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The effect of the nitrification inhibitor dicyandiamide (DCD) on herbage production when applied at different times and rates in the autumn and winter

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ABSTRACT

The high rate of urine excreted during animal grazing in late autumn provides a source of nitrogen (N) to the growing sward and also provides the potential for losses of N over the winter months. This study was established to evaluate the potential of applying a nitrification inhibitor, dicyandiamide (DCD), to urine patches to increase N use efficiency in grassland. Four simulated grazing plot experiments were undertaken across two experimental sites, one a free-draining acid brown earth (Experiments 1 and 3) and the other a moderate to heavy brown earth soil (Experiments 2 and 4). Experiments 1 and 2 received no fertiliser N application, and Experiments 3 and 4 received a split application of 350 kg N fertiliser ha^{-1} year^{-1}. The effect of applying the nitrification inhibitor dicyandiamide (DCD) at 5 or 10 kg DCD ha^{-1} in autumn and winter to plots receiving synthetic urine or zero urine on spring and annual herbage production was examined in all experiments. The application of DCD did not increase spring herbage production in any of the experiments. Over the two years, the application of 5 or 10 kg DCD ha^{-1} increased annual herbage production in Experiment 1 when applied to October and November deposited urine patches. Urine application increased herbage production in spring and annually in Experiments 1 and 2, and increased herbage crude protein content and herbage N uptake in all experiments. The application of urine increased soil ammonium and TON content in the 0–100 mm horizon at both sites. The application of 10 kg DCD ha^{-1} reduced surplus N in Experiment 1 when applied to October and November deposited urine. Overall the effects of DCD on herbage production, surplus N and other parameters in this study were not consistent.

1. Introduction

Increasing the proportion of grazed grass in the diet of the dairy cow, particularly in early spring, reduces milk production costs and can increase the profitability of grass based milk production systems in Ireland and other temperate climates (Shalloo et al., 2004; Dillon et al., 2005; Kennedy et al., 2005). Nitrogen (N) availability is one of the key factors driving grass growth. Increasing N availability in spring through fertiliser or slurry application can result in increased grass growth and therefore herbage availability for grazing. Urine and dung are also sources of N in grazed swards, although their deposition is localised. Nitrogen concentration under urine patches is very high, equivalent to a fertiliser N application rate of up to 1000 kg N ha^{-1} (Whitehead, 1995). The majority of this N is in excess of sward requirements and is often lost by nitrate (NO_3^-) leaching through the soil profile or nitrous oxide (N_2O) emissions, particularly over winter when grass growth rates are low.

Nitrification inhibitors are being investigated in many countries as a strategy to mitigate NO_3^- leaching, denitrification and N_2O emissions under urine patches (Serna et al., 1995). They therefore have the potential to increase N availability in the soil for grass growth, thereby increasing the N use efficiency of grazed swards, as well as reducing N losses to the environment (O'Connell et al., 2004). Dicyandiamide (DCD; $C_2H_4N_4$) is one such nitrification inhibitor. It is a white crystalline nitrogenous powder naturally broken down in the soil, with no traces of residue remaining beyond the cropping year (Amberger, 1989). Dicyandiamide slows the conversion of ammonium (NH_4^+) to NO_3^- in the soil by interfering with the cytochrome oxidase in the respiratory electron transport system of *Nitrosomonas* bacteria, which are responsible for the first step of the nitrification process (Serna et al., 1995). Reductions in NO_3^- leaching and N_2O emissions following the application of DCD have been reported by many authors including Moir et al. (2007) and Dennis et al. (2008). Di and Cameron (2002) reported reductions in annual NO_3^- leaching of 59% from urine

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patches (lysimeter study) following DCD application; in addition Di and Cameron (2005) reported a 68% reduction in NO_3^- leaching from dairy cow urine N when DCD was applied in autumn (Di and Cameron, 2005). Selbie et al. (2011) reported that DCD (10 kg ha^{-1}) reduced NO_3^- leaching by 45% and N_2O emissions by 70% on dairy cow urine (1000 kg N ha^{-1}) treatments on Irish soils. Richards et al. (2008) also reported that DCD application on urine patches reduced NO_3^- leaching, especially on Irish soils.

In addition to the reductions in environmental N losses, Di and Cameron (2002) also observed an 18% increase in herbage production following DCD application to spring deposited urine patches, and an average increase in herbage production of 49% following DCD application to autumn deposited urine patches. The same authors observed that the application of DCD to autumn deposited urine patches, followed by a second application in spring, increased herbage production by 33% annually (Di and Cameron, 2005). Zaman and Blennerhassett (2010) reported that the application of DCD to spring deposited urine increased herbage production by an average of 12%.

An N balance can be used to describe the potential for N loss to the environment; it gives an indication of the quantity of N that may be lost through leaching, denitrification and volatilization or immobilization into soil organic N. Research has identified the importance of some individual loss processes, such as N losses by ammonia volatilization in pastures grazed by dairy cows (Bussink and Oenema, 1996), NO_3^--N leaching (Scholefield et al., 1993), N_2O emissions and N removal by immobilization (Ledgard et al., 1999).

The efficacy of DCD is influenced by several factors, including soil and environmental factors. Temperature is the most influential environmental factor; an increase in temperature can have a negative effect on the persistence of DCD in the soil, reducing the time frame in which it can provide effective nitrification inhibition. The half life of DCD at 6 °C is 100 days (Williamson et al., 1996), and 18–25 days at 20 °C (Di and Cameron, 2004). As a consequence, DCD should be applied in cool conditions such as late autumn, winter and early spring in temperate climates to maximise its potential effectiveness in inhibiting nitrification in the soil (Kelliher et al., 2008). Therefore, the hypothesis of this experiment was that the application of DCD in autumn and winter will increase N availability for grass growth in spring and, therefore, increase spring herbage production.

The objective of this study was to establish if herbage production was increased following the application of DCD to autumn and winter deposited urine patches, and to determine the appropriate rate and time of application of DCD on grass swards receiving zero or 350 kg N ha^{-1} $year^{-1}$.

2. Materials and methods

2.1. Soil type and pasture

Four experiments were undertaken using simulated grazing plots at the Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland, on two contrasting soil types. The soils were a free-draining acid brown earth of sandy loam to loam in texture with a pH of 6.02 and bulk density of 1.00 g cm^3 at Moorepark Research Farm, hereafter referred to as MPK (50°07′N, 08°16′W) in Experiments 1 and 3 and a moderate to heavy brown earth with evidence of an iron pan with a pH of 5.52 and bulk density of 0.83 g cm^3 at Ballydague Research Farm, hereafter referred to as BD (52°12′N, 08°13′W) in Experiments 2 and 4. Ballydague Research Farm is approximately 8 km from MPK. Swards were predominately perennial ryegrass (*Lolium perenne* L.) and were previously rotationally grazed by dairy cows at MPK and by dairy heifers at BD. In Experiments 1 and 2 no fertiliser N was applied, and in Experiments 3 and 4 split applications of N fertiliser (total 350 kg N ha^{-1} $year^{-1}$) were applied between mid–January and mid September (as specified in the Irish Governments Nitrates Action Plan (S.I. 378, 2006)) as urea (46% N) from February to April (120 kg N ha^{-1} $year^{-1}$), and calcium ammonium nitrate (CAN; 27% N) from May to September (230 kg N ha^{-1} $year^{-1}$).

2.2. Experimental design

Apart from the N fertiliser application strategy (zero N in Experiments 1 and 2, and 350 kg N ha^{-1} in Experiments 3 and 4), all experiments had the same design. Each experiment had three replications (blocks), and within each replication five factors (details below) were manipulated resulting in 28 treatments in total. The 28 treatments were each applied to one plot within each block; plots were 5 m × 1 m at BD and 5 m × 1.5 m at MPK. Experiments were established in September 2008 and completed in November 2010. A cleaning cut was undertaken in November 2008 and the recording of experimental data began in February 2009. Each plot was harvested ten times in year 1 (2009) and eight times in year 2 (2010).

2.3. Treatments

Within each experiment, the five factors manipulated were urine rate, date of application of urine, DCD rate, date of first application of DCD and date of second application of DCD (Table 1). The two urine application rates were 0 and 1000 kg N ha^{-1} (0U or U, respectively) applied on one of three occasions in autumn – late September, October or November. Synthetic urine was used (urea and water mix) so that a known quantity of N was applied. Synthetic urine was deposited using 10 L watering cans with rose caps (cap with small openings). Dicyandiamide was applied at rates of 0, 5 and 10 kg ha^{-1} to all designated plots within 24 h of urine application in either late September, October or November. Of the plots that received a first application of DCD > 0 kg ha^{-1}, half received a second application of the same rate approximately 90 days later. The DCD was applied as a fine particle suspension (FPS) using a walk behind sprayer (Kestrel Spray-Master Sprayer, R&J Hay, St Johnston, Cavanacaw, Co. Donegal, Ireland).

Plots receiving DCD at rates of 0, 5 and 10 kg ha^{-1} will hereafter be referred to as 0, 5 and 10. Plots receiving a single application of urine and DCD in late September, October or November will hereafter be referred to as S, O or N, and those receiving a second application of DCD 90 days later will hereafter be referred to as S + 90, O + 90 and N + 90. Plots that received zero urine and zero DCD are called control and hereafter be referred to as C. In total there are 28 unique combinations of factor levels or treatments (including control) listed in Table 1. Treatments applied in year 1 were applied between September 2008 and March 2009 and treatments applied in year 2 were applied between September 2009 and March 2010.

2.4. Measurements

2.4.1. Herbage production

Herbage was mechanically harvested every four weeks from February to November 2009 and in 2010 using an Agria auto-scythe mower (Agria Werke GmbH, Bittelbronnerstr 42, Moeckmuehl 74219, Germany) at MPK and a Honda rotary blade lawnmower (Honda HRH 536 HX Pro Hydrostatic 4-wheel mower, Honda, Swepsonville, NC, USA) at BD. The Honda lawnmower was used at BD due to the heavy soil at the site (Hennessy et al., 2008). All fresh samples were weighed and a sub-sample (100 g) was dried at 40 °C for 48 h to determine dry matter (DM) content. Dry matter yield was calculated by multiplying DM% of the subsample by

Table 1
Experimental treatments 1–28; for each of the four experiments, each treatment appeared once in each of three blocks.

Treatment #	Treatment code	Urine (kg N ha^{-1})	Date of urine application[a]	DCD rate (kg ha^{-1})	Date first DCD application[a,b]	Date of second DCD application (days post first application)[a]
1	C	0	0	0	0	0
2	S0U5	0	0	5	September	0
3	S0U10	0	0	10	September	0
4	S + 900U5	0	0	5	September	+90
5	S + 900U10	0	0	10	September	+90
6	O0U5	0	0	5	October	0
7	O0U10	0	0	10	October	0
8	O + 900U5	0	0	5	October	+90
9	O + 900U10	0	0	10	October	+90
10	N0U5	0	0	5	November	0
11	N0U10	0	0	10	November	0
12	N + 900U5	0	0	5	November	+90
13	N + 900U10	0	0	10	November	+90
14	SU0	1000	September	0	0	0
15	SU5	1000	September	5	September	0
16	SU10	1000	September	10	September	0
17	S + 90U5	1000	September	5	September	+90
18	S + 90U10	1000	September	10	September	+90
19	OU0	1000	October	0	0	0
20	OU5	1000	October	5	October	0
21	OU10	1000	October	10	October	0
22	O + 90U5	1000	October	5	October	+90
23	O + 90U10	1000	October	10	October	+90
24	NU0	1000	November	0	0	0
25	NU5	1000	November	5	November	0
26	NU10	1000	November	10	November	0
27	N + 90U5	1000	November	5	November	+90
28	N + 90U10	1000	November	10	November	+90

[a] In each of the three columns related to date of application 0 indicates that there was no application.

[b] DCD was applied within 24 h of urine application.

the fresh weight (kg^{-1}) recorded and converted to hectares (Ansah et al., 2010). The responses of annual herbage production (DM was summed over all harvest dates within each year) and spring (February–April, inclusive) herbage production (DM was summed over spring harvest dates within each year) were computed for each plot for each year of the experiments.

2.4.2. Herbage crude protein content and herbage N uptake

The dried herbage was subsequently milled through a 1 mm screen (Tecator Cyclotec 1093 Mill) for chemical analysis. Herbage crude protein (CP) content was determined using near infra-red spectroscopy (NIRS) analysis (NIRS, Model 6500, FOSS-NIR System, 3400 Hillerød, Denmark) and the equation developed by Burns et al. (2010). Herbage N uptake was computed using the following equations:

$$\text{Herbage N content} = \frac{\text{herbage mass (kg DM ha}^{-1}) \times \text{herbage CP content (g kg}^{-1})}{6.25 \times 1000}$$

$$\text{Herbage N uptake} = \text{treatment herbage N content} - \text{control herbage N content}$$

Spring herbage crude protein (averaged over spring harvest dates within each year) and spring herbage N uptakes (summed over spring harvest dates within each year) were computed for each treatment for the two years of the experiments.

2.4.3. Soil ammonium and total oxidised nitrogen content

Soil sampling took place in year 2 on day 7, 14, 28, 56 and 84 post treatment application to determine NH_4^+-N and total oxidised nitrogen (TON) content. Total oxidised nitrogen is NO_3^- plus nitrite (NO_2^-). Two soil cores (0–100 mm depth and 22.5 mm diameter) were removed from each plot. Soil samples from each plot were composited and then sieved through a 2 mm screen to remove debris. Subsamples of 100 g were dried at 105 °C for 24 h to determine DM content. Another subsample of 30 g was extracted with 2 M KCl (Merck KGaA, Frankfurter StraBe 250, 64293 Darmdtadt, Germany) solution by shaking for 2 h using the methodology described by Zaman et al. (1999). The extractant was filtered and frozen until analysis to determine NH_4^+-N and TON content. The NH_4^+-N and TON content in the extractant were measured using an Aquakem 600 Discrete Analyser (Thermo Scientific, Ratastie 2, P.O. Box 100, FI-01621 Vantaa, Finland). Soil bulk density (g m^3) was calculated using methodology described by Hao et al. (2008). Soil NH_4^+-N and TON content (kg N ha^{-1}) were calculated by multiplying NH_4^+-N (mg kg^{-1}) or TON (mg kg^{-1}) by the bulk density. Soil NH_4^+-N and TON were recorded on each plot repeatedly on five sampling occasions in year 2 of the experiments only.

2.4.4. Nitrogen balance

An N balance was calculated for the urine treatments with and without DCD, and the zero urine treatments, again with and without DCD. An N balance was also calculated for a hypothetical farm system in which 2.77% of the land area would receive urine patches treated with DCD; 2.77% represents the land area on which urine was deposited in a single grazing month. Nitrogen surplus was calculated by subtracting N outputs from N inputs.

2.5. Nitrogen inputs

The N inputs consisted of fertilizer (0 or 350 kg N ha^{-1}) and livestock urine deposition (0 or 208.3 kg N ha^{-1}). Annual urine deposition per hectare was calculated using a stocking rate (SR) of 2.5 LU ha^{-1}, urine patch coverage of 20.83% of pasture area per year, and urine deposition of 1000 kg N ha^{-1} at each urination

(Haynes and Williams, 1993). Atmospheric N deposited each year was assumed to be 9 kg N ha^{-1} as estimated by Ryan et al. (2006).

2.6. Nitrogen outputs

The only N output measured in this study was spring herbage N uptake, as calculated above. The N uptake from June to November was calculated using herbage CP content previously measured at Moorepark by O'Donovan and Kennedy (2007).

2.7. Meteorological data

Rainfall, mean daily temperature and soil temperature (measured at 100 mm soil depth) (Table 2) were recorded at climatological station located at the Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark.

2.8. Data analysis

Spring herbage production, annual herbage production, spring herbage CP content, spring herbage N uptake and N balance data were analysed with linear mixed models that allowed for the two repeated measurements (Year 1 and Year 2) fitted using the MIXED procedure in SAS (SAS, 2003). The main factors included in each model were urine rate, urine application date, DCD rate, DCD first application date and DCD second application date. There were two urine rates, 0 and 1000 kg N ha^{-1}. Urine application date had four levels which were 0 (i.e. no application), September, October and November. DCD rate had three levels which were 0, 5 and 10 kg ha^{-1}. DCD first application date had four levels which were 0 (i.e. no application), September, October and November. DCD second application date had two levels which were 0 (i.e. no application) and 90 days after the first application of DCD. Interactions among the variables were also tested. Note that there was some confounding of main effects (Table 1) which restricted how some interactions could be tested. For example, when urine was 0, urine application date also had to be 0 as it was not possible to manipulate date of application when there was no application. A similar situation arises when comparing DCD rate with the two DCD application date variables. Soil $\text{NH}_4{}^+$-N and TON content were also analysed with linear mixed models that allowed for the repeated measures over the five sampling dates (within the one year of sampling for these responses) and were fitted using the MIXED procedure in SAS. The model included main factors as described above and post treatment sampling day. Data for each experiment were analysed individually.

3. Results

3.1. Meteorological data

Table 2 shows the mean daily air temperature; mean monthly soil temperature at a depth of 100 mm and total monthly rainfall for 2008–2010, and the 30 year average (1981–2010) at the Moorepark site. Mean daily air temperatures observed following DCD application in this study were greater than the 30 year average for the months of October of Year 2, November Years 1 and 2, and March Year 1; and was similar or lower than the 30 year average for all other dates. Mean soil temperatures observed in this study were lower than the 30 year average for all application dates except for September Year 1 and March Year 2. Total rainfall in September Year 1, October Years 1 and 2, November Year 2, January Year 1 and March Year 2 were greater than the 30 year average, and was similar or lower to the 30 year average for all other dates.

3.2. Experiments 1 and 2

3.2.1. Herbage production

Spring herbage production was significantly ($P < 0.001$) reduced in Year 2 compared to Year 1 in both experiments (Tables 3 and 4). In Experiment 2 annual herbage production was significantly ($P < 0.001$) greater in Year 2 compared to Year 1 (Table 4).

Spring herbage production was significantly ($P < 0.001$) increased following urine application in Experiment 1 by +90% and in Experiment 2 by +21% compared to the 0U treatments (Tables 3 and 4). Annual herbage production was +36 and +17% greater at MPK and BD, respectively, on U treatments compared to 0U treatments (Tables 3 and 4).

There was no significant effect of urine application date on spring herbage production in either experiment (Tables 3 and 4). Applying urine in November significantly ($P < 0.01$) increased annual herbage production (+18%) in Experiment 1 compared with the September application date (Table 3). Applying urine in September significantly ($P < 0.01$) increased annual herbage production in Experiment 2 compared to the two other application dates (Table 4). Applying DCD at a rate of 5 or 10 kg ha^{-1} significantly ($P < 0.01$) increased annual herbage production in Experiment 1 by +25 and +16%, respectively, compared to applying zero DCD (Table 3). There was no significant effect of DCD first application date or DCD second application date on spring or annual herbage production in either Experiment 1 or 2.

There was a significant ($P < 0.05$) interaction between urine application date and DCD rate at MPK on annual herbage production. Treatment 0U10 significantly increased annual herbage

Table 2
Total rainfall (mm), mean daily air temperature (°C) and mean soil temperature at 100 mm (°C) at Moorepark in 2008, 2009, 2010 and 30 year average (1981–2010).

	Total rainfall (mm)				Mean daily air temperature (°C)				Mean soil temperature at 100 mm (°C)			
	2008	2009	2010	30 year average	2008	2009	2010	30 year average	2008	2009	2010	30 year average
January	145.0	193.7	106.8	109.2	6.7	4.5	2.2	5.4	6.2	4.1	2.3	5.1
February	42.3	15.6	39.0	80.7	6.0	5.1	3.0	5.7	6.1	4.9	3.2	5.2
March	110.5	56.4	88.2	85.6	6.1	7.1	5.4	6.9	6.6	7.3	5.7	6.7
April	37.7	106.7	59.3	64.8	7.9	8.9	8.5	8.5	9.1	10.2	9.9	8.9
May	51.0	88.6	38.3	67.8	12.8	11.0	10.9	11.0	14.7	13.0	13.1	12.3
June	94.4	51.9	52.5	69.8	13.6	14.6	15.4	13.6	16.5	17.1	17.9	15.3
July	134.8	153.8	142.7	65.9	15.1	14.8	15.8	15.5	17.2	17.6	18.2	16.8
August	117.7	116.5	23.1	83.8	15.4	14.9	14.3	15.3	16.8	17.3	17.1	16.3
September	89.9	41.2	102.1	80.9	12.4	12.9	13.6	13.2	13.9	14.9	15.4	13.9
October	113.2	126.7	82.6	113.0	9.2	11.8	9.9	10.2	10.2	12.9	11.1	10.9
November	65.6	259.5	97.7	104.6	8.0	7.8	5.4	7.5	7.7	8.4	6.5	7.8
December	50.1	82.7	36.5	104.3	5.3	3.2	0.6	5.8	4.7	4.0	1.7	6.0
Total	1052	1293	869	1030								

Table 3
The effect of DCD applied following urine application on herbage production (kg DM ha^{-1}), crude protein (g kg^{-1} DM) and nitrogen uptake (kg N ha^{-1}) in Experiment 1.

	Herbage production (kg DM ha^{-1})		Crude protein (g kg ha^{-1})	Nitrogen Uptake (kg ha^{-1})	Surplus N (kg ha^{-1})
Measurement period	Spring	Annual	Spring	Spring	Annual
Year					
Year 1	3027	8067	246	115	22
Year 2	2617	8078	234	89	45
s.e.m.	60	137	1.9	2.6	16.1
Significance	***	NS	***	***	NS
Urine					
Zero urine	1688	6341	211	51	-33
Urine	3200	8649	232	119	89
s.e.m.	97	222	2.6	3.9	8.2
Significance	***	***	***	***	***
DCD rate (kg ha^{-1})					
0	2714	7076	228	93	129
5	2838	8200	243	104	28
10	2914	8840	251	110	15
s.e.m.	79	184	2.2	3.2	19.5
Significance	NS	**	***	**	***
Urine application date					
September	3175	7991	234	114	114
October	3125	8538	252	119	91
November	3300	9420	265	125	55
s.e.m.	98	225	2.7	3.9	16.6
Significance	NS	***	***	NS	*
Urine application date × DCD rate (kg ha^{-1})					
September 0	3112	7397	223	106	137
September 5	2843	6943	235	104	116
September 10	3225	7796	239	113	101
October 0	3107	7997	238	114	139
October 5	3244	8858	253	122	95
October 10	3275	9960	271	127	63
November 0	3306	8578	241	121	111
November 5	3288	9811	266	133	55
November 10	3402	10503	286	135	28
s.e.m.	240	381	5.0	6.7	20.4
Significance	NS	*	***	NS	**

NS, not significant.
[*] $P < 0.05$.
[**] $P < 0.01$.
[***] $P < 0.001$.

production in Experiment 1 by +25%, compared to the OU0 treatment, and treatment NU5 and NU10 significantly increased annual herbage production compared to the NU0 treatment in Experiment 1 by +14 and +22%, respectively (Table 3).

3.2.2. *Herbage crude protein content and nitrogen uptake*

Spring herbage CP content was significantly ($P < 0.001$) greater in Experiment 1 (+5%) (Table 3) and Experiment 2 (+5%) in Year 1 compared with Year 2 (Table 4).

Spring herbage CP content was significantly ($P < 0.001$) greater following urine application in Experiment 1 (+10%) (Table 3) and in Experiment 2 (+18%) (Table 4).

There was a significant ($P < 0.001$) effect of urine application date on spring herbage CP content in both Experiments (Tables 3 and 4). Applying urine in October and November significantly ($P < 0.001$) increased spring herbage CP content compared to September application in Experiment 1 (Table 3). Urine application in September significantly ($P < 0.01$) increased spring herbage CP content by +7 and +4%, respectively, compared to October application in Experiment 2 (Table 4).

Applying 5 or 10 kg DCD ha^{-1} significantly ($P < 0.001$) increased spring herbage CP content by +7 and +10%, respectively, compared to applying zero DCD in Experiment 1 (Table 3). There was no significant effect of DCD first application date or DCD second application date on spring herbage CP content in either Experiment 1 or 2.

There was a significant ($P < 0.01$) interaction between urine application date and DCD rate on spring herbage CP content in Experiment 1 (Table 3). Treatment OU5 and OU10 had significantly ($P < 0.05$) greater spring herbage CP content compared to the OU0 treatment, and treatment NU5 and NU10 had significantly ($P < 0.05$) greater spring herbage CP content compared to the NU0 treatment (Table 3).

Herbage N uptake was significantly greater in Year 1 compared to Year 2 (Tables 3 and 4).

Herbage N uptake was significantly ($P < 0.001$) greater on U treatments compared to 0U treatments in Experiment 1 (+133%) and in Experiment 2 (+44%) (Tables 3 and 4). September urine application significantly ($P < 0.01$) increased herbage N uptake compared to all other application dates in Experiment 2 (Table 4). There was a significant ($P < 0.001$) effect of DCD rate on herbage N uptake in Experiment 1 but not in Experiment 2 (Tables 3 and 4). Applying

Table 4
The effect of DCD applied following urine application on herbage production (kg DM ha^{-1}), crude protein (g kg^{-1} DM) and nitrogen uptake (kg N ha^{-1}) in Experiment 2.

	Herbage production (kg DM ha^{-1})		Crude protein (g kg ha^{-1})	Nitrogen Uptake (kg ha^{-1})	Surplus N (kg ha^{-1})
Measurement period	Spring	Annual	Spring	Spring	Annual
Year					
Year 1	1685	5177	272	72	11
Year 2	1268	7360	258	47	13
s.e.m.	35	96	1.9	1.7	18.2
Significance	***	***	***	***	NS
Urine					
Zero urine	1274	5548	234	45	-51
Urine	1543	6508	275	65	67
s.e.m.	66	163	3.2	2.9	12
Significance	***	***	***	***	***
DCD rate (kg ha^{-1})					
0	1515	6388	265	60	62
5	1457	6155	266	60	5
10	1457	6262	264	59	2
s.e.m.	54	132	2.6	2.4	23.4
Significance	NS	NS	NS	NS	NS
Urine application date					
September	1623	7013	284	73	30
October	1486	6014	265	63	115
November	1521	6497	276	58	55
s.e.m.	66	163	3.2	2.9	23.4
Significance	NS	***	***	**	**
Urine application date × DCD rate (kg ha^{-1})					
September 0	1707	5951	279	76	51
September 5	1553	5258	265	72	25
September 10	1446	5273	270	72	25
October 0	1576	7060	288	72	105
October 5	1467	5942	267	63	114
October 10	1529	6340	276	61	121
November 0	1586	7029	286	72	93
November 5	1439	5844	262	60	66
November 10	1589	6878	282	62	26
s.e.m.	162	397	7.7	5.0	46.3
Significance	NS	NS	*	NS	NS

NS, not significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

DCD at a rate of 5 or 10 kg ha^{-1} significantly ($P < 0.001$) increased herbage N uptake by 11 and 17 kg N ha^{-1}, respectively, compared to applying zero DCD (Table 3). There was no significant effect of DCD first application date or DCD second application date on herbage N uptake in either Experiment 1 or 2.

There was no significant interaction between urine application date and DCD rate on herbage N uptake in either Experiment 1 or 2 (Tables 3 and 4).

3.2.3. *Soil ammonium and total oxidised nitrogen content*

Urine application had a significant effect ($P < 0.001$) on soil NH_4^+-N content compared to 0U treatments in Experiments 1 and 2 (Fig. 1A and B). Soil NH_4^+-N content declined from day 7 to day 28 in Experiment 1 (Fig. 1A), and from day 7 to 84 in Experiment 2 (Fig. 1B) following urine application. There was no significant effect of DCD rate on NH_4^+-N content in either Experiment 1 or 2 (data not shown).

Urine application had a significant effect ($P < 0.001$) on soil TON content compared to 0U treatments in Experiments 1 and 2 (Fig. 1A and B). There was a significant ($P < 0.001$) increase in soil TON content from sampling day 7 to 84 following urine application in Experiment 1 (Fig. 1A) and to day 84 in Experiment 2 (Fig. 1B). There was a significant ($P < 0.05$) effect of DCD rate on soil TON content in Experiment 1 (data not shown); treatment U5 had significantly lower soil TON content on sampling day 7 compared to the treatment U0.

3.2.4. *Nitrogen balance*

Surplus N was significantly ($P < 0.001$) lower on treatments receiving zero urine compared to those receiving urine in Experiments 1 and 2 (Tables 3 and 4). September applied urine significantly ($P < 0.05$) increased surplus N compared to November applied urine in Experiment 1 (Table 3). October applied urine significantly ($P < 0.01$) increased surplus N compared to September applied urine in Experiment 2 (Table 4).

The application of 5 and 10 kg DCD ha^{-1} significantly ($P < 0.001$) reduced N surplus in Experiment 1 (Table 3); there was no effect in Experiment 2.

There was a significant ($P < 0.01$) effect of the interaction between urine application date and DCD rate on N surplus in Experiment 1 only (Table 3). The N surplus on treatment OU10 was

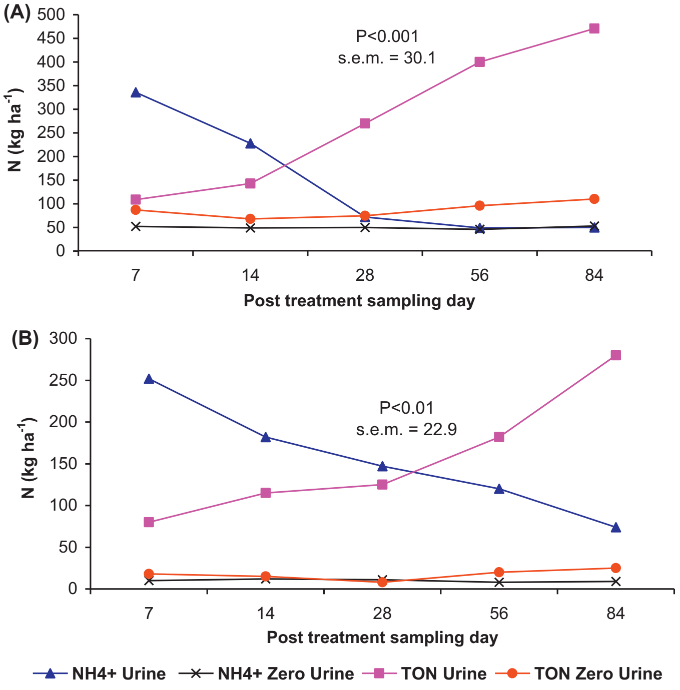

Fig. 1. The effects of urine or zero urine applied at N equivalent rates of 1000 or 0 kg N ha^{-1} on NH_4^+-N and TON content in the upper layers (0–100 mm) of the soil profile in Experiment 1 (A) and in Experiment 2 (B).

significantly lower than treatment OU0, and was significantly lower on treatment NU10 compared to treatment NU0 (Table 3).

3.3. Experiments 3 and 4

3.3.1. Herbage production

Spring herbage production was significantly ($P < 0.001$) reduced in Year 2 compared to Year 1 in Experiments 3 and 4 (Tables 5 and 6). In Experiment 3 annual herbage production was significantly ($P < 0.001$) reduced in Year 2 compared to Year 1 (Table 5), while in Experiment 4 annual herbage production was significantly ($P < 0.001$) greater in Year 2 than in Year 1 (Table 6).

Urine application had no significant effect on spring herbage production in Experiment 3 (Table 5). Annual herbage production was significantly ($P < 0.05$) increased (+5%) following urine application compared to zero urine application in Experiment 3 (Table 5). In Experiment 4, spring herbage production was significantly ($P < 0.001$) reduced following urine application, but there was no significant effect on annual herbage production (Table 6).

In Experiment 3 September urine application significantly ($P < 0.01$) increased spring herbage production compared to November urine application (Table 5).

There was no significant effect of DCD rate, DCD first application date or DCD second application date on spring or annual herbage production in either Experiment 3 or 4 (Tables 5 and 6).

3.3.2. Herbage crude protein content and nitrogen uptake

Spring herbage CP content was significantly ($P < 0.001$) greater in Year 1 (+5%) compared to Year 2 in Experiment 4 (Table 6).

Urine application significantly ($P < 0.001$) increased spring herbage CP content in Experiments 3 and 4 (Tables 5 and 6) compared to the 0U treatment. October and November applied urine significantly ($P < 0.01$) increased spring herbage CP content compared to September applied urine in Experiment 3 (Table 5).

Applying DCD at a rate of 5 or 10 kg ha^{-1} significantly ($P < 0.001$) increased spring herbage CP content compared to applying zero DCD in Experiments 3 and 4 (Tables 5 and 6). There was no significant effect of DCD first application date or DCD second application date on spring herbage CP content in either Experiment 3 or 4.

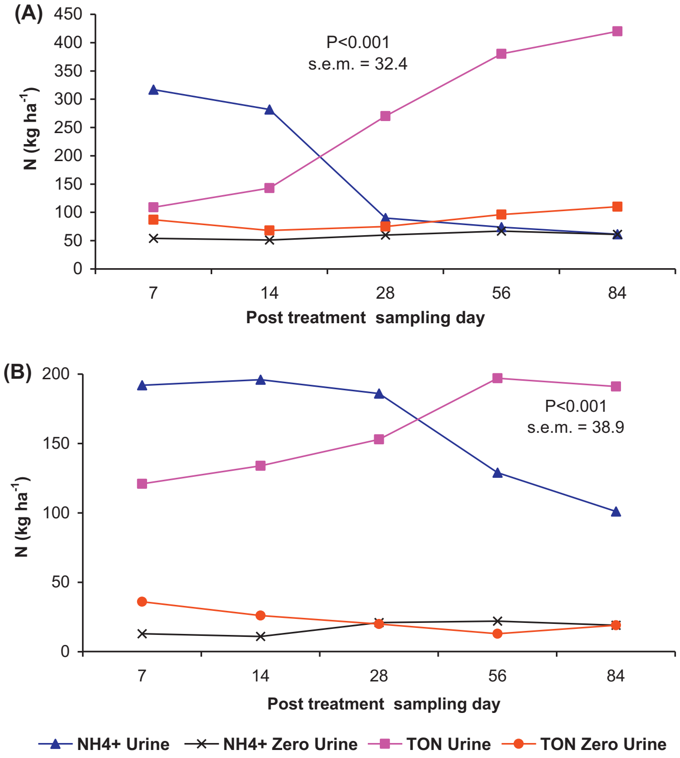

Fig. 2. The effects of urine or zero urine applied at N equivalent rates of 1000 or 0 kg N ha^{-1} on NH_4^+-N and TON content in the upper layers (0–100 mm) of the soil profile in Experiment 3 (A) and in Experiment 4 (B).

There was a significant interaction ($P < 0.001$) between urine application date and DCD rate on spring herbage CP content in Experiment 3 (Table 5); treatment NU0 had a significantly ($P < 0.01$) lower spring herbage CP content than NU10.

Herbage N uptake was significantly ($P < 0.001$) reduced in Year 2 (−55%) compared to Year 1 in Experiment 3 (Table 5). In Experiment 4, herbage N uptake was significantly ($P < 0.001$) greater in Year 2 compared to Year 1 (Table 6).

Urine application significantly ($P < 0.001$) increased herbage N uptake in both Experiments 3 and 4 compared to 0U treatments (Tables 5 and 6). There was no significant effect of urine application date on herbage N uptake in either Experiment 3 or 4.

Applying DCD at a rate of 5 or 10 kg ha^{-1} significantly ($P < 0.001$) increased herbage N uptake by +19 and +22%, respectively, compared to applying zero DCD in Experiment 4 (Table 6). There was no significant effect of DCD first application date or DCD second application date on herbage N uptake in either Experiment 3 or 4.

Treatments SU5 and SU10 significantly ($P < 0.01$) increased herbage N uptake in Experiment 4 by +27 and 29%, respectively, compared to SU0 treatment (Table 6).

3.3.3. Soil ammonium and total oxidised nitrogen content

Urine application significantly ($P < 0.001$) increased soil NH_4^+-N content compared to the 0U treatments up to day 14 in Experiment 3 and up to day 56 in Experiment 4 (Fig. 2A and B). The NH_4^+-N content declined from day 7 to day 84 post urine application. There was no significant effect of DCD rate on soil NH_4^+-N content in both Experiments 3 and 4 (data not shown).

Urine application significantly ($P < 0.001$) increased soil TON content compared to 0U treatments across all sampling days in Experiments 3 and 4 (Fig. 2A and B). There was no significant effect

Table 5
The effect of DCD applied following urine application on herbage production (kg DM ha^{-1}), crude protein (g kg^{-1} DM) and nitrogen uptake (kg N ha^{-1}) in Experiment 3.

	Herbage production (kg DM ha^{-1})		Crude protein (g kg ha^{-1})	Nitrogen Uptake (kg ha^{-1})	Surplus N (kg ha^{-1})
Measurement period	Spring	Annual	Spring	Spring	Annual
Year					
Year 1	4437	13467	293	204	396
Year 2	2122	9682	290	92	438
s.e.m.	59	138	1.7	2.5	22.6
Significance	***	***	NS	***	NS
Urine					
Zero urine	3142	11170	279	134	307
Urine	3325	11710	296	153	496
s.e.m.	103	241	2.8	4.2	5.9
Significance	NS	*	***	***	***
DCD rate (kg ha^{-1})					
0	3345	11588	279	145	498
5	3256	11637	296	150	413
10	3238	11499	300	149	401
s.e.m.	84	199	2.3	3.4	30
Significance	NS	NS	***	NS	NS
Urine application date					0.08
September	3565	11583	289	156	499
October	3301	11648	297	152	494
November	3108	11898	302	150	493
s.e.m.	103	243	2.8	4.2	12.5
Significance	**	NS	**	NS	NS
Urine application date × DCD rate (kg ha^{-1})					
September 0	3781	11940	279	157	494
September 5	3123	10841	278	152	504
September 10	3440	12480	288	159	498
October 0	3350	11239	295	137	524
October 5	3478	12060	302	160	488
October 10	3008	11937	302	159	485
November 0	3565	11569	292	156	477
November 5	3304	12043	311	150	497
November 10	2877	11277	317	144	496
s.e.m.	245	671	6.8	7.2	24.9
Significance	NS	NS	*	NS	NS

NS, not significant.
[*] $P < 0.05$.
[**] $P < 0.01$.
[***] $P < 0.001$.

of DCD rate on soil TON content in Experiments 3 and 4 (data not shown).

3.3.4. Nitrogen balance

Urine application significantly ($P < 0.001$) increased surplus N compared to zero urine application by 62 and 59% in Experiments 3 and 4, respectively (Tables 5 and 6).

The application of DCD at a rate of 5 and 10 kg ha^{-1} significantly ($P < 0.05$) reduced N surplus by 20 and 21%, respectively, in Experiment 4 (Table 6).

4. Discussion

4.1. Herbage production, herbage crude protein content and herbage N uptake

Whitehead and Bristow (1990) reported that the recovery of urinary N by the soil/plant component of the sward is subject to a number of factors including rainfall amount, which affects plant growth and leaching, and variation in temperature, which contributes to evaporation and to plant growth. In Experiment 1 (zero N fertiliser) average spring and annual herbage production was increased at both sites when urine was applied, similar to Qiu et al. (2010), due to high N content in urine (Haynes and Williams, 1993). In Experiment 3 (350 kg N ha^{-1} year^{-1}) there was a positive effect of urine application on annual herbage production. While some of the additional N applied in the urine would have been used for herbage growth, it is likely that most of the N was surplus to the requirements of the sward. Frame (1992) and Van Burg et al. (1981) suggest that N supplies greater than 350–400 kg N ha^{-1} year^{-1} are surplus to the requirements of the growing plant. Lantinga et al. (1987) reported that grass is more susceptible to urine scorch at N fertilisation levels similar to the rate applied in Experiments 3 and 4. Middelkoop and Deenan (1990) observed that urine had a negative effect on herbage production in the treated areas due to scorching; this effect was strongest at 400 kg N ha^{-1} and increased with increasing N concentration. The application of urine to N fertiliser treated swards may have inhibited herbage production as a result of some scorching in Experiments 3 and 4.

The application of DCD at 5 or 10 kg ha^{-1} significantly increased annual herbage production in Experiment 1; similar to Di and Cameron (2002, 2005). In a whole paddock scenario receiving zero

Table 6
The effect of DCD applied following urine application on herbage production (kg DM ha^{-1}), crude protein (g kg^{-1} DM) and nitrogen uptake (kg N ha^{-1}) in Experiment 4.

	Herbage production (kg DM ha^{-1})		Crude protein (g kg ha^{-1})	Nitrogen Uptake (kg ha^{-1})	Surplus N (kg ha^{-1})
Measurement period	Spring	Annual	Spring	Spring	Annual
Year					
Year 1	2038	7254	299	21	429
Year 2	1146	8507	285	52	440
s.e.m.	35	117	1.4	1.6	24.0
Significance	***	***	***	***	NS
Urine					
Zero urine	1800	8083	279	25	327
Urine	1592	7880	296	40	521
s.e.m.	60	167	1.9	1.7	7.4
Significance	***	NS	***	***	***
DCD rate (kg ha^{-1})					
0	1544	7776	288	32	530
5	1560	7894	294	38	426
10	1672	7971	294	39	420
s.e.m.	50	147	1.5	1.6	31.2
Significance	NS	NS	*	**	*
Urine application date					
September	1470	7643	294	41	520
October	1459	7678	296	40	525
November	1639	8119	299	39	517
s.e.m.	61	176	1.9	1.9	7
Significance	NS	NS	NS	NS	NS
Urine application date × DCD rate (kg ha^{-1})					
September 0	1489	7271	289	34	536
September 5	1337	7435	292	43	517
September 10	1630	8134	298	44	516
October 0	1342	7615	293	37	538
October 5	1504	7804	299	44	519
October 10	1512	7092	300	40	525
November 0	1578	8043	299	37	517
November 5	1537	7794	296	38	520
November 10	1774	8131	300	43	514
s.e.m.	151	410	4.7	3.0	13.4
Significance	NS	NS	*	NS	NS

NS, not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

N fertiliser, growing 8000 kg DM ha^{-1} and grazed by dairy cows the addition of DCD at either rate would increase annual herbage production by between 200 and 400 kg DM ha^{-1}, assuming that 25% of the paddock receives urine deposition per year plus 5 or 10 kg DCD ha^{-1} (Haynes and Williams, 1993). The application of DCD did not increase herbage production in Experiment 2, 3 or 4, similar to Cookson and Cornforth (2002), Menneer et al. (2008) and Monaghan et al. (2009), and therefore the hypothesis that the application of DCD at different rates and times in autumn and winter would increase herbage production in Experiment 2, 3 and 4 is rejected. Previous authors report that DCD should be applied in cool temperatures and periods of low rainfall to maximise its effectiveness (Vallejo et al., 2005). The rainfall experienced following DCD application in this experiment may have leached the DCD beyond the rooting zone, therefore reducing its effectiveness. High soil and air temperatures recorded in October and November may have reduced the half life of the DCD, therefore reducing its capacity to slow the conversion of NH_4^+ to TON. As mentioned temperature is one of the most influential environmental factors affecting the effectiveness of DCD (Williamson et al., 1996). An increase in temperature can have a negative effect on the persistence of DCD in the soil, reducing the time frame in which it can provide effective nitrification reduction.

Developing a DCD application strategy to contribute to increased herbage production and help to mitigate environmental emissions associated with increased agricultural production in Ireland would most likely require consideration of factors other than soil temperature. It may be worth considering DCD application at a time when rainfall is lower than in autumn/winter so that the DCD is less likely to be leached beyond the rooting zone. In such instances the DCD will reduce nitrification in a soil zone in which herbage could benefit from the availability of additional N for plant growth. The soil sampling undertaken in Experiments 3 and 4 indicates that DCD had almost no effect on soil mineral N content in the 0–100 mm soil horizon. However, as DCD has been shown to be effective at reducing N_2O emissions and NO_3^- leaching in Irish soils (Selbie et al., 2011; Richards et al., 2008) it is quite possible that the DCD is inhibiting nitrification beyond a soil depth of 100 mm.

During this study urine deposition increased spring herbage CP content in all experiments similar to other authors (Ledgard et al., 1982; Williams and Haynes, 1994). Similar to Moir et al. (2007), the application of DCD had an effect on spring herbage CP content in

Experiments 1 and 3 at MPK as a result of increased N uptake. The application of DCD may have provided additional N in the rooting zone for plant uptake; but due to low temperatures in spring this did not result in increased grass growth but rather resulted in the accumulation of N in the plant foliage.

Throughout this study urine application increased herbage N uptake compared to zero urine application, similar to Cuttle and Bourne (1993). The increase in herbage N uptake may be due to the uptake of urea into the plant by roots; this mode of N uptake is small compared to the uptake of NO_3^--N and NH_4^+-N. However, foliar adsorption of urea can occur at a high rate and can contribute substantially to plant N accumulation and not result in herbage production (Camberato, 2001).

Also, N uptake is reported to increase with increasing rates of N applied, including rates of N above which herbage production is maximised (Lkhagvasuren, 2007). Nitrogen uptake is also reported to occur in periods of low grass growth (Blombäck and Eckersten, 1997), also resulting in increased CP content but not herbage production. The ability of perennial ryegrasses to increase N uptake below the top 100 mm of the soil profile are limited as a small proportion of the roots occur below the top 100 mm of the soil profile (Garwood, 1967) and also 90% of the root activity of perennial ryegrass occurs in the top 125 mm of the soil profile (Syers et al., 1984), thereby, the capability of perennial ryegrasses to utilise the available N for herbage production below the top 100 mm of the soil profile is greatly reduced.

4.2. Soil ammonium and total oxidised nitrogen content

Urine application increased soil NH_4^+-N and TON content compared to zero urine application. In urine treated soils, urea is rapidly hydrolysed to NH_4^+-N and reaches maximum values soon after urine application (Zaman et al., 2009). Nitrification then oxidises the NH_4^+-N to nitrite and the nitrite is further oxidised to NO_3^- (Estavillo et al., 2002). In the experiments reported here, soil NH_4^+-N content declined with time following urine application. The period following urine application was one of low sward N demand due to low rates of grass growth and as a result N was likely to be lost by leaching through the soil profile or through immobilization (Woodmaness et al., 1981). At MPK the elevated NH_4^+-N content following urine application declined and was similar to the zero urine treatments by day 84 and so no additional N was available for spring grass growth. At BD, there was considerably more soil NH_4^+-N present in the rooting zone (0–100 mm) at sampling day 84 on the urine treatments. The rapid movement of N to below the rooting zone at MPK can be attributed to the free draining soil characteristics at this site, while at BD the drainage capabilities are more restricted, resulting in reduced leaching potential (Decau et al., 2003; Richards et al., 2008).

Moir et al. (2007) reported that the application of 10 kg DCD ha^{-1} to urine patches inhibited urine sourced NH_4^+-N in the soil for several weeks over the autumn/winter period, however, in Experiments 1 and 2 reported here DCD provided inhibition of urine sourced NH_4^+-N for a short period of 14 days. The soil sampling undertaken in the four experiments indicate that DCD had almost no effect on soil mineral N content to a soil depth of 100 mm suggesting that the DCD may have leached below this soil depth.

4.3. Nitrogen balance

Research has shown that N losses from a growing sward increase exponentially with an increase in N inputs (Scholefield et al., 1993), this was observed in the experiments reported here when urine and fertiliser were applied to a growing sward. As it is not possible to quantify the total quantity of surplus N in this study as the only N output measured was herbage N uptake, the N balances reported are a crude estimate of N surplus. As DCD can reduce NO_3^- leaching and N_2O emissions, as measured by other authors, it will have a positive effect on a farm N balance. However, DCD is most effective on urine patches and in cool temperatures, and so the over all effect in reducing N surplus will be proportional to the area of the farm affected by urine patches, particularly in autumn. For example, using an annual farm-gate N balance scenario where DCD was applied in September following urine deposition on a farm applying 250 kg inorganic N ha^{-1} year^{-1} compared to a farm applying zero DCD, the N surplus would be reduced by 3 kg N based on the effect of DCD on herbage production.

5. Conclusions

Urine increased spring and annual herbage production when applied to swards receiving zero fertiliser; however when urine was applied to swards receiving fertiliser, spring herbage production was not significantly increased. Urine application also increased N uptake and spring herbage CP content. DCD applied at 5 and 10 kg ha^{-1} increased annual herbage production at MPK by 200 to 400 kg DM ha^{-1}. Dicyandiamide increased spring herbage CP content at MPK in Experiments 1 and 3. The addition of DCD also increased herbage N uptake at MPK in Experiment 1 and at BD in Experiment 4. In Experiments 1 and 3, soil NH_4^+-N content on the urine treatments declined over the sampling period, while soil TON content was significantly increased on the urine treatments compared to the zero urine treatments in both experiments. Dicyandiamide applied to October and November deposited urine reduced N surplus in Experiments 1 and 4. Overall the effects of DCD on herbage production, surplus N and other parameters in this study were not consistent.

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