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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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 NH₄ClNO₃ 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-containing 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$_2$O and N$_2$ emissions. In moist soil conditions, cumulative N$_2$O emissions were up to 4 times greater from NH$_4$NO$_3$ followed by CS than from NH$_3$NO$_3$ alone (McTaggart et al., 1997). When CS was supplemented with NH$_3$NO$_3$, the loss of N$_2$O was 2.2 % compared with 1.2 % for NH$_4$NO$_3$ alone (Clayton et al., 1997). In studies conducted on four occasions in 1997, cattle slurry increased the flux of N$_2$O 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$_2$O 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$_2^-$. 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$_2$O and N$_2$). Nitrification inhibitors are thought to act specifically on the enzyme ammonium mono-oxygenase, 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 (Hendriksen 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$_2$O, a potent greenhouse gas (GHG), can be produced. Physical, chemical and biological factors influence the reduction of N$_2$O 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$_2$O, 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$_2$O 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°46′ N; 6°08′ 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* L.) 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.

<table>
<thead>
<tr>
<th>Application Date</th>
<th>Raw CS properties</th>
<th>Amended CS properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH$_3$-N mg kg$^{-1}$</td>
<td>Acetic Acid g L$^{-1}$</td>
</tr>
<tr>
<td>Mar-09</td>
<td>2845</td>
<td>5.54</td>
</tr>
<tr>
<td>Jun-09</td>
<td>2001</td>
<td>7.98</td>
</tr>
<tr>
<td>Oct-09</td>
<td>1545</td>
<td>5.74</td>
</tr>
<tr>
<td>Mar-10</td>
<td>1960</td>
<td>3.99</td>
</tr>
<tr>
<td>Jun-10</td>
<td>1671</td>
<td>4.28</td>
</tr>
<tr>
<td>Oct-10</td>
<td>1068</td>
<td>2.14</td>
</tr>
<tr>
<td>Mar-11</td>
<td>2485</td>
<td>5.18</td>
</tr>
</tbody>
</table>

### 2.2 Experimental treatments

Cattle slurry (33 m$^3$ ha$^{-1}$) amended with KNO$_3$ (65 kg N ha$^{-1}$), with or without DCD (at 15 % NH$_3$-N content of the CS), was surface applied to grassland with either the NH$_3$ or the NO$_3$ pool 15N labelled (15CS$^{14}$NO$_3$, 14CS$^{15}$NO$_3$) at 50 atom %, according to the procedure of Stevens et al. (1997). Ammonium chloride (NH$_4$Cl) amended with KNO$_3$ (65 kg N ha$^{-1}$), with or without DCD, with either the NH$_3$ or the NO$_3$ pool 15N labelled, served as controls for 14CS$^{15}$NO$_3$ and 15CS$^{14}$NO$_3$, respectively, having the same amount of NH$_3$-N as the CS but no degradable carbon. In summary, there were eight treatments: (i) 15CS$^{14}$NO$_3$ without DCD, (ii) 15CS$^{14}$NO$_3$ with DCD, (iii) 14CS$^{15}$NO$_3$ without DCD, (iv) 14CS$^{15}$NO$_3$ with DCD, (v) 15NH$_4$Cl$^{14}$NO$_3$ without DCD, (vi) 15NH$_4$Cl$^{14}$NO$_3$ with DCD, (vii) 14NH$_4$Cl$^{15}$NO$_3$ without DCD, and (viii) 14NH$_4$Cl$^{15}$NO$_3$ with DCD. The eight treatments were replicated four times in a randomised block design. Immediately prior to application aliquots of CS/NH$_4$Cl, KNO$_3$, and DCD/water were mixed together and uniformly applied directly inside the chamber (area of 0.16 m$^2$).

### 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 NH$_3$-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 N$_2$ and N$_2$O 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 15N$_2$, 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 N$_2$O were conducted on 104 occasions over the total experimental period from the CSN03 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 ($R^2$) for the accepted datasets. Eighty-four percent of the accepted data had an $R^2$ 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 N$_2$O and N$_2$

The concentration and 15N content of N$_2$O and the 15N content of the N$_2$ was determined by automated isotope ratio mass spectrometry (IRMS) as described by Stevens et al. (1993), using a Europa Scientific 20-20 stable isotope
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 $^{45}\text{R} (^{45}\text{I}^{44}\text{I})$ and $^{46}\text{R} (^{46}\text{I}^{44}\text{I})$ to be calculated for N$_2$O. The sources of N$_2$O were then apportioned into the fraction ($d'_\text{D}$) derived from the denitrifying pool of enrichment $d_\text{D}$ and the fraction $d'_\text{K} = (1 - d'_\text{D})$ derived from the pool or pools at natural abundance (Arak, 1997). For N$_2$, the ion currents at m/z 28, 29 and 30 enabled molecular ratios $^{29}\text{R} (^{29}\text{I}^{28}\text{I})$ and $^{30}\text{R} (^{30}\text{I}^{28}\text{I})$ 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 $d_\text{D}$ (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 NH$_4^+$-N form (two levels: CS or NH$_4$Cl) 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$_2$O, N$_2$, and N$_2$O mole fraction, and on the values of $d'_\text{D}$ and $d_\text{D}$. 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 NH$_4^+$-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 NH$_4^+$-N applied varied between 73.9 to 140.6 kg N ha$^{-1}$. The same rate of NO$_3^-$-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$_2$O 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$_2$O. On each of the seven application times, N$_2$O fluxes were higher for the CSNO$_3$ treatment than the NH$_4$CINO$_3$ 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 N$_2$O-N and N$_2$-N and the mole fraction of N$_2$O (N$_2$O/(N$_2$O + N$_2$)) for each measurement period are given in Table 2. The flux of N$_2$O 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. $^{15}$CS$^{14}$NO$_3$ and $^{14}$CS$^{15}$NO$_3$) were averaged. It was only possible to measure the flux of N$_2$ from treatments where the $^{15}$NO$_3$ pool was labelled.

Cumulative N$_2$O 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$_4$CINO$_3$ 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$_4$CINO$_3$ with DCD treatment cumulative N$_2$O 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 N\textsubscript{2}O and N\textsubscript{2} evolved over 20 days after treatment application for CSNO\textsubscript{3} and NH\textsubscript{4}ClNO\textsubscript{3} with and without DCD.

<table>
<thead>
<tr>
<th>Application Date</th>
<th>Treatment</th>
<th>N\textsubscript{2}O (kg N ha\textsuperscript{-1})</th>
<th>N\textsubscript{2} (kg N ha\textsuperscript{-1})</th>
<th>N\textsubscript{2}O mole fraction (N\textsubscript{2}O/(N\textsubscript{2}O + N\textsubscript{2}))</th>
<th>Rainfall 5 days post- average</th>
<th>Temperature over 20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar-09</td>
<td>CSNO\textsubscript{3}</td>
<td>17.28</td>
<td>12.79</td>
<td>**</td>
<td>42.38</td>
<td>21.77</td>
</tr>
<tr>
<td></td>
<td>NH\textsubscript{4}ClNO\textsubscript{3}</td>
<td>8.79</td>
<td>6.70</td>
<td>NS</td>
<td>34.92</td>
<td>14.54</td>
</tr>
<tr>
<td>Jul-09</td>
<td>CSNO\textsubscript{3}</td>
<td>12.91</td>
<td>12.73</td>
<td>NS</td>
<td>34.27</td>
<td>19.83</td>
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<tr>
<td></td>
<td>NH\textsubscript{4}ClNO\textsubscript{3}</td>
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<td>4.43</td>
<td>NS</td>
<td>30.11</td>
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</tr>
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<td>6.54</td>
<td>NS</td>
<td>10.42</td>
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<td></td>
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<td>5.15</td>
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<td></td>
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<td>2.98</td>
<td>4.85</td>
<td>NS</td>
<td>3.27</td>
<td>4.32</td>
</tr>
</tbody>
</table>

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.
Cumulative N₂ emissions over 20 days in the CSNO₃ treatments, without DCD treatment, ranged from 3.27 kg N ha⁻¹ in March 2011 to 42.38 kg N ha⁻¹ in March 2009; CSNO₃ with DCD treatment ranged from 3.43 kg N ha⁻¹ in June 2010 to 21.77 kg N ha⁻¹ in March 2009; NH₄ClNO₃ without DCD treatment ranged from 3.03 kg N ha⁻¹ in June 2010 to 34.92 kg N ha⁻¹ in March 2009; and in the NH₄ClNO₃ with DCD treatment cumulative N₂ emissions ranged from 1.32 kg N ha⁻¹ in March 2011 to 18.19 kg N ha⁻¹ in July 2009.

3.4.1 Effect of CS on the cumulative emissions of N₂O and N₂

On each of the seven application times, the presence of CS significantly increased cumulative N₂O emissions (when compared to the NH₄Cl 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 N₂ emissions on only two occasions: in March 2009 (P < 0.05) and in March 2011 (P < 0.01).

When CS was applied, the N₂O mole fraction (N₂O/(N₂O + N₂)) increased on all occasions. The N₂O mole fraction averaged over the seven measurement periods, was significantly higher (P < 0.001) for CSNO₃ at 0.34 (± 0.03 standard error) compared to NH₄ClNO₃ at 0.24 (± 0.03 standard error). The average N₂O 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 N₂O and N₂

When data were averaged over the CSNO₃ and NH₄ClNO₃ treatments, there was an overall DCD effect on two occasions, where the application of DCD was shown to decrease cumulative N₂O emission in March 2010 (P < 0.001) and October 2010 (P < 0.05) (results not shown). When the DCD effect on CSNO₃ was examined, there was a significant decrease (P < 0.01) in cumulative N₂O emissions in March 2009 from 17.28 to 12.79 kg N ha⁻¹. However, DCD did not significantly decrease N₂O emissions in CSNO₃ at any other time. There was a significant decrease (P < 0.05) in cumulative N₂O emissions from NH₄ClNO₃ in March 2011 from 1.18 to 0.70 kg N ha⁻¹, but DCD did not significantly decrease N₂O emissions at any other time.

Across all treatments there was a decrease in N₂ 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 N₂ emissions from CSNO₃ in March 2009, where the cumulative N₂ emission decreased from 42.38 to 21.77 kg N ha⁻¹ (Table 2). DCD did not significantly decrease N₂ emission in CSNO₃ on any other occasion. There was a significant decrease in N₂ emissions from NH₄ClNO₃ in March 2009 and March 2011 from 34.92 to 14.54 kg N ha⁻¹ and from 2.47 to 1.32 kg N ha⁻¹, respectively; DCD did not significantly decrease N₂ emissions in NH₄ClNO₃ at any other time (Table 2).

DCD significantly increased the N₂O mole fraction (N₂O/N₂O+N₂) in March 2009, but had no significant effect at other times.

4 Source of N₂O

Following the procedure of Arah (1997), the fraction (d¹⁵O) of the N₂O flux that was derived from the ¹⁵NO₃ pool and the ¹⁵N atom fraction (αD) of that pool were calculated. Values of αD indicate the enrichment of the ¹⁵N-labelled denitrifying pool, and its change over time is indicative of the rate of nitrification. Calculations of d¹⁵O and αD can only be performed when the nitrate pool is labelled and when there is a detectable N₂O flux. Therefore only values obtained from the ¹⁴CNO₃ and ¹⁴NH₄Cl¹⁵NO₃ treatments are presented. As the N₂O peak emission pattern was different for each application event, N₂O αD values, when the N₂O flux was above 2 ppmv, and N₂O d¹⁵O values are given at times of maximum peak N₂O emissions. Values of αD are presented in Table 3 for ¹⁴C¹⁵NO₃ and ¹⁴NH₄Cl¹⁵NO₃ with and without DCD.

N₂O αD value at the first sampling time after application (after approximately 2 h) was 0.50, which was the...
same as the theoretical enrichment of 0.50 atom fraction $^{15}$N (50 atom% $^{15}$N). At all application times $\delta_D$ values for CSNO$_3$ were higher than $\delta_D$ for NH$_4$ClNO$_3$, indicating that the rate of nitrification was slower for CSNO$_3$ than for NH$_4$ClNO$_3$ (March 2009, $P < 0.001$; July 2009, $P < 0.01$; October 2009, $P < 0.01$; October 2010, NS; March 2011, $P < 0.001$). Examining the change in N$_2$O $\delta_D$ 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 $\delta_D$ 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 $\delta_D$ was slower in the presence of DCD. When DCD was applied to NH$_4$ClNO$_3$, the values of $\delta_D$ in NH$_4$ClNO$_3$ were significantly higher than the NH$_4$ClNO$_3$ without DCD treatment on the same 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$_2$O $\delta_D$ in either the CSNO$_3$ or NH$_4$ClNO$_3$ treatments in July and October 2009. Enrichment of N$_2$O from the $^{15}$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 $^{15}$N$_2$O was evolved.

$N_2O \delta_D$ is the fraction of the emitted N$_2$O which is derived from the $^{15}$N-labelled denitrifying nitrate pool, with a N$_2$O $\delta_D$ value of unity (1.00) indicating that 100% of the N$_2$O emitted is from the nitrate pool. Values of N$_2$O $\delta_D$ (Table 4) for CSNO$_3$ with and without DCD were not significantly different from unity; therefore the source of the N$_2$O emitted from the CSNO$_3$ treatments was the nitrate pool. Values of N$_2$O $\delta_D$ for NH$_4$ClNO$_3$ 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$_2$O emitted was derived from a natural abundance nitrate pool. DCD did not significantly change N$_2$O $\delta_D$ in either the CSNO$_3$ or NH$_4$ClNO$_3$ treatments.
Table 4. The fraction of N\textsubscript{2}O derived from the labelled nitrate pool (d\textsubscript{15}N) during peak N\textsubscript{2}O emissions for CSNO\textsubscript{3} and NH\textsubscript{4}CINO\textsubscript{3} with and without DCD.

<table>
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<th>Mar-09</th>
<th>Jul-09</th>
<th>Oct-09</th>
<th>Mar-10</th>
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<th>Oct-10</th>
<th>Mar-11</th>
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<td>1.00</td>
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<td>0.96</td>
<td>0.88</td>
<td>0.99</td>
<td>0.94</td>
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<tr>
<td>CSNO\textsubscript{3} with DCD</td>
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<td>1.00</td>
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<td>0.96</td>
<td>0.89</td>
<td>0.99</td>
<td>0.96</td>
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<tr>
<td>NH\textsubscript{4}CINO\textsubscript{3} without DCD</td>
<td>0.96</td>
<td>0.97</td>
<td>0.95</td>
<td>0.90</td>
<td>0.65</td>
<td>0.98</td>
<td>0.87</td>
</tr>
<tr>
<td>NH\textsubscript{4}CINO\textsubscript{3} with DCD</td>
<td>0.98</td>
<td>0.93</td>
<td>0.96</td>
<td>0.88</td>
<td>0.65</td>
<td>0.97</td>
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Difference from unity:

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<tbody>
<tr>
<td>CSNO\textsubscript{3} without DCD</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
<td>&lt; 0.05</td>
<td>&lt; 0.001</td>
<td>&lt; 0.05</td>
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<tr>
<td>NH\textsubscript{4}CINO\textsubscript{3} without DCD</td>
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<td>NS</td>
<td>&lt; 0.05</td>
<td>&lt; 0.001</td>
<td>NS</td>
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<td>NH\textsubscript{4}CINO\textsubscript{3} with DCD</td>
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<td>NS</td>
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<td>&lt; 0.001</td>
<td>NS</td>
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<tr>
<td>DCD effect on CSNO\textsubscript{3}</td>
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<tr>
<td>DCD effect on NH\textsubscript{4}CINO\textsubscript{3}</td>
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NS = not significant at P < 0.05; LSD at P = 0.05.

5 Discussion

5.1 N\textsubscript{2}O and N\textsubscript{2} cumulative emissions

N\textsubscript{2}O and N\textsubscript{2} 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 (NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}) was lost as N\textsubscript{2}O and between 2.1–23.2 % was lost as N\textsubscript{2} in the CSNO\textsubscript{3} treatment. When NH\textsubscript{4}CINO\textsubscript{3} was applied, between 0.3–4.8 % was lost as N\textsubscript{2}O and 1.7–20.4 % was lost as N\textsubscript{2}. Lowest emissions were measured when soil moisture was low. The current IPCC default N\textsubscript{2}O 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 N\textsubscript{2}O and N\textsubscript{2} 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 N\textsubscript{2}O-N plus N\textsubscript{2}-N, averaged over the seven measurement periods, expressed as a percentage of the available N applied was 15.9 % for CSNO\textsubscript{3} without DCD, 12.4 % for CSNO\textsubscript{3} with DCD, 12.4 % for NH\textsubscript{4}CINO\textsubscript{3} with DCD, and 8.7 % for NH\textsubscript{4}CINO\textsubscript{3} without DCD.

The simultaneous application of cattle slurry and NO\textsubscript{3} induced a large increase in cumulative N\textsubscript{2}O emissions compared to those measured from NH\textsubscript{4}Cl combined with NO\textsubscript{3}, 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 N\textsubscript{2}O emissions derived from NO\textsubscript{3}. Lampe et al. (2006) found that the application of slurry and mineral fertiliser increased N\textsubscript{2}O 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 \textsuperscript{15}N tracer technique allowed the simultaneous measurement of N\textsubscript{2}O and N\textsubscript{2}, and hence the N\textsubscript{2}O mole fraction (N\textsubscript{2}O/(N\textsubscript{2}O + N\textsubscript{2})) to be calculated. The application of CS and NO\textsubscript{3} significantly increased N\textsubscript{2} emissions in March 2009 and March 2011. CS did not have an effect on N\textsubscript{2} emission at any other time. Previous studies have reported a range of N\textsubscript{2}O mole fractions: Mathieu et al. (2006) reported a range of 0.15–0.94 measured from undisturbed soil cores following the application of \textsuperscript{15}N-nitrate after 2 h. Stevens and Laughlin (2001a) measured an average N\textsubscript{2}O mole fraction of 0.31 for NH\textsubscript{4}HCO\textsubscript{3}NO\textsubscript{3} and 0.66 for CSNO\textsubscript{3}. Despite the high variability of reported values for N\textsubscript{2}O mole fractions, the current study showed that an increase in the N\textsubscript{2}O mole fraction occurred on each of the seven occasions when CS was applied with nitrate to grassland soils, compared to NH\textsubscript{4}CINO\textsubscript{3}. This increase in N\textsubscript{2}O mole fraction was due to the increase in N\textsubscript{2}O emissions in the presence of CS. Averaged across the seven application dates, the mole fraction was 0.34 for CSNO\textsubscript{3} compared to 0.24 for NH\textsubscript{4}CINO\textsubscript{3}. The average N\textsubscript{2}O mole fraction was lower (0.19) in June 2010, under relatively dry conditions, than at other times (average 0.34), indicating a lower N\textsubscript{2}O mole fraction under conditions more conducive to nitrification.

5.2 N\textsubscript{2}O a\textsubscript{D} and N\textsubscript{2}O d\textsubscript{15}N

As the occurrence of nitrification can be inferred from the rate of dilution of the labelled nitrate pool, N\textsubscript{2}O a\textsubscript{D} results have demonstrated that the rate of nitrification in CSNO\textsubscript{3} was slower than in NH\textsubscript{4}CINO\textsubscript{3}, with a delay in the onset of nitrification occurring in the CSNO\textsubscript{3} treatments. The application of a readily available organic carbon source to the soil stimulates microbial respiration and causes a subsequent decrease
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 dD 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 dD 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 dD 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 dD 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 dD, 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 NH3+ -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

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**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.
0.24 for the NH$_4$ClINO$_3$ treatment, indicating the dominance of N$_2$ emissions.

The rate of nitrification in CSNO$_3$ was slower than in NH$_4$ClINO$_3$, and DCD effectively inhibited nitrification in both treatments. However, the effect of DCD in lowering N$_2$O emissions is limited in the presence of a NO$_3$ fertiliser, when denitrification is the main source of N$_2$O. To obtain the maximum cost-benefit of DCD in lowering N$_2$O 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.

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