



The effect of cattle slurry in combination with nitrate and the nitrification inhibitor dicyandiamide on in situ nitrous oxide and dinitrogen emissions

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Received: 22 June 2012 – Published in Biogeosciences Discuss.: 24 July 2012

Revised: 5 November 2012 – Accepted: 6 November 2012 – Published: 4 December 2012

Abstract. A field study was conducted to determine the effect of the nitrification inhibitor dicyandiamide (DCD) on N₂O and N₂ emissions after cattle slurry (CS) application in the presence of nitrate (NO₃) fertiliser on seven different occasions (between March 2009 and March 2011).

N₂O emissions from CS in the presence of NO₃ fertiliser were very high (0.4–8.7 % of applied N) over a 20-day period, under mild moist conditions. Emissions were significantly larger from the CS treatment compared to an NH₄⁺-N source, supplying the same rate of N as in the slurry. This study supports the view that organic fertilisers should not be applied at the same time as nitrate-based fertilisers, as significant increases in N₂O emissions occur. The average N₂O mole fraction (N₂O/(N₂O + N₂)) over all seven application dates was 0.34 for CSNO₃ compared to 0.24 for the NH₄ClNO₃ treatment, indicating the dominance of N₂ emissions.

The rate of nitrification in CSNO₃ was slower than in NH₄ClNO₃, and DCD was found to be an effective nitrification inhibitor in both treatments. However, as N₂O emissions were found to be predominantly associated with the NO₃ pool, the effect of DCD in lowering N₂O emissions is limited in the presence of a NO₃ fertiliser. To obtain the maximum cost-benefit of DCD in lowering N₂O emissions, under mild moist conditions, it should not be applied to a nitrate contain-

ing fertiliser (e.g. ammonium nitrate or calcium ammonium nitrate), and therefore the application of DCD should be restricted to ammonium-based organic or synthetic fertilisers.

1 Introduction

Ammonium nitrate (AN) and calcium ammonium nitrate (CAN) are the dominant forms of fertiliser applied to grassland in UK and Ireland. AN contains nitrogen as NH₄⁺-N and NO₃⁻-N, and CAN contains in addition dolomite or limestone. Fertiliser usage for grassland has been declining since the mid-1980s by 38 % in UK and 33 % in Ireland. Spiralling fertiliser prices (Farmers Weekly, 2012) and nitrate regulations have mostly caused the observed decline. There is now more of an incentive to use organic fertilisers to supply nitrogen (N) in a nutrient management plan on farms, and cattle slurry is by far the most common form of organic fertiliser. On-farm nutrient management planning encourages the integrated use of both cattle slurry and inorganic fertilisers. Therefore the practice of cattle slurry being applied in the presence of NO₃ is regionally relevant.

Reviews of field studies where N₂O emissions were measured suggested that mineral N fertilisers plus organic manures resulted in higher losses than with mineral N fertilisers

alone (Bouwman, 1990; Granli and Bøckman, 1994). Field studies have shown that whenever fertilisers containing NO_3^- and CS are applied together, or within a few days of each other, the potential exists for enhanced N_2O and N_2 emissions. In moist soil conditions, cumulative N_2O emissions were up to 4 times greater from NH_4NO_3 followed by CS than from NH_4NO_3 alone (McTaggart et al., 1997). When CS was supplemented with NH_4NO_3 , the loss of N_2O was 2.2 % compared with 1.2 % for NH_4NO_3 alone (Clayton et al., 1997). In studies conducted on four occasions in 1997, cattle slurry increased the flux of N_2O by an average factor of 3 (Stevens and Laughlin, 2001a). Lampe et al. (2006) found that the combined application of slurry and mineral fertiliser increased N_2O emissions by between 30 to 150 % compared to emissions from CAN alone. It has been reported that denitrifying bacteria are capable of utilising the volatile fatty acids (VFAs) (acetate, propionate and butyrate) present in slurry as C sources (Paul et al., 1989).

Nitrification is the general term for the aerobic oxidation of reduced nitrogen (NH_3) to NO_2^- and further to NO_3^- by autotrophic or heterotrophic microorganisms (Coyne and Frye, 2005). Autotrophic nitrification is a two-step process carried out by chemolithotrophic bacteria, which obtain energy by oxidizing NH_3 to NO_3^- . The first step is carried out by *Nitrosomonas* bacteria and involves the sequential oxidation of NH_3 to hydroxylamine and then to NO_2^- . The second step is the oxidation of NO_2^- to NO_3^- by *Nitrobacter* bacteria.

Heterotrophic nitrification is the oxidation of organic N and NH_3 to NO_3^- by chemoheterotrophic bacteria and fungi who gain their energy from organic C instead of CO_2 . Oxidation of NH_4^+ is a key process in the N cycle having implications for the environment as non-mobile NH_4^+ is converted to mobile NO_3^- , which, if produced in excess of plant needs, is either leached out of the root zone or is denitrified to produce N-gases (NO , N_2O and N_2). Nitrification inhibitors are thought to act specifically on the enzyme ammonium monooxygenase, by blocking the site where ammonium is converted to NO_2^- . This slows down the microbial conversion of NH_4^+ to NO_3^- and hence reduces NO_3^- build-up and associated loss processes. The rate of degradation and hence the efficacy of nitrification inhibitors are affected by soil temperature (Zerulla et al., 2001; Edmeades, 2004), pH (Keeney, 1986), moisture content (Hendrikson and Keeney, 1979; Puttanna et al., 1999) and soil organic matter content (Briggs, 1975). The mobility of nitrification inhibitors in soil may also be a factor affecting their efficacy. As DCD is highly soluble, it can be readily leached through the profile (Zerulla et al., 2001).

Denitrification can be a major N loss process in grassland soils. Although the final product of denitrification is the benign atmospheric gas N_2 , this process is not always completed and variable amounts of N_2O , a potent greenhouse gas (GHG), can be produced. Physical, chemical and biological factors influence the reduction of N_2O to N_2 (Weier et

al., 1993) including soil moisture content (Davidson, 1991; Ruser et al., 2006), pH (Simek and Cooper, 2002; Cuhel et al., 2010), temperature (Bailey, 1976; Keeney et al., 1979), C supply (Parkin, 1987; Mathieu et al., 2006), soil redox conditions (Firestone and Tiedje, 1979; Tiedje, 1988) and management (Frolking et al., 1998; Liu et al., 2007).

Most attention over the last decade has been concentrated on the intermediate gaseous products of denitrification, NO and N_2O , because of their importance in tropospheric and stratospheric processes of ozone production and consumption, and radiative forcing (Davidson et al., 2000; Hall et al., 1996; Seitzinger and Kroeze, 1998). The anthropogenic inputs of reactive N to the environment, resulting from food and energy production, have been increasing during the last few decades and reactive N has been accumulating in the environment. The largest gap in knowledge is the amount of reactive N converted back to N_2 by denitrification. Without this information it is impossible to determine the rate of accumulation of reactive N in all environmental reservoirs. At present reliable quantification of N_2 produced in the field is rare, and estimates of how much reactive N is denitrified are highly uncertain.

In this study we used the ^{15}N gas flux method in the field to determine the effect of CS and DCD in the presence of nitrate on N_2 and N_2O emissions on seven occasions between March 2009 and March 2011.

2 Materials and methods

2.1 Study site characteristics

The experimental grassland site was located in Hillsborough, County Down, Northern Ireland ($54^\circ 46' \text{N}$; $6^\circ 08' \text{W}$), where experiments were conducted on seven different occasions (March, July, October 2009, March, June, October 2010 and March 2011). A new area of grassland was used for each of the seven experimental applications. The sward was dominated by perennial ryegrass (*Lolium perenne* L.). White clover (*Trifolium repens*) and broad-leaved docks (*Rumex obtusifolius*) were present in low amounts (clover < 2 %), and, in order to prevent any confounding effect of clover, the sward was sprayed using an appropriate herbicide one month prior to each treatment application. The long-term (1995–2010) average rainfall and daily temperature for the site were 916 mm and 9.3°C , respectively. The soil is a sandy clay loam with moderate drainage. The site was managed as a low N input system to maintain the same sward for all experiments. A basal dressing of P, K and S was applied prior to each experiment so that these major nutrients were not limiting grass growth.

Table 1. Properties and application rates of the cattle slurries.

Application Date	Raw CS properties												Amended CS properties			
	NH ₄ ⁺ -N mg kg ⁻¹	Acetic Acid g L ⁻¹	Ethanol g L ⁻¹	i-Butyric Acid g L ⁻¹	i-Valeric Acid g L ⁻¹	Lactic Acid g L ⁻¹	n-Butyric Acid g L ⁻¹	n-Valeric Acid g L ⁻¹	Propanol g L ⁻¹	Propionic Acid g L ⁻¹	Total VFAs g L ⁻¹	Dumas C g kg ⁻¹ dry matter	pH	Dry Matter %	NH ₄ ⁺ -N rate kg N ha ⁻¹	NO ₃ ⁻ -N rate kg N ha ⁻¹
Mar-09	2845	5.54	0.00	0.16	0.30	0.00	0.14	0.00	0.00	1.66	7.80	409.9	7.88	5.00	135.1	65
Jun-09	2001	7.98	0.00	0.26	0.38	0.00	0.88	0.14	0.00	2.72	12.36	430.2	7.88	5.00	82.7	65
Oct-09	1545	5.74	0.00	0.18	0.34	0.00	0.36	0.00	0.00	1.70	8.32	370.2	7.40	3.79	103.4	65
Mar-10	1960	3.99	0.00	0.07	0.17	0.00	0.16	0.00	0.00	0.51	4.90	415.8	8.27	5.02	140.6	65
Jun-10	1671	4.28	0.00	0.12	0.20	0.00	0.46	0.00	0.00	1.06	6.12	421.3	7.14	5.39	114.7	65
Oct-10	1068	2.14	0.10	0.00	0.14	0.00	0.08	0.00	0.00	0.08	2.54	393.7	7.52	3.16	80.0	65
Mar-11	2485	5.18	0.00	0.16	0.30	0.00	0.50	0.06	0.00	1.42	7.62	424.9	7.43	5.00	73.9	65

2.2 Experimental treatments

Cattle slurry (33 m³ ha⁻¹) amended with KNO₃ (65 kg N ha⁻¹), with or without DCD (at 15 % NH₄⁺-N content of the CS), was surface applied to grassland with either the NH₄⁺ or the NO₃⁻ pool ¹⁵N labelled (¹⁵CS¹⁴NO₃, ¹⁴CS¹⁵NO₃) at 50 atom %, according to the procedure of Stevens et al. (1997). Ammonium chloride (NH₄Cl) amended with KNO₃ (65 kg N ha⁻¹), with or without DCD, with either the NH₄⁺ or the NO₃⁻ pool ¹⁵N labelled, served as controls for ¹⁴CS¹⁵NO₃ and ¹⁵CS¹⁴NO₃, respectively, having the same amount of NH₄⁺-N as the CS but no degradable carbon. In summary, there were eight treatments: (i) ¹⁵CS¹⁴NO₃ without DCD, (ii) ¹⁵CS¹⁴NO₃ with DCD, (iii) ¹⁴CS¹⁵NO₃ without DCD, (iv) ¹⁴CS¹⁵NO₃ with DCD, (v) ¹⁵NH₄Cl¹⁴NO₃ without DCD, (vi) ¹⁵NH₄Cl¹⁴NO₃ with DCD, (vii) ¹⁴NH₄Cl¹⁵NO₃ without DCD, and (viii) ¹⁴NH₄Cl¹⁵NO₃ with DCD. The eight treatments were replicated four times in a randomised block design. Immediately prior to application aliquots of CS/NH₄Cl, KNO₃, and DCD/water were mixed together and uniformly applied directly inside the chamber (area of 0.16 m²).

2.3 Slurry collection and analysis

Dairy CS was collected prior to each of the seven treatment application dates. The properties of the raw and amended CS and application rates for each of the seven application dates are detailed in Table 1. CS was amended by adding either unlabelled urea or urea enriched at 99 atom % and incubating the CS for 3 days at 35 °C to hydrolyse the urea to NH₄⁺-N (Stevens and Laughlin, 2001a). Slurry dry matter content was determined by drying a representative slurry sample at 105 °C for 24 h. Total ammoniacal N in slurry was determined by steam distillation into boric acid solution, in the presence of magnesium oxide. The boric acid solution was titrated with 0.1 M sulphuric acid to determine the ammonium N in the sample. Slurry pH was determined by Orion pH meter Model 420A; volatile fatty acids (VFAs) by capillary gas-liquid chromatography and detected by a flame ionization detector; and total C by the Dumas method (Bremner and Mulvaney, 1984) with quantitative analysis by an Elemental Vario Max CN elemental analyser.

2.4 Flux measurement technique

Gaseous N₂ and N₂O emissions were measured using the static chamber method by deploying square stainless steel chambers (0.4 × 0.4 m wide and 0.15 m high). The chamber collar was inserted into the ground to a depth of ≥ 5 cm at least 3 days prior to commencing each experiment, and left in position for the duration of the experiment. The collars had a water-filled trough into which the chamber lid was placed when sampling, thus ensuring a gas-tight seal. Gas sampling occurred between 10:00 and 12:00 GMT on 10 occasions over a 20-day period following treatment application. In order to detect evolved ¹⁵N₂, Stevens and Laughlin (1998) showed that a chamber closure time of 2 h was necessary. After 2 h, samples of the chamber headspace were taken through a silicone septa positioned on the centre of the chamber lid, using a 20-mL polypropylene syringe equipped with a 25-gauge luer lock needle (0.5 × 16 mm). The syringe was flushed once with headspace air before sampling. A 15-mL sample was withdrawn from the chamber and injected into a 12-mL pre-evacuated glass vials fitted with a 3-mm butyl rubber septa (Labco, UK). Linearity checks on N₂O were conducted on 104 occasions over the total experimental period from the CSNO₃ without DCD treatment by sampling the chamber headspace four times over the chamber closure period. Chamber datasets were not examined for linearity if the T_{2h} sample was not significantly higher than the T_{0h} sample, or occasionally if apparent leaks occurred in the vials. From the 104 chamber datasets, 27 % were discarded for these reasons. In Fig. 1 the percentage of data is depicted for ranges of linear coefficient of determination (R²) for the accepted datasets. Eighty-four percent of the accepted data had an R² of > 0.85. As this experiment was a comparative study, it was deemed suitable to apply a linear regression model to all data. Cumulative fluxes were calculated by linear interpolation between sampling times.

2.5 Analysis of N₂O and N₂

The concentration and ¹⁵N content of N₂O and the ¹⁵N content of the N₂ was determined by automated isotope ratio mass spectrometry (IRMS) as described by Stevens et al. (1993), using a Europa Scientific 20-20 stable isotope

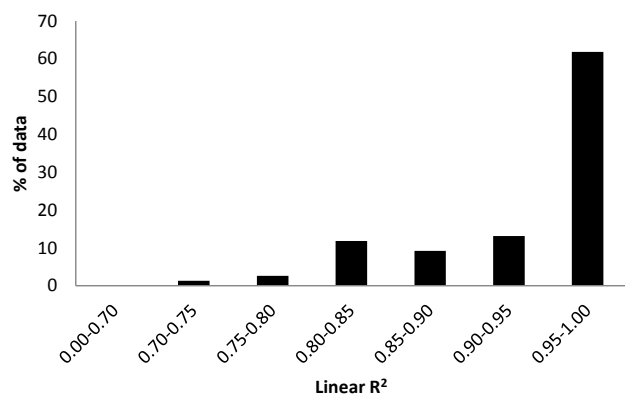


Fig. 1. Percentage of data for given ranges of coefficient of determination (R^2) of the linear regression, $n = 76$.

analyser interfaced to a Europa Scientific Trace Gas Preparation System ANCA-TG (Crewe, UK) with Gilson autosampler (Anachem, Luton, UK). The ion currents (I) at mass-to-charge ratio (m/z) 44, 45, and 46 enabled concentrations and molecular ratios ^{45}R ($^{45}\text{I}/^{44}\text{I}$) and ^{46}R ($^{46}\text{I}/^{44}\text{I}$) to be calculated for N_2O . The sources of N_2O were then apportioned into the fraction (d'_D) derived from the denitrifying pool of enrichment a_D and the fraction $d'_\text{N} = (1 - d'_\text{D})$ derived from the pool or pools at natural abundance (Arah, 1997). For N_2 , the ion currents at m/z 28, 29 and 30 enabled molecular ratios ^{29}R ($^{29}\text{I}/^{28}\text{I}$) and ^{30}R ($^{30}\text{I}/^{28}\text{I}$) to be determined. Differences between the molecular ratios in enriched and normal atmospheres were calculated as $\Delta^{29}\text{R}$ and $\Delta^{30}\text{R}$. The flux of N_2 was calculated using $\Delta^{30}\text{R}$ data only and the equation of Mulvaney (1984), assuming that the enrichment of the denitrifying pool was a_D (Stevens and Laughlin, 2001b). This method of N_2 flux calculation improved the sensitivity of N_2 emission measurement by a factor of 16, and without this method we could not have detected the N_2 flux.

2.6 Statistical methods

Analyses were carried out using GenStat version 14 software. Each of the seven application times was analysed separately. The experiment was analysed using analysis of variance as a randomised block experiment with a full factorial design incorporating two factors. The factors used were $\text{NH}_4^+\text{-N}$ form (two levels: CS or NH_4Cl) and DCD (2 levels: with and without DCD). The ANOVA model was used to ascertain the significance of treatments on the cumulative fluxes of N_2O , N_2 , and N_2O mole fraction, and on the values of d'_D and a_D . A significance level of 0.05 was used, unless otherwise stated. Estimates of means, standard error of means, standard error of differences in means and Fisher's least significant difference (LSD) were calculated.

3 Results

3.1 Composition of applied cattle slurries

The $\text{NH}_4^+\text{-N}$ contents of the amended slurries used over the course of this experiment varied considerably over the seven application times (Table 1). The rate of $\text{NH}_4^+\text{-N}$ applied varied between 73.9 to 140.6 kg N ha^{-1} . The same rate of $\text{NO}_3^-\text{-N}$ was applied on each occasion to give a rate of 65 kg N ha^{-1} . The slurries used had a mean dry matter content of 4.6 %, and the pH ranged from 7.14 to 8.27 (Table 1).

3.2 Rainfall and soil temperature

Daily rainfall and soil temperature at 5 cm were collected at a weather station located within 1 km of the site. Figure 2 shows the daily data for 20 days post-treatment application for each of the seven application times. The total rainfall for five days post-application ranged from 0.0 mm in June 2010 to 33.0 mm in July 2009, indicating a wide range of rainfall conditions.

3.3 Overview of chamber fluxes

The N_2O and N_2 emitted over each measurement period showed a similar pattern, with peak emissions occurring in the first five days after treatment application. Figure 3 illustrates a typical pattern of emissions for July 2009 for N_2 and N_2O . On each of the seven application times, N_2O fluxes were higher for the CSNO_3 treatment than the NH_4ClNO_3 treatment. Fluxes were considerably lower in June 2010 compared to other measurement periods, probably due to lower soil moisture contents, as there was no rainfall for 10 days post-treatment application.

3.4 Cumulative emissions

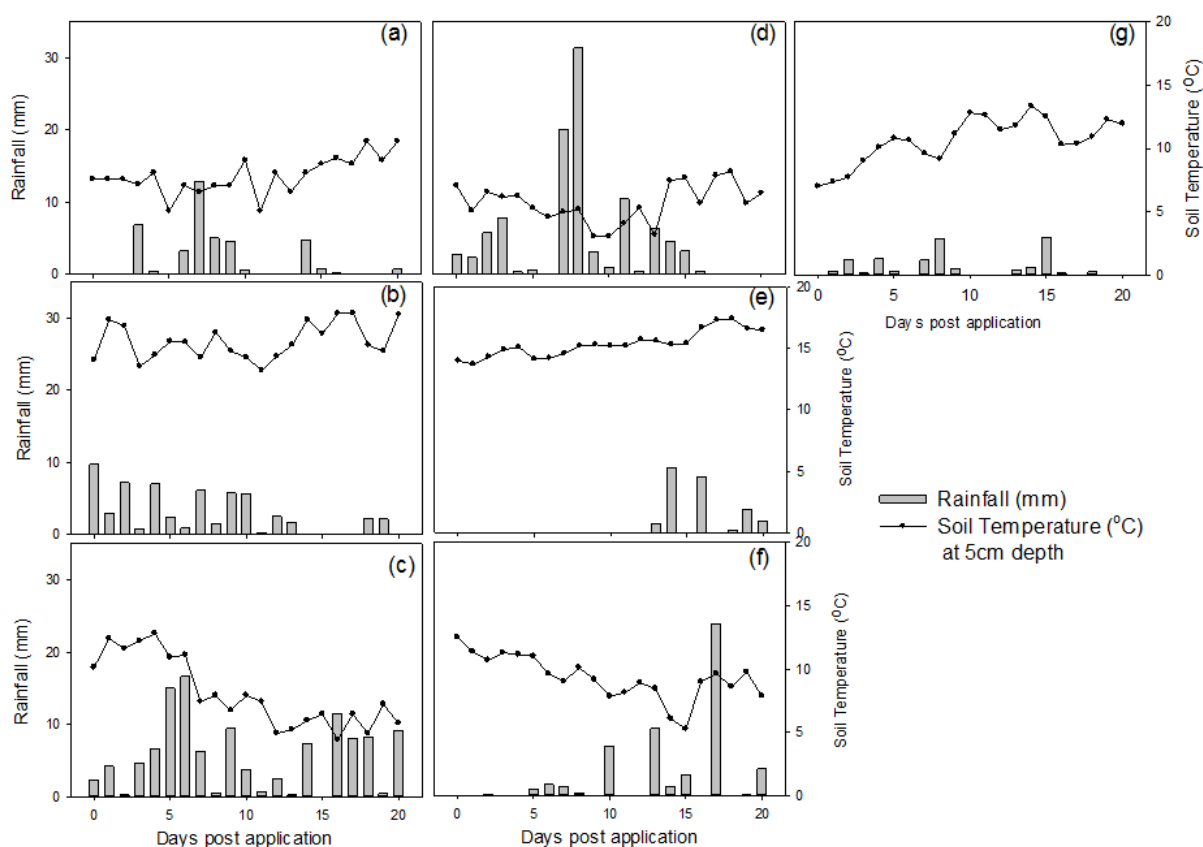
The cumulative fluxes of $\text{N}_2\text{O-N}$ and $\text{N}_2\text{-N}$ and the mole fraction of N_2O ($\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$) for each measurement period are given in Table 2. The flux of N_2O was independent of the ^{15}N labelling; therefore, fluxes from treatments with either the NH_4^+ or the NO_3^- pool ^{15}N labelled (e.g. $^{15}\text{CS}^{14}\text{NO}_3$ and $^{14}\text{CS}^{15}\text{NO}_3$) were averaged. It was only possible to measure the flux of N_2 from treatments where the $^{15}\text{NO}_3$ pool was labelled.

Cumulative N_2O emissions over 20 days in the CSNO_3 without DCD treatment ranged from 0.76 kg N ha^{-1} in June 2010 to 17.28 kg N ha^{-1} in March 2009; CSNO_3 with DCD treatment ranged from 0.88 kg N ha^{-1} in June 2010 to 12.79 kg N ha^{-1} in March 2009; NH_4ClNO_3 without DCD treatment ranged from 0.51 kg N ha^{-1} in June 2010 to 8.79 kg N ha^{-1} in March 2009; and in the NH_4ClNO_3 with DCD treatment cumulative N_2O emissions ranged from 0.47 kg N ha^{-1} in June 2010 to 6.70 kg N ha^{-1} in March 2009.

Table 2. Cumulative fluxes of N₂O and N₂ evolved over 20 days after treatment application for CSNO₃ and NH₄ClNO₃ with and without DCD.

Application Date	Treatment	N ₂ O (kg N ha ⁻¹)			N ₂ (kg N ha ⁻¹)			N ₂ O mole fraction (N ₂ O/(N ₂ O + N ₂))			Rainfall 5 days post-application (mm)	Temperature average over 20 days (°C)
		without DCD	with DCD	Signif.	without DCD	with DCD	Signif.	without DCD	with DCD	Signif.		
Mar-09	CSNO ₃	17.28	12.79	**	42.38	21.77	**	0.28	0.36	**	7.1	7.8
	NH ₄ ClNO ₃	8.79	6.70	NS	34.92	14.54	*	0.19	0.31	**		
Jul-09	CSNO ₃	12.91	12.73	NS	34.27	19.83	NS	0.29	0.38	NS	30.0	15.3
	NH ₄ ClNO ₃	6.12	4.43	NS	30.11	18.19	NS	0.19	0.19	NS		
Oct-09	CSNO ₃	6.09	6.54	NS	10.42	14.97	NS	0.36	0.32	NS	33.0	8.2
	NH ₄ ClNO ₃	4.18	5.15	NS	11.10	9.67	NS	0.29	0.36	NS		
Mar-10	CSNO ₃	9.71	9.01	NS	23.91	21.52	NS	0.30	0.29	NS	19.4	5.6
	NH ₄ ClNO ₃	4.49	3.98	NS	21.35	16.99	NS	0.16	0.19	NS		
Jun-10	CSNO ₃	0.76	0.88	NS	3.77	3.43	NS	0.17	0.21	NS	0.0	15.4
	NH ₄ ClNO ₃	0.51	0.47	NS	3.03	3.08	NS	0.15	0.13	NS		
Oct-10	CSNO ₃	8.60	6.60	NS	13.55	8.47	NS	0.39	0.44	NS	1.0	9.3
	NH ₄ ClNO ₃	6.52	4.26	NS	12.23	9.49	NS	0.36	0.33	NS		
Mar-11	CSNO ₃	2.98	4.85	NS	3.27	4.32	NS	0.46	0.50	NS	5.5	10.1
	NH ₄ ClNO ₃	1.18	0.70	*	2.47	1.32	*	0.33	0.24	*		

Significance levels: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS = no significant difference.

**Fig. 2.** Rainfall and Soil Temperature at 5 cm depth for 20 days post-treatment application for (a) March 2009, (b) July 2009, (c) October 2009, (d) March 2010, (e) June 2010, (f) October 2010, and (g) March 2011.

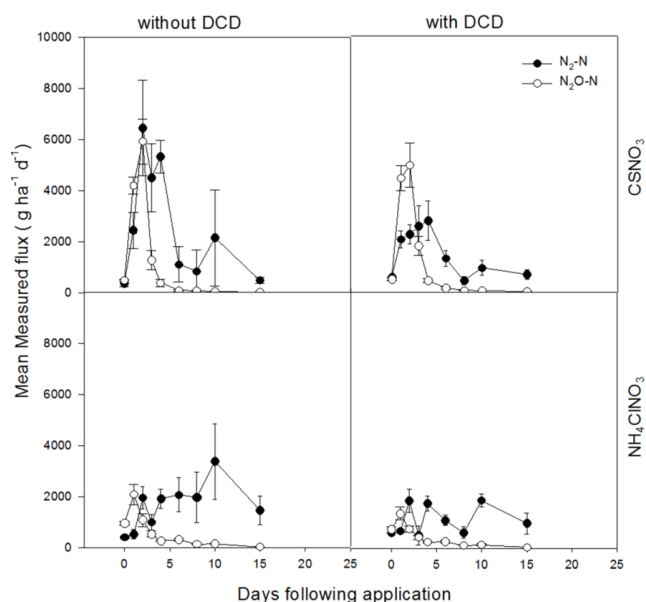


Fig. 3. Mean measured $\text{N}_2\text{O-N}$ and $\text{N}_2\text{-N}$ fluxes versus time for CSNO_3 and NH_4ClNO_3 with and without DCD for July 2009.

Cumulative N_2 emissions over 20 days in the CSNO_3 without DCD treatment ranged from $3.27 \text{ kg N ha}^{-1}$ in March 2011 to $42.38 \text{ kg N ha}^{-1}$ in March 2009; CSNO_3 with DCD treatment ranged from $3.43 \text{ kg N ha}^{-1}$ in June 2010 to $21.77 \text{ kg N ha}^{-1}$ in March 2009; NH_4ClNO_3 without DCD treatment ranged from $3.03 \text{ kg N ha}^{-1}$ in June 2010 to $34.92 \text{ kg N ha}^{-1}$ in March 2009; and in the NH_4ClNO_3 with DCD treatment cumulative N_2 emissions ranged from $1.32 \text{ kg N ha}^{-1}$ in March 2011 to $18.19 \text{ kg N ha}^{-1}$ in July 2009.

3.4.1 Effect of CS on the cumulative emissions of N_2O and N_2

On each of the seven application times, the presence of CS significantly increased cumulative N_2O emissions (when compared to the NH_4Cl control) on all occasions (March 2009, $P < 0.001$; July 2009, $P < 0.001$; October 2009, $P < 0.01$; March 2010, $P < 0.001$; June 2010, $P < 0.001$; October 2010, $P < 0.05$; March 2011, $P < 0.001$). This increase ranged from a factor of between 1.27 and 6.93.

The presence of CS significantly increased cumulative N_2 emissions on only two occasions: in March 2009 ($P < 0.05$) and in March 2011 ($P < 0.01$).

When CS was applied, the N_2O mole fraction ($\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$) increased on all occasions. The N_2O mole fraction averaged over the seven measurement periods, was significantly higher ($P < 0.001$) for CSNO_3 at 0.34 (± 0.03 standard error) compared to NH_4ClNO_3 at 0.24 (± 0.03 standard error). The average N_2O mole fraction was

lower (0.19) in June 2010, under relatively dry conditions, than at other times (average 0.34) (Table 2).

3.4.2 Effect of DCD on the cumulative emissions of N_2O and N_2

When data were averaged over the CSNO_3 and NH_4ClNO_3 treatments, there was an overall DCD effect on two occasions, where the application of DCD was shown to decrease cumulative N_2O emission in March 2010 ($P < 0.001$) and October 2010 ($P < 0.05$) (results not shown). When the DCD effect on CSNO_3 was examined, there was a significant decrease ($P < 0.01$) in cumulative N_2O emissions in March 2009 from 17.28 to $12.79 \text{ kg N ha}^{-1}$. However, DCD did not significantly decrease N_2O emissions in CSNO_3 at any other time. There was a significant decrease ($P < 0.05$) in cumulative N_2O emissions from NH_4ClNO_3 in March 2011 from 1.18 to $0.70 \text{ kg N ha}^{-1}$, but DCD did not significantly decrease N_2O emissions at any other time.

Across all treatments there was a decrease in N_2 cumulative emissions in March 2009 ($P < 0.001$), July 2009 ($P < 0.01$), and October 2010 ($P < 0.05$). There was a significant decrease ($P < 0.001$) in N_2 emissions from CSNO_3 in March 2009, where the cumulative N_2 emission decreased from 42.38 to $21.77 \text{ kg N ha}^{-1}$ (Table 2). DCD did not significantly decrease N_2 emission in CSNO_3 on any other occasion. There was a significant decrease in N_2 emissions from NH_4ClNO_3 in March 2009 and March 2011 from 34.92 to $14.54 \text{ kg N ha}^{-1}$ and from 2.47 to $1.32 \text{ kg N ha}^{-1}$, respectively; DCD did not significantly decrease N_2 emissions in NH_4ClNO_3 at any other time (Table 2).

DCD significantly increased the N_2O mole fraction ($\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$) in March 2009, but had no significant effect at other times.

4 Source of N_2O

Following the procedure of Arah (1997), the fraction (d'_D) of the N_2O flux that was derived from the $^{15}\text{NO}_3$ pool and the ^{15}N atom fraction (a_D) of that pool were calculated. Values of a_D indicate the enrichment of the ^{15}N -labelled denitrifying pool, and its change over time is indicative of the rate of nitrification. Calculations of d'_D and a_D can only be performed when the nitrate pool is labelled and when there is a detectable N_2O flux. Therefore only values obtained from the $^{14}\text{CS}^{15}\text{NO}_3$ and $^{14}\text{NH}_4\text{Cl}^{15}\text{NO}_3$ treatments are presented. As the N_2O peak emission pattern was different for each application event, N_2O a_D values, when the N_2O flux was above 2 ppmv, and N_2O d'_D values are given at times of maximum peak N_2O emissions. Values of a_D are presented in Table 3 for $^{14}\text{CS}^{15}\text{NO}_3$ and $^{14}\text{NH}_4\text{Cl}^{15}\text{NO}_3$ with and without DCD.

N_2O a_D value at the first sampling time after application (after approximately 2 h) was 0.50, which was the

Table 3. The fraction of the ^{15}N -labelled nitrate pool (a_D) for CSNO_3 and NH_4ClNO_3 with and without DCD.

	Mar-09	Jul-09	Oct-09	Mar-10	Jun-10	Oct-10	Mar-11
Time to reach minimum detectable flux (days)	10	4	3	10	3	11	11
CSNO_3 without DCD	0.37	0.47	0.51	0.46	0.45	0.36	0.38
CSNO_3 with DCD	0.47	0.48	0.48	0.50	0.49	0.44	0.44
NH_4ClNO_3 without DCD	0.25	0.40	0.42	0.36	0.43	0.33	0.30
NH_4ClNO_3 with DCD	0.45	0.43	0.44	0.47	0.48	0.39	0.39
CSNO_3 vs. NH_4ClNO_3	< 0.001	< 0.01	< 0.01	< 0.001	< 0.01	NS	< 0.001
DCD effect on CSNO_3	< 0.001	NS	NS	< 0.05	< 0.001	< 0.05	< 0.01
DCD effect on NH_4ClNO_3	< 0.001	NS	NS	< 0.001	< 0.001	< 0.05	< 0.01

NS = not significant at $P < 0.05$; LSD at $P = 0.05$.

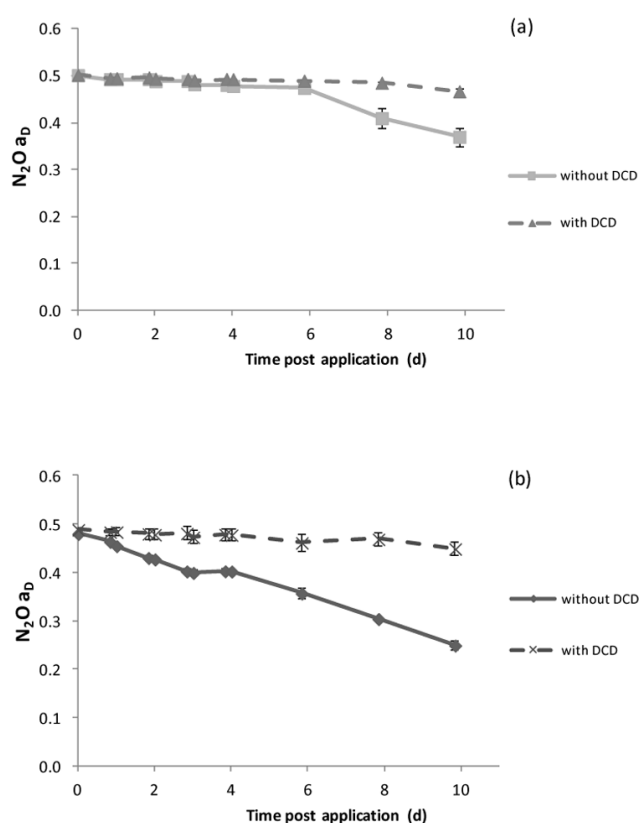


Fig. 4. Change in the ^{15}N mole fraction of the labelled nitrate pool (a_D) over time in March 2009 for $^{14}\text{CS}^{15}\text{NO}_3$ (a) and for $^{14}\text{NH}_4\text{Cl}^{15}\text{NO}_3$ (b) with and without DCD. Error bars indicate standard error.

same as the theoretical enrichment of 0.50 atom fraction ^{15}N (50 atom % ^{15}N). At all application times a_D values for CSNO_3 were higher than a_D for NH_4ClNO_3 , indicating that the rate of nitrification was slower for CSNO_3 than for NH_4ClNO_3 (March 2009, $P < 0.001$; July 2009, $P < 0.01$; October 2009, $P < 0.01$; March 2010, $P < 0.001$; June 2010,

$P < 0.01$; October 2010, NS; March 2011, $P < 0.001$). Examining the change in N_2O a_D with time, it was apparent that the presence of CS caused a delay in nitrification (Fig. 4). The application of DCD caused the values of a_D in CSNO_3 to be significantly higher compared to the CSNO_3 without DCD treatment on five occasions (March 2009, $P < 0.001$; March 2010, $P < 0.05$; June 2010, $P < 0.001$; October 2010, $P < 0.05$; March 2011, $P < 0.01$) as the rate of decrease in a_D was slower in the presence of DCD. When DCD was applied to NH_4ClNO_3 , the values of a_D in NH_4ClNO_3 were significantly higher than the NH_4ClNO_3 without DCD treatment on the same five occasions (March 2009, $P < 0.001$; March 2010, $P < 0.001$; June 2010, $P < 0.001$; October 2010, $P < 0.05$; March 2011, $P < 0.01$). DCD did not have a significant effect on N_2O a_D in either the CSNO_3 or NH_4ClNO_3 treatments in July and October 2009. Enrichment of N_2O from the $^{15}\text{NH}_4$ -labelled treatments increased with time (Fig. 5) suggesting that ^{15}N -labelled ammonium was oxidized through the nitrification process, enriching the NO_3 pool, from which $^{15}\text{N}_2\text{O}$ was evolved.

N_2O d_D is the fraction of the emitted N_2O which is derived from the ^{15}N -labelled denitrifying nitrate pool, with a N_2O d_D value of unity (1.00) indicating that 100% of the N_2O emitted is from the nitrate pool. Values of N_2O d_D (Table 4) for CSNO_3 with and without DCD were not significantly different from unity; therefore the source of the N_2O emitted from the CSNO_3 treatments was the nitrate pool. Values of N_2O d_D for NH_4ClNO_3 were significantly lower than unity in March 2009, March 2010, June 2010 and March 2011 with values of 0.96, 0.90, 0.65, and 0.87 being measured, indicating that 4, 10, 35 and 13%, respectively, of the N_2O emitted was derived from a natural abundance nitrate pool. DCD did not significantly change N_2O d_D in either the CSNO_3 or NH_4ClNO_3 treatments.

Table 4. The fraction of N₂O derived from the labelled nitrate pool (*a_D*) during peak N₂O emissions for CSNO₃ and NH₄ClNO₃ with and without DCD.

	Mar-09	Jul-09	Oct-09	Mar-10	Jun-10	Oct-10	Mar-11
CSNO ₃ without DCD	0.99	1.00	0.98	0.96	0.88	0.99	0.94
CSNO ₃ with DCD	0.99	1.00	0.96	0.96	0.89	0.99	0.96
NH ₄ ClNO ₃ without DCD	0.96	0.97	0.95	0.90	0.65	0.98	0.87
NH ₄ ClNO ₃ with DCD	0.98	0.93	0.96	0.88	0.65	0.97	0.76
Difference from unity:							
CSNO ₃ without DCD	NS	NS	NS	NS	NS	NS	NS
CSNO ₃ with DCD	NS	NS	NS	NS	NS	NS	NS
NH ₄ ClNO ₃ without DCD	< 0.01	NS	NS	< 0.05	< 0.001	NS	< 0.05
NH ₄ ClNO ₃ with DCD	< 0.05	NS	NS	< 0.05	< 0.001	NS	< 0.05
CSNO ₃ vs. NH ₄ ClNO ₃	< 0.001	NS	NS	< 0.05	< 0.001	NS	< 0.01
DCD effect on CSNO ₃	NS	NS	NS	NS	NS	NS	NS
DCD effect on NH ₄ ClNO ₃	NS	NS	NS	NS	NS	NS	NS

NS = not significant at $P < 0.05$; LSD at $P = 0.05$.

5 Discussion

5.1 N₂O and N₂ cumulative emissions

N₂O and N₂ cumulative emissions were expressed as a percentage of the applied N (ammonium-N and nitrate-N). In our study, over the seven treatment dates, between 0.4–8.7 % of the applied N (NH₄⁺ and NO₃⁻) was lost as N₂O and between 2.1–23.2 % was lost as N₂ in the CSNO₃ treatment. When NH₄ClNO₃ was applied, between 0.3–4.8 % was lost as N₂O and 1.7–20.4 % was lost as N₂. Lowest emissions were measured when soil moisture was low. The current IPCC default N₂O emission factor is 1 % of the applied N, regardless of N source (organic-N or fertiliser-N) (IPCC, 2006). The current study did not include unfertilized control plots; therefore, emission factors could not be calculated. However, the average percentage N₂O and N₂ losses expressed in terms of N applied indicate that N gas losses are large, with increased losses in the presence of CS. The total cumulative emission of N₂O-N plus N₂-N, averaged over the seven measurement periods, expressed as a percentage of the available N applied was 15.9 % for CSNO₃ without DCD, 12.4 % for CSNO₃ with DCD, 12.4 % for NH₄ClNO₃ with DCD, and 8.7 % for NH₄ClNO₃ without DCD.

The simultaneous application of cattle slurry and NO₃ induced a large increase in cumulative N₂O emissions compared to those measured from NH₄Cl combined with NO₃, with increases of between 1.27 and 6.93 fold being measured. This increase indicates that the easily mineralisable carbon components in cattle slurry promoted N₂O emissions derived from NO₃. Lampe et al. (2006) found that the application of slurry and mineral fertiliser increased N₂O emissions by between 30 to 150 % compared to emissions from CAN alone following the application in spring to a grassland soil. This effect has been reported in a number of other studies (Stevens

and Laughlin, 2001a, 2002; Dittert et al., 2005; Velthof and Oenema, 1993).

The use of the ¹⁵N tracer technique allowed the simultaneous measurement of N₂O and N₂, and hence the N₂O mole fraction (N₂O/(N₂O + N₂)) to be calculated. The application of CS and NO₃ significantly increased N₂ emissions in March 2009 and March 2011. CS did not have an effect on N₂ emission at any other time. Previous studies have reported a range of N₂O mole fractions: Mathieu et al. (2006) reported a range of 0.15–0.94 measured from undisturbed soil cores following the application of ¹⁵N-nitrate after 2 h. Stevens and Laughlin (2001a) measured an average N₂O mole fraction of 0.31 for NH₄HCO₃NO₃ and 0.66 for CSNO₃. Despite the high variability of reported values for N₂O mole fractions, the current study showed that an increase in the N₂O mole fraction occurred on each of the seven occasions when CS was applied with nitrate to grassland soils, compared to NH₄ClNO₃. This increase in N₂O mole fraction was due to the increase in N₂O emissions in the presence of CS. Averaged across the seven application dates, the mole fraction was 0.34 for CSNO₃ compared to 0.24 for NH₄ClNO₃. The average N₂O mole fraction was lower (0.19) in June 2010, under relatively dry conditions, than at other times (average 0.34), indicating a lower N₂O mole fraction under conditions more conducive to nitrification.

5.2 N₂O *a_D* and N₂O *d_D*

As the occurrence of nitrification can be inferred from the rate of dilution of the labelled nitrate pool, N₂O *a_D* results have demonstrated that the rate of nitrification in CSNO₃ was slower than in NH₄ClNO₃, with a delay in the onset of nitrification occurring in the CSNO₃ treatments. The application of a readily available organic carbon source to the soil stimulates microbial respiration and causes a subsequent decrease

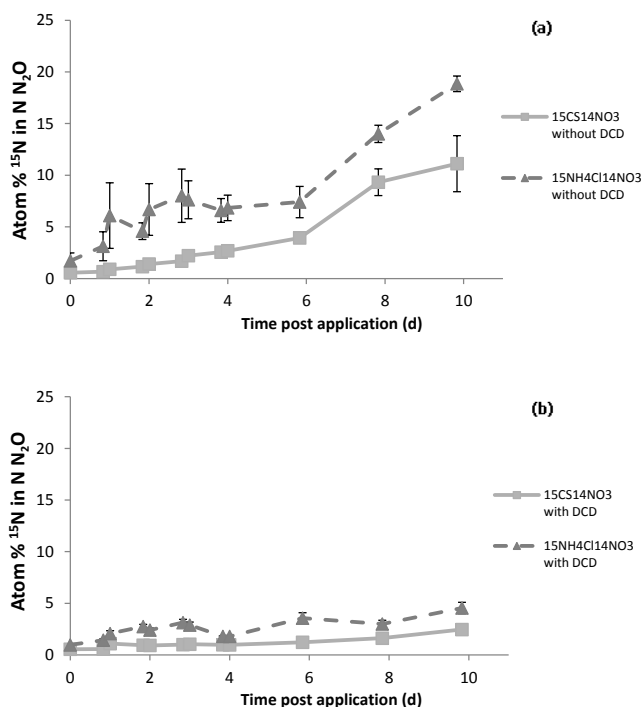


Fig. 5. Change in atom % ¹⁵N in N₂O over time in March 2009 for ¹⁵CS¹⁴NO₃ and ¹⁵NH₄Cl¹⁴NO₃ without DCD (a) and with DCD (b). Error bars indicate standard error.

in oxygen concentration in the soil pore space (Tiedje, 1988). Stevens and Laughlin (2001a) also found that the application of CS increased soil respiration, thus creating conditions where NO₃ would be used as the terminal-electron acceptor instead of O₂.

In the current study, although nitrification often occurred it only made a significant contribution (35 %) to the N₂O flux in June 2010. At this time there was zero rainfall indicating that the dry soil conditions favoured nitrification. CS *d_D* values were not different from unity; hence, all N₂O came from the denitrifying nitrate pool, as the metabolism of the carbon source in the slurry enhanced anaerobic conditions. When CS was not present, the N₂O *d_D* values for NH₄ClNO₃ treatments were lower than those for CSNO₃ providing further evidence that nitrification rates were more rapid in the NH₄ClNO₃ treatments.

5.3 Effect of DCD

The N₂O *a_D* values in both the CSNO₃ and NH₄ClNO₃ treatments were significantly higher with than without DCD on five occasions; therefore DCD appeared to be effective in inhibiting the nitrification process. DCD did not affect the N₂O *a_D* values in both CSNO₃ and NH₄ClNO₃ treatments in July or October 2009. Cumulative rainfall over five days post-application was 30.0 and 33.0 mm for July and October 2009, respectively. This suggested that either anaerobic conditions

inhibited nitrification or that DCD moved down the soil profile post-application during this heavy rainfall, and therefore was not acting as a nitrification inhibitor on these occasions. Zerulla et al. (2001) highlighted that a shortcoming of using DCD was that it is susceptible to losses through leaching due to its high water solubility. Monaghan et al. (2009) showed that between 2 and 16 % of the DCD applied annually could be lost in drainage water.

Overall DCD significantly lowered ($P < 0.01$) both N₂O and N₂ fluxes in March and October 2009, but only N₂ fluxes in July 2009. It had no significant effect on cumulative emissions at other times. Although this study has found DCD to be an effective nitrification inhibitor by examining the changes in N₂O *a_D*, its effectiveness was not always translated into a reduction in N₂O emissions, when an ammonium-N (as mineral N or CS) and nitrate-N fertiliser source were applied together. DCD did not alter the fraction of the emitted N₂O which was derived from the denitrifying nitrate pool at any time in either the CSNO₃ or the NH₄ClNO₃ treatments. As the source of N₂O was found to be predominantly from the NO₃ pool, the benefit in inhibiting NH₄⁺-N oxidation by DCD was too small to be seen against the large nitrate pool already present. Other studies have shown that DCD was effective in reducing N₂O emissions from ammonium-based fertilisers (Dobbie and Smith, 2003; Skiba et al., 1993) and cattle slurries (Hatch et al., 2005; Merino et al., 2002). Skiba et al. (2003) found that N₂O was predominantly produced by nitrification when soils were dry and DCD reduced emissions by at least 40 %. A recent study conducted at two grassland sites in Ireland found DCD to be highly effective, reducing N₂O emissions from CS by up to 82 % (Cahalan et al., 2012). The effect of DCD in lowering N₂O emissions is limited in the presence of a NO₃ source, when denitrification is the main source of N₂O.

6 Conclusions

The current study used the ¹⁵N tracer technique to simultaneously measure N₂O and N₂ emissions from cattle slurry in the presence of fertiliser NO₃. N₂O emissions were predominantly from denitrification of the NO₃ pool. N₂O emissions from CS in the presence of NO₃ fertiliser were very high (0.4–8.7 % of applied N) over a 20-day period, under mild moist conditions in Northern Ireland. Emissions were significantly larger from the CS treatment compared to the NH₄Cl treatment, supplying the same rate of N as in the slurry. This was probably due to the easily mineralisable C components in the CS promoting N₂O emissions from the added fertiliser NO₃ pool. This study supports the view that organic fertilisers should not be applied at the same time as nitrate-based fertilisers, as significant increases in N₂O emissions occur. The average N₂O mole fraction (N₂O/(N₂O+N₂)) over all seven application dates was 0.34 for CSNO₃ compared to

0.24 for the NH_4ClNO_3 treatment, indicating the dominance of N_2 emissions.

The rate of nitrification in CSNO_3 was slower than in NH_4ClNO_3 , and DCD effectively inhibited nitrification in both treatments. However, the effect of DCD in lowering N_2O emissions is limited in the presence of a NO_3 fertiliser, when denitrification is the main source of N_2O . To obtain the maximum cost-benefit of DCD in lowering N_2O emissions, under mild moist conditions, it should not be applied to a nitrate containing fertiliser (e.g. AN or CAN), and therefore the application of DCD should be restricted to ammonium-based organic or synthetic fertilisers.

Acknowledgements. This research was funded by the Irish National Development Plan, through the Research Stimulus Fund (RSF 07 519), administered by the Irish Department of Agriculture, Food and the Marine. The authors would like to thank Michael Nicholson, Gareth Ridgway, Raymond Stewart, Brieghe McCarney, Elizabeth Anne Wasson and Jill Mellon (Agri-Food and Biosciences Institute) for their help with field and lab work. We also thank Dave Mathews and Sally Dawson (Agri-Food and Biosciences Institute) for carrying out the statistical analyses.

Edited by: U. Skiba

References

- Arah, J. R. M.: Apportioning nitrous oxide fluxes between nitrification and denitrification using gas-phase mass spectrometry, *Soil Biol. Biochem.*, 29, 1295–1299, 1997.
- Bailey, L. D.: Effects of temperature and roots on denitrification in a soil, *Can. J. Soil Sci.*, 56, 79–87, 1976.
- Bouwman, A. F.: Exchange of greenhouse gases between terrestrial ecosystems and the atmosphere, in: *Soils and the greenhouse effect*, edited by: Bouwman, A. F., John Wiley and Sons, Chichester, 100–120, 1990.
- Bremner, J. M. and Mulvaney, C. S.: Nitrogen total, in: *Methods of soil analysis Part 2*, edited by: Page, A. L., Am. Soc. Agron., Madison, WIA, 595 pp., 1982.
- Briggs, G. G.: Behaviour of nitrification inhibitor N-Serve in broadcast and incorporated applications to soil, *J. Sci. Food Agric.*, 26, 1083–1092, 1975.
- Cahalan, E., Ernfors, M., Müller, C., Devaney, D., Laughlin, R. J., Watson, C. J., Khalil, M. I., Hennessy, D., McGeough, K. L., and Richards, K. G.: Effects of the nitrification inhibitor dicyandiamide (DCD) on nitrous oxide and methane emissions after cattle slurry application, *Agric. Ecosys. Environ.*, submitted, 2012.
- Clayton, H., McTaggart, I. P., Parker, J., Swan, L., and Smith, K. A.: Nitrous oxide emissions from fertilised grassland: a 2-year study of the effects of N fertiliser form and environmental conditions, *Biol. Fert. Soils*, 25, 252–260, 1997.
- Coyne, M. S. and Frye, W. W.: Nitrogen in soils: The N cycle, in: *Encyclopaedia of Soils in the Environment*, Vol. III., edited by: Hillel, D., Elsevier Ltd., 13–20, 2005.
- Cuhel, J., Simek, M., Laughlin, R. L., Bru, D., Chenaby, D., Watson, C. J., and Philippot, L.: Insights into the effect of soil pH on N_2O and N_2 emissions and denitrifier community size and activity, *Appl. Environ. Microbiol.*, 76, 1870–1878, 2010.
- Davidson, E. A., Hart, S. C., Shanks, C. A., and Firestone, M. K.: Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores, *J. Soil Sci.*, 42, 335–349, 1991.
- Davidson, E. A., Keller, M., Erickson, H. E., Verchot, L. V., and Veldkamp, E.: Testing a conceptual model of soil emissions of nitrous and nitric oxide, *Bioscience*, 50, 667–680, 2000.
- Dittert, K., Lampe, C., Gasche, R., Butterbach-Bahl, K., Wachendorf, M., Papen, H., Sattelmacher, B., and Taube, F.: Short-term effects of single or combined application of mineral N fertilizer and cattle slurry on the fluxes of radiatively active trace gases from grassland soil, *Soil Biol. Biochem.*, 37, 1665–1674, 2005.
- Dobbie, K. E. and Smith, K. A.: Impact of different forms of N fertilizer on N_2O emissions from intensive grassland, *Nutr. Cycl. Agroecosys.*, 67, 37–46, 2003.
- Edmeades, D. C.: Nitrification and urease inhibitors, A review of the national and international literature on their effects on nitrate leaching, greenhouse gas emissions and ammonia volatilisation from temperature legume based pastoral systems, Environment Waikato Technical Report 2044/22, 2004.
- Farmers Weekly, Reed Business Information Ltd., Surrey, England, 2012.
- Firestone, M. K. and Tiedje, J. M.: Temporal change in nitrous oxide and dinitrogen from denitrification following onset of anaerobiosis, *Appl. Environ. Microbiol.*, 38, 673–679, 1979.
- Frolking, S. E., Mosier, A. R., Ojima, D. S., Li, C., Parton, W. J., Potter, C. S., Priesack, E., Stenger, R., Haberbosch, C., Dorsch, P., Flessa, H., and Smith, K. A.: Comparison of N_2O emissions from soils at three temperate agricultural sites: simulations of year-round measurements by four models, *Nutr. Cycl. Agroecosyst.*, 52, 77–105, 1998.
- Granli, T. and Bøckman, O. C.: Nitrous oxide from agriculture, *Norw. J. Agric. Sci.*, 12, 1–128, 1994.
- Hall, S. J., Matson, P. A., and Roth, P. M.: NO_x emissions from soil: implications for air quality modeling in agricultural regions, *Annu. Rev. Energ. Env.*, 21, 311–346, 1996.
- Hatch, D., Trindade, H., Cardenas, L., Carneiro, J., Hawkins, J., Scholefield, D., and Chadwick, D.: Laboratory study of the effects of two nitrification inhibitors on greenhouse gas emissions from a slurry-treated arable soil: impact of diurnal temperature cycle, *Biol. Fert. Soils*, 41, 225–232, 2005.
- Hendrickson, L. L. and Keeney, D. R.: Effect of some physical and chemical factors on the rate of hydrolysis of nitrapyrin (n-serve), *Soil Biol. Biochem.*, 11, 47–50, 1979.
- IPCC: IPCC Guidelines for national greenhouse gas inventories, prepared by the National Greenhouse Gas Inventories Programme, edited by: Eggleston, H. S., Buendia, L., Miwa, K., Ngara, T., and Tanabe, K., Volume 4, Chapter 11, N_2O emissions from managed soils, and CO_2 emissions from lime and urea application, IGES, Hayama, Japan, 11 pp., 2006.
- Keeney, D. R., Fillery, I. R. P., and Marx, G. P.: Effect of temperature on the gaseous nitrogen products of denitrification in a silt loam soil, *Soil Sci. Soc. Am. J.*, 43, 1124–1128, 1979.
- Lampe, C., Dittert, K., Sattelmacher, B., Wachendorf, M., Loges, R., and Taube, F.: Sources and rates of nitrous oxide emissions from grazed grassland after application of ^{15}N -labelled mineral fertilizer and slurry, *Soil Biol. Biochem.*, 38, 2602–2613, 2006.

- Liu, X. J., Mosier, A. R., Halvorson, A. D., Curtis, A. R., and Fusuo, S. Z.: Dinitrogen and N₂O emissions in arable soils: Effect of tillage, N source and soil moisture, *Soil Biol. Biochem.*, 39, 2362–2370, 2007.
- Mathieu, O., Lévêque, J., Hénault, C., Milloux, M. J., Bizouard, F., and Andreux, F.: Emissions and spatial variability of N₂O, N₂ and nitrous oxide mole fraction at the field scale, revealed with ¹⁵N isotopic techniques, *Soil Biol. Biochem.*, 38, 941–951, 2006.
- McTaggart, I. P., Douglas, H., Clayton, H., and Smith, K. A.: Nitrous oxide emission from slurry and mineral nitrogen fertiliser applied to grassland, in: *Gaseous nitrogen emissions from grasslands*, edited by: Jarvis, S. C. and Pain, B. F., CAB International, New York, 201–209, 1997.
- Merino, P., Estavillo, J. M., Graciollo, L. A., Pinto, M., Lacuesta, M., Muñoz-Rueda, A., and Gonzalez-Murua, C.: Mitigation of N₂O emissions from grassland by nitrification inhibitor and Actilith F2 applied with fertilizer and cattle slurry, *Soil Use Manage.*, 18, 135–141, 2002.
- Monaghan, R. M., Smith, L. C., and Ledgard, S. F.: The effectiveness of a granular formulation of dicyandiamide (DCD) in limiting nitrate leaching from a grazed dairy pasture, *New Zeal. J. Agr. Res.*, 52, 145–149, 2009.
- Mulvaney, R. L.: Determination of N-15-labeled dinitrogen and nitrous-oxide with triple-collector mass spectrometers, *Soil Sci. Soc. Am. J.*, 48, 690–692, 1984.
- Parkin, T. B.: Soil microsites as a source of denitrification variability, *Soil Sci. Soc. Am. J.*, 51, 1194–1199, 1987.
- Paul, J. W., Beauchamp, E. G., and Trevors, J. T.: Acetate, propionate, butyrate, glucose, and sucrose as carbon sources for denitrifying bacteria in soil, *Can. J. Microbiol.*, 35, 754–759, 1989.
- Puttanna, K., Gowda, N. M. N., and Rao, E.: Effect of concentration, temperature, moisture, liming and organic matter on the efficacy of the nitrification inhibitors benzotriazole, o-nitrophenol, m-nitroaniline and dicyandiamide, *Nutr. Cycl. Agroecosys.*, 54, 251–257, 1999.
- Ruser, R., Flessa, H., Russow, R., Schmidt, G., Buegger, F., and Munch, J. C.: Emission of N₂O, N₂ and CO₂ from soil fertilized with nitrate: Effect of compaction, soil moisture and rewetting, *Soil Biol. Biochem.*, 38, 263–274, 2006.
- Seitzinger, S. P. and Kroeze, C.: Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems, *Global Biogeochem. Cy.*, 12, 93–113, 1998.
- Simek, M. and Cooper, J. E.: The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years, *Eur. J. Soil Sci.*, 53, 345–354, 2002.
- Skiba, U., Smith, K. A., and Fowler, D.: Nitrification and denitrification as sources of nitric-oxide and nitrous-oxide in a sandy loam soil, *Soil Biol. Biochem.*, 25, 1527–1536, 1993.
- Stevens, R. J. and Laughlin, R. L.: Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils, *Nutr. Cycl. Agroecosys.*, 52, 131–139, 1998.
- Stevens, R. J. and Laughlin, R. L.: Cattle slurry affects nitrous oxide and dinitrogen emissions from fertilizer nitrate, *Soil Sci. Soc. Am. J.*, 65, 1307–1314, 2001a.
- Stevens, R. J. and Laughlin, R. L.: Lowering the detection limit for dinitrogen using the enrichment of nitrous oxide, *Soil Biol. Biochem.*, 33, 1287–1289, 2001b.
- Stevens, R. J. and Laughlin, R. J.: Cattle slurry applied before fertilizer nitrate lowers nitrous oxide and dinitrogen emissions, *Soil Sci. Soc. Am. J.*, 66, 647–652, 2002.
- Stevens, R. J., Laughlin, R. J., Atkins, G. J., and Prosser, S. J.: Automated determination of nitrogen-15 labeled dinitrogen and nitrous oxide by mass spectrometry, *Soil Sci. Soc. Am. J.*, 57, 981–988, 1993.
- Stevens, R. J., Laughlin, R. J., Arah, J. R. M., and Hood, R. C.: Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil, *Soil Biol. Biochem.*, 29, 139–151, 1997.
- Tiedje, J. M.: *Ecology of denitrification and dissimilatory nitrite reduction to ammonium*, Biology of anaerobic microorganisms, New York, Wiley, 179–244, 1988.
- Velthof, G. L. and Oenema, O.: Nitrous oxide flux from nitric-acid-treated cattle slurry applied to grassland under semi-controlled conditions, *Neth. J. Agr. Sci.*, 41, 81–93, 1993.
- Weier, K. L., Doran, J. W., Power, J. F., and Walters, D. T.: Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate, *Soil Sci. Soc. Am. J.*, 57, 66–72, 1993.
- Zerulla, W., Barth, T., Dressel, J., Erhardt, K., von Locquenghien, K. H., Pasda, G., Rädle, M., and Wissemeier, A. H.: 3,4-Dimethylpyrazole phosphate (DMPP) – a new nitrification inhibitor for agriculture and horticulture, *Biol. Fertil. Soils*, 34, 79–84, 2001.