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Multiple small monthly doses of dicyandiamide (DCD) did not reduce denitrification in Waikato dairy pasture

NL Watkins[a], LA Schipper[a], GP Sparling[a]*, B Thorrold[b] and M Balks[a]

[a]*Department of Earth and Ocean Sciences, University of Waikato, Hamilton, New Zealand;* [b]*DairyNZ, Hamilton, New Zealand*

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The effectiveness of multiple small doses of the nitrification inhibitor dicyandiamide (DCD) to decrease denitrification under warm moist conditions was tested in a 1-year field trial on a grazed dairy pasture. DCD was applied approximately every 4 weeks as an aqueous spray onto ten replicate plots 3 days after rotational grazing by dairy cows. Each application was at the rate of 3 kg DCD ha^{-1}, with a total annual application of 33 kg ha^{-1}. Denitrification was assessed 5 days after each DCD application using the acetylene block method. At the end of the trial, the rate of degradation of DCD under summer conditions was measured. DCD significantly decreased the mean annual nitrate concentration by about 17%. Denitrification and denitrification enzyme activity were highly variable and no significant effect of DCD in decreasing denitrification was detected. In the summer month of December, DCD degraded rapidly with an estimated half-life of 5 ± 3 days (mean and standard deviation).

Keywords: denitrification; nitrous oxide; pastures; cattle urine; nitrification inhibitor; Dicyandiamide (DCD); New Zealand

Introduction

New Zealand is unusual among developed countries in that about half of the national greenhouse gas (GHG) emissions are produced from soil and animals in the rural sector rather than from urban industry and vehicles (MFE 2012). A further distinction is that 14.6% of total GHG emissions are attributable to nitrous oxide (N_2O). Pastoral agriculture is the dominant agricultural land use in New Zealand, and N_2O emissions from pastoral soils have increased by 23% since 2009 (MFE 2012). N_2O is a particularly potent GHG, being persistent in the atmosphere and with a 100-year global warming potential some 300 times greater than CO_2 (MFE 2012). The main sources of N_2O emissions from pastures are urine spots from grazing animals. Under New Zealand climate conditions, animals are normally kept on pasture the whole year and urine is thus consistently deposited directly on the soil (de Klein & Ledgard 2005). Emissions of N_2O from pastures are expected to increase due to the expansion in dairy farming, with larger dairy herds and higher stocking rates (SNZ 2012), coupled with greater use of N fertiliser (PCE 2004).

Nitrification inhibitors such as DCD (dicyandiamide) and N-Serve (nitrapyrin) were originally intended to improve N retention in soil by blocking the microbial oxidation of ammonium to nitrate. However, the inhibitors also have the potential to alter other components of the nitrogen cycle such as denitrification. Saggar et al. (2007) suggest most N_2O emitted from soil is formed during the

*Corresponding author. Email: sparling@waikato.ac.nz

microbial process of denitrification; that is, when nitrate in soil, in the presence of a C substrate and low aeration, is partly denitrified by soil microbes to N_2O. Di & Cameron (2008) showed that up to 40% of the N_2O emitted from urine patches could be from nitrification, with 60% from denitrification. Substantial decreases in nitrate content and N_2O emissions from New Zealand pasture soils with simulated urine spots treated with DCD were reported by Di & Cameron (2005) and Di et al. (2007). There was an average 70% reduction in N_2O from the simulated urine spots, following application of DCD in a fine particle suspension. In further work, Di & Cameron (2008) showed DCD decreased N_2O emissions during both nitrification and denitrification, resulting in a decrease of 72% in total emissions.

The persistence and effectiveness of DCD in soil are strongly influenced by soil temperature (Kelliher et al. 2008). Di & Cameron (2004) reported a half-life of 111–116 days at 8 °C, which dropped to 18–25 days at 20 °C. Di & Cameron (2004) suggested DCD would be most effective on well-drained soils in late autumn and spring, when soil temperatures would be less than 10 °C. However, in a further study which included two soils and sites in North Island, Di et al. (2007) reported that DCD reduced N_2O emissions by around 50% in both North Island and South Island sites. This was despite the generally warmer climate in the North Island Waikato region, although they did note that the weather pattern at the time of the trial had been unusually cool and dry.

If widespread use of DCD is to be encouraged to reduce NO_3 leaching and/or N_2O emissions, it is important to know what other aspects of the N cycle are affected by this compound. Denitrification is dependent on anaerobic conditions in the soil, available carbon and a sufficient supply of nitrate to act as electron acceptor. The authors were interested to test whether DCD would decrease denitrification (and hence N_2O emissions) by limiting the nitrate supply during the warm and moist months typical of the Waikato region of New Zealand. The Waikato region in North Island contains 30% of the national dairy herd (SNZ 2012) and hence is a major contributor to GHG emissions. It was reasoned that most denitrification would occur during spring and early summer when the soils are typically warm, moist and heavily stocked with cattle. Waikato has one of the highest cattle stocking rates of 2.96 cows per hectare (DairyNZ 2011). The soil temperature at 5 cm depth during the summer is 20–25 °C (Harris et al. 1999, Mudge et al. 2011), with a mean winter temperature of 13.6 °C (Environment Waikato 2006). These are within the range of soil temperatures used in a synthesis by Kelliher et al. (2008) to derive the temperature dependence of DCD degradation. If DCD can persist under warm and moist climate regimes, it was hypothesised that DCD would reduce denitrification by limiting the amount of nitrate available for denitrification.

This paper reports on a 12-month field study carried out on a grazed dairy pasture in the Waikato to determine the effectiveness of DCD in reducing denitrification. DCD was applied in multiple small doses approximately every 4 weeks at a rate equivalent to a total annual application of 33 kg ha^{-1}. This approach differs from that used by other researchers who have normally followed the recommendation that DCD be applied in two or three applications between autumn and late winter. The reason for using multiple monthly small doses was that rapid degradation of DCD during the summer months was anticipated and maintenance of a baseline concentration in the treated plots was sought. Also wanting the application method to follow normal farm practices, in contrast to other studies the plots were not irrigated following DCD application. Irrigation is not common practice in the Waikato region with less than 5000 ha of irrigated land. This contrasts greatly to other areas in New Zealand such as Canterbury where in 2009 there were an estimated 363,614 ha of irrigated land (New Zealand Government 2010).

Rates of denitrification on treated and non-treated plots were assessed using the acetylene block technique and ancillary measures of soil nitrate and ammonium contents, soil moisture and temperature, respirable C and denitrifying enzyme activity (DEA). We also measured the persistence of DCD in the field during summer at the end of the trial.

Methods

Field site and soil

The field trial was located at the Scott Research Farm (37°47′, 175°19′, 40 m a.s.l.) managed by DairyNZ near Hamilton, New Zealand. The soil at the study site was a Horotiu silt loam derived from volcanic ash (Typic Orthic Allophanic Soil; Hewitt 1998). The Horotiu soils have medium to low dry bulk densities, moderate permeability and high phosphate retention. Typical Horotiu topsoil contains 8.2% C, 0.75% N, has a pH of 5.3, total P of 2.4 g kg^{-1} and a bulk density of 0.8 Mg m^{-3} (Singleton 1991).

An experimental area for the DCD study was established on the control plots of a field experiment investigating grazing intensity, stocking rates, fertiliser use and supplementary feeds (Jensen et al. 2005). The control plots represented current average farming practice. The trial had been running since 2001 and carried 3.0 dairy cows per hectare, had an annual pasture production of 17.5 Mg dry matter per hectare, was not irrigated and received 200 kg N fertiliser each year. The pasture composition was dominated by ryegrass, paspalum and white clover.

Soil moisture content at the field site was continuously measured using time domain reflectometery (TDR) model CS 625 (Campbell Scientific Inc.). Rainfall at the site was measured using a tipping bucket. A CR200 data logger collected the soil moisture content and rainfall data every 10 seconds; these data were averaged for over 30 minutes and stored for later use.

Trial design

Two treatments were used: (1) control pasture plots that were grazed and fertilised as described above; (2) treatment pasture plots that also received DCD. DCD was applied at the rate of 33 kg ha^{-1} yr^{-1}: this was similar to annual rates used in other New Zealand studies (e.g. Williamson et al. 1996, Di & Cameron 2005). Twenty replicated field plots on a uniform area of pasture were established in a randomised plot design. Each plot measured 4 m by 4 m and had a 0.5 m guard strip between the plots. Each plot was randomly assigned one of the two treatments and there were ten replicates of each treatment. For each 4 m by 4 m plot, 4.8 g of DCD was dissolved in 5 litres of water (equivalent to 3 kg DCD ha^{-1} per application) and applied using a backpack sprayer. Plots receiving DCD were sprayed approximately every 4 weeks (Table 1), giving a total annual application of 33 kg ha^{-1} yr^{-1}. DCD was usually applied 3–5 days after rotational grazing of the paddocks. However, depending on pasture growth, on some occasions rotational grazing was longer than the normal 20–25 day cycle (January, June and August) and in that case DCD was reapplied before grazing so as to maintain the weekly pattern (Table 2). The application of DCD 3 days after grazing was preferred as this ensured short pasture to maximise the quantity of DCD reaching the soil surface. Natural rainfall was used to wash any DCD on foliage into the soil. Monthly rainfall varied from 30 to 145 mm, the driest months being September (31 mm) and February (48 mm) and the wettest May, August and January (139, 139 and 143 mm respectively).

Denitrification rates were measured 5 days after the application of DCD using the acetylene inhibition method described later.

Degradation of DCD in the field

Duplicate soil cores 2 cm diameter by 10 cm depth were collected in December 2006 from

Table 1 Timetable indicating the dates cows grazed the field trial, dates of application of DCD (3 kg ha^{-1}) after grazing and dates of soil sampling 5 days after DCD application

Sampling month (2006)	Cows grazed	DCD application	Sampling date
January	6 January	12 January	–
February	22 January	26 January	1 February
March	19 February	23 February	1 March
April	Not sampled	Not sampled	Not sampled
May	22 April	26 April	2 May
June	26 May	1 June	–
July	26 June	30 June	4 July
August	26 July	1 Aug	–
September	1 September	5 September	11 September
October	24 September	28 September	4 October
November	30 October	3 November	9 November
December	21 November	5 November	1 December

four replicate plots that had received DCD for 11 months previously. Cores were collected daily for the first 6 days following DCD application (3 kg ha^{-1}) and then every second day for a further 12 days. For each day, cores were further bulked to give two replicates per day. The cores were sieved to <4 mm, stored at 4 °C overnight and analysed for the presence of DCD as described below.

Analytical methods

Field measurements of in situ denitrification rates were made using the static soil core incubation system described by Ryden et al. (1987). Briefly, from each replicate plot, four intact soil cores (3.2 cm diameter, 16 cm depth) were collected using perforated PVC liners that allowed gaseous exchange. Four replicate cores were incubated in a 1.8 l glass preserving jar fitted with a gas sampling septum. Acetylene was added to the jar to obtain 10% v/v concentration and mixed with the headspace air by flushing with a 60 ml syringe. The jars were then placed in a temperature-controlled room set to the soil temperature at the time of sampling. Headspace gas samples (22 ml) were taken from the jars 0.5, 3, 6 and 24 h after the addition of acetylene, injected into Vacutainer tubes to obtain positive pressure and stored for subsequent analysis. The concentration of N_2O in the headspace gas was obtained using a Philips gas chromatograph with an electron capture detector as described by Schipper et al. (2005). The accumulation of N_2O in the presence of acetylene represents the total production of N_2O and N_2 from denitrification (Tiedje et al. 1989). Hourly denitrification rates were calculated from headspace concentration at each sampling time, and corrected for the solubility of N_2O in the soil water using the temperature-dependent Bunsen absorption coefficients (Tiedje 1994).

Denitrifying enzyme activity (DEA) was measured following the methods of Tiedje et al. (1989). DEA is a commonly used method to determine relative amounts of microbes in soil capable of denitrifying (Tiedje et al. 1989). Soil (10 g) was incubated in 100 ml Schott bottles in the presence of 20 ml of glucose–nitrate solution (0.2 g glucose and 0.1 g KNO_3 per litre) and containing 0.125 g chloramphenicol per litre to prevent protein synthesis. The bottles were sealed with lids fitted with a gas sampling septum and flushed for 2 minutes with nitrogen gas. Acetylene (10 ml) was added to inhibit the conversion of N_2O to N_2. The samples were incubated at 25 °C on a rotary shaker and 5 ml of headspace was removed after 15 and 75 min and injected into 3 ml Vacutainer tube to generate

Table 2 Monthly averages (ten replicates ± standard error) of soil moisture content, temperature at 10 cm depth, respirable C, ammonium and nitrate concentrations of Horotiu silt loam with and without amendment with DCD every 4 weeks at the rate of 3 kg ha $^{-1}$.

			Respirable C (μg CO_2–C g $^{-1}$ h $^{-1}$)		Ammonium-N (μg N g $^{-1}$)		Nitrate-N (μg N g $^{-1}$)	
Month (2006)	Moisture content (% w/w)	Temperature at 10 cm depth (°C)	Control	DCD	Control	DCD	Control	DCD
January	59.6	19	3.19 ± 0.3	3.91 ± 0.2	2.8 ± 0.4	3.0 ± 0.3	23.6 ± 5.1	32.3 ± 6.6
February	65.4	23	5.84 ± 0.6	6.39 ± 0.7	6.3 ± 0.6	6.0 ± 0.5	51.5 ± 8.2	55.4 ± 7.5
March	55.4	19	5.06 ± 0.2	5.31 ± 0.4	16.4 ± 9.3	22.9 ± 9.8	64.6 ± 7.8	58.2 ± 6.1
April	NT	NT	NT	NT	NT	NT	NT	NT
May	74.7	15	4.49 ± 0.2	3.96 ± 0.2	5.7 ± 1.9	9.8 ± 2.6	23.5 ± 3.5	19.7 ± 1.8
June	79.9	10	4.55 ± 0.5	5.78 ± 0.4	1.4 ± 0.2	2.9 ± 0.7	4.6 ± 0.5	4.1 ± 0.4
July	78.1	4	3.87 ± 0.4	3.91 ± 0.3	50.8 ± 7.8	63.5 ± 6.4	7.9 ± 0.8	5.1 ± 0.4
August	74.0	12	2.98 ± 0.2	3.02 ± 0.2	21.0 ± 4.2	42.3 ± 7.2	17.6 ± 3.0	11.1 ± 1.9
September	78.3	14	4.13 ± 0.7	4.33 ± 0.4	30.5 ± 7.4	46.5 ± 7.2	22.5 ± 2.1	8.5 ± 0.8
October	79.8	15	4.24 ± 0.4	4.77 ± 0.5	26.7 ± 11.3	25.8 ± 8.8	17.4 ± 3.7	15.1 ± 4.6
November	82.5	16	4.46 ± 0.2	4.46 ± 0.4	4.8 ± 0.3	5.7 ± 0.5	27.9 ± 8.0	15.1 ± 2.0
December	76.9	16	3.34 ± 0.6	3.96 ± 0.4	8.6 ± 0.4	12.9 ± 2.2	43.5 ± 7.2	28.8 ± 2.6
Mean ± SE	73.1 ± 0.63	14.8 ± 1.5	4.20 ± 0.15	4.53 ± 0.15	15.9 ± 2.14	21.9 ± 2.45	27.7 ± 2.27	23.0 ± 2.07

NT = not tested.

positive pressure and stored for later analysis. The headspace gas samples were analysed for N_2O as described earlier.

Analysis for DCD followed the method of Schwarzer & Haselwandter (1996). Sieved soil samples were extracted by shaking 20 g dry weight equivalent with 100 ml distilled water at 20 °C for 1 h on an end-over-end shaker. The sample was then centrifuged at 14,500 rpm for 5 minutes, filtered through Whatman #42 paper and frozen until subsequent analysis using a Shimadzu high-performance liquid chromatograph (HPLC), with a 300 × 7.80 mm Aminex organic acid column HPX-87H.

Respirable C was estimated by incubating moist soil (35 g equivalent dry weight) in a sealed 1.8 l preserving jar fitted with a gas sampling septum. The jars were incubated for 7 days at 25 °C and the CO_2 concentration in a sample of the headspace gas measured on a LiCor infra red gas analyser (Sparling & Zhu 1993).

The total C and N content of the soil samples were determined by dry combustion on air-dry, finely ground soils using a Laboratory Equipment Corporation (LECO) TruSpec carbon/nitrogen determinator, using software version 1.6 × (LECO Corporation 2006).

Soil nitrate and ammonium concentrations were measured by shaking 10 g soil with 100 ml of 2 M KCl for 1 h and filtering through Advantec 5C filter paper into extraction bottles. The samples were then frozen until subsequent analysis using standard AutoAnalyser methods (Blakemore et al. 1987). Soil pH was measured in a 1 part soil 2.5 parts water paste using a calibrated glass electrode as described by Blakemore et al. (1987). Soil moisture content was determined gravimetrically on each sampling date, from the weight loss of a sub-sample dried overnight at 105 °C (Blakemore et al. 1987). Soil temperature was measured at a depth of 10 cm at 0830 h on the day of field sampling.

Data analysis

Analysis of variance (ANOVA) was performed on denitrification rates, ammonium and nitrate concentrations, DEA, carbon availability and pH to determine whether there were significant differences ($P < 0.05$) between control plots and DCD-amended plots using Statistica version 7.1 (StatSoft Inc. 2007). Denitrification rates, DEA, and ammonium and nitrate data were log-transformed prior to analysis, while respirable C and soil pH values were squared prior to analysis to normalise the data.

Results

Soil temperature and moisture

Mean soil temperature at 10 cm depth was lowest (5–10 °C) in the winter months of June and July and highest (19–24 °C) in the summer months of January, February and March (Table 2). Rainfall was distributed throughout the year, but February (48 mm) and September (31 mm) were markedly drier than the other months. Ignoring those two dry months, the average rainfall was 117 ± 26 mm per month (mean and SD). The soil moisture content fluctuated very little, remaining consistently around 73% w/w (Table 2).

Denitrification

Denitrification rates were highly variable. They were highest in November (46–168 kg N ha^{-1} yr^{-1}) and lowest in January (0.7–0.8 kg N ha^{-1} yr^{-1}). Annual rates of denitrification were 14 ± 3.56 kg N ha^{-1} (mean and standard error, $n = 110$) for the control plots and 28 ± 8.26 kg N ha^{-1} for the DCD-treated plots. There were no significant differences between the two treatments at any of the sampling times (Fig. 1A).

Denitrification enzyme activity

DEA was highly variable, being greatest in summer (January) and least in August and September. Annual hourly rates were 311 ± 38 ng N g^{-1} for the control plots (mean and standard error, $n = 110$) and 368 ± 48 ng N g^{-1}

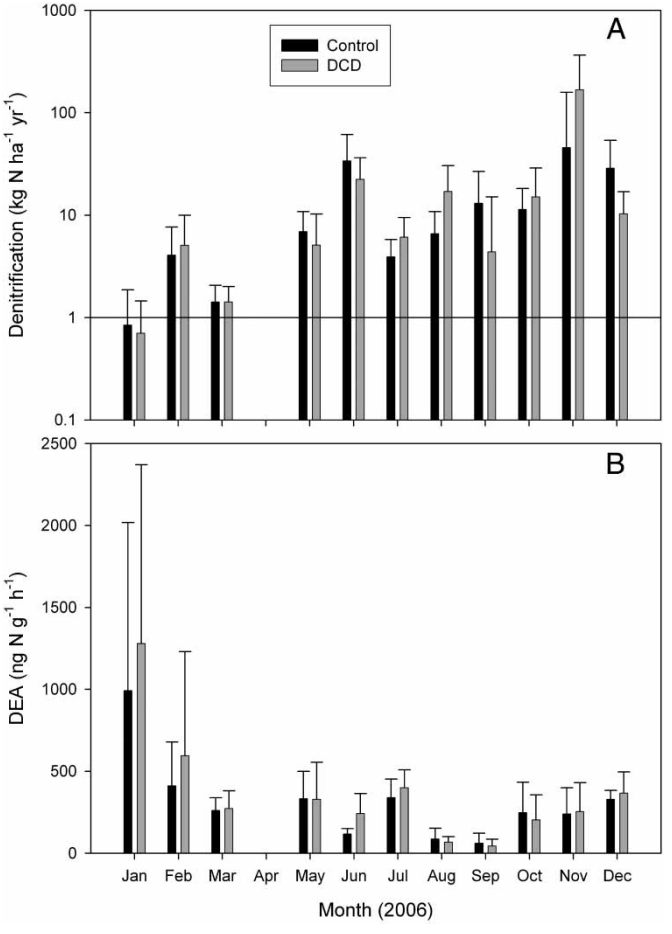

Figure 1 Monthly rates of **A** denitrification and **B** denitrification enzyme activity in grazed pasture plots receiving DCD at an annual rate of 33 kg ha^{-1} yr^{-1}, applied every 3 weeks at the rate of 3 kg ha^{-1}, compared with non-treated control pasture plots. No sampling was done in April. Note logarithmic scale in graph **A**. Bars show standard error.

for the DCD-treated plots. There were no significant differences between the treatments at any of the sampling times (Fig. 1B).

Respirable C and soil pH

Respirable C was reasonably constant throughout the year, fluctuating between 2.98 and 6.39 μg C g^{-1} h^{-1}. There was no significant difference between the DCD and control plots (Table 2). Soil pH fluctuated between 5.5 and 6.4, with no clear trend relating to season or treatment and no significant differences between treatments (data not presented).

Ammonium and nitrate concentrations

There was considerable seasonal variation in ammonium and nitrate concentrations (Table 2). Ammonium was generally higher in the winter months (July–September) while nitrate was generally higher in the summer (January–March). Overall, ammonium-N concentrations were significantly greater ($P < 0.01$) on the

plots receiving DCD (annual mean 21.9 μg g^{-1}, compared to 15.9 μg g^{-1} on the control plots), and nitrate-N concentrations on the DCD plots were significantly ($P < 0.01$) lower than the controls (annual mean 23.0 μg g^{-1} on the DCD plots and 27.7 μg g^{-1} on the control plots).

Degradation of DCD in the field

The average soil temperature during December 2006 when the degradation study was undertaken was 16 °C at a soil depth of 10 cm. In the first 2 days of the experiment, 3 mm of rain fell. The calculated amount of DCD added to the soil was 3.7 μg g^{-1} soil, assuming even distribution through the 10 cm soil depth. The amount recovered 1 day after application was 3.69 μg g^{-1} soil, representing almost full recovery (99%) of the calculated amount. Beyond 1 day, the amount of DCD recovered declined in a logarithmic manner (Fig. 2) and after 15 days was no longer detectable. The half-life of DCD calculated for each of the sampled times over 0–15 days, and using a starting concentration of 3.69 μg g^{-1} soil, was 5.5 ± 3.2 days (mean and standard deviation).

Discussion

In common with other studies (e.g. Thompson 1989; Di & Cameron 2004, 2008) DCD changed the N cycle in grazed pasture soil; ammonium concentrations were significantly higher by around 38% and nitrate concentrations significantly lower by around 17% in the plots receiving DCD. However, this study did not detect any significant reduction in the monthly rates of denitrification or on DEA, despite the lower nitrate concentration. It has been hypothesised that a reduced level of nitrate in pasture plots receiving DCD would limit denitrification. This hypothesis was not supported, even though nitrate levels were lower in the plots receiving DCD. Ryden (1983) suggested that, for nitrate to limit denitrification, the concentration must be below 5 μg NO_3–N g^{-1}. In the current study, the nitrate concentration fell below this value in only one month (June), even on the plots receiving DCD. For 10 of the 11 months sampled, nitrate concentrations were well above the 5 μg NO_3–N g^{-1} threshold and this is one reason why a decrease in denitrification was not detected.

Overall annual rates of denitrification (14–28 kg N ha^{-1}) were close to the mean of 13 kg N ha^{-1} reported by Barton et al. (1999)

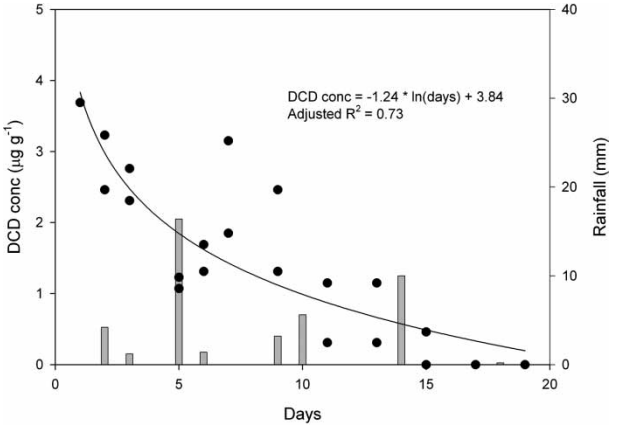

Figure 2 Degradation of DCD after application to dairy farm soils during the month of December fitted to an exponential decay curve (DCD μg $g^{-1} = 1.24$ ln(days) + 3.84). Vertical bars show amount (mm) and occurrence of rainfall.

for agricultural soils. The generally low rate of denitrification could possibly be attributed to the moisture content of the soil, which was consistently around 70%, and the soil may not have reached the waterfilled pore space of 62–83% v/v, above which denitrification is considered to be enhanced in loam soils (Barton et al. 1999).

The total annual amount of DCD applied (33 kg DCD ha^{-1}) in the study was at the upper end of the ranges of 10–30 kg ha^{-1} used in other works (Merino et al. 2001, Cookson & Cornforth 2002, Macadam et al. 2003) and considerably more than the 10–20 kg ha^{-1} used in recent New Zealand studies by Di & Cameron (2005) and Di et al. (2007). DCD has usually been applied in one or two large doses, and one difference with the current study was that the DCD was applied in multiple small doses of 3 kg ha^{-1} every 3–4 weeks. The intention was to maintain the concentration in surface soil to compensate for an anticipated rapid rate of degradation in the soil. However, Di & Cameron (2005) reported that a single application of 5 kg DCD ha^{-1} was not effective in obtaining the desired benefits on nitrification and denitrification. While the monthly rate of DCD application in this research was less than in other studies, the annual amount was greater; greater ammonium concentrations and lower nitrate concentrations were detected in the DCD plots, suggesting that DCD was at least partially effective at blocking nitrification. In contrast to these findings, Di et al. (2007) reported nearly 70% reduction in N_2O emissions from a Waikato Horotiu soil when DCD was applied in two annual doses of 10 kg ha^{-1}, which suggests that the DCD was persistent and effective at reducing denitrification when applied at higher rates.

The rates of degradation of DCD in this study were very much faster than those reported in New Zealand literature. Di and Cameron (2004) reported that the half-life of DCD was 18–25 days at 20 °C whereas, with an average field soil temperature at 10 cm depth of 16 °C, the half-life is this study was 5.5 days. Williamson et al. (1996) reported a half-life of 39 days at 22 °C. Rajbanshi et al. (1992), working on soils in Germany, reported that between 10 and 30 °C, the decomposition rate of DCD was doubled by a 10 °C rise in temperature and, at 30 °C, half-lives were 2.9–11.5 days. The data from the current study fall within that latter range, although the temperature at 10 cm depth was about half of the maximum used by Rajbanshi et al. (1992).

This study relied on natural rainfall to wash any DCD retained on foliage into the soil, and the high recovery of DCD at early stages of the degradation experiment suggests that DCD had indeed been incorporated into the soil. However, it is possible that it was not distributed to the full depth of soil sampled and may have been retained in the top few centimetres. It is likely that the soil temperature in the top few centimetres of soil would have been considerably greater than the 16 °C measured in the Horotiu soil at 10 cm depth. Mudge et al. (2011) recorded surface soil temperatures (5 cm) at Scott Farm of 20–25 °C during summer and Harris et al. (1999) reported that the soil temperature during summer could reach a maximum of 36 °C at 5 cm depth. The surface Horotiu soil would also be likely to support a very active microbial population and to have a high organic matter content (Sarathchandra et al. 1984; Sparling et al. 2001). Organic matter can sorb DCD and reduce its effective concentration (Kelliher et al. 2008), and an active microbial community would assist with rapid degradation. The combination of a high soil temperature in the surface, DCD being retained in surface soil, organic matter sorption and rapid microbial degradation are likely explanations for the decline in DCD concentration and the shorter half-life than have previously been reported. The persistence of DCD for only 15 days during summer strongly suggests that for half of that month there would not have been any DCD present for it to be effective. Our estimate of a half-life of 5.5 days for DCD suggests that any month

with a temperature greater than 25 °C in the surface soil could be expected to show a similar trend (Kelliher et al. 2008). The soil temperature at 10 cm depth fell below 10 °C in only one month (July) and was 15 °C or above for 7 months of the year.

A further complication in demonstrating any effects of DCD was the high variability in our dataset, which meant that differences between treatments were seldom significant. The plots were rotationally grazed by dairy cows and dung and urine was naturally deposited on the experimental plots. Such deposits are known to be randomly distributed, leading to highly variable concentrations of soluble nitrogen (Moir et al. 2011). Over a 12-month rotational grazing we would expect about a quarter of the plot surface to have received deposits of dung and urine. The soil sampling routine used here was random, but avoided obvious dung deposits. The high number of replicates and bulking of soil cores was intended to reduce this soil variability but, even so, on a monthly basis, no significant differences were detectable. It was only by combining all the monthly data that we were able to demonstrate significant annual differences in nitrate and ammonium concentrations between the DCD-treated and control plots.

The rapid degradation of DCD in the field, sorption to soil organic matter and our approach of using low monthly applications may be some reasons why DCD was only partly effective in blocking nitrification and hence limiting the supply of nitrate to the denitrifying microbes. In view of the apparently rapid degradation of DCD under summer conditions in this volcanic ash soil, to achieve a substantial reduction in nitrate levels and influence denitrification, it is likely that much larger amounts of DCD will need to be applied than used here, and several applications may still be required. Small monthly applications do not appear effective, even if the total annual application is large. Further work is needed to determine whether nitrification inhibitors will be sufficiently effective to limit denitrification under warm and moist climate conditions and whether such approaches are economically feasible and practical. The reliance on natural rainfall to wash DCD into soil also needs further investigation, as does the distribution of DCD through the soil following application to non-irrigated soils.

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