FACTORS CONTROLLING DISAPPEARANCE OF NITRIFICATION INHIBITOR, DICYANDIAMIDE (DCD) IN A GRAZED PASTURE SOIL IN MANAWATU

Dong-Gill Kim[a*], Donna Giltrap[a], Surinder Saggar[a], Thilak Palmada[a], Peter Berben[a], Doug Drysdale[b]

[a]Landcare Research, Palmerston North 4442, New Zealand
[b]Royal Society of New Zealand Primary Science Teacher Fellow, Bunnythorpe School, Bunnythorpe 4867, New Zealand
**Corresponding author, KimD@landcareresearch.co.nz; donggillkim@gmail.com*

Abstract
Nitrification inhibitors (NIs) including dicyandiamide (DCD) slow nitrogen (N) turnover by retarding the oxidation of ammonium (NH_4^+) to nitrate (NO_3^-), providing more time for plant uptake of NH_4^+. While studies evaluating the efficacy of DCD on reducing nitrous oxide (N_2O) emissions have been widely conducted, the characteristics of biophysical disappearance of DCD (i.e., biological decomposition, plant uptake and physical loss through surface run-off and leaching) and its longevity in soil are not well understood. The objectives of this study were to improve our understanding of seasonal variations in the biophysical disappearance of DCD in soil and the key control factors regulating the variations. Changes in DCD concentrations in soil and plant canopy were measured following its application in dairy-grazed pasture soil. The treatments included two levels of DCD alone (10 and 20 kg ha^{-1}) applied to non-grazed pasture field plots and a single level DCD (10-kg ha^{-1}) applied with urine and with urea fertiliser. DCD (10-kg ha^{-1}) was also applied in grazed farmlets following grazing. Our measurements show 4 to 40% of applied DCD was intercepted and stayed on plant canopy from <6 up to 16 days, depending on timing and intensity of rainfall following DCD application. In this poorly drained soil <10 % of applied DCD leached below 10 cm depth. Our results suggest that neither the level of DCD nor the N source had any significant effect on the half-life of DCD in soil. Seasonal variations in soil temperature affected the half-life of DCD in soil. The DCD half-life showed a linear decrease with increased temperature over the observed range of average seasonal temperatures (10.7 to 16.5°C). The results suggest that to maintain an optimum effective DCD concentration in soil, different DCD application rates and frequency may be required in different seasons.

1. Introduction
Nitrous oxide contributes to the enhanced greenhouse effect with a global warming potential 298 times greater than of carbon dioxide (CO_2) in a 100-year time horizon (Forster et al. 2007). Of the 5.7 Tg N_2O–N yr^{-1} anthropogenic N_2O emissions, agricultural soils contribute 3.5 Tg N_2O–N yr^{-1} (IPCC 2006). Nitrification inhibitors such as DCD, nitropyrin, and 3,4 dimethyl pyrazole phosphate (DMPP) deactivate the enzyme ammonia monooxygenase of *Nitrosomonas* and/or *Nitrosospira*, the genus of nitrifying bacteria responsible for the oxidation of NH_4^+ to NO_3^-. This helps retain N in the NH_4^+ form longer in soil, providing more opportunity for plants to uptake NH_4^+ (e.g., Abbasi and Adams 2000; Di et al. 2007, 2009). Thus NI can reduce N_2O emissions both from nitrification and from denitrification of NO_3^-.

While a number of studies have estimated the reductions in N_2O emissions with DCD, the nature and longevity of the biophysical disappearance of DCD in soil that control DCD effectiveness are not well understood. In an incubation experiment with New Zealand grassland soils, the DCD degradation rate differs between different types of soils (Singh et al. 2008). DCD is known to decompose more rapidly in soils with high organic matter content (Amberger and Vilsmeier 1979; Kutzova et al. 1993; Singh et al. 2008) and the fate of DCD in soil might be affected by sorption of DCD onto soil organic matter (Sahrawat et al. 1987). In incubation experiments with grassland soils, the half-life of DCD was longer at the higher rate of DCD application (Rajbanshi et al. 1992; Singh et al. 2008). Published data from incubation experiments indicate that the half-life of DCD was strongly affected by soil temperature, showing an exponential decrease of half-life of DCD as soil temperature increased (Kelliher et al. 2008). However, these findings are mainly based on controlled laboratory experiments, and there are still uncertainties in our understanding of the biophysical disappearance of DCD in field conditions. These suggest that soil properties, DCD application rates, and climate factors affect the biophysical disappearance of DCD and further studies are now needed to improve our understanding of the characteristics of the biophysical disappearance of DCD.

The objectives of this study were (a) to examine seasonal variations in the biophysical disappearance of DCD in soil, and (b) to determine the major factors controlling this variation. It should be noted that in this study the measured rate of the biophysical disappearance of DCD was the net result of several processes, including the biological decomposition of DCD in soil, the physical movement of DCD from plant canopy to soil, and DCD movement in the soil (e.g., run-off and leaching following rainfall). In this study, we had three major hypotheses:

1. The half-life of DCD in soil does not differ with the amount of DCD application.
2. The addition of different sources of N (Urine or synthetic N fertiliser) has no influence on the half-life of DCD in soil.
3. Seasonal variations in soil temperature affect the half-life of DCD in a soil. Rainfall is also likely to affect the rate of leaching of DCD in soil (however, this effect was not quantified).

2. Materials and methods

2. 1. Study site

Field experiments were set up on a Tokomaru silt loam soil and permanent ryegrass-clover pasture managed for grazing dairy cows (3 cows ha^{-1}) at Massey University Research Dairy Farm 4, Palmerston North, Manawatu, New Zealand (40º 23' 40" S, 175º 36' 28" E) in 2010 and 2011. The soil is a Tokomaru silt loam and is classified as an Argillic-fragic Perch-gley Palllic Soil (Hewitt 1998) or Typic Fragiaqualf (Soil Survey Staff 1998) derived from deep deposits of loess-brown river sediments. The Tokomaru soil consists of a weakly to moderately developed brown silt loam A-horizon, a weakly developed grey strongly mottled, clay loam B-horizon, and a highly compacted, weakly developed pale gray, silt loam fragipan C-horizon that acts as a natural barrier to drainage (Hewitt 1998). Soil pH is 5.8, soil bulk density ranges from 1.1 to 1.3 g cm^{-3} and soil C and N contents range from 3.2 to 3.6 % and 0.26 to 0.27 %, respectively (Table 1).

Table 1. Soil properties of study site

Soil properties	Unit	Soil depth	
		0−10 cm	10−20 cm
Soil type[†]	-	Silt loam	Silt loam
Sand[†]	%	8.5	NA[‡]
Silt[†]	%	68.4	NA
Clay[†]	%	23.0	NA
Soil pH[§]	-	5.8	5.8
Bulk density[†]	g cm^{-3}	1.1	1.3
Soil C	%	3.6	3.2
Soil N	%	0.27	0.26
Soil CEC[†]	$cmol_c$ kg^{-1}	22.3	NA
Field capacity[†]	%	48	45

[†]Singh et al. 2008
[‡]NA: Not available
[§]1:2 soil to water ratio

2. 2. Experimental design

A preliminary laboratory experiment was performed to evaluate the DCD recovery rate of the water extraction method which was used to quantify soil DCD in this study. The field studies were conducted using two different experimental sites within the farm: a field-plot experiments site (non-grazed) and a cattle-grazed site. The non-grazed site was fenced off for a month before the application of treatments to minimise the effect of previous grazing events

2. 2. 1. DCD recovery with water extraction method

Tests were initially conducted to evaluate DCD recovery using the water extraction method described in section 2. 3. Briefly, the 6 replicate subsamples of 10 g field-moist sieved soils (0–10 and 10–20 cm soil depths) were spiked with 5 ml of 2, 5, 10, 20 and 40 mg L^{-1} DCD solution in 35 ml tubes. The tubes were vigorously shaken by hand and stored at 4 °C for 2 hrs to provide sufficient time for DCD and soil to equilibrate. Then, 15 ml of distilled water was added to the tubes. The resultant soil solution was centrifuged (9000 rpm for 3 min), filtered (Whatman No.42) and the extract analysed for DCD as described in section 2. 3. The amount of DCD recovered was calculated after accounting for dilution factors and % recovery was determined using Equation 1:

$$\text{DCD recovery rate}\,(\%) = \frac{\text{Amount of DCD recovered}}{\text{Amount of DCD added originally}} \times 100 \qquad \text{(Equation 1)}$$

2. 2. 2. Field-plot experiments

The field-plot experiments outlined below were conducted to determine the effects on biophysical disappearance of DCD of the amount, N source and season.:

(i) Effect of the rates of DCD application on biophysical disappearance of DCD in soil

To investigate the effect of the rates of DCD application on biophysical disappearance of DCD in soil, two rates of DCD (10 and 20 kg DCD ha^{-1}) were applied with no N input in 6 replicated plots (2.5 × 2.5 m) on three occasions between early and late spring (August, October, November 2010; southern hemisphere). The permanent ryegrass-clover pasture in the treatment plots was cut to 5-cm height with a John Deere JX80 mower to mimic a grazing effect. After cutting, a solution of DCD (12.5 g DCD in 1 L of water) was evenly sprayed on plots by a hand sprayer. Soil sampling (soil depth 0–10 cm) for all plots was initially conducted 1 or 2 days after the DCD application and later every 3 days for the first week, and then weekly or bi-weekly for the rest of the study period.

(ii) Effect of type of N input on biophysical disappearance of DCD in soil

To investigate the effect of type of N input on biophysical disappearance of DCD in soil, 10 kg DCD ha^{-1} (control), 10 kg DCD ha^{-1} with synthetic urine (700 kg N ha^{-1}; synthesized based on Clough et al., 1998) and 10 kg DCD ha^{-1} with urea fertiliser (25 kg N ha^{-1}) were applied in six replicated plots (2.5 × 2.5 m) during April 2010. Three months later, in June 2010, 10 kg DCD ha^{-1} (control) and 10 kg DCD ha^{-1} with synthetic urine (700 kg N ha^{-1}) were applied in six replicated plots (2.5 × 2.5 m),. The permanent ryegrass-clover pasture in the treatment plots was cut to 5-cm height to mimic a grazing effect. After cutting, synthesized urine (Clough et al. 1998) was evenly sprayed by a water sprayer and urea fertiliser (46% N) was applied by hand to the plots. A solution of DCD (12.5 g DCD in 1 L of water) was then evenly sprayed on the plots with a hand sprayer. Soil sampling (soil depth 0–10 cm) for all plots was initially conducted 1 or 2 days after the DCD application, and later, every 3 days for the first week, and then weekly or bi-weekly for the rest of the study period.

(iii) Seasonal variation of biophysical disappearance of DCD from different soil depth

To quantify seasonal variation of biophysical disappearance of DCD from the different soil depths (0–10 and 10–20 cm), 10 kg DCD ha^{-1} was applied in 6 replicated plots (2.5 × 2.5 m) in March, June, August and October 2010 and April, June and August 2011. As decribed in (ii), the permanent ryegrass-clover pasture in the treatment plots was cut to 5-cm height to mimic a grazing effect. Then a solution of DCD (12.5 g DCD in 1 L of water) was evenly sprayed on the plots by a hand sprayer. Soil sampling (soil depths 0–10 and 10–20 cm) for all plots was initially conducted 1 or 2 days after the DCD application and later every 3 days for the first week, and then weekly or bi-weekly for the rest of the study period.

2. 2. 3. Seasonal biophysical disappearance of DCD in soil under cattle grazing

At the cattle-grazing farmlet site, experiments were conducted to assess the seasonal differences in biophysical disappearance of DCD as follows:

At the cattle-grazing site, 10 kg DCD ha^{-1} was applied to 9 replicated farmlets (each plot size 600–1000 m^2) with a tractor-mounted spray unit on 6 occasions (March, April, October 2010 and March, April and June 2011) 2–3 days after cattle grazing (160–300 cow ha^{-1}). Soil sampling (soil depth 0–10 cm) on all plots was initially conducted 1 or 2 days after the DCD application and then at weekly or bi-weekly intervals for the rest of the study period.

2. 2. 4. DCD persistence on plant canopy

To quantify DCD persistence on the plant canopy, 3 plots were randomly selected in the 10 kg DCD ha^{-1} with no N input applied- plots described in section 2. 2. 2. (iii). Plant sampling was initially conducted 1 or 2 days after the DCD application (August, October and November 2010), and later, every 3 days for the first week, and then weekly or bi-weekly for the rest of the study period. In addition, in April 2011, after the DCD application, plant sampling was conducted in 3 randomly selected farmlets at the cattle-grazing site (described in 2. 2. 3.). Plant sampling was conducted 1 or 2 days after the DCD application and later every 3 days for the first week, and then weekly or bi-weekly for the rest of the campaign period.

2. 3. Soil and plant sampling and soil and plant canopy DCD analysis

At the non-grazing site, 10 intact soil cores (diameter 25 mm) were collected in each plot with a soil auger to soil depths of 0–10 and 10–20 cm. In the cow-grazing site, 24 intact soil cores (diameter 25 mm) were collected in each plot in soil depths of 0–10 and 10–20 cm. For plant sampling, a 20 cm-diameter ring was randomly located in each plot and all plants inside the rings were cut to 2–3 cm. The soils and plant samples were then transferred to the laboratory and processed within 3 hours.

Field-moist soil samples were sieved through a 4 mm sieve, and sub-samples were used to determine soil moisture contents. DCD was extracted from the soil by shaking 10 g of moist soil in 20 ml of deionised water for 1 hr on an end-over-end shaker in the laboratory at room temperature (20−21 °C). The extract was then centrifuged (9000 rpm for 10 min.) and the supernatant filtered through No. 42 Whatman filter paper. Recovery of DCD from the plant canopy was conducted by shaking 25 g of plants in 1000 ml of deionised water for 20 min. on an end-over-end shaker and the solution was then filtered through No. 42 Whatman filter paper. Five ml of the soil or plant extract was then acidified with 0.2 ml of 0.66 M H_2SO_4 and allowed to stand for 30 minutes before centrifuging (4500 rpm for 10 min.) to remove precipitated material. The concentration of DCD in the acidified supernatant was determined on a Waters 2695 high pressure liquid chromatography (Waters Co., Milford, MA, USA)using a cation-H guard column (30 × 4.6 mm) with a 0.025M H_2SO_4 mobile phase at a flow rate of 0.6 ml min^{-1} and a 210 nm UV detector (Schwarzer and Haselwandter 1996). The detection limit for DCD s was 0.005 ppm.

2. 4. Quantifying the half-life of DCD

The half-life of DCD in soils was quantified using regression analysis by fitting the DCD concentration in the soil as a function of time to a first-order exponential model as follows (Kelliher et al. 2008; Singh et al. 2008) (Equation 2):

$$C(t) = C_0 e^{-kt} \qquad \text{(Equation 2)}$$

where, C (t) is the DCD concentration in the soil as a function of time (t); C_0 is the initial DCD concentration; k is a constant

The DCD half-life (the time taken for C_0 to decline to $C_0/2$) was calculated as follows:

$$\text{DCD half-life time} = 0.693/k \qquad \text{(Equation 3)}$$

Throughout this study, 4–6 different soil DCD concentration points were used to determine k values and the model was accepted if $R^2 > 0.8$.

2. 5. Soil properties

Soil pH was determined on a 1:2 (soil: H_2O) diluted soil solution using a pH meter (Accument 910, Fisher Scientific Ltd., Pittsburgh, PA, USA). Soil moisture content was determined by oven drying a subsample at 105°C for 24 h and bulk density was determined by the core method (Grossman and Reinsch 2002). For Total C and N analysis, soils were air dried at room temperature, sieved (2 mm), and gravimetric moisture contents determined. Total C and N in the soil were measured by combustion in a Leco FP-2000 CNS (LECO Corp., MI, USA).

2. 6. Soil microclimate and climate data collection

On-site instrumentation was used to collect half-hourly averaged values of soil temperature (at 5 cm, a thermistor probe, CS107, Campbell Scientific, USA), soil moisture (at 5 cm depth, time domain reflectometry probes, CS615, Campbell Scientific, USA), air temperature (107-L Temperature Sensor, Campbell Scientific, USA) and precipitation (CS700-L, Campbell Scientific, USA) throughout the study period. Long-term (1971–2010) air and soil (0–10-cm soil depth) temperature and rainfall data in Palmerston North, Manawatu, were obtained from the National Institute of Water and Atmospheric Research, New Zealand (http://www.niwa.co.nz/education-and-training/schools/resources/climate/earthtemp) and New Zealand's National Climate Database (http://cliflo.niwa.co.nz/).

2. 7. Statistical analysis

For all datasets the normality of the distribution of the data was first analysed using the Shapiro–Wilk normality test (Shapiro and Wilk 1965). When data had normal distribution, mean ± standard error values were presented for data summary. However, when the standard assumptions of normality were violated, median values with lower 25% and upper 75% values were presented.

T-test was used to evaluate the significance of difference (at the $P < 0.05$ level) in 1) DCD recovery rates at two different soil depths (0–10 vs 10–20 cm) and 2) recovered DCD concentrations and half-life of DCD at two different DCD application rates (10 vs 20 kg DCD ha^{-1}) and soil depths (0–10 vs 10–20 cm). When the standard assumptions of normality were violated, a Mann-Whitney rank sum test (Mann and Whitney, 1947) was used instead of a t-test, , One-way analysis of variance (ANOVA) was used to evaluate the differences (at the $P < 0.05$ level) in means of 1) half-life of DCD by different N types and seasons, and 2) soil microclimate variables by seasons. When the standard assumptions of normality were violated, non-parametric Kruskal–Wallis one-way ANOVA on ranks (Kruskal and Wallis 1952) was used. Dunn's test was used for all pairwise comparisons following Kruskal–Wallis one-way ANOVA on ranks.

To determine the relationship between 1) initial recovered DCD concentrations and half-life of DCD and 2) weather and soil microclimate and half-life of DCD, Pearson correlation analysis was applied. The NONLIN procedure of SAS software (SAS Institute, 2009) was used for deriving the best fit of N half-life of DCD models for the relationship between weather and soil microclimate variables and half-life of DCD. These statistical analyses were conducted using SAS ver. 9.2 (SAS Institute, Cary, NC, USA) and SigmaPlot ver. 11.0 (Systat Software Inc., San Jose, CA, USA).

3. Results and discussion

3. 1. Climate condition

Long-term average annual rainfall (1971–2009) at this region is about 997 mm, which is fairly evenly distributed throughout the year, with the driest months being January–March (Fig. 1). Long-term average annual air temperature (1971–2009) at this region is 13.2°C, and the coldest and warmest months are July (8.7°C) and February (18.1°C) (Fig. 1) (National Institute of Water and Atmospheric Research, New Zealand, http://www.niwa.co.nz/education-and-training/schools/resources/climate/earthtemp).
Observed annual rainfall at this site is 968 and 1224 mm in 2010 and 2011, respectively, and observed mean annual air temperature is 13.6 and 13.1 in 2010 and 2011, respectively (Fig.1).

3. 2. DCD recovery rate by water extraction

The DCD recovery rate was 95.8 ± 0.3 % (n = 30) for soil DCD concentrations ranging from 1 to 30 mg DCD kg^{-1} soil. In our experiments the DCD concentration in our soil samples was always less than 20 mg DCD kg^{-1} soil, so these results suggest that recovery of DCD by water extraction should provide reliable data. However, the DCD recovery rate determined in this study may not be applicable to other soils. Further research is clearly needed to reveal the mechanisms by which DCD concentrations decline in soil, and to better explain the DCD recovery rate for the soils we studied.

3. 3. Effect of the rates of DCD application on biophysical disappearance of DCD in soil

The half-lives of DCD for 10 kg and 20 kg DCD treatments (0–10 cm soil depth) were not significantly different each other for the applications made in three different seasons. In the August application, initial concentration of DCD in the 0–10 cm soil after DCD application (10 kg and 20 kg DCD treatments) was 6.6 ± 0.6 and 10.7 ± 2.0 kg DCD ha^{-1}, respectively. These values were significantly different (P = 0.041) but the half-lives for 10 kg and 20 kg DCD treatments were not different (10.0 ± 0.9 d and 10.1 ± 1.2 d, respectively) (P = 0.941) (Table 2). Similarly, in the October and November applications, initial concentration of DCD in the 0–10 cm soil after DCD application (10 kg and 20 kg DCD treatments) was significantly different (all P = 0.005) but the half-lives for 10 kg and 20 kg DCD treatments did not differ (all P > 0.5) (Table 2). Combining all the results from the three treatments it was found that the observed initial DCD concentrations in the soil after DCD applications were not significantly correlated with half-life of DCD (Pearson correlation coefficient r = –0.252, P = 0.63). The results suggest that the biophysical disappearance rate of DCD was not affected by the amount of DCD applied. Overall, further studies are needed to further clarify these differences on the effect of DCD application rate on biophysical removal or degradation of DCD.

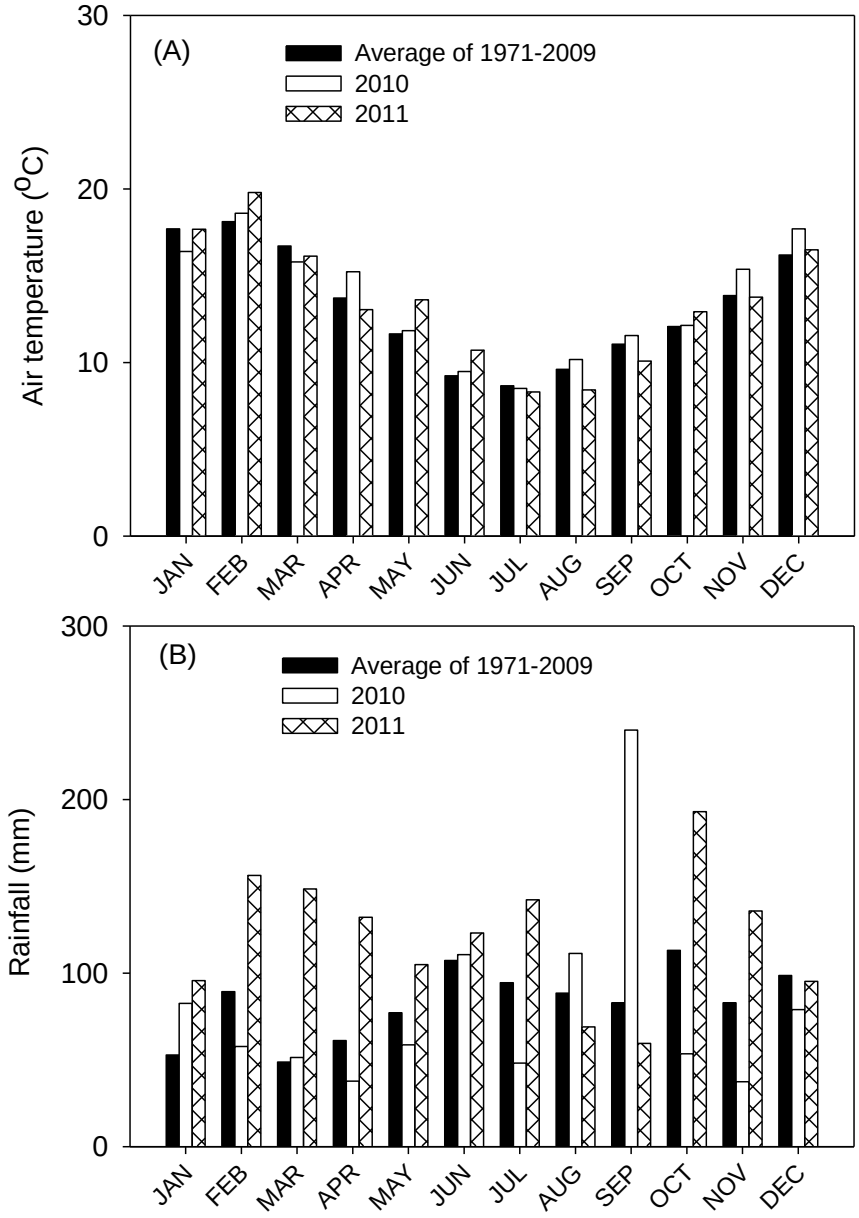

Figure 1. Monthly average air temperature and cumulative rainfall in 2010 and 2011 in the study site and long-term average (1971–2009) in the region.

Table 2. Initial DCD concentrations (kg ha^{-1}) after DCD application and half-life of DCD (d, mean ± standard error) in soil (0−10 cm soil depth). Two different amounts of DCD (10 and 20 kg ha^{-1}) were applied with no nitrogen treatment in non-grazing site on August (n = 6), October (n = 6) and November 2010 (n = 6).

	Initial DCD concentrations (kg ha^{-1})			Half-life of DCD (d)		
DCD Application date	DCD 10 kg ha^{-1}	DCD 20 kg ha^{-1}	P[#]	DCD 10 kg ha^{-1}	DCD 20 kg ha^{-1}	P[#]
August 2010	6.6 ± 0.6	10.7 ± 2.0	0.041	10.0 ± 0.9	10.1 ± 1.2	0.941
October 2010	4.5 ± 1.1	8.9 ± 1.0	0.005	9.1 ± 1.2	10.0 ± 0.2	0.437
November 2010	7.5 ± 0.1	15.4 ± 0.7	0.005	8.2 ± 2.3	8.3 ± 1.8	0.957

[#]Testing significant difference in DCD (10 and 20 kg ha^{-1}) application

3. 4. Effect of type of N input on biophysical disappearance of DCD in soil

The half-life of DCD with different N treatments was not significantly different in two different seasons. In the March application, there was no significant difference in the half-life of DCD (0–10 cm soil depth) relating to different N treatments (control, urea fertiliser and urine) (P = 0.732) (Table 3). Similarly, in the June application there was no significant difference in the half-life of DCD in 0–10 cm soil depth between different N treatments (control and urea fertiliser) (P = 0.520) (Table 3). These results suggest that biophysical disappearance of DCD were not affected by type of N input (urea fertiliser and urine).

Table 3. Half-life (d, mean ± standard error) of DCD in soil (0−10 cm soil depth) treated with DCD (10 kg ha^{-1}) combined control, urea fertiliser (25 kg N ha^{-1}) and urine (700 kg N ha^{-1}) in non-grazing site on April (n = 6) and June 2010 (n = 6).

Application date	Control + DCD	Urea fertiliser + DCD	Urine + DCD	P value[#]
April 2010	14.5 ± 1.5	13.7 ± 0.7	11.3 ± 2.4	P = 0.732
June 2010	11.6 ± 1.0	14.0 ± 2.5	ND[†]	P = 0.520

[†]ND: no data

[#]Testing significant difference in DCD with different N treatments.

3. 5. Initial DCD concentrations and half-life of DCD at different soil depths

Initial concentrations of DCD at 10–20 cm soil depth after DCD application were significantly lower than those at 0–10 cm soil depth (all $P < 0.05$) (Table 4).

Table 4. Initial DCD concentrations (mean ± standard error) after DCD application and half-life (d, mean ± standard error) of DCD in soil (0–10 and 10–20 cm soil depth) following DCD (10 kg ha^{-1}) application in non-grazed site in March, June, August and October 2010 and April, June and August 2011.

DCD application date	Initial DCD concentrations (kg ha^{-1})			Half-life of DCD (d)		
	0–10 cm	10–20 cm	P[#]	0–10cm	10–20 cm	P[#]
March 2010	4.34 ± 0.6	0.50 ± 0.2	0.003	6.5 ± 0.5	NA[†]	NA
June 2010	3.67 ± 0.5	0.71 ± 0.1	0.004	12.9 ± 0.9	25.1 ± 2.6	< 0.001
August 2010	6.01 ± 0.5	0.62 ± 0.2	0.002	10.0 ± 0.9	NA	NA
October 2010	2.97 ± 0.4	0.41 ± 0.2	0.001	9.1 ± 1.2	NA	NA
April 2011	2.23 ± 0.6	0.2 ± 0.1	0.004	12.0 ± 1.0	NA	NA
June 2011	3.89 ± 0.4	0.18 ± 0.03	0.004	13.8 ± 0.9	NA	NA
August 2011	4.40 ± 0.4	0.18 ± 0.03	0.001	11.9 ± 1.5	NA	NA

[#]Testing significant difference in 0–10 and 10–20 cm

[†]NA: not available, pattern of biophysical disappearance of DCD was not fitted with either a linear model or a first-order exponential model.

The initial concentrations in 10–20 cm soil depth were 10–20% of those at 0–10 cm soil depth, and less than 10% of DCD applied reached depths below 10 cm (Table 4). The pattern of biophysical disappearance of DCD at 10–20 cm depth did not closely fit either a linear model or a first-order exponential model. Furthermore, the half-life of DCD could not be determined at 10–20 cm depth, except for the June 2010 application. In this case, the half-life of DCD at 10–20 cm depth was significantly longer than at 0–10 cm depth ($P < 0.001$) (Table

4). The lower DCD concentration in the 10–20 cm layer may result from small amount of DCD leached from the top 10 cm, and simultaneously existing biological decomposition of DCD in the soil. A combination of the leaching and the biological decomposition of DCD may also explain why the concentration does not show an exponential decrease with time in the 10–20 cm, as would be expected from a typical decay process. Overall, these results suggest that only a small amount of the DCD applied may reach beyond soil depths of 10 cm.

3. 6. DCD residence on plant canopy

Initial % DCD (n = 3) on plant canopy of the applied DCD (10 kg DCD ha^{-1}) in August, October and November 2010 and April 2011 ranged from 4.3% up to 39.8% (Table 5). The DCD persisted on the plant canopy for less than 6 days in August and October, and for up to 16 days in November (Fig. 2). It appears that plant height was related to % DCD on plant canopy: there was a higher initial % DCD on plant canopy of the applied DCD in taller plants (4.3 – 9.2% on < 5 cm height plant vs 39.8% on 5–10 cm plant height) (Table 5).

Table 5. Initial % DCD (n = 3) on plant canopy of the applied DCD (10 kg DCD ha^{-1}), DCD residence time on plant canopy, air temperature and cumulative rainfall (6 days following DCD application). DCD was applied in August, October and November 2010 and April 2011.

DCD application date	Plant height (cm)	Initial % DCD on plant surface of the applied DCD (mean ± standard error)	DCD residence time on plant canopy (d)	Air temperature (°C) (mean ± standard error)	Rainfall (mm)
August 2010	< 5	4.3 ± 0.8	< 6	10.8 ± 0.4	29.7
October 2010	< 5	9.2 ± 1.5	< 6	11.7 ± 0.9	24
November 2010	< 5	4.9 ± 1.8	16	12.6 ± 0.9	5.2
April 2011	5 to 10	39.8 ± 3.3	15	17.4 ± 0.3	0.3

This can be explained by the taller plant having a larger canopy area on which the DCD can be intercepted and retained. In the four periods tested, mean air temperatures were not significantly different (P = 0.281) but rainfall was higher in August and October (Table 6). It appears that the amount of rainfall was related to the DCD residence time on the plant canopy: residence time was longer under lower rainfall conditions (< 6 days in 24–30 mm rainfall vs 15–16 days in 0.3 – 5.2 mm rainfall; rainfall values are accumulated amount within 6 days after DCD application). The DCD application followed by a rainfall event within 12 h in August and November showed relatively lower % DCD on the plant canopy (Table 5) and higher recovered soil DCD concentrations (Tables 2 and 4). These results suggest that applied DCD is washed from the plant canopy into the soil by rainfall. Our results suggest that plant height and rainfall events need to be considered in selecting DCD application dates to maximise the effectiveness of DCD.

3. 7. Seasonal variation of half-life of DCD in soil

The median half-life of DCD (0–10-cm soil depth) applied to cow-grazed farmlets in March, April and October was 6.9 d (lower 25%: 5.0 & upper 75%: 8.6), 10.3 d (lower 25%: 9.2 & upper 75%: 11.2) and 8.4 d (lower 25%: 6.9 & upper 75%: 13.1), respectively (the half-life data showed signs of skewness and failed the Shapiro–Wilk normality test). The half-life of DCD applied in April was significantly longer than applications in March and October (P = 0.011) (Table 6). There was no significant difference between the half-life of DCD applied on cow-grazing farmlets and non-grazing sites for the same period (all P > 0.05).

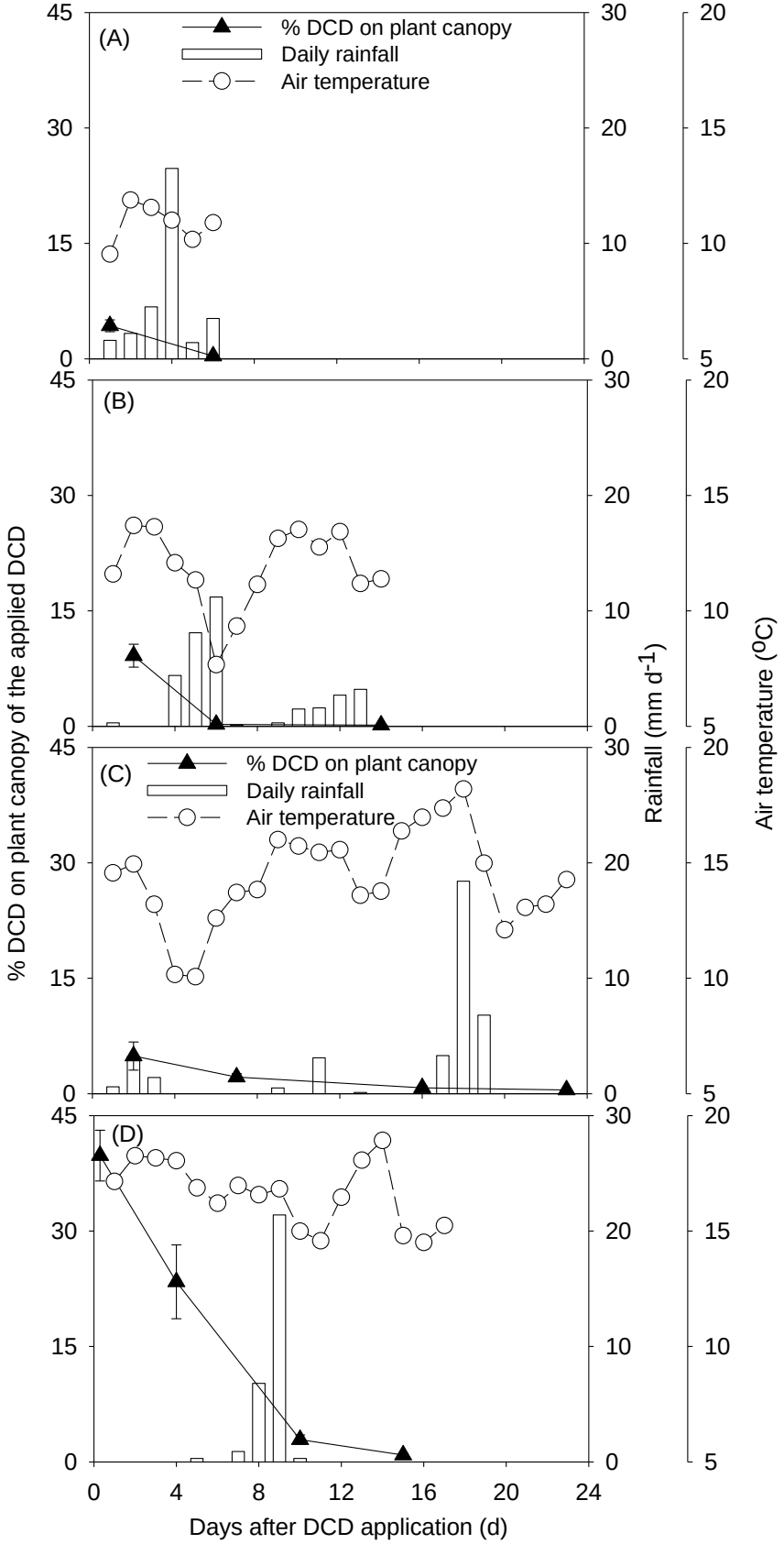

Figure 2. Variation in the proportion of applied DCD retained on plant canopy, daily rainfall and air temperature. DCD was applied in August (A), October (B) and November (C) 2010 (*n* = 6).

Table 6. Half-life of DCD (d) in soil (0−10 cm soil depth) on cow-grazed farmlets (n = 9). DCD (10 kg ha^{-1}) was applied in March, April and October 2010 and March and June 2011.

DCD application date	Half-life of DCD (d)				
	Median	Mean	Standard error	Lower 25%	Upper 75%
March 2010	6.9[b#]	6.8	0.7	5.0	8.6
April 2010	10.3[a]	12.3	0.6	9.2	11.2
October 2010	8.4[ab]	9.6	0.5	6.9	13.1
March 2011	6.0[ab]	7.4	2.4	5.8	9.8
June 2011	10.8[a]	11.0	0.7	9.5	12.4

[#]Identical letters in a column indicate values that are not significantly different

Combining all the data from non-grazing and cow-grazing sites, it was found that the half-life of DCD varied seasonally from 6.8 to 12.9 d (Table 7), and was shortest (6.8 – 8.3 d) in March and November 2010 and longest (12.3 – 12.9 d) in April and June 2010. The half-life of DCD is significantly and negatively correlated with soil temperature (Pearson correlation coefficient r = –0.8, P = 0.05) (Fig. 3). Based on this relationship, the half-life of DCD can be estimated from soil temperature as follows (Equation. 4, standard errors in parentheses).

$Y = a X + b$ (Equation. 4)

a = –0.734 (± 0.18), P = 0.004

b = 20.110 (± 2.5), P < 0.0001

R^2 = 0.66

where, Y is the half-life of DCD (d) and X is soil temperature (0−10 cm soil depth, °C)

Table 7. Half-life of DCD (d, mean ± standard error) in soil (0–10 cm soil depth) and mean soil temperature and moisture and cumulative rainfall during DCD lifetime (from initial to the time DCD is not detected). Soil temperature is the mean of values recorded at 30 min intervals (0-10 cm soil depth), soil moisture is the mean of determined values from collected soil DCD samples and rainfall is the cumulative value recorded in each period.

DCD application date	Half-life	Soil temperature (°C)	Soil moisture (%)	Rainfall (mm)
March 2010	6.8 ± 0.7	16.1 ± 0.3	19.2 ± 0.3	17.0
April 2010	12.3 ± 0.6	13.5 ± 0.7	25.8 ± 0.7	20.6
June 2010	12.9 ± 0.9	9.3 ± 1.4	34.9 ± 0.2	53.9
August 2010	10.1 ± 0.7	11.5 ± 1.4	41.1 ± 0.5	145.0
October 2010	9.6 ± 0.5	13.3 ± 1.4	36.0 ± 0.5	42.9
November 2010	8.3 ± 1.3	16.4 ± 1.4	23.2 ± 0.5	8.9
March 2011	7.4 ± 2.2	16.5 ± 0.3	32.0 ± 1.2	29.7
April 2011	12.0 ± 1.0	13.1 ± 0.2	44.0 ± 1.7	156.5
June 2011	11.8 ± 0.7	10.7 ± 0.2	39.4 ± 0.7	213.9
August 2011	11.9 ± 1.5	13.1 ± 0.2	40.4 ± 0.3	156.5

[#]Identical letters in a column indicate values that are not significantly different

Using Equation 4 and the monthly average of soil temperature (40 year average) in Palmerston North, Manawatu, the half-life of DCD for each month was estimated (Table 8).

The half-life was shortest (6.6 d) in January when soil temperature was highest (40 year average 18.3°C), and longest (15.0 d) in July when soil temperature was lowest (40 year average 7°C). The annual average of DCD half-life was 12.7 ± 1.2 d. These results suggest that to optimize the effectiveness of DCD (i.e. inhibiting nitrification and mitigating N_2O emission), different DCD application rates and frequency of application may be needed in different seasons to take account of variation in temperature and rainfall. Further work is also now needed to quantify the effectiveness of DCD at different concentrations of DCD, and taking account of the height of the pasture sward. This will provide better information for managing DCD application to minimise leaching losses and N_2O emissions, as well as enhancing pasture production.

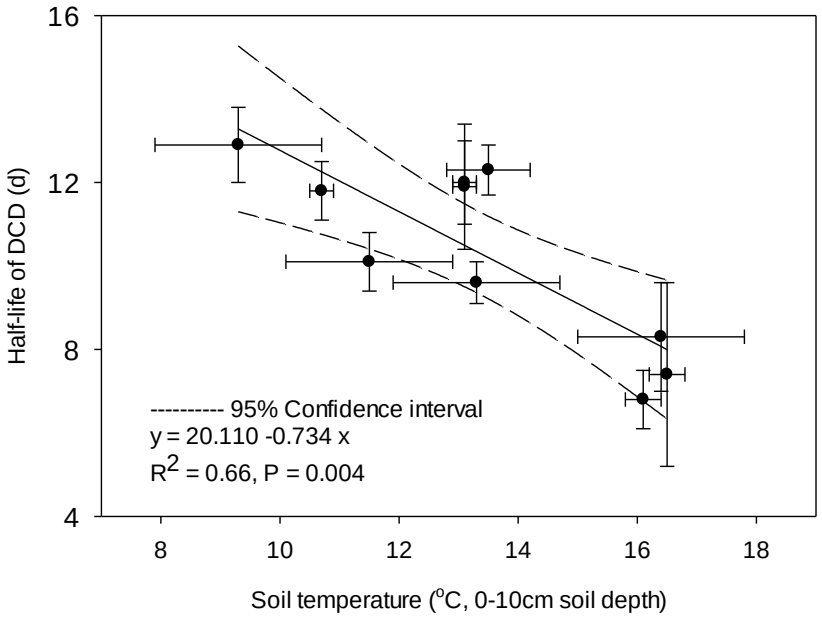

Figure 3. Relationship between the half-life of DCD and soil temperature (°C, 0–10 cm soil depth).

Table 8. Long-term average monthly soil temperature (1971–2010; 0–10 cm soil depth) and estimated half-life of DCD (d) in a Tokomaru silt loam soil.

Month	Soil temperature (°C, 40 years aver.)	Half-life of DCD (d)
Jan.	18.3	6.6
Feb.	18.2	6.7
Mar.	16.1	8.3
Apr.	12.7	10.8
May	10.3	12.5
Jun.	7.8	14.4
Jul.	7.0	15.0
Aug.	8.2	14.1
Sep.	10.2	12.6
Oct.	11.8	11.5
Nov.	14.3	9.6
Dec.	16.9	7.7

4. Conclusions

The results of this study show that 4–40% of applied DCD stayed on plant canopy from less than 6 days and up to 16 days, depending on plant height and the timing of rainfall following DCD application. Half-life of DCD in soil was not affected by either the amount of DCD application (10 or 20 kg ha^{-1}) or the source of N applied (synthetic fertiliser or urine) in a poorly-drained New Zealand dairy-grazed pasture soil. However, half-life of DCD differed with the season and was between 7 and 13 days during March to November. Soil temperature was a major control factor for the variation, and the half-life was longer in lower soil temperature condition. The monthly half-life of DCD in soil was calculated using the derived relationship with soil temperature. DCD half-life in soil ranged from 6.6 days in January to 15.0 days in July (annual average 12.7 ± 1.2 days). These results suggest that to sustain certain levels of DCD concentration in soil to optimize its effectiveness, different amounts of DCD and frequencies of application may be required to account for temperature differences.

An extended version of this paper is also submitted to a peer-refereed journal for dispersion to the international scientific community.

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