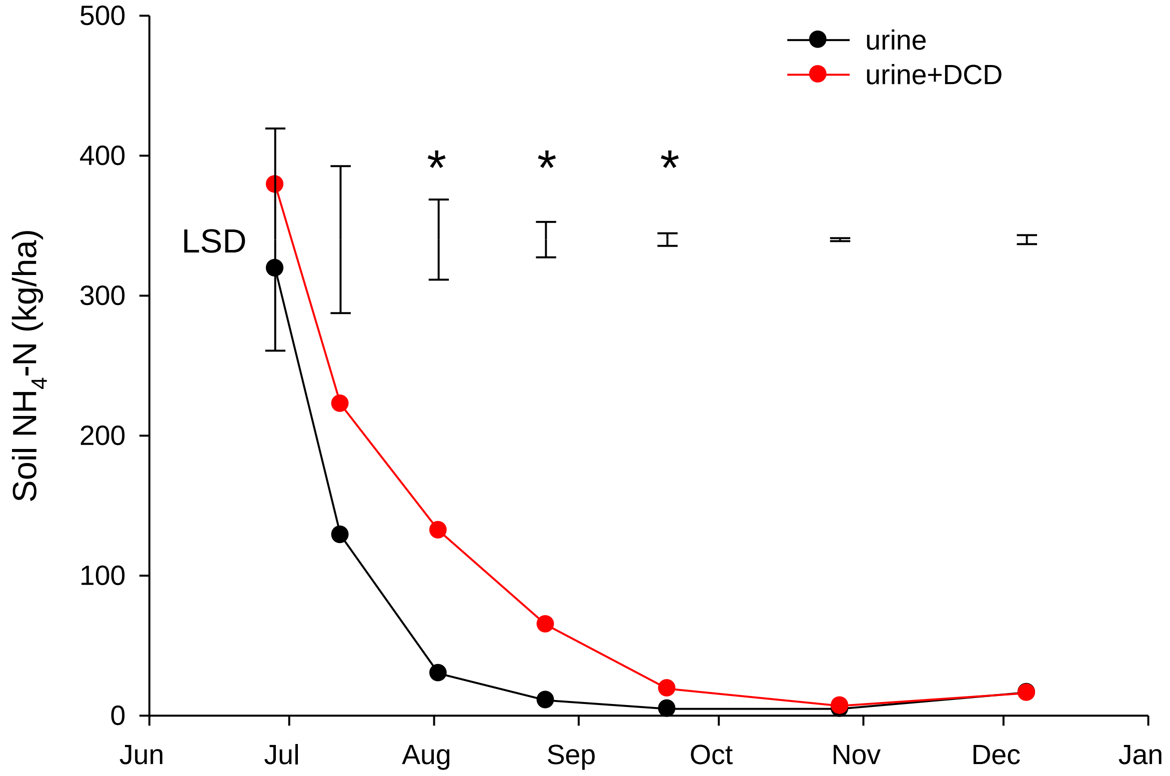

Figure 5. Soil NH_4-N in the urine and urine+DCD treatments (0-40 cm). The error bars represent the least significant difference ($LSD_{p<0.05}$) between treatments. * Difference significant at $p<0.05$.

From the early-August sampling until mid-September there was an average of 32 kg/ha more inorganic N present in the top 40 cm of soil in the urine+DCD treatment than in the urine-only treatment (this difference was significant only at the $p<0.05$ level at the September sampling; Figure 6). The difference between urine treatments with and without DCD was most pronounced in the top 10 cm of soil ($p<0.05$) from July to September ranging from 79 to 4 kg N/ha, respectively (Figure 7). There were no differences between the control and control+DCD treatments in soil inorganic N concentrations (results not shown).

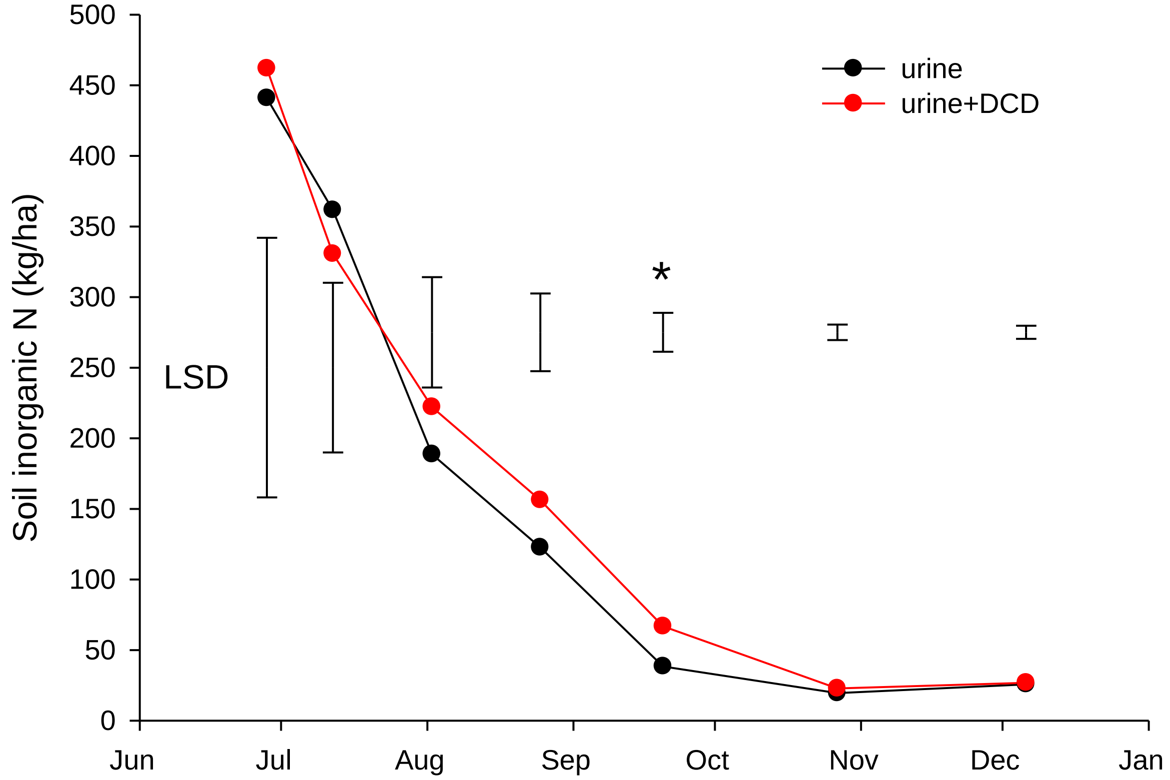

Figure 6. Soil inorganic N in the urine treatments (0-40 cm). The error bars represent the least significant difference ($LSD_{p<0.05}$) between treatments. * Difference significant at $p<0.05$.

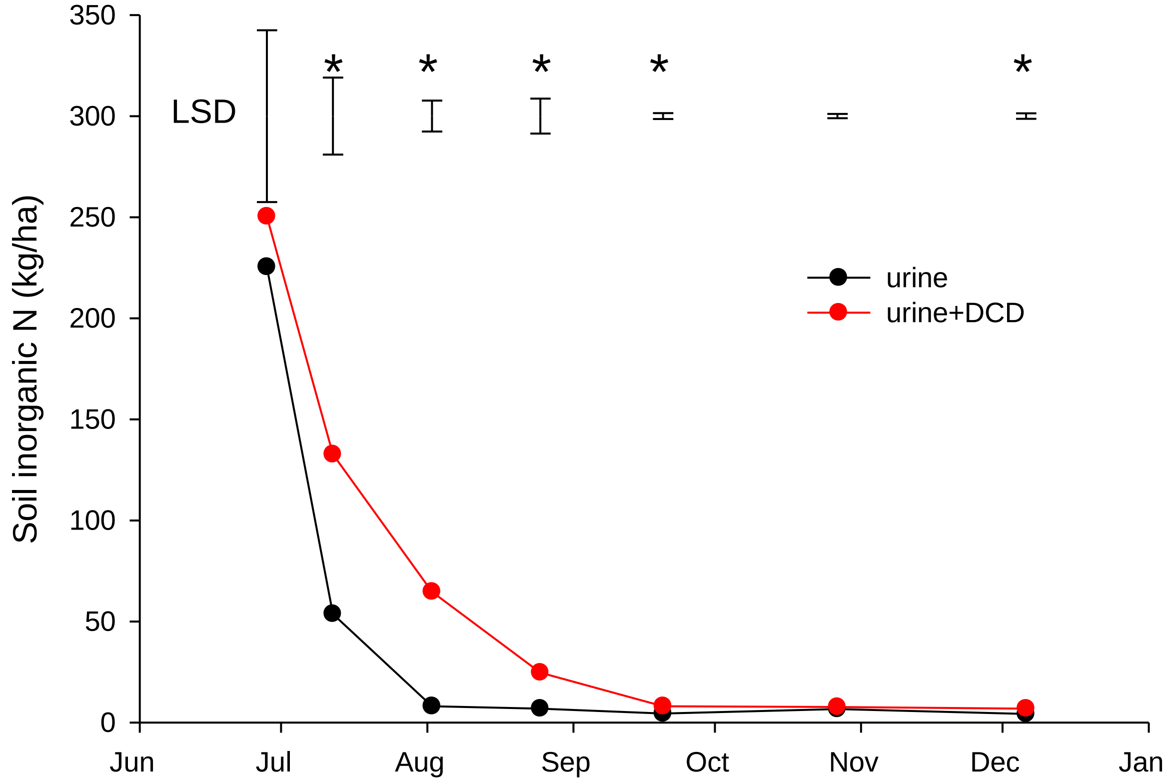

Figure 7. Soil inorganic N in the urine treatments (0-10 cm depth). The error bars represent the least significant difference ($LSD_{p<0.05}$) between treatments. * Difference significant at $p<0.05$.

One week after application, 7.4 kg/ha of DCD was measured in the top 40 cm of soil (Figure 8) with 6.5 kg/ha of this being in the top 10 cm (Figure 9). By 66 days after application the amount of DCD present in the top 40 cm of soil had fallen below 0.5 kg/ha, and at 129 days after application had fallen below the limit of detection. There were no significant differences between the amount of DCD in the control+DCD treatment and the urine+DCD treatment.

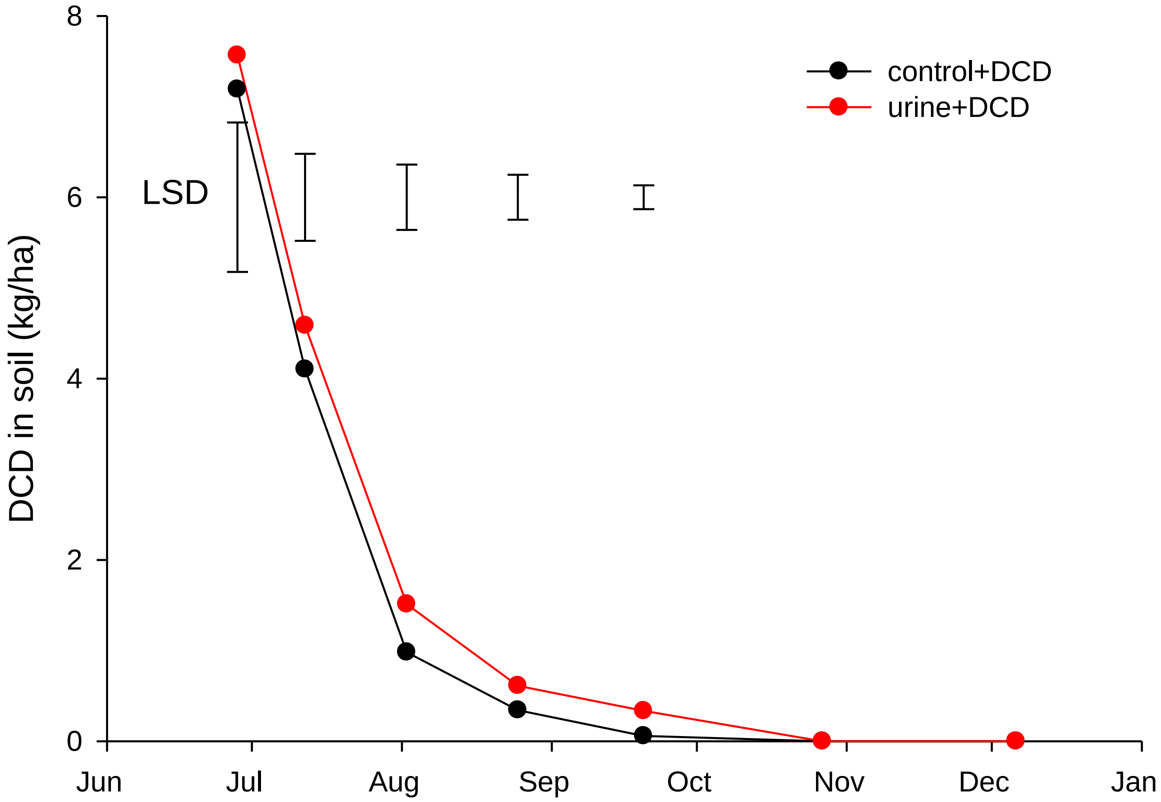

Figure 8. Soil DCD in the control+DCD and urine+DCD treatments (0-40 cm depth). The error bars represent the least significant difference ($LSD_{p<0.05}$) between treatments.

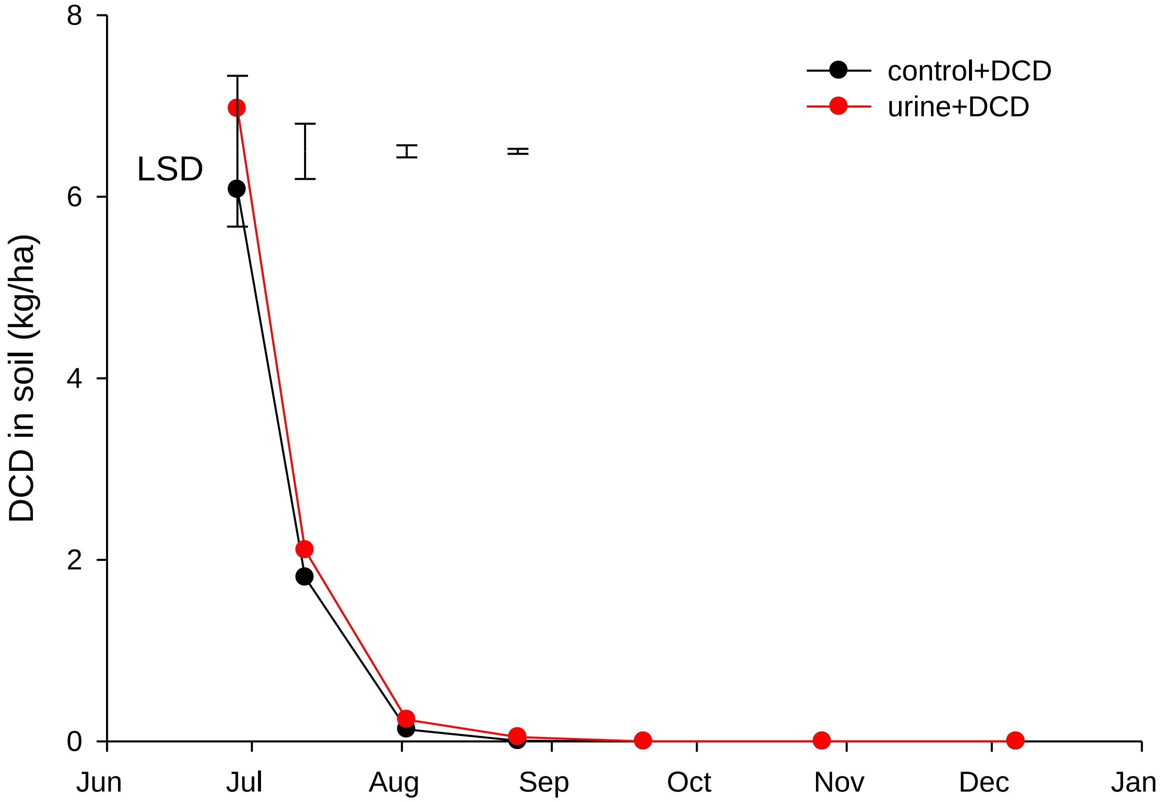

Figure 9. Soil DCD in the control+DCD and urine+DCD treatments (0-10 cm depth). The error bars represent the least significant difference ($LSD_{p<0.05}$) between treatments.

Pasture

Urine treatments produced an average of 15.4 t DM/ha which was 42% more dry matter ($p<0.001$) than the control treatments, in the period 17th June 2011 to 26th April 2012 (Figure 10). The urine+DCD treatment produced 11% more dry matter than the urine-only treatment at the first harvest (2735 versus 2466 kg DM/ha, respectively. $p<0.01$). However, the difference between the urine+DCD and urine-only treatments in total dry matter produced over the period of June 2011-April 2012 was not significant. There was no significant difference in dry matter yield between the control and control+DCD treatments.

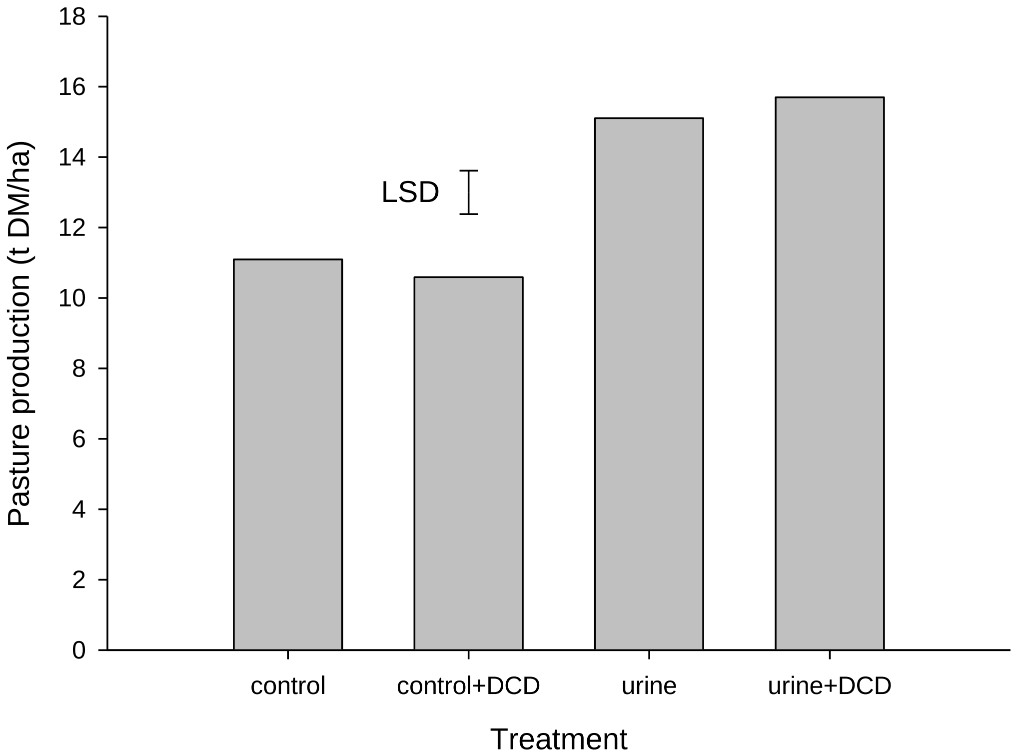

Figure 10. Pasture production from 16th June 2011 to 26th April 2012. The error bar represents the least significant difference ($LSD_{p<0.05}$) between individual treatments.

Clover component in pasture

Application of urine had a large impact on clover content (% by weight) in the pasture. Prior to the application of urine in June, the average clover content of pasture in the trial was 15%. At the August harvest this had fallen to 9.5% on the non-urine plots and 2% of pasture on the urine plots. Clover content in the urine treatments was still less than 10% in February 2012, by which time the average clover content in the non-urine treatments was more than 50%.

The control+DCD treatments tended to have a lower pasture clover content than the control treatment (Figure 11) with the differences being statistically significant ($p<0.05$) at the September, November and late February harvests. The clover yield data (not shown) followed the same pattern with no significant differences between DCD treatments on the urine plots and a trend toward less clover growth on the control+DCD plots than the control plots with statistically significant differences at the second and fourth harvests.

Over the ten months from June 2011 to April 2012 the control and urine treatments with DCD produced 18% and 40% less clover than their non-DCD counterparts, respectively (Figure 12). These differences were not statistically significant and neither was the average yield reduction due to DCD across both urine and non-urine treatments.

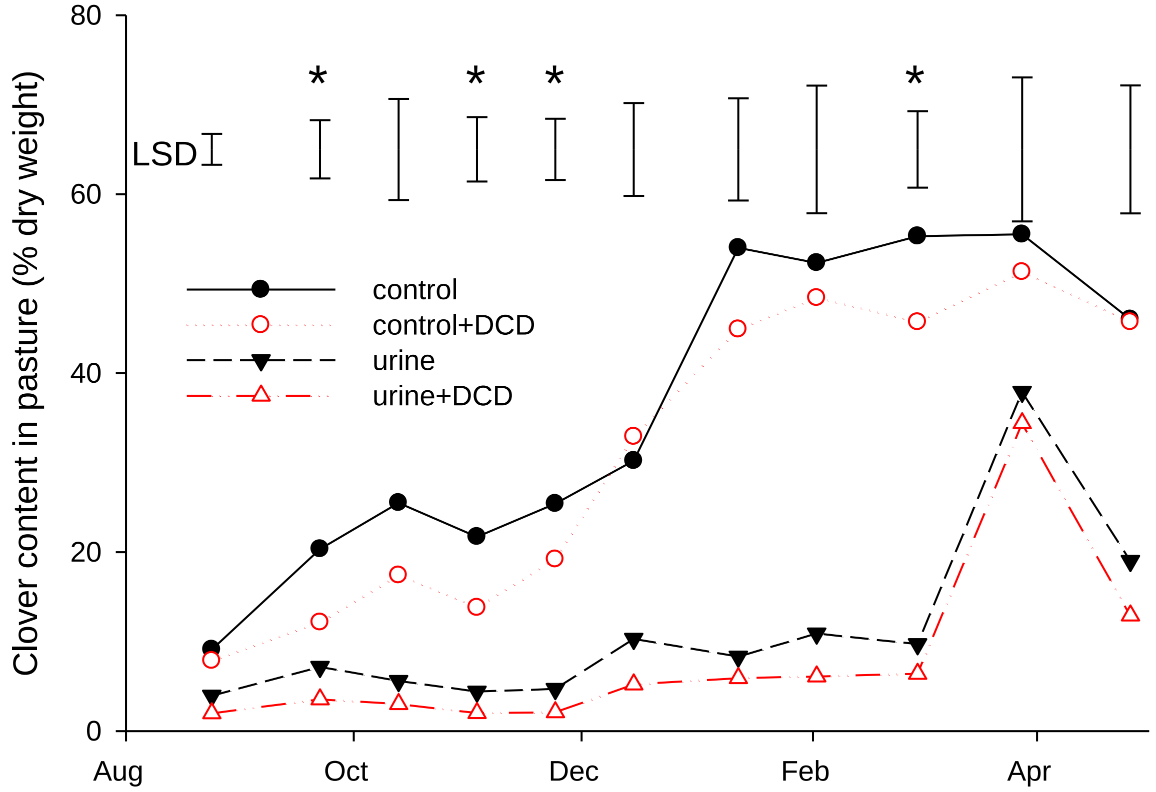

Figure 11. Clover content in pasture from August 2011 to April 2012 (adjusted for June covariate analysis). The error bars represent the least significant difference ($LSD_{p<0.05}$) between individual treatments. * indicates significant difference ($p<0.05$) between control and control+DCD treatments.

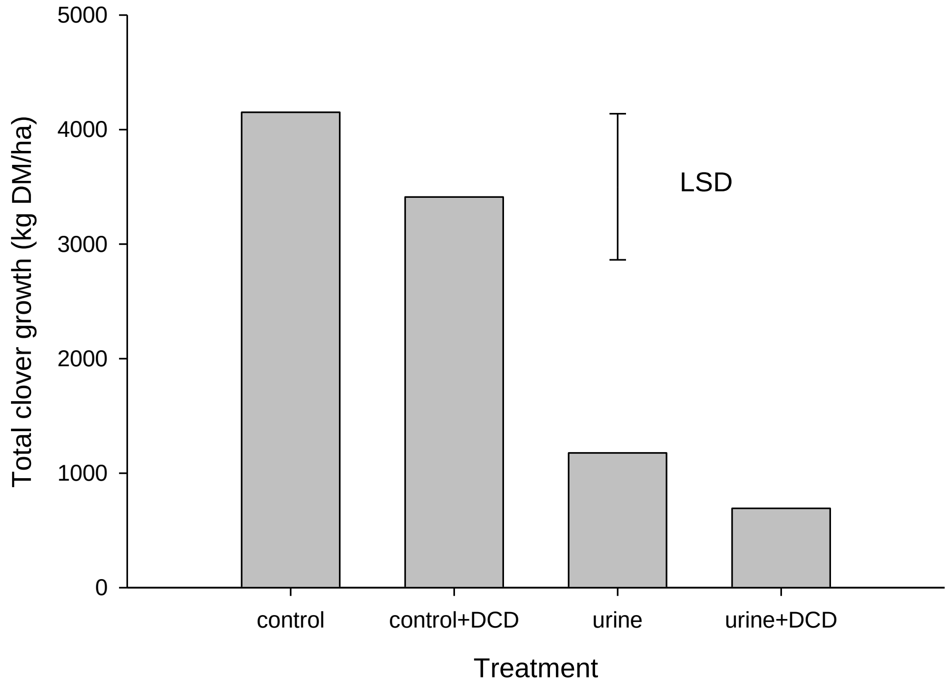

Figure 12. Total clover harvested from June 2011 to April 2012 (adjusted for June covariate analysis). The error bar represents the least significant difference ($LSD_{p<0.05}$) between individual treatments.

There was no significant difference in the proportion of clover N fixed between the control and control+DCD treatments (Figure 13). Normally 70-80% of the N in the clover herbage was derived from atmospheric N_2, with the remaining 20-30% being derived from soil or added N. The proportions of N fixed were lower and more variable at the August and March samplings. These samplings occurred soon after ^{15}N application and there may have been insufficient time for the ^{15}N to spread evenly through the plant root zone. Nevertheless, there was no effect of DCD on the proportion of clover N fixed at any of the samplings.

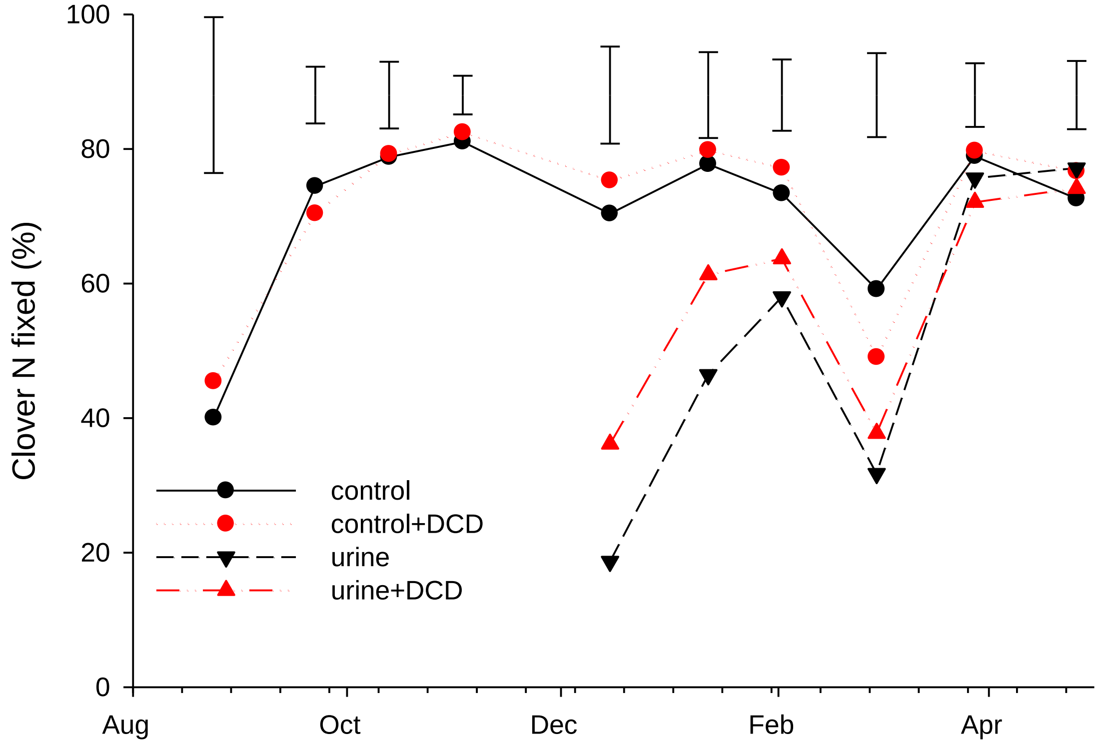

Figure 13. Proportion of N in clover herbage derived from atmospheric N_2. The error bars represent the least significant difference ($LSD_{p<0.05}$) between treatments.

The initial results from the urine treatments indicated that the grass, with its deeper rooting system, took up much of its N from the large amount of unlabelled urine that had moved some distance down the soil profile, while the clover took up N from more shallow soil depths where most of the ^{15}N labelled isotope was located. This was evident from the higher ^{15}N concentrations in clover than in grass. Since the isotope dilution technique relies on grass and clover accessing the same pool of labelled N (Ledgard *et al*. 1985; Witty 1983) the results up until November from the urine treatments did not give an accurate measurement of N_2 fixation. Later results showed application of urine reduced the proportion of N in clover from N_2 fixation compared to the non-urine treatments (60% reduction in December) and the difference remained significant until April (Figure 13). This will have been due to uptake of N derived from urine by the clover, resulting in a corresponding relative reduction in N derived from N_2 fixation. Application of DCD did not significantly reduce the proportion of N in clover from N_2 fixation in the urine plots.

The control+DCD treatment showed a significantly lower amount of atmospheric N_2 fixation than the control treatment at two of the harvests (data not presented). However, this was due to a reduction in clover content in the pasture and not to any change in the proportion of fixed N in the clover. Over the full course of the trial, there was no significant difference in the total amount of fixed N harvested between the control treatment and the control+DCD treatment (155 kg/ha versus 110 kg/ha, respectively; $LSD_{p<0.05}$ =59). Using the factor of 1.7 suggested by Jorgensen and Ledgard (1997) to account for the unmeasured N in the clover roots and stubble provides an estimate of total clover N_2 fixation of 264 and 187 kg N/ha in the control and control+DCD plots, respectively. It was only possible to measure the amount of N fixed in the urine treatments from December to May but there was no significant difference in the total amount of fixed N harvested in that period between the urine and urine+DCD treatment (8.5 kg/ha versus 10 kg/ha respectively; $LSD_{p<0.05}$ =10.6).

Modelling

The APSIM simulations showed no effect of additional N on clover content of pasture in non urine-affected areas except at the 100 kg N/ha rate (Figure 14). When modelled pasture yields and clover content were combined, the simulations resulted in total clover yields estimated at 2859, 2875, 2902, 2970 and 3130 kg DM/ha for the 0, 7, 15, 30 and 100 kg N/ha rates, respectively (Table 8) . Thus, there was little predicted effect of N fertiliser on clover content or yield as simulated using APSIM on a non urine-affected area. For urine-affected areas, the model showed no effect of additional N on clover content in pasture even when 150 kg N/ha was added (Figure 15). When the modelled pasture yields and clover contents were combined for a urine-affected area, they indicated no effect on clover yield (Table 7). This analysis indicated an increase in clover yield in urine-affected areas compared to non urine-affected areas.

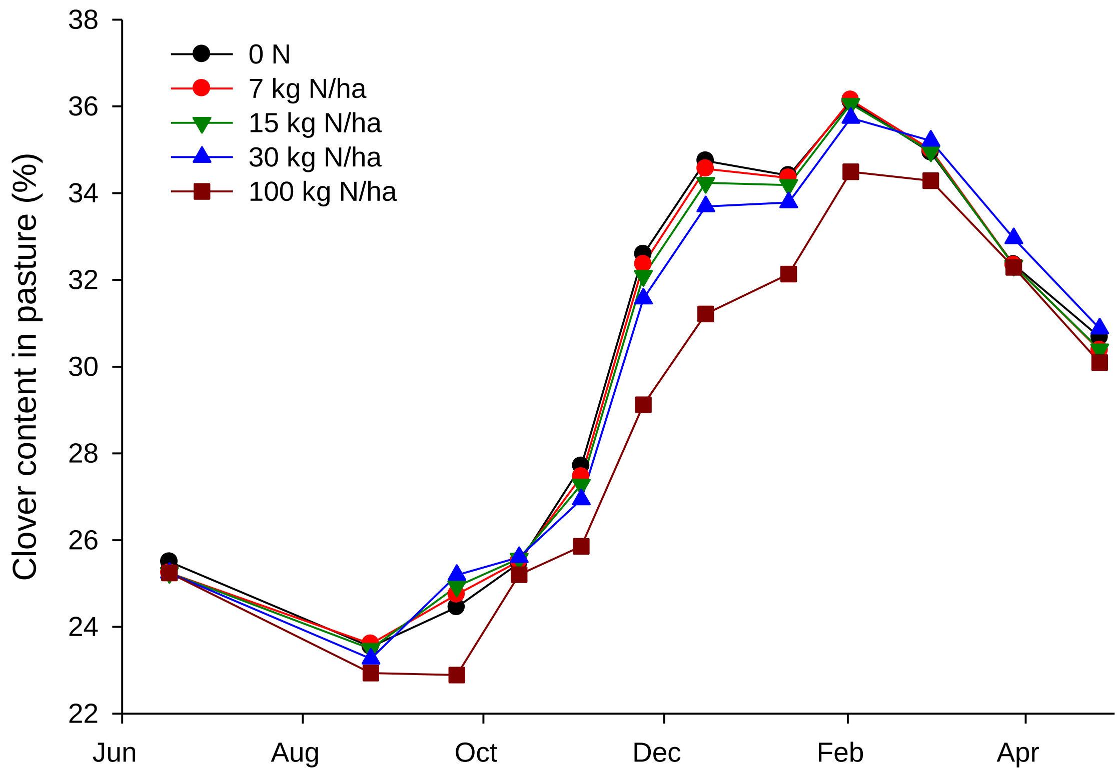

Figure 14. Effect on clover content of pasture from applying additional NH_4-N in July to a non urine-affected area using the APSIM model to simulate changes over time.

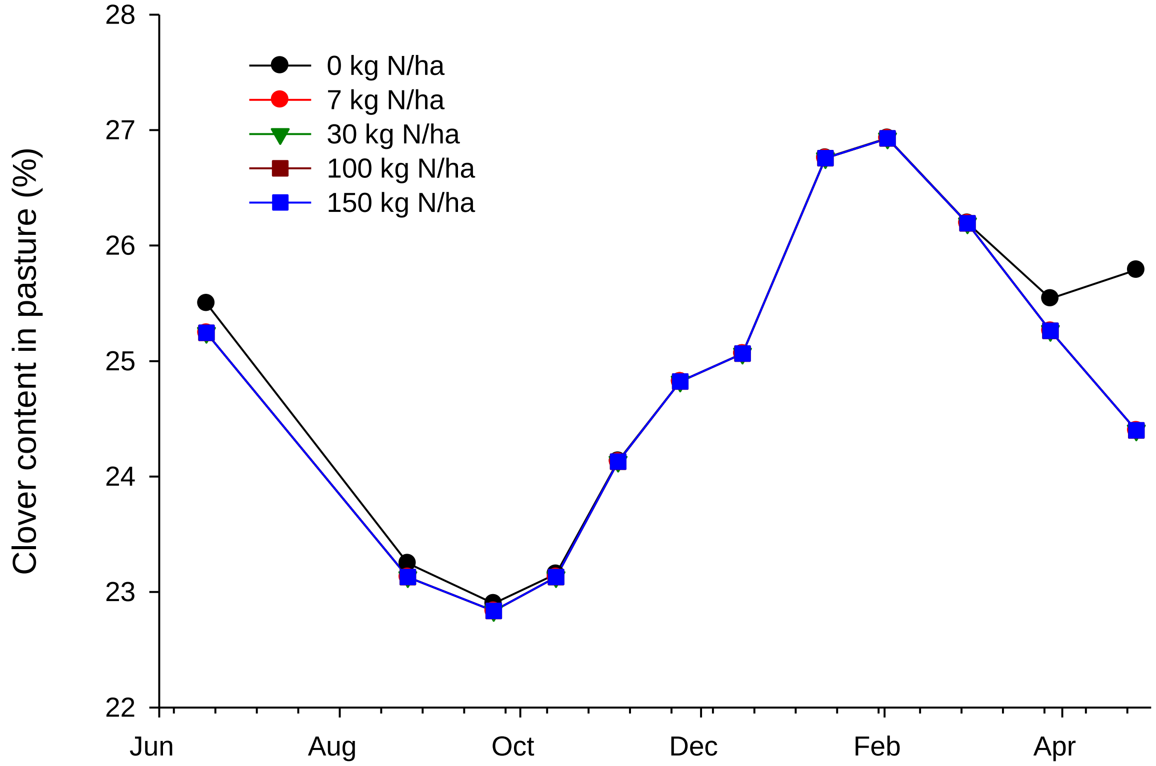

Figure 15. Effect on clover content of pasture from applying additional NH_4-N in July to a urine-affected area using the APSIM model to simulate changes over time.

Table 7. Simulated effects of applying additional N on pasture production, clover content and clover growth using the APSIM model (n.a. = not analysed).

	Additional N (kg N/ha)					
	0	**7**	**15**	**30**	**100**	**150**
Control						
Pasture growth (t DM/ha)	9.26	9.35	9.49	9.77	10.94	n.a.
Clover in pasture (%)	30.7	30.7	30.6	30.4	28.6	n.a.
Clover growth (t DM/ha)	2.86	2.88	2.9	2.97	3.13	n.a.
Urine-affected						
Pasture growth (t DM/ha)	17.68	17.78	n.a.	17.78	17.78	17.78
Clover in pasture (%)	25.0	24.8	n.a.	24.8	24.8	24.8
Clover growth (t DM/ha)	4.44	4.41	n.a.	4.41	4.41	4.41

GRAZING TRIALS

Waikato

There was no significant difference in total pasture production between the three treatments over the thirteen months from May 2011 to May 2012 (Table 8).

Table 8. Pasture production (t DM/ha) on the Tokanui grazing trial from 5 May 2011 to 29 May 2012.

control	DCDx2	DCDx4	$LSD_{(p<0.05)}$
15.37	15.35	14.85	1.36

The clover content of the pasture was low (less than 5%) in June and August, but by January all treatments had more than 10% clover. There were no significant differences in pasture clover content (Figure 16) or total amount of clover grown between any of the treatments.

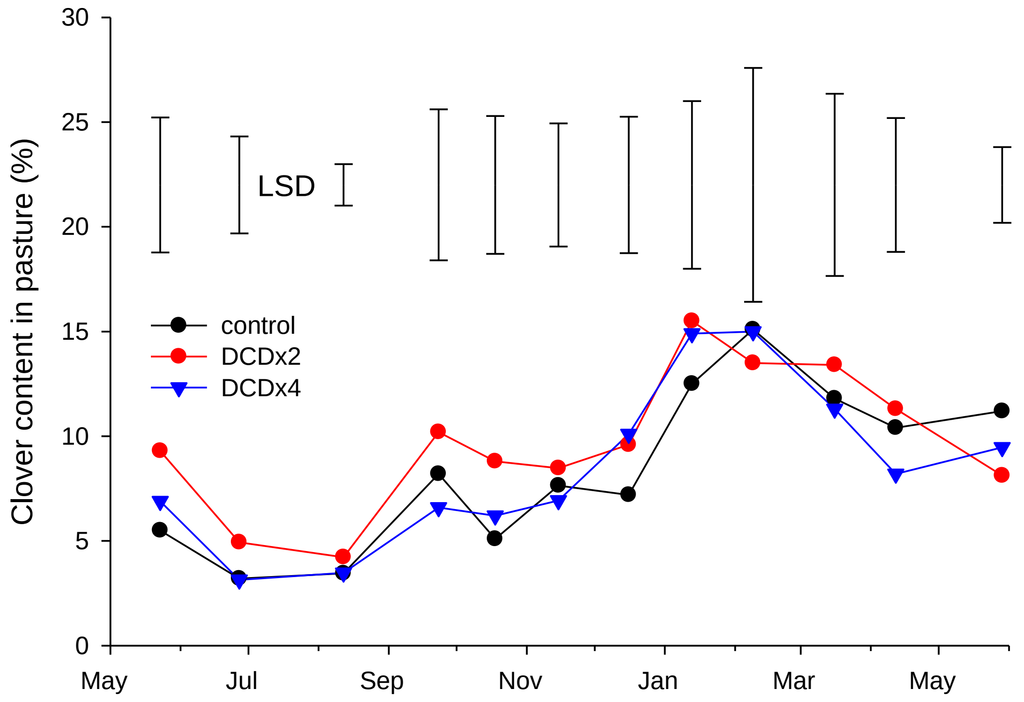

Figure 16. Pasture clover content immediately prior to each grazing at the Tokanui trial (covariate adjusted). The error bars represent the least significant difference ($LSD_{p<0.05}$) between treatments.

Insufficient clover in the ^{15}N plots made accurate N_2 fixation measurements impossible in June and August. However, from September 2011 through to April 2012 there were no significant differences in the proportion of clover N derived from N_2 fixation between the control treatment and the DCD treatments (Figure 17). The average proportion of clover N from N_2 fixation was lower than that measured on the non-urine plots in the mowing trial, probably due to the effect of urine patches on some of the ^{15}N microplots and the application of fertiliser N. This occurs because clover will utilise inorganic N in soil to partly substitute for N_2 fixation in meeting its total N requirements.

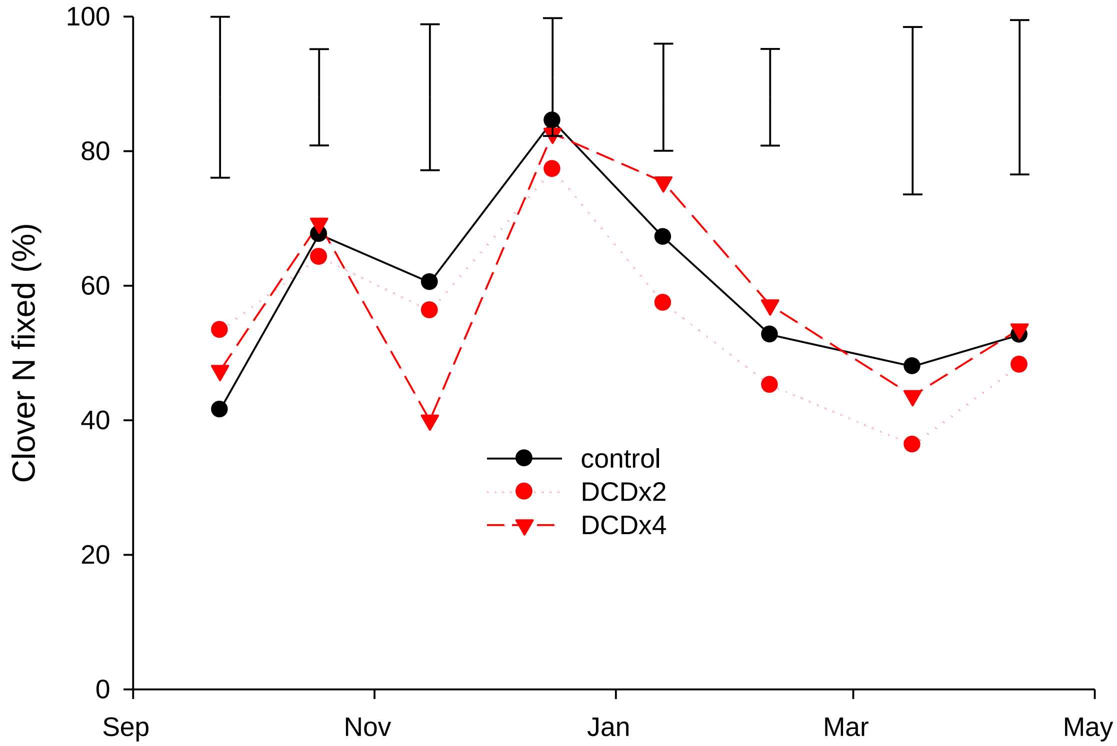

Figure 17. Proportion of N in clover herbage derived from atmospheric N_2. The error bars represent the least significant difference ($LSD_{p<0.05}$) between treatments.

From late-September to mid-April there was no significant difference between treatments in the total amount of fixed N harvested (31, 38 and 21 kg N/ha in the control, DCDx2 and DCDx4 treatments, respectively; $LSD_{p<0.05}$ =27). The reduced amount of N fixed relative to the mowing trial was partly due to the lower proportion of fixed N in the clover but mostly due to there being less clover in the Tokanui pasture compared to the mowing trial. After adjusting for the unmeasured N in the clover roots and stubble (Jorgensen and Ledgard 1997), total clover N_2 fixation was 53, 65 and 36 kg N/ha respectively, for the control, DCDx2 and DCDx4 treatments.

Southland

There was no significant difference in total pasture production between the control and DCD treatments at the Tussock Creek site (10.97 and 10.73 t/ha, respectively; LSD 0.87 t/ha). White clover content in pasture was also unaffected by DCD use (Figure 18).

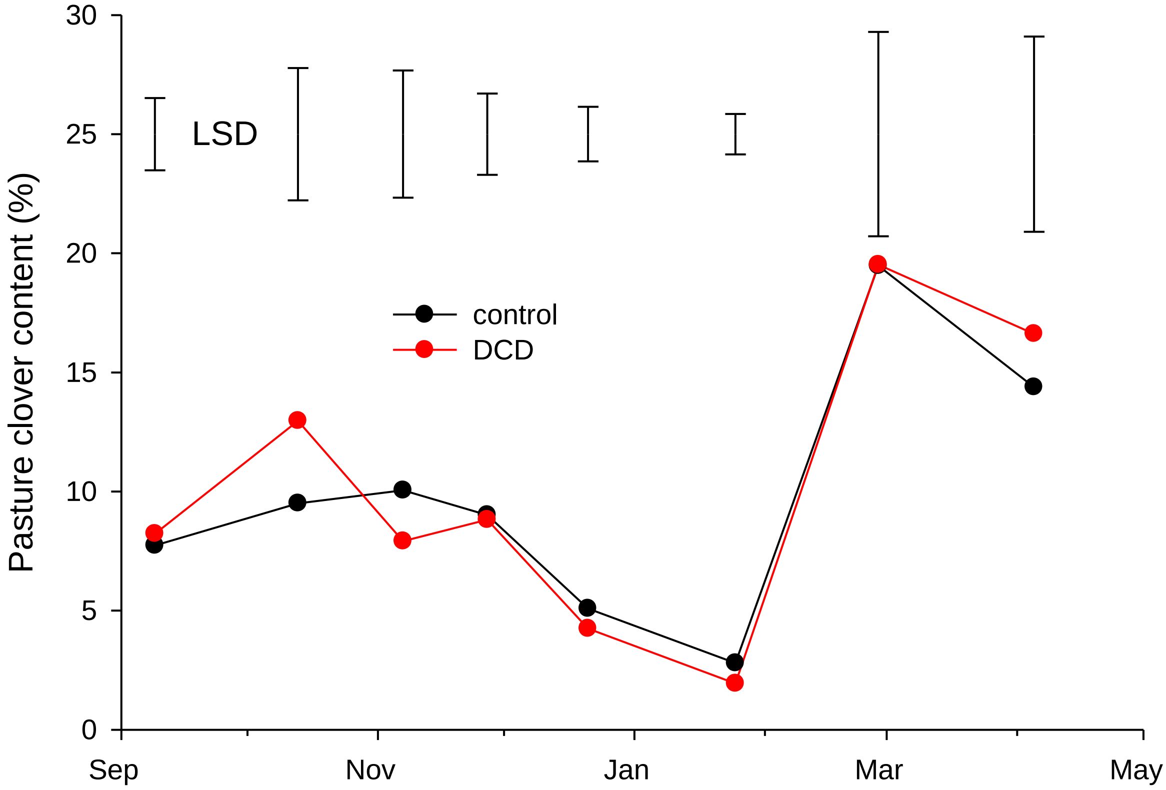

Figure 18. Pasture clover content immediately prior to each grazing at the Tussock Creek trial. The error bars represent the least significant difference ($LSD_{p<0.05}$) between treatments.

Lack of white clover in the ^{15}N microplots meant accurate measurement of the fixation of atmospheric N_2 by clover was only possible on four occasions (Table 9). The values for the proportion of clover N derived from N_2 fixation were lower than those measured on the Tokanui grazing trial, probably due to the higher rate of fertiliser N applied at Tussock Creek. There was no significant difference in the proportion of N fixed in clover or in the amount of fixed N in clover herbage between the control and DCD treatments.

Table 9. Proportion and amount of N in clover herbage derived from atmospheric N_2 estimated prior to various grazings at the Tussock Creek trial.

	Proportion of clover N fixed (%)			Amount of clover N fixed (kg/ha)		
Harvest	**control**	**DCD**	**$LSD_{(p<0.05)}$**	**control**	**DCD**	**$LSD_{(p<0.05)}$**
13 October	27	25	18.5	0.8	1.4	1.2
7 November	54	50	25.3	3.8	3.2	3.4
21 December	45	35	24.2	0.4	0.4	0.5
28 February	45	33	25.6	8.9	6.6	7.3

Discussion

Mowing trial

A mowing trial in the Waikato showed the effectiveness of DCD in inhibiting nitrification in soil. Soil sampling in this trial showed that more NH_4-N and more total inorganic N was retained in the soil following the application of DCD to urine treated plots. No differences were detected between the amounts of soil ammonium and total inorganic N in the control and control+DCD treatments.

However, the control treatments showed significant reductions in white clover content in pasture due to DCD application. The control+DCD treatment had less clover content on 9 of the 11 pasture sampling occasions and the differences were statistically significant at four of these sampling dates. No significant differences in white clover content were apparent between the urine and urine+DCD treatments but the white clover contents in these treatments were greatly reduced following the application of 700 kg N/ha as urine and statistically significant differences were difficult to detect. There was a trend for less total clover growth on both the DCD treatments (23% less over ten months), but this difference was not statistically significant.

The ^{15}N isotope analyses carried out on the control treatments showed no effect of DCD on the proportion of N in white clover derived from fixation of atmospheric N_2. In the urine treatments, the applied ^{15}N was not distributed evenly throughout the plant root zone with the applied urine, and relatively more was available to clovers than to deeper rooting grasses making initial measurements inaccurate. Later results showed a decrease in the proportion of clover N derived from N_2 fixation in the urine treatments that lasted nine months. However, there was no significant difference in N_2 fixation between the urine treatments with and without DCD.

The mowing trial indicated that DCD has the potential to at least temporarily reduce white clover content and consequently the amount of N fixed in pasture in some situations. This apparent effect appeared to be due directly to DCD application rather than indirectly through an increase in available soil inorganic N following DCD application. The reduction occurred in the non-urine treatments where no additional soil inorganic N was measured in the DCD treatment and there was no evidence in any of the trials of a reduction in the proportion of clover N derived from atmospheric N_2 when DCD was applied.

The APSIM model was used in an attempt to simulate the effect of increased inorganic N in soil on production, clover content in pasture and clover yield. In the non urine-affected pasture, it suggested little effect on clover content and clover yield (or even an increase with N rate in clover yield) with rates of N application up to 100 kg N/ha. Similarly, it suggested no effect in urine-affected pasture from retained N at up to 150 kg N/ha. However, it also predicted an increase in clover yield due to urine application, which contrasts greatly with the field study and other research. This suggests the model needs further developmental and validation work before it can be used reliably for this type of evaluation.

Grazing trial

Neither grazing trial showed any adverse effect of DCD on white clover content in pasture or on total white clover growth. Although, the low proportions of white clover in the pastures of both the grazing trials made detection of differences difficult, DCD has been used on the Southland (Tussock Creek) trial for eight years and in only one year was it associated with reduced clover growth (Monaghan *et al*. 2009). The ^{15}N isotope analyses showed no effect of DCD on the proportion of clover N derived from fixation of atmospheric N_2 or on the amount of N fixed by white clover in either of the grazing trials.

Conclusion

The conclusion to date from this limited series of mowing and grazing trials is that the application of DCD may have an initial negative effect on clover content and clover production in pasture, but any reductions are unlikely to be significant on an annual basis. DCD appears to have no effect on the proportion of clover N fixed from atmospheric N_2 in non urine-affected pasture. While no medium term effect of DCD application on N_2 fixation by clover in urine patches was detected, this trial did not measure clover N_2 fixation immediately following urine application. Any possible reduction in N_2 fixation during that period would be unlikely to significantly affect the total amount of N fixed because the low clover contents in urine-affected areas means these areas contribute relatively little to the total amount of atmospheric N_2 fixed in a pasture. Additionally, clover is likely to derive most of its N from urine during this initial period after urine application when soil inorganic N is high. Further research on a range of grazed pastures with higher clover contents would help to determine whether adverse white clover responses to DCD are isolated events or pose a realistic risk to clover growth in dairy pastures. However, many modern NZ dairy pastures have relatively low clover content as a result of frequent applications of N fertiliser and therefore a temporary reduction in clover growth is unlikely to result in a large reduction in N input from atmospheric N_2.

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Appendix 1. Literature review

INTRODUCTION

Cow urine patches are the main source of nitrogen (N) loss from grazed pastures on New Zealand dairy farms (Ball *et al*. 1979; Ryden & Garwood 1984). The nitrification inhibitor dicyandiamide (DCD) is recognised as a key mitigation option in reducing N losses from grazed pastures through nitrous oxide (N_2O) emissions and nitrate (NO_3) leaching (Di & Cameron 2007; Monaghan *et al*. 2009). Apart from its inhibiting effect on ammonium (NH_4) oxidising bacteria, DCD does not appear to affect other soil microbiota, and eventually breaks down entirely to leave no residue (Amberger 1989; Di & Cameron 2004; Aalders & Bell 2008; O'Callaghan *et al*. 2010).

White clover (Trifolium repens) provides New Zealand pastoral farming with a significant competitive advantage primarily due to its input of N into the soil through the fixation of atmospheric N_2. In 1996 its total financial contribution to the New Zealand economy was estimated at over $3 billion (Caradus *et al*. 1996). The benefits of DCD to New Zealand agriculture could be significantly reduced if DCD had an adverse effect on white clover.

The aim of this report is to provide a review of published research on the effects of DCD on soil inorganic N and white clover growth.

EFFECT OF DCD ON SOIL INORGANIC N

The N in a urine patch is rapidly converted into NH_4 which is strongly retained in the soil profile. However, NH_4 is gradually oxidized by soil bacteria, firstly to nitrite (NO_2) and then to NO_3 (Jarvis *et al*. 1995). Negatively charged NO_3 is relatively easily leached down the soil profile or lost to the atmosphere as N_2O. DCD works by deactivating the enzyme that enables the soil bacteria to convert NH_4 to NO_2 (Amberger 1989). Consequently, the N stays in the soil longer, allowing more time for plants to utilise it or for immobilisation into soil organic N to occur.

Various New Zealand studies have confirmed that DCD applied to urine-affected soil results in the N remaining in the form of NH_4 for longer (Cookson & Cornforth 2002; Di & Cameron 2004; Smith *et al*. 2005; Sprosen *et al*. 2009). Factors influencing the effect of DCD on soil inorganic N include rate of application, temperature, moisture and drainage. Rates of DCD applied in the above trials ranged from 7.5 to 28 kg/ha. Cookson and Cornforth (2002) and Sprosen *et al*. (2009) found increased effectiveness at higher rates but Di and Cameron (2004) found no difference between rates of 7.5 and 15 kg DCD/ha. Kim *et al*. (2011) found no difference in the half-life of DCD whether it was applied at 10 or 20 kg/ha. Temperature has a strong effect on DCD breakdown. Di and Cameron (2004) found the half-life of DCD at 8ºC was 113 days, while Kelliher *et al*. (2008) calculated a 72 day half -life at 10ºC and Williamson *et al.* (1996) found a half life of only 39 days at 22ºC. Kim *et al*. (2011) found a strong correlation between increased soil moisture and a longer half-life of DCD. However, unlike NH_4, DCD is not held strongly in the soil and excessive soil moisture can lead to leaching. A study on a free-draining pumice soil that received 600 mm of rainfall, found that over half the applied DCD was leached below 50 cm (Menneer *et al*. 2008). Multiple applications of DCD can extend the period of effectiveness in reducing nitrate leaching (Di & Cameron 2002) but since DCD comprises two thirds nitrogen and eventually breaks down to ammonium (Amberger 1989), each application adds further inorganic N to the soil.

EFFECT OF DCD ON WHITE CLOVER

DCD has been observed to have phytotoxic effects and to reduce yields on a number of crops, including cotton, sorghum, maize and soybeans (Reddy 1964; Maftoun & Sheibany 1979; Reeves & Touchton 1986). Toxicity was related to plant species (Reddy 1964) and the amount of DCD applied to the plant (Reeves & Touchton 1989).

Studies with another nitrification inhibitor (nitrapyrin) used with ammonium fertilisers have shown reduced concentrations of calcium (Ca) and magnesium (Mg) in plants (English *et al.* 1980; Mathers *et al.* 1982) due to competition for absorption with NH_4. Reeves & Touchton (1989) found that DCD had the same effect on reducing Ca and Mg concentrations in a cotton crop.

Most studies examining DCD toxicity have been conducted on individual crops. In New Zealand dairy pastures, white clover is normally grown in combination with perennial ryegrass (Lolium perenne). One overseas study (Macadam *et al*. 2003) showed a direct deleterious effect on white clover when DCD was applied to a ryegrass/white clover pasture. In this study, damage to white clover leaves was noted when DCD was applied at a rate of 25 kg/ha to a pasture receiving 80-85 kg N/ha in the form of either calcium ammonium nitrate (CAN) or cattle slurry. A separate solution culture trial with plants receiving DCD at rates equivalent to 0, 25 and 50 kg DCD/ha confirmed a reduction in white clover growth with DCD, mostly due to a reduction in petiole mass. In this case, since the amount of NH_4 in the growth solution was the same in both the control and DCD treatments, Macadam *et al*. (2003) concluded that the phytotoxicity was due directly to the DCD and not to an induced nutrient imbalance although this conclusion has been questioned (Edmeades 2004).

In New Zealand, a grazing trial in Canterbury from 2002-2006 (Moir *et al*. 2007) showed no adverse effect on white clover when DCD was applied twice a year at a rate of 10 kg/ha to a ryegrass/white clover dairy pasture. Two other New Zealand trials on ryegrass/white clover pasture have shown instances of reduced white clover growth following DCD application. In a four year grazing trial in Southland (Monaghan *et al.* 2009), DCD was applied at a rate of 10 kg/ha, either twice or three times per year. In the 2005-2006 year, white clover growth was significantly lower on the DCD-treated plots than on the untreated control plots (904 versus 1326 kg DM/ha/year) but no significant difference was measured in any of the other years. In a Waikato mowing trial, Sprosen *et al*. (2009) found significantly less white clover in DCD-treated plots (10 versus 25% white clover content in the control versus the average of 7, 14 and 28 kg DCD/ha treatments) six months after a single application of 600 kg N/ha in the form of artificial urine. None of the New Zealand studies noted any visible damage to white clover.

In ryegrass/white clover pastures the competitive balance between the two species is governed by the N status of the soil (Brock & Hay 2001). Where available N in soil is low, white clover has an advantage due to its ability to fix atmospheric N_2, but where soil available N is relatively high grass is more competitive. Macadam *et al*. (2003) noted that in retaining more N in the soil, by reducing losses from N_2O and N leaching, DCD may disadvantage white clover by increasing the competitiveness of ryegrass. However, if this is the only mechanism of DCD effect on white clover, it is likely to be a transient effect that disappears when soil available N declines to background levels.

CONCLUSIONS

DCD delays the conversion of NH_4 to NO_3 in soil. This can result in reduced losses of inorganic N from the soil and an increase in the N available for plant uptake. DCD can potentially have phytotoxic effects on plants. One overseas study showed an apparent phytotoxic effect on white clover when used at a rate of 25 kg/ha but the clover was grown in a solution culture and the outcome may have been an artefact of this approach. The rate used in the study was above that recommended for use on New Zealand pastures (10 kg/ha). No New Zealand studies have reported visible damage to white clover following DCD application but two have recorded reductions in clover yield. However, only one of those studies was conducted under grazing conditions and in that trial there was a significant reduction in clover growth in only one out of four years. This highlights the need for further investigation to determine how and under what circumstances DCD might adversely affect clover growth in New Zealand pastures.

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