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Manatū Ahu Matua

Effect of soil aeration status on the degradation of DCD in soil

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Executive summary

- Dicyandiamide (DCD) is a nitrification inhibitor that has been used in New Zealand's agriclutural systems to reduce nitrate leaching and nitrous oxide (N_2O) emissions. The efficacy of DCD at reducing both nitrate leaching and N_2O can vary with season and soil type. One reason for this is the variation in seasonal soil temperature, since biological degradation of DCD is influenced by temperature. Other possibilities included the level of organic matter in the soil and the degree of soil aeration. Few studies have examined how soil organic matter influences DCD degradation while no studies have performed controlled experiments to determine the effect of soil aeration on DCD.
- Thus the objectives of the studies performed here were firstly to assess how a soil's aerobic status and organic matter content affected DCD degradation in the absence of ruminant urine being present. Then secondly, to determine how a soils aeration status affected DCD degradation when ruminant urine was present.
- Two experiments were performed. In the first factorial experiment DCD degradation was followed over 40 days in a silt loam soil with two levels of organic matter x four levels of aeration, using repacked soil cores. Soil aeration was determined by setting set levels of soil moisture and was measured by recording relative gas diffusivity of the soil cores at the varying soil moisture levels. The results of experiment 1 clearly show that DCD degraded faster when more soil organic matter was present under aerobic soil conditions. When soils were anaerobic DCD degradation did not differe with level of organic matter present.
- In the second experiment, DCD degradation was again followed over 40 days in repacked soil cores, in the same silt loam soil with the higher level of organic matter, at two levels of aeration, with and without ruminant urine present. The degradation of DCD was again lower under anaerobic conditions but lower still under anaerobic conditions with urine present. When soil was aerobic with urine present the rate of DCD degradation was higher. Measures of inorganic-N, soil pH, and dissolved organic matter corresponded to expected time trends as previously seen from urine studies. The efficacy of DCD in reducing urine-N derived cumulative N_2O-N emissions was higher under anaerobic conditions (95±3% (stdev)) compared to the aerobic conditions (57±10%(stdev)) with this variance in efficacy due to the variation observed in the DCD degradation constants.
- The data collated here also explain why seasonal variation occurs with regard to DCD efficacy. Not only does soil temperature have a role to play in degradation but so too does the soil moisture status and its organic matter content.

Literature review

INTRODUCTION

The loss of nitrogen (N) as nitrous oxide (N_2O) and nitrate (NO_3^-) from grazed pasture systems, particularly under urine patches, is of environmental concern. Nitrous oxide is a greenhouse gas and also a precursor to compounds that deplete ozone layer (Crutzen, 1981). Nitrate, being negatively charged, is not retained by the soil and is carried by drainage waters and is the dominant form of N leached, which consequently leads to surface water and ground water contamination (Di and Cameron, 2002, Di and Cameron, 2007, Mc Dowell and Houlbrooke, 2009). Silva et al. (1999) showed that NO_3^- concentrations in the drainage water under a urine patch ranged from 95 to 155 mg N L^{-1}.

Thus, to mitigate these N losses, major efforts have been devoted towards developing methodologies for N management under intensively grazed pasture systems. Many countries have been investigating nitrification inhibitors as a strategy to reduce NO_3^- leaching and N_2O emissions from agricultural soils (Serna et al. 1995). The production of synthetic nitrification inhibitors gained momentum in the 1960s, particularly in United States, Japan and West Germany (Amberger, 1989). Dicyandiamide (DCD) is one such nitrification inhibitor and in recent decades it has received significant attention due to its lower cost and effectiveness (Trenkel, 1997). However, DCD's effectiveness in reducing N_2O and NO_3^- leaching losses from soil depends on its duration in the soil, which is a function of soil type and environmental conditions. Most of the New Zealand studies to date have focused on DCD's efficacy at inhibiting nitrification, and as a consequence N_2O and NO_3^- leaching (Di and Cameron 2002, 2003, 2006, 2007; Smith et al. 2008; Moir et al. 2007). Relatively few studies have examined the biophysical loss of DCD in soil via biological degradation and physical loss through leaching and runoff. Moreover, there are no studies examining the effect of soil aeration status and organic matter availability on its degradation in soil and subsequently its effectiveness in reducing urine-derived N_2O emissions.

DICYANDIAMIDE

Dicyandiamide ($C_4H_4N_4$, DCD) is a white crystalline powder with relatively high water solubility (23 g L^{-1} at 13°C). It is a dimer of cyanamide which consists of about 65% N and is a physically and chemically stable compound (Amberger, 1989). It is abiotically stable in water and is not hydrolysed regardless of pH (UNEP publication). It is a non-toxic compound with an LD-50 value 3 times higher than that of table salt (NaCl). DCD is also non- volatile and non-hygroscopic compound (Amberger, 1989, Serna et. al, 1994). All these properties make DCD one of the more environmentally benign nitrification inhibitors (Callaghan et al. 2010) and allow it to be easily and effectively formulated with various N fertilizers (Serna et al. 1994). DCD is also known by different names such as cyanoguanidine, N-cyanoguanidine, Didin (Ashworth and Rodgers 1981; OECD, 2003; Schwarzer et al.1998; Zang et al. 2004).

MODE OF ACTION

Dicyandiamide (DCD) is known to inhibit the first stage of nitrification i.e. the conversion of NH_4^+ to NO_2^- which results in accumulation of ammonium ions in the soil (Amberger, 1989). DCD works by deactivating the enzyme ammonia monooxygenase which is responsible for converting ammonia to hydroxyl amine (Amberger, 1989, Di et al; 2009). DCD particularly inhibits *Nitrosomonas europaea*. This inhibition occurs due to the reaction between the nitrile group on the DCD molecule with either sulfhydryl or a heavy metal group of the respiratory enzymes of the bacteria (Amberger 1989). In an experiment using a pure culture of *Nitrosomonas europaea*, it was found that NO_2^- formation was inhibited at DCD concentrations of 200-300 mg L^{-1}. However, in DCD free medium, the ability of the same bacterial culture to oxidise NH_4^+ was increased to 90%. This indicated that DCD is bacteriostatic and not a bactericidal compound (Amberger, 1989). Thus DCD has the potential to reduce both, nitrification and denitrification as less oxidation of NH_4^+ means less NO_3^-, which is an essential substrate for denitrification. Application of DCD to soil also helps in retaining N in the NH_4^+ form for a longer time, providing greater opportunity for plant uptake of NH_4^+. Other studies indicate that DCD does not have adverse effects on soil agrobacteria, methanotrophs, soil respiratory activity and microbial biomass (O' Callaghan 2010; Di et al. 2011; Singh et al. 2008). Another recent study by Gou et al. (2013) confirmed that DCD does not affect non-target microbial and enzyme activities in soil.

USE OF DCD IN NEW ZEALAND

In New Zealand, pastoral agriculture dominates and animals are grazed all year round. Nitrogen is regularly deposited onto soils in the form of animal excreta. Agricultural N_2O emissions in New Zealand contributed about 95% of the total N_2O emissions in 2010 (Ministry for the Environment, 2012). Deposition of animal excreta, particularly urine, on the intensively grazed grasslands is a major source of direct N_2O emissions and NO_3^- leaching (Di et al. 2007). These N losses are likely to increase further with increased fertilizer inputs and stocking rates. Almost 70-90% of N ingested by ruminants is returned to the pasture in the form of animal dung and urine (Haynes and Williams, 1993). The N loading rate under a urine patch may vary and can reach 1000 kg N ha^{-1} (Whitehead, 1995). This rate of N is too high for immediate utilization by plants or microorganisms and is either leached as NO_3^- via drainage or emitted as N_2O via nitrification and denitrification. Di and Cameron (2008) showed that almost 40% of the N_2O emissions derived from nitrification while 60% were attributed to denitrification. Most of the NO_3^- leaching in New Zealand occurs during the high risk period which extends from the late autumn to early spring when the temperatures are low and drainage is high (Di and Cameron, 2005).

In New Zealand, the first reported study with DCD was by Francis et al. (1985) who showed that DCD was effective in reducing NO_3^- leaching, during pasture renovation, by 30-45%. However, the use of DCD as a mitigation strategy to reduce N_2O and NO_3^- leaching losses only became common from 2004 when Di and Cameron (2002, 2003) showed that DCD was effective in reducing NO_3^- leaching and N_2O emissions from urine patches. Di and Cameron (2002) showed in a lysimeter study that the NO_3^- leaching from a Lismore stony silt loam soil decreased by 76% for urine-N applied in autumn while it decreased by 42% for urine-N applied in spring following DCD application. They also showed that the use of DCD also increased herbage production by >30%. In another study by Di and Cameron (2003), it was shown that urine-derived N_2O emissions were decreased by 76% after DCD application in autumn while these emissions decreased by 78% in spring. In New Zealand, prior to its removal from the market place, DCD was generally applied to soil at a rate of 10-15 kg ha^{-1} per application using spray application technology (Di and Cameron, 2005).

FATE OF DCD IN SOIL

Dicyandiamide reportedly undergoes photo-degradation via indirect photo oxidation by hydroxyl radicals present in the atmosphere (UNEP, 2003). However, in soil Amberger (1989) suggested that DCD was initially decomposed as a consequence of the interaction with metal oxides such as iron oxides and hydroxides, which results in the formation of guanyl urea with it being further decomposed to produce CO_2, NH_3 and H_2O via guanidine and urea. However, Hallinger et al. (1990) showed that the process of DCD degradation in soil was controlled by soil microorganisms. Schwarzer and Haselwandter (1991) also proved that the degradation of DCD was enzyme based and was not due to the interaction with metal oxides. Rajbanshi et al. (1992) produced similar results and showed that under sterile conditions DCD concentrations did not change for 36 days while DCD degraded in 7 days when soil was re-inoculated with bacteria.

FACTORS AFFECTING DEGRADATION OF DCD IN SOIL AND ITS NITRIFICATION INHIBITION EFFICACY

Since the rate of DCD decomposition in soil is influenced by microbial activity (Schwarzer and Haselwandter, 1991; Hallinger et al. 1990) it will be altered by soil factors such as temperature, parent material, moisture, pH, and the DCD application rate.

Soil temperature

Many published studies have reported the effect of soil temperature on the degradation of DCD. Vilsmeier (1980) reported the effects of temperature on the degradation of DCD in a sandy silt loam soil with a pH of 6.2. In that study it was shown that 0.67 mg DCD-N 100 g $soil^{-1}$ was degraded to 0.60, 0.40, and 0.10 mg 100 g $soil^{-1}$ at 8ºC, 14ºC and 20ºC, respectively. Bronson et al. (1989) showed in a study using two contrasting soil types (silt loam and loamy sand) that the degradation rate of DCD increased with increasing temperature with greater half-life at lower temperature. Another study by Amberger et al. (1989) showed similar findings. Rajbanshi et al. (1992) also examined the decomposition kinetics of DCD at 3 different concentrations and at varying temperatures of 10, 20 and 30ºC. They showed that the decomposition of DCD in soil followed zero order-kinetics and a 10ºC increase in soil temperature doubled the degradation rate of DCD in a grassland soil. A seasonal difference in the degradation of DCD in soil has also been shown by Corre and Zwart (1995) in a study that found the degradation of DCD was slower during the winter and faster in the spring. Willamson et al. (1996) also showed a clear relationship between soil temperature and DCD degradation following application of dairy effluent. A study by Di and Cameron (2004) examined the effect of soil temperature on DCD degradation by incubating a Lismore silt loam soil at two temperatures, 8º and 20º C. They found that the degradation of DCD at 20ºC was faster with a half- life of 18-25 days while at 8ºC, the half-life was111-116 days. Guiraud et al. (1992) studied the influence of temperature on the nitrification inhibition activity of DCD and found that only 10% of ^{15}N applied was nitrified over a period of 6 months at a temperature of 10ºC while at higher temperature there was an increase in the degradation of DCD. Menneer et al. (2008) also found that the effectiveness of DCD as a nitrification inhibitor was greater in winter while it was limited in the autumn period. This decrease in effectiveness of DCD in inhibiting nitrification was attributed to the rapid degradation of DCD in autumn due to higher soil temperatures. Irigoyen et al. (2003) examined the effects of temperature on the ammonium (NH_4^+) oxidation kinetics in soil in the presence of DCD and found that the inhibitory capacities of DCD prevailed for 1 week, 1 month or more than 3 months when soil temperatures were 30, 20 and 10ºC, respectively.

In a data synthesis, Kelliher et al. (2008) reported an exponential decrease in the half-life of DCD as the soil temperature increased. They concluded that DCD should be applied when the soil temperature is low (< 10°C) in order to maximise its longevity in soil and effectiveness in inhibiting nitrification. In a more recent study changes in DCD concentrations were measured following its application to New Zealand dairy-grazed pasture and non-grazed pasture soils and it was shown that the half-life of DCD varied with season from March to November and decreased linearly with increases in soil temperature from 10.7 to 16.5°C (Kim et al. 2012).

Soil moisture and aeration

Very few studies in the literature report on the effect of soil moisture and soil aeration with respect to DCD's fate in soil. In a laboratory study Puttanna et al. (1999) showed that DCD was more effective in inhibiting nitrification at 40% WHC (water holding capacity) where it showed 52% inhibition while at higher WHC of 60% and 80%, the nitrification inhibition was 39% and 32 %, respectively. In another study by Amberger and Vilsmeier (1988), it was shown that when 20 mg L^{-1} DCD was applied to sediments flooded with water to a height of 10 to 60 cm it was found that DCD was completely decomposed within 1 year under aerobic conditions while only 2/3rd of DCD was degraded under anaerobic conditions. More studies are required to completely comprehend the effect of soil moisture and aeration status on the fate of DCD in soil.

Soil type, pH and organic matter

Soil type has been shown to effect the degradation of DCD in soil. In a series of laboratory experiments by Reddy (1964) it was shown that the decomposition of DCD was faster in a fine textured Cecil sandy loam soil with relatively more organic matter content that coarse textured Lakeland sandy soil with low organic matter content. They concluded that both soil type and organic matter content of the soil should be evaluated in order to examine the fate of DCD in soil. In an incubation study by Singh et al. (2008) it was found that the degradation of DCD was fastest in a brown loam allophanic soil and was slowest in a silt loam non-allophanic soil. These differences were attributed to the differences in the adsorption of DCD and microbial activities in soils. Zang et al. (2004) examined the sorption-adsorption behaviour of DCD on different soils and found that the adsorption of DCD was higher on peat humus and was lowest on the soil with organic matter removed. They suggested that the hydrophobic domains of organic matter play a vital role in DCD sorption. Rodgers et al. (1985) compared the mineralization of DCD in a near-neutral soil with acidic soils and found that in a near-neutral soil (pH 6.8) 50% of the DCD-N was mineralised after 60 days while only10-25% was mineralised in an acidic soil over the same period. The same study found that the mineralisation of DCD-N correlated with soil pH but not with organic matter or total N. Puttanna et al. (1999) studied the effect of liming on the nitrification inhibitor efficiency of DCD and found that increase in pH from 5.4 to 8.3 decreased the efficacy of DCD.

Application rate, concentration and leaching

There are contradictory reports on the effects of repeated application on the fate of DCD in soil. Rodgers (1986) did not observe any differences in the degradation of DCD in soils at a temperature of 25°C. However, Mohanty et al. (2009) found that degradation of DCD in soil increased after repeated applications to an alluvial soil incubated at a temperature of 28°C with the half-life of DCD 13.6 days after the first application which decreased to 10.7 and 7.9 days after the second and third applications, respectively. Many studies have shown different threshold concentrations of DCD for effective nitrification inhibition. Prakasa Rao and

Puttanna (1987) found that in a sandy loam soil, 15-20 mg kg^{-1} of DCD was required for effective inhibition of nitrification of urea-N, while Reddy (1964) showed that this value was 25 mg kg^{-1} for a sandy soil. Puttanna et al. (1999) showed that increasing the concentration of DCD from 0 to 15 mg kg soil prolonged the nitrification for up to 60 days in a sandy loam soil.

Dicyandiamide is mobile in drainage water (Shepherd et al. 2012). Thus leaching of DCD with drainage water is another factor that might affect its fate in soil. Few studies in the literature have quantified the DCD in leachates. Corre and Zwart (1995) collected leachate samples following DCD application at 90-100 cm deep and detected and quantified the presence of DCD in the samples which was 7% in November and 2% in December. Menneer et al. (2008) showed that 58% of DCD-N was lost in the leachate during the period of 76 days and it was suggested that leaching of DCD was influenced by the macropore flow processes in soil. Monaghan et al. (2009) quantified the amount of DCD in leachate and found that losses of DCD in leachate were highest soon after rainfall with the total loss of DCD equal to 7% of the applied DCD over a period of 4 years. In one recent lysimeter study by Shepherd et al. (2012), the effects of three soil types (silt loam, sandy loam and clay) on the movement of DCD in soil were studied and it was found that movement of DCD was due to convective-dispersive in all the three soils. However, in the clay soil some preferential flow was also detected. There were significant differences due to soil type on the amount of DCD lost in the drainage water. Shepherd et al. (2012) also concluded that rainfall could to result in substantial losses of DCD via leaching which have implications for its effectiveness in decreasing in nitrogen losses, particularly under urine patches.

Experiment 1: Influence of soil aeration on DCD degradation

Changing the soil oxygen status potentially changes the soil microbial community structure and function. Denitrifiers are a classic example of this happening, with reductase enzymes becoming synthesised as soils become anaerobic. The change in microbial function also applies to those organisms that are able to degrade synthetic chemicals in the soil. For example, bioremediation of soil contaminated by poly-aromatic hydrocarbons (PAH) can occur under aerobic and anaerobic soil conditions (Ambrosoli et al. 2005). Ball et al. (2012) conducted a soil trampling experiment to determine its impact on the efficacy of dicyandiamide (DCD) for reducing N_2O emissions from urine. Trampling reduced air permeability and pore continuity in the soil, reducing aeration. While DCD was equally effective at reducing N_2O emissions under the trampling treatment less DCD was recovered from the soil in this more anaerobic treatment. The reason given was that the trampled soil may have been warmer and that this may have enhanced DCD degradation rates. No consideration was given to anaerobic conditions enhancing biodegradation of DCD. Besides soil aeration being a possible cause of differing DCD degradation rates there have also been suggestions that the level of soil organic matter has also been shown to influence DCD degradation (Puttanna et al. 1999). Therefore the objective of this experiment was to test the hypotheses that:

- The rate of DCD degradation in soil will be faster under anaerobic conditions.
- The rate of DCD degradation in soil will be faster as soil organic matter increases.

EXPERIMENTAL SET UP

A Wakanui silt loam soil was collected (0-15 cm) from two sites (A and B) with differing cropping histories from the Millennium Trial site, Lincoln, New Zealand. Site A was a grass sward and site B was fallow. Soil texture comprised of sand, 33%; silt 48% and clay 19%. The soil sites were selected in order to obtain soil samples with different carbon levels while still having the same parent material. Selected properties of the soil at the two sites are provided in the Table 1. Soil from each site was air-dried and sieved to < 2 mm. Soil cores were then constructed by packing sieved soil into stainless steel (SS) rings (internal diameter 7.3 cm) to a bulk density of 1.1 Mg m^{-3} and a depth of 4.1 cm. Treatments included soil site (A or B) and four levels of soil water matric potential, (-1.0, -3.0, -6.0 and -10 kPa), replicated four times. Soil cores were arranged in a randomised block design on tension tables. A total of 296 soil cores were made. Prior to placing soil cores on the tension tables at the designated kPa levels 232 soil cores were pre-saturated with DCD solution (30 µg ml^{-1}) and 64 control cores were saturated with DI water. Eight of the DCD-treated soil cores (4 replicates of each soil, A and B) were destructively sampled on day zero to measure soil DCD concentrations immediately after saturation. The remaining DCD-treated soil cores (224) were sub-sampled on days 1, 4, 8, 12, 20, 30 and 40 by taking 32 soil cores (2 sites x 4 kPa levels x 4 replicates). Thirty two control soil cores (2 sites x 4 kPa levels x 4 replicates) were destructively sampled on both day 1 and day 40. The mean room temperature for the experiment was 21.8°C (±Stdev. 1.9).

Table 1 Selected soil properties of soil from sites A and B used in the experiments

Soil properties	Soil A	Soil B
pH	5.25 (0.03)	5.03 (0.04)
Organic matter (%)	5.34 (0.26)	3.44 (0.09)
Total Carbon (%)	3.10 (0.14)	2.00 (0.05)
Total Nitrogen (%)	0.30	0.30

ANALYSES

Measurements of relative gas diffusivity (*Dp*/*Do)* were performed using the method of Rolston and Moldrup (2002). Dicyandiamide analyses were performed by extracting 5 g subsamples (oven-dry equivalent) of soil with 25 mL of DI water. The extract was then shaken for 1 h on an end over end shaker followed by centrifugation at 4000 rpm for 20 minutes. The supernatant was then filtered using a Whatman 42 grade filter paper (particle retention to 2.5 µm) and followed by another filtration using Phenomenex syringe filters with a particle retention size of 0.22 µm. Then an HPLC system comprising of a Prominence Degasser (DGU-20A3); LC-20AB/Prominence Liquid Chromatograph (LC-20AB); Prominence Auto Sampler (SIL-20A HT); Prominence UV/Vis Detector (SPD-20A); Prominence Column Oven (CTO-20A) was used for the analyses. The column was a Rezex RHM-Monosaccharid (50 x 7.80 mm, Phenomenex) and the eluent was 0.0025M H_2SO_4. The flow rate was 1 mL min^{-1} and the analysis temperature was 45°C. The detector wavelength was 210 nm and the inject volume was 50 µL. The standards were made from high purity

dicyandiamide (99%, Sigma-Aldrich) with deionised water to establish an appropriate standard curve.
A first order exponential function was fitted to the time series of the DCD concentrations as follows:

$C(t) = C_o e^{-kt}$

where; C = concentration of DCD (mg kg^{-1}) at time t (days)
C_o = initial DCD (mg kg^{-1}) concentration
k = degradation constant (day^{-1})
The half-life, $T_{1/2}$ (time required for the DCD concentration to reduce by half (days)) was calculated as follows:

$T_{1/2} = Ln(0.5)/-k$

Data were checked for normality by checking the normality of the residuals in Minitab 16 and when the data showed deviation from the normality, they were log-transformed before analysis. However, the graphs and tables include the original data. The 5% confidence level was considered statistically significant. Analysis of variance was performed using the General Linear Model in Minitab 16.

RESULTS

The soil cores were initially saturated with either the DCD solution or deionised water and because it took nearly four days to reach the designated matric potential (ψ) levels, the DCD concentrations measured on days 0 and 1 were not included when fitting the exponential function to the data. The DCD concentrations on days 0 and 1 along with the respective values for the soil volumetric water contents (θ_v) are provided in Table 2. The volumetric water content (θ_v) from day 4 to day 40 remained constant for soil from both sites. Mean values of θ_v for site A at -10, -6.0 , -3.0 and -1.0 kPa were 0.24 (0.01),0.30 (0.01),0.37 (0.02) and 0.47 (0.02), respectively, while for site B at -10, -6.0, -3.0 and -1.0 kPa θ_v values were 0.25 (0.01), 0.30 (0.01), 0.36 (0.01) and 0.47 (0.02), respectively. No DCD was detected in the controls.

Table 2 Soil DCD concentrations (mg kg^{-1} soil) and volumetric water contents (m^3 m^{-3}) measured on day 0 and after one day at varying matric potentials (kPa). Standard deviations are in brackets.

DCD (mg kg^{-1})

	Day 0	Day 1			
		-10 kPa	-6.0 kPa	-3.0 kPa	-1.0 kPa
Site A	14.84 (0.86)	7.08 (0.28)	8.41 (0.13)	9.40 (0.330	11.10 (0.38)
Site B	15.12 (1.21)	6.63 (0.21)	8.53 (0.34)	9.99 (0.38)	10.78 (0.34)

θ_v (m^3 m^{-3})

	Day 0	Day 1			
		-10 kPa	-6.0 kPa	-3.0 kPa	-1.0 kPa
Site A	0.56 (0.10)	0.37 (0.01)	0.42 (0.01)	0.44 (0.01)	0.51 (0.02)
Site B	0.52 (0.02)	0.36 (0.01)	0.41 (0.01)	0.44 (0.01)	0.50 (0.01)

The concentrations of DCD in soil from both sites, A and B, decreased exponentially with time from day 4 to day 40 irrespective of matric potential (Figure 1a and 1b). Higher DCD concentrations ($p<0.05$) were observed under wetter soil conditions (-1.0 kPa) throughout the measurement period. The values of the estimated parameters (C_o and k) derived from the regression analysis and the measured *Dp/Do* values measured at different ψ levels are shown in Table 3. For each site, the matric potential (ψ) affected the degradation (k) constant with significantly lower values at -1.0 kPa. When the degradation constants (k) were compared between site A and B for each level of ψ level, it was found that the k values differed significantly ($p < 0.05$) only at -10 and -6.0 kPa (Figure 2). The calculated half-lives ($T_{1/2}$) of DCD for soil at site A increased as soil became wetter and were 15.4 (± 2.4), 16.9 (± 2.8), 21.0 (± 3.4), and 27.6 (± 3.5) days at -10, -6.0, -3.0 and -1.0 kPa, respectively. For soil from site B the half-lives of DCD also increased as soil became wetter with values of 22.4 (± 5.8), 23.1 (± 4.4), 24.7 (±4.8), 31.5 (± 5.5) days, at -10, -6.0, -3.0 and -1.0 kPa. Measured *Dp/Do* values varied with kPa level but did not vary due to soil site (Table 3).

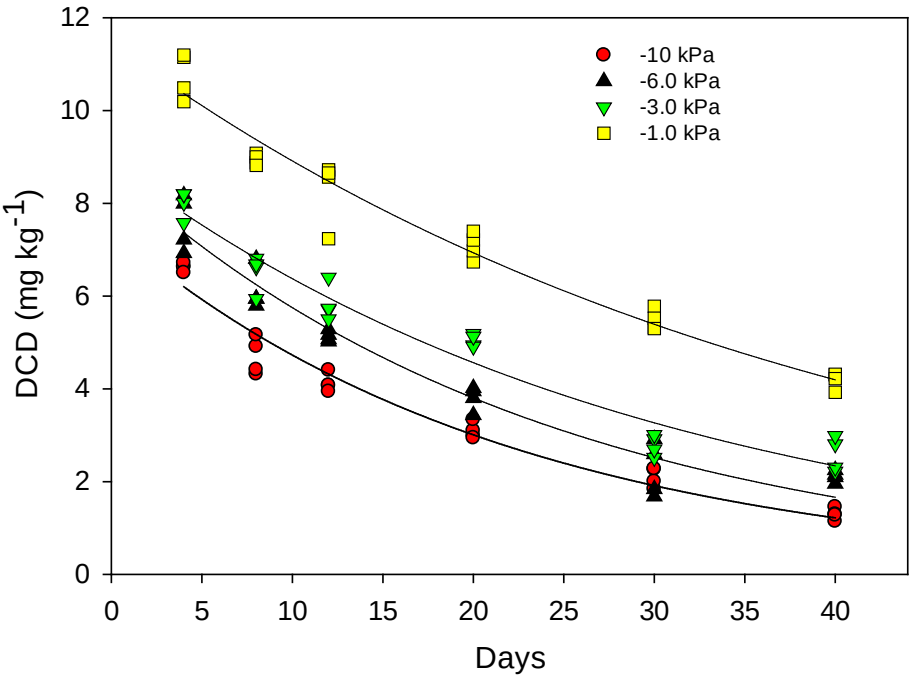

Figure 1a Soil DCD concentrations in Soil A over a period of 40 days. Data points are individual values from four replicates.

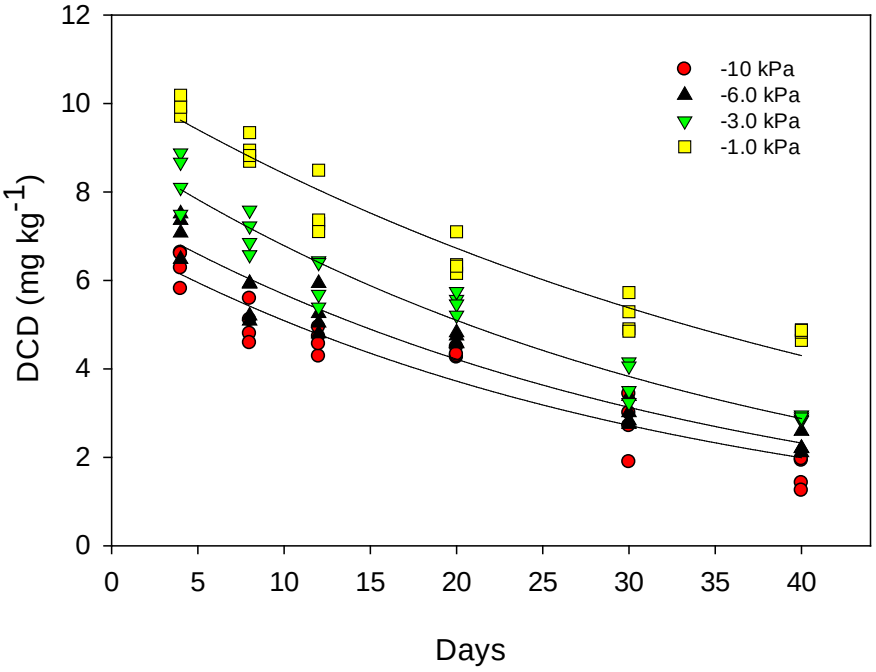

Figure 1b Soil DCD concentrations in Soil B over a period of 40 days. Data points are individual values from four replicates.

Table 3 Regression parameters (*Co* and *k*) and measured *Dp/Do* values for soil from site A and site B. Values in a column that do not share a common letter are significantly different at 5% level. Standard deviations (SD) are in brackets.

Matric potential (-kPa)	Soil A			Soil B		
	C_o	*k*	*Dp/Do*	C_o	*k*	*Dp/Do*
10	7.43a	0.045a	0.07	6.96a	0.031a	0.08
	(0.44)	(0.005)	(0.008)	(0.54)	(0.005)	(0.005)
6.0	8.69b	0.041a	0.03	7.65b	0.029a	0.03
	(0.53)	(0.005)	(0.004)	(0.48)	(0.004)	(0.003)
3.0	8.90b	0.033b	0.01	9.03c	0.029a	0.01
	(0.48)	(0.004)	(0.003)	(0.48)	(0.003)	(0.002)
1.0	11.45c	0.025c	0	10.52d	0.022b	0
	(0.44)	(0.002)		(0.47)	(0.002)	

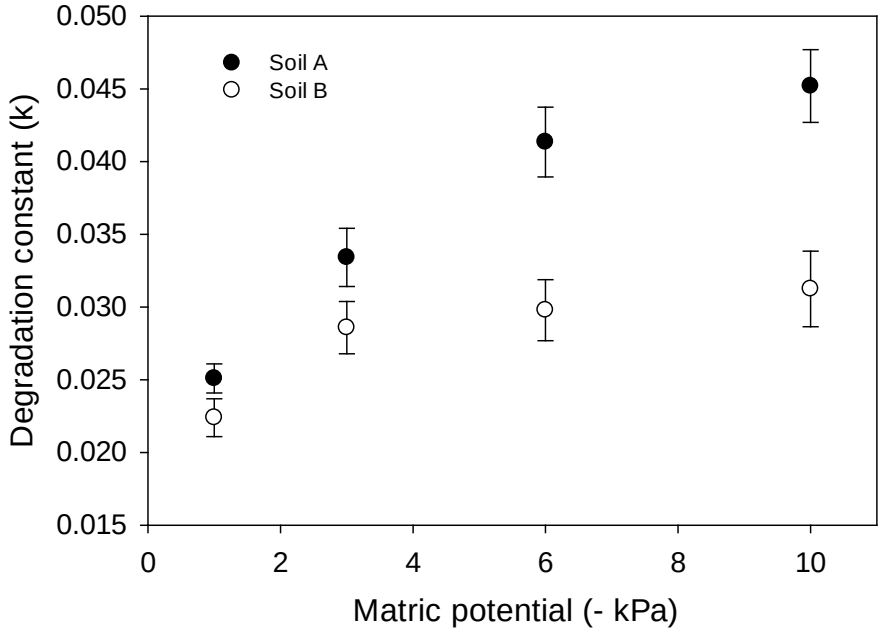

Figure 2 DCD degradation constant (*k*) **for soil A and soil B measured at different ψ. Error bars represent the standard error of 4 replicates.**

EXPERIMENT 1 - DISCUSSION

The DCD degradation rate and the calculated DCD half-life at -10 kPa and 22ºC in soil from both sites A and B, observed in this current study, compared well with the DCD half-life in the data synthesis performed by Kelliher et al. (2008). Measured DCD degradation rate constants were lower under wetter soil conditions. Stepniewski (1981) provided a threshold for anaerobic conditions in soil and suggested that soils start becoming anaerobic when the relative gas diffusivity (*Dp/Do*) falls within the range of 0.02-0.005. Values of *Dp/Do* obtained in this study at -1.0 and -3.0 kPa (0.01 to 0) fall within this 'anaerobic' range described by Stepniewski (1981). Conversely, the *Dp/Do* values for soil from both sites A and B show the soil was aerobic at -6.0 and -10 kPa (Table 3). Under these aerobic soil conditions the degradation of DCD was higher (larger rate constant (*k*)) in soil from site A under aerobic conditions. Differences between soil at site A and B included a higher soil pH, higher soil C and more organic matter at site A (Table 1). Thus it is feasible to conclude that one or more of these factors caused the faster rate of DCD degradation observed for soil from site A. Puttanna et al. (1999) added fresh organic matter (undecomposed dried and ground residue, from the steam distillation of citronella (*Cymbopogon* sp.)), to a sandy loam soil with a pH of 8.3 at a rate of 1000 mg kg^{-1} soil, with DCD applied at a rate of 10 mg/kg^{-1} soil, and incubated the mixture at 30ºC and observed no change in DCD efficacy. However, Amberger and Vilsmeier (1979) found DCD decomposed faster when the soil organic matter content was high. Reddy et al. (1964) also proposed DCD decomposed faster as organic matter content increased, since ammonium was found to nitrify faster in a sandy soil with 0.9% organic matter than in a sandy loam with 0.3% organic matter maintained at 27ºC. It should be noted, however, that DCD was not directly measured and faster decomposition was inferred due to reduced DCD efficacy (Reddy et al. 1964). To further evaluate this finding Reddy et al. (1964) ran another experiment using sucrose as a source of organic matter and concluded from the increased rate of nitrification, observed in the presence of sucrose, that sucrose was able to provide soil microorganisms with energy to utilize the DCD as a source of N, thus reducing its concentration and efficacy. The current experimental measurements of the DCD degradation rate constants, which appear to be the only such measurements of DCD in soils of varying organic matter content, clearly show that DCD is degraded under a higher soil organic matter content, and support the earlier findings showing that DCD efficacy is reduced when more soil organic matter is present. However, the possibility that soil pH, which was higher in the soil with more organic matter, also promoted DCD degradation cannot be ruled out. While nitrification rates are promoted with liming (e.g. Clough et al. 2004) few studies have examined DCD efficacy under liming. Reddy et al. (1964) took a soil at pH 5.4 and limed it to pH 8.2, and found that DCD efficacy decreased with liming possibly due to increased nitrification activity and/or more rapid degradation of the inhibitor due to enhanced microbial activity. In the current study the pH gap between the soil from site A and B was much less than the study cited (Reddy et al. 1964) and no additional N was added and so nitrification activity wasn't stimulated. Thus we believe the main influence on the higher degradation rate of DCD was the variation in soil organic matter. Further studies need to evaluate this potential pH effect. Thus, the hypothesis that "the rate of DCD degradation in soil is faster as soil organic matter increases" is accepted.

In the current experiment degradation of DCD clearly increased as soil conditions became more aerobic. Slower degradation of DCD was also reported by Amberger and Vilsmeier (1988) and Kim et al. (2011) who both found that DCD had a longer half-life under wetter soil conditions. Thus the hypothesis that "The rate of DCD degradation in soil would be faster under anaerobic conditions." is rejected.

In summary experiment 1 has shown that DCD degrades faster as soil organic matter content increases and as soil moisture decreases.

Experiment 2: Effect of soil aeration and urine on DCD degradation.

In experiment one the degradation of DCD was examined in the absence of any N addition. Given that the nitrification inhibitor DCD has been used in New Zealand to mitigate N_2O and NO_3^- losses from ruminant urine hot-spots in pastures it was considered that the rate of DCD degradation in a soil affected by urine should also be examined. Ruminant urine deposition onto pasture soil produces an increase in dissolved organic carbon (Monaghan and Barraclough, 1993) which is available to soil microbes, as demonstrated by soil carbon priming responses following urine addition to soil (Clough et al. 2004). Thus, following the results of experiment 1, it was hypothesised that DCD degradation would still be higher under aerobic conditions, regardless of urine application, but that DCD degradation would be higher under urine due to more organic matter being available to the microbes. The objective of this experiment was to test the hypothesis that:

- The rate of DCD degradation in urine-affected soil would be faster under aerobic soil conditions.

EXPERIMENTAL SET UP

Soil A (Table 1) was used to make soil cores by compacting the soil into SS cylinders to a depth of 4.1 cm in order to obtain a bulk density of 1.1 Mg m^{-3} which was representative of field conditions. The total number of soil cores made was 256, and these were divided into two levels of soil moisture (-1.0 and -10.0 kPa) with 128 cores in each moisture regime (4 treatments x 4 replicates x 8 destructive sampling events). Within each moisture regime the four treatments were; control (DI water); urine only (700 kg ha^{-1}); urine (700 kg ha-1) + DCD (16 mg kg^{-1} soil); DCD only (16 mg kg^{-1} soil). These treatments are, hereafter referred to as control, U, DU and D, respectively. Urine had been previously collected from the Lincoln Research Dairy and had been frozen prior to use, where upon it was thawed and brought to room temperature. Dicyandiamide was dissolved either in deionised water (100 µg mL^{-1}; solution 1) or in the urine (100 µg mL^{-1}; solution 2). For the control and U treatments, 30 mL of either DI water or urine were applied to the soil cores, respectively, while the D and DU treatments received 30 mL of either solution 1 or 2, respectively. Prior to treatment application the soil cores were wetted with DI water to a moisture content that still allowed

for the subsequent addition of treatments so that the final soil moisture content of the soil cores equalled the water-filled pore space at -1.0 and -10 kPa (previously calculated using the data from the experiment 1). Then the soil cores were placed on tension tables at either -1.0 or -10.0 kPa. The experiment was run for 40 days with destructive measurements taken after 0, 3, 7, 14, 21, 28, 35 and 40 days. The mean temperature of the room in which the experiment was performed was 22.4ºC (±Stdev. 2.1).

ANALYSES

Soil surface pH was measured, prior to core destruction, using a calibrated flat surface pH electrode (Broadley- James Corp, Irvine, CA.). During destructive soil core analyses the soil was extruded from the cores and mixed well prior to sub-sampling. Dissolved organic carbon (DOC) analyses were performed by adding 30 mL of DI water to 5 g of sub-sampled moist soil and extracting the DOC by shaking the sample on an end over end shaker for 30 min (Ghani et al., 2003). The samples were then centrifuged for 20 min at 3500 rpm. The supernatant was then filtered through a pre-leached filter paper (Advantec 5C) prior to DOC determination using a Shimadzu TOC analyser TOC 5000A (Shimadzu Oceania Ltd, Sydney, Australia). Soil gravimetric water content (θ_g) was measured by oven drying 10 g of wet soil at 105ºC for 24 h. Soil inorganic N concentrations were measured using Flow Injection Analysis (FIA) (Blakemore et al., 1987). The analysis for inorganic N was performed by extracting sub-sampled soil with 2M KCl at a 1:10 ratio. The extract was shaken for 1 h on a reciprocal shaker followed by filtration. Measurements of *Dp*/*Do* were performed using the method of Rolston and Moldrup (2002).

Soil surface fluxes of N_2O were obtained by placing soil cores inside 1 L paint tins. The lids of the paint tins were equipped with pre-fitted gas-tight rubber septa. After sealing the headspace, ambient air samples were taken at time zero and then headspace gas samples (10 mL) for N_2O determinations were taken at 15 and 30 minutes, using a 20 mL glass syringe fitted with a 3-way tap and 25 gauge 0.5 x 16 mm needles (Precision Glide, Becton-Dickinson, NJ). Each gas sample was transferred into a pre-evacuated (-1 atm.) 6 mL vial (Exetainer® tubes, Labco Ltd, UK). Nitrous oxide concentrations were determined using gas chromatography as described by Clough et al. (2006) on an automated GC (8610, SRI Instruments, Torrance, CA) interfaced to an autosampler (Gilson 222XL, Middleton, WI). Increases in N_2O concentrations over time were linear. The N_2O fluxes were calculated according to the equation given by Hutchinson and Mosier (1981).

As in experiment 1, a first order exponential function was fitted to the time series of the DCD concentrations. Data were checked for normality by checking the normality of the residuals in Minitab 16 and when the data showed deviation from the normality, they were log-transformed before analysis. However, the graphs and tables include the original data. The 95% confidence level was considered statistically significant. Analysis of variance was performed using the General Linear Model in Minitab 16.

RESULTS

Soil surface pH in the control and DCD only (D) treatments ranged from 5.3 to 6.3 over a period of 40 days with no effect of DCD and surface soil pH in these treatments was significantly ($p < 0.01$) lower than in urine-treated cores (Figure 3). Following urine application soil pH increased to be 9.3 at day 3 with no effect of DCD at this time (Figure 3). Soil surface pH declined after day 14 in urine-treated soils but with a significant ($p < 0.01$) effect of DCD addition observed from day 21 to 40 where soil surface pH in the DU treatments, both -1.0 and -10 kPa, declined at a slower rate. However, there was no consistent effect of soil kPa on soil surface pH in the urine-treated soil cores.

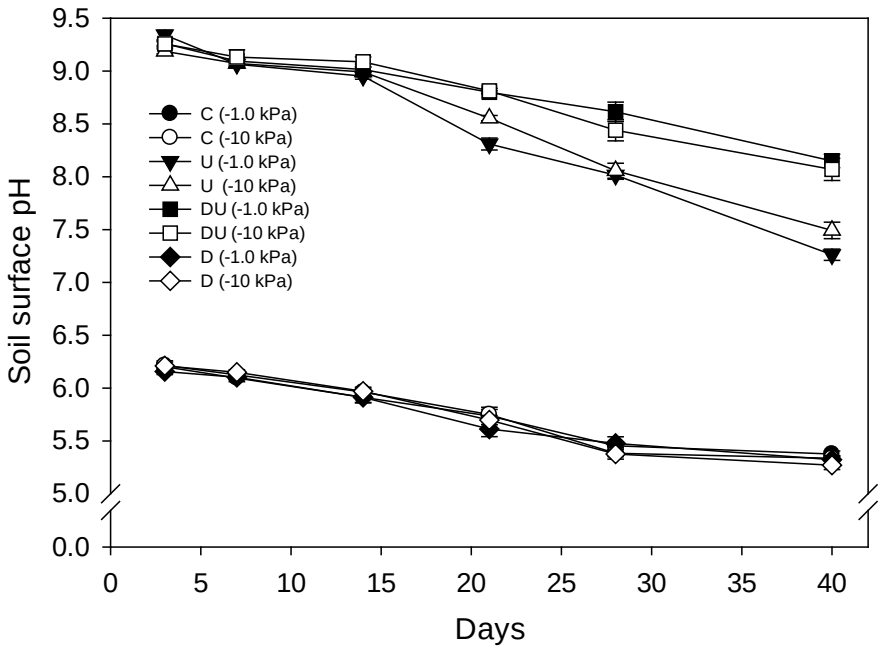

Figure 3 Soil surface pH over a period of 40 days. Error bar = s.e.m, n = 4.

Mean DOC concentrations in the control and D treatments ranged from 9 to 122 µg g^{-1} soil with no effect of kPa (Figure 4). Urine application increased ($p<0.01$) mean DOC concentrations regardless of soil kPa and DCD presence and by day 3 they ranged from 1173 to 1423 µg g^{-1} soil, before declining to a range of 312 to 700 µg g^{-1} soil on day 40 (Figure 4). In the absence of DCD soil DOC concentrations under urine were lower ($p < 0.01$) on days 28 and 40 than in the urine treatment alone, with no effect of kPa treatment.

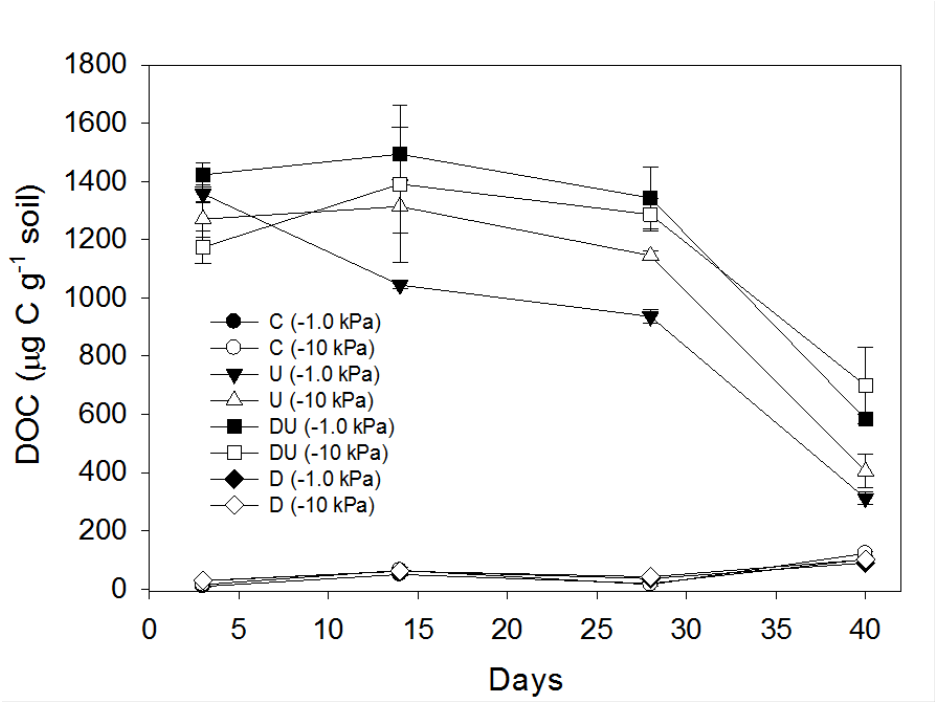

Figure 4 Soil dissolved organic carbon (DOC) concentrations measured over a period of 40 days. Error bar = s.e.m, n = 4.

Soil DCD concentrations decreased with time in all treatments (Figure 5). The calculated half-lives of the different treatments were 53.3 (±7.2), 23.5 (±2.9), 14.9 (±2.7) and 16.7 (±2.5) for DU (-1.0), D (-1.0), DU (-10) and D (-10), respectively. There was a significant effect (Table 4) of urine addition on the DCD degradation constant (*k*) at both kPa levels. At -10 kPa, the value of *k* was higher while at -1.0 kPa, it was lower in the DU treatments when compared with D treatments. Soil kPa also affected the *k* values in both DU and D treatments as these values were significantly lower at -1.0 kPa than at -10 kPa (Table 4). No DCD was detected in the controls.

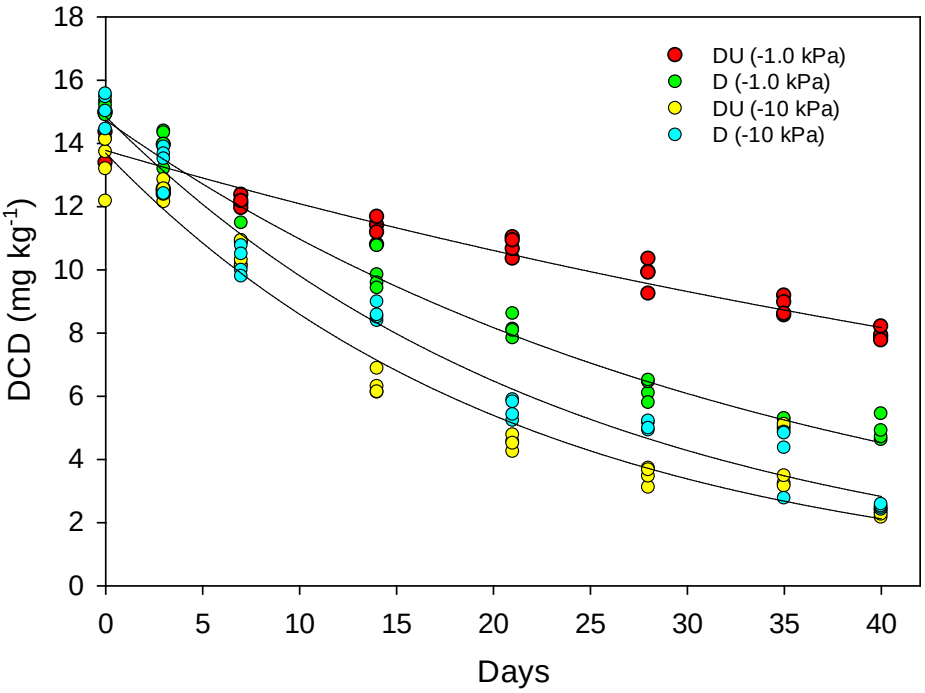

Figure 5 Soil DCD concentrations over a period of 40 days. Data points are individual replicates.

Table 4 Regression parameters (*Co* and *k*) obtained after fitting a first-order exponential function to the DCD data, and the measured *Dp/Do* values for D and DU treatments. Values in a column that do not share a common letter are significantly different at 5% level. Standard deviations (SD) are in the brackets.

Treatments	C_o	*k*	*Dp/Do*
DU (-1.0 kPa)	13.77a (0.42)	0.013a (0.002)	0
D (-1.0 kPa)	14.73b (0.45)	0.030b (0.002)	0
DU (-10 kPa)	13.69a (0.49)	0.047c (0.003)	0.073 (0.007)
D (-10 kPa)	14.85b (0.51)	0.041d (0.003)	0.071 (0.009)

Non-urine treated soil cores had NH_4^+-N concentrations ≤ 54 µg g^{-1} soil. Soil NH_4^+-N concentrations were higher ($p < 0.01$) in urine-treated soil and ranged from 1380 to 1520 µg g^{-1} soil on day 3, decreasing to a range of 889 to 1094 µg g^{-1} soil on day 40 (Figure 7a). In urine-treated soil cores the presence of DCD elevated soil NH_4^+-N concentrations ($p<0.01$) from day 21 to 40 when compared to the urine only treatment, regardless of the soil's kPa.

Soil NO_3^--N concentrations in non-urine treated soil cores were ≤ 59 µg g^{-1} soil. Urine-treated soil cores had mean NO_3^--N concentrations ranging from 2 to 242 µg g^{-1} soil and started increasing after day 14 (Figure 6b). In urine-treated cores there was an interaction between the DCD and the kPa treatments from day 21 to 40 ($p<0.001$) with lower soil NO_3^--N concentrations with DCD present, but within the DCD treatment the wetter soil at -1 kPa had lower soil NO_3^--N concentrations (Figure 6b).

For non-urine treated soil cores the NO_2^--N concentrations were < 0.6 µg g^{-1} soil with no consistent treatment effects. Higher ($p < 0.01$) NO_2^--N concentrations occurred in the urine-treated soil, ranging from 0.5 to 2.6 µg g^{-1} soil on day 3 and from 0.4 to 1.0 µg g^{-1} soil on day 40 (Figure 6c). In the urine-treated soil cores an interaction between the DCD and kPa treatments occurred from days 3 to 21 ($p<0.05$) and caused NO_2^--N concentrations to be lower when DCD was present at -1 kPa but in the absence of DCD NO_2^--N concentrations were lower at -10 kPa.

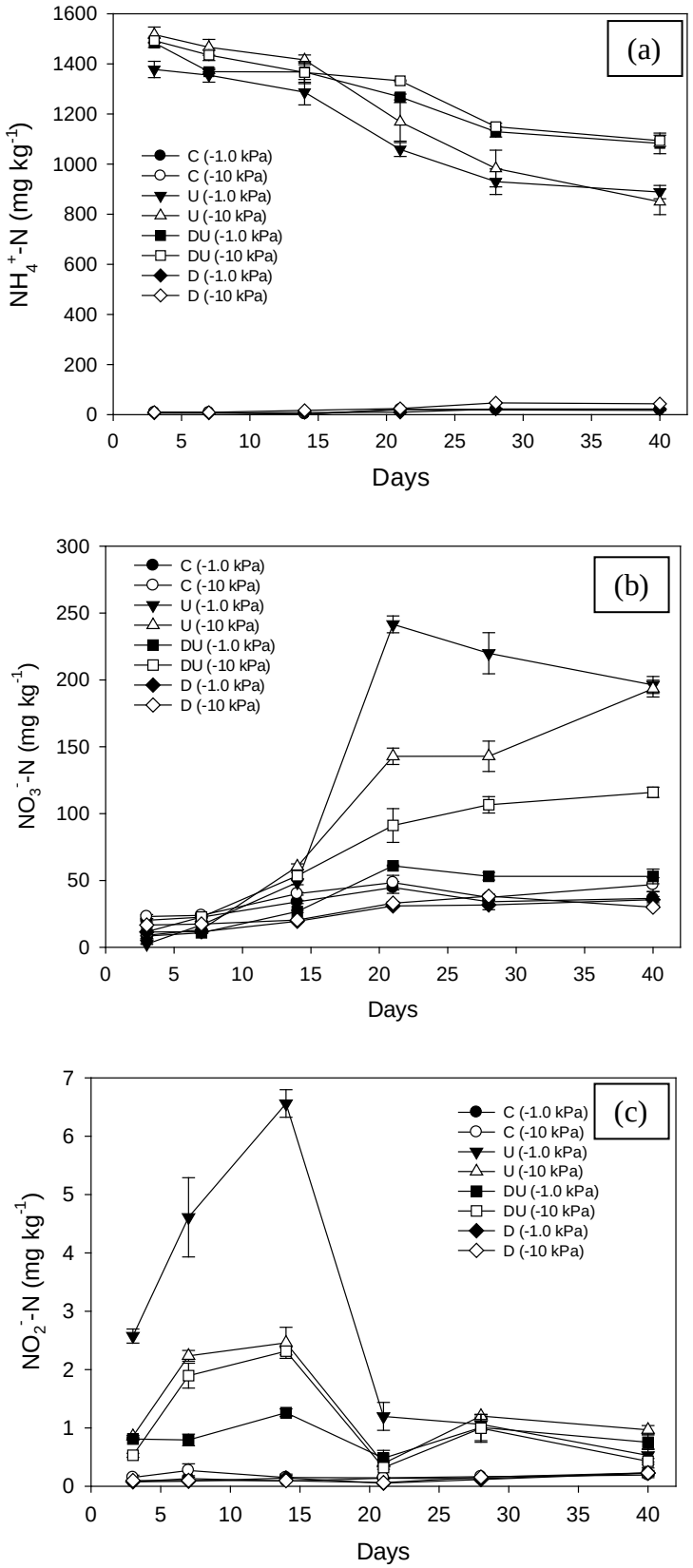

Figure 6 Soil inorganic N concentrations over days. Error bar = s.e.m, n = 4.

Application of urine elevated N_2O-N fluxes at both -1.0 kPa and -10 kPa with the highest N_2O-N fluxes, 1341 µg m^{-2} h^{-1} at -1.0 kPa and 729 µg m^{-2} h^{-1} at -10 kPa, occurring on day 30 and day 24, respectively (Figure 7). Non-urine treated cores had N_2O-N fluxes ranging from 1 to 29 µg m^{-2} h^{-1} with no consistent treatment effects. Mixing DCD with the urine resulted in lower N_2O-N fluxes ($p < 0.01$) on all days except day 21. The cumulative N_2O-N fluxes after 40 days were highest under urine application at -1.0 kPa followed by the urine application at -10 kPa (Table 5). The reduction in cumulative N_2O-N fluxes due to DCD application with urine was higher at -1.0 kPa (95±3%) than at -10 kPa (57±10%) with no difference in cumulative N_2O-N fluxes due to DCD application in the absence of urine (Table 5).

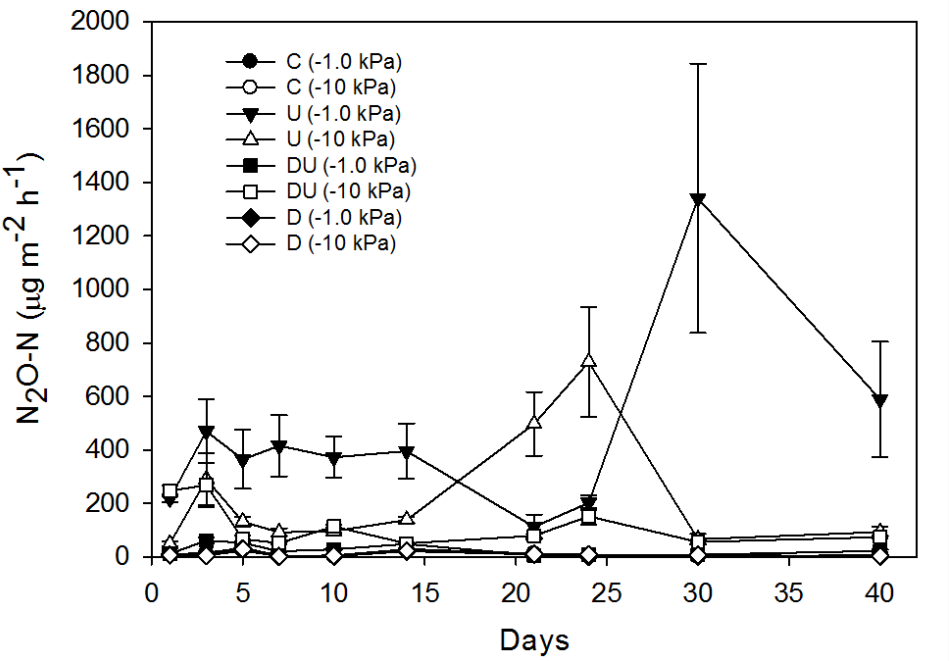

Figure 7 Mean N_2O-N fluxes over days. Error bar = s.e.m, n = 4.

Table 5 Cumulative N_2O-N (mg kg^{-1}) fluxes over the 40 day period and the % reduction in the cumulative N_2O-N fluxes following DCD application at -1.0 or -10 kPa. For each treatment, lower case letters indicate significant differences between means (Tukey's Test, $p<0.05$).

Treatments	Cumulative N_2O-N (mg kg^{-1})	% reduction in N_2O-N flux with DCD
U (-1.0 kPa)	11.53 (4.29) a	
DU (-1.0 kPa)	0.50 (0.09) d	95 (3.1)
U (-10 kPa)	4.80 (1.35) b	
DU (-10 kPa)	1.99 (0.21) c	57 (9.7)
D (-1.0 kPa)	0.17 (0.04) e	
D (-10 kPa)	0.21 (0.06) e	
C (-1.0 kPa)	0.20 (0.02) e	
C (-10 kPa)	0.24 (0.06) e	

EXPERIMENT 2 - DISCUSSION

The increase in the surface soil pH in all the urine-treated soils was due to the hydrolysis of the carbonate ions formed during urea hydrolysis (Sherlock 1984). During the nitrification process a net release of H^+ ions occurs resulting in a soil pH decrease. Thus, in the DU treatments the slower rate of decline in soil surface pH was due to that fact that the DCD inhibited nitrification as shown by the higher $NH4^+$-N concentrations in the DU treatments at both kPa levels. The high soil pH level following urine deposition to soil results in the dissolution of soil organic matter as seen by the higher levels of DOC measured under the urine-treated soil. It is worth noting that the soil pH was similar in urine treatments with or without DCD present yet there were still differences in degradation rate constants (Table 4) as a result of soil aeration which suggests soil aeration had more impact than soil pH on DCD degradation.

The half-life of DCD derived from the DCD treatments at both kPa levels and the effects of kPa on the degradation constant (*k*) were similar to the results obtained in experiment 1, with a longer half-life under more anaerobic conditions. However, the effect of urine was to decrease *k* even further (increase in DCD half-life) at -1.0 kPa (anaerobic conditions with

Dp/Do = 0) and to further increase *k* (decrease in half-life) at -10 kPa (aerobic conditions with *Dp/Do* = 0.07).

The increase in DOC with urine present at -10 kPa will have resulted in more aerobic microbial activity and this may potentially have caused the observed increase in the degradation rate (higher *k* value) of DCD. Why the degradation rate constant decreased yet further in the already anaerobic soil, at -1.0 kPa, under urine is not immediately obvious. Addition of urine might have further enhanced anaerobic conditions in soil, due to CO_2 production from urea hydrolysis, or maybe NH_3 toxicity further repressed microbial activity due to the higher soil water content slowing gas release from the soil. Kim et al. (2012) in a field experiment assessed the effect of N source via urine addition on the DCD degradation on a Tokomaru silt loam soil and suggested that the biophysical disappearance of DCD was not affected by urine addition. The likely reason for obtaining results differing from the current study might be that the experiments were performed in the field where soil moisture was not controlled.

Application of DCD at both kPa levels was effective in inhibiting nitrification, as shown by the lower concentrations of NO_3^--N in the DU treatments, and consequently in reducing N_2O-N fluxes. Such effects were expected and have been observed in many studies (Di et al. 2002, 2003, Singh et al. 2008). The occurrence of some nitrification activity at -1.0 kPa suggests that even though *Dp/Do* values were zero, soil was aerobic enough, possibly at the soil surface, for some nitrification to occur.

In this current study, the inhibitory effects of DCD on nitrification and N_2O-N fluxes were more pronounced at -1.0 kPa where the cumulative N_2O fluxes were reduced by 95% while they were reduced by 57% at -10 kPa. This concurs with the fact that degradation of DCD in the soil was slower at -1.0 kPa than at -10 kPa which resulted in higher concentrations of DCD remaining in soil to inhibit nitrification and consequently N_2O-N fluxes. Further confirming this was the enhanced decrease in DOC concentrations in the urine only treatments after day 21 resulting from increased denitrification of the elevated soil NO_3^--N concentrations. When DCD was present with urine the slower appearance of NO_3^--N meant that less DOC was utilised and as a consequence N_2O emissions declined. The variability in cumulative N_2O emissions seen here under urine plus DCD, as a result of varying soil aeration helps to explain, along with soil temperature variation, the seasonal variability observed in DCD efficacy. Warm and drier soils will promote DCD degradation.

Conclusions

These studies have systematically confirmed that both soil aeration status AND soil organic matter levels affect the degradation of DCD in soil. Secondly, these study show that the efficacy of DCD, following urine deposition to soil, is also affected by the soil's aeration status, with efficacies of 95 (±3) and 57 (± 10)% at -1.0 and -10 kPa, respectively, in the current work. Further studies are required to understand in more detail the degradation of DCD in soil. For example, how does the range in soil organic matter contents of grazed pastures around New Zealand relate to the magnitude and range of DCD degradation rate constants? Likewise, the current studies were performed in the absence of plants but in theory plant presence and associated microbial communities may also impact on DCD degradation.

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