

# INHIBITION OF AMMONIA OXIDISERS TO CONTROL NITRIFICATION RATE UNDER SIMULATED WINTER DAIRY FORAGE GRAZING CONDITIONS: AN INCUBATION STUDY

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## Abstract

The microbial process of nitrification plays a key role within the soil nitrogen cycle. Nitrification is the process where ammonia is oxidised to nitrite and then to nitrate and this process can have major negative environmental effects. It has previously been determined that both ammonia oxidising bacteria (AOB) and ammonia oxidising archaea (AOA) mediate the first step of the nitrification process, i.e. the ammonia oxidation process in soil, but it is unclear which group is important under wet winter forage grazing conditions. The reduction of nitrification rates is an important factor in reducing  $\text{NO}_3^-$  leaching from winter forage systems and a key mitigation tool is the use of the nitrification inhibitor dicyandiamide (DCD). The aim of this study was to investigate the effect of cow urine and dicyandiamide (DCD), on AOA and AOB population abundance in dairy winter forage grazed soils.

While this study indicated that AOA were present within the soil, it was AOB that played the dominant role in ammonia oxidation in the urine treated soil. In the urine only treatment (applied at  $500 \text{ kg N ha}^{-1}$ ), the AOB *amoA* gene copy numbers were 11.7 times that of the control on day 21. The urine plus DCD treatment applied at  $10 \text{ kg DCD ha}^{-1}$  and urine plus DCD treatment applied at  $20 \text{ kg DCD ha}^{-1}$ , respectively, showed a 91.3 % and 96.6 % reduction in AOB *amoA* gene copy numbers at the same point in time. By day 112, the nitrate concentration for the urine only treatment was 8.4 times the control. Whereas, the urine plus DCD ( $10 \text{ kg ha}^{-1}$ ) and urine plus DCD ( $20 \text{ kg ha}^{-1}$ ) treatments had an 84.4 % and an 88.5 % reduction in nitrate concentration, respectively, compared to the urine only treatment.

These results illustrate that while both AOA and AOB were present within the soil only one group of microbes was actively involved in the ammonia oxidation process. The results also show that using the nitrification inhibitor DCD is a highly effective way to inhibit the growth of AOB, leading to a reduction in  $\text{NO}_3^-$  concentration in the soil under a winter forage grazing system.

## Introduction

Nitrification plays a key role within the soil nitrogen cycle. Nitrification converts ammonia to nitrate via nitrite. Nitrate is weakly held within the soil and can be readily lost from the soil. This leads to significant environmental and ecological consequences. Of most concern are: surface water eutrophication, production of the greenhouse gas nitrous oxide and human health issues if present in drinking water at high concentrations (Smith *et al.*, 1999; de Klein & Eckard, 2008; Powlson *et al.*, 2008).

The most common form of nitrogen being lost from the soil is nitrate, through nitrate leaching. This primarily occurs during the cooler months of the year when drainage is high

but pasture growth rates and nitrogen uptake are low. Within a pastoral farming system, the majority of nitrate being leached comes from animal urine deposited on the pastures. Animal urine patches have the greatest effect on the amount of nitrate being leached because they are highly concentrated areas of nitrogen. A single dairy cow urine patch contains between 700-1300 kg N ha<sup>-1</sup> (Haynes & Williams, 1993; Ledgard, 2001; Moir *et al.*, 2007; de Klein & Eckard, 2008). This high nitrogen concentration occurs because dairy cows partition 70-90 % of ingested nitrogen into urine (Di & Cameron, 2002a). There is also a small impact through the addition of nitrogen based fertilisers.

With nitrogen having such a significant impact on the environment, measures need to be put in place to help reduce both the environmental and ecological impacts. To date, a number of mitigation tools and best management practices have been developed to help farmers reduce their environmental footprint. A key mitigation tool is the nitrification inhibitor dicyandiamide (DCD) which has been researched and developed in New Zealand over the last decade. Adding DCD to a soil can lead to a 42-76 % reduction in the amount of nitrate being leached from the soil profile (Di & Cameron, 2002b; Di & Cameron, 2007).

It has previously been determined that both AOA and AOB mediate nitrification in soil (Leininger *et al.*, 2006; Di *et al.*, 2009; Di *et al.*, 2010; Tourna *et al.*, 2011) but it is unclear which group of microbes mediate nitrification in winter forage grazing systems, where there are high nitrogen concentrations and the soil is wet, for the majority of the time. The aim of this study was to investigate the effect of cow urine and dicyandiamide (DCD), on AOA and AOB in dairy winter forage grazed soils.

## Materials and Methods

### *Trial Setup*

20 kg of Balmoral stony silt loam was collected from the Lincoln University Ashley Dene farm. The soil collected was from the top 0.1 m of the soil profile and was sieved through a 5 mm sieve for use in the incubation study.

For the incubation study, each plastic pottle required 500 g soil based on dry weight. A lid with two aeration holes, 0.5 cm diameter, was then placed on top of each pottle. The pottles were placed into an incubator set at 10 °C for one week so that pre-incubation equilibrium could occur.

### *Treatments*

**Table 1** Treatments that were applied to the soil

Control	No urine/DCD
Urine	500 kg N ha <sup>-1</sup>
Urine & DCD	500 kg N ha <sup>-1</sup> + 20 kg DCD ha <sup>-1</sup>
Urine & DCD	500 kg N ha <sup>-1</sup> + 10 kg DCD ha <sup>-1</sup>

The treatments (Table 1) were assigned randomly to pottles and applied after the pre-incubation period. The urine used was fresh dairy cow urine. Following the application of all treatments, the soil moisture content was adjusted to 100 % water-holding capacity and was maintained at this content throughout the incubation period.

### Chemical analysis

Samples of soil were collected on days 1, 7, 14, 21, 28, 42, 56, 70, 86 and 112 after treatment application and analysed for  $\text{NO}_3^-$ , and  $\text{NH}_4^+$  concentrations using flow injection analysis (FIA), and DCD using HPLC (Waters Inc).

### AOB and AOA assays

The methodology for the soil AOB and AOA assays followed that of Di *et al.* (2009). The population abundance of AOB and AOA present within the soil was determined using real-time quantitative PCR (qPCR), by targeting the functional *amoA* gene (Di *et al.*, 2009). All fresh soil samples that had been subsampled from the bulk soil sample were stored at  $-80^\circ\text{C}$  before DNA extraction and analysis, unless analysed immediately following sampling.

### Results

The amount of  $\text{NH}_4^+$ -N in the urine only treatment (applied at  $500\text{ kg N ha}^{-1}$ ) decreased over the study period. The urine and DCD treatments showed little change over time and no differences between DCD application rates were observed.

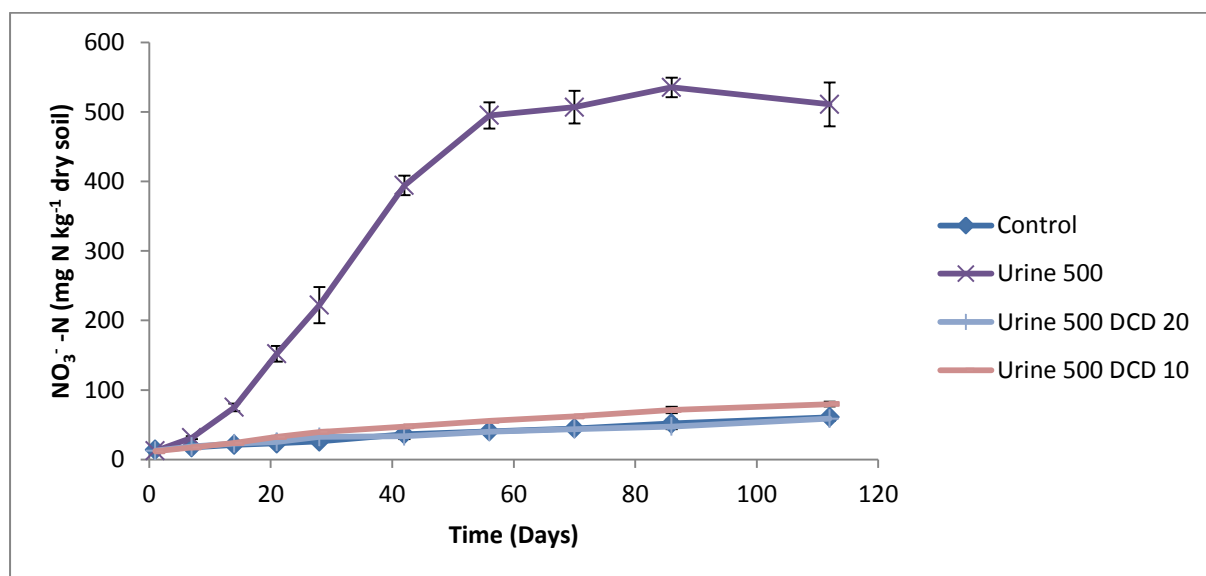


Figure 1 Soil nitrate concentration in a soil that has been treated with animal urine and DCD

The concentration of soil nitrate versus time for each treatment is shown in Figure 1. The urine only treatment (applied at  $500\text{ kg N ha}^{-1}$ ) produced a higher  $\text{NO}_3^-$  concentration within three weeks from the start of the study. The addition of DCD resulted in low  $\text{NO}_3^-$  concentrations for the entire study period. Little difference was observed between the two DCD application rates of  $10\text{ kg DCD ha}^{-1}$  and  $20\text{ kg DCD ha}^{-1}$ .

## *Ammonia oxidising bacteria abundance*

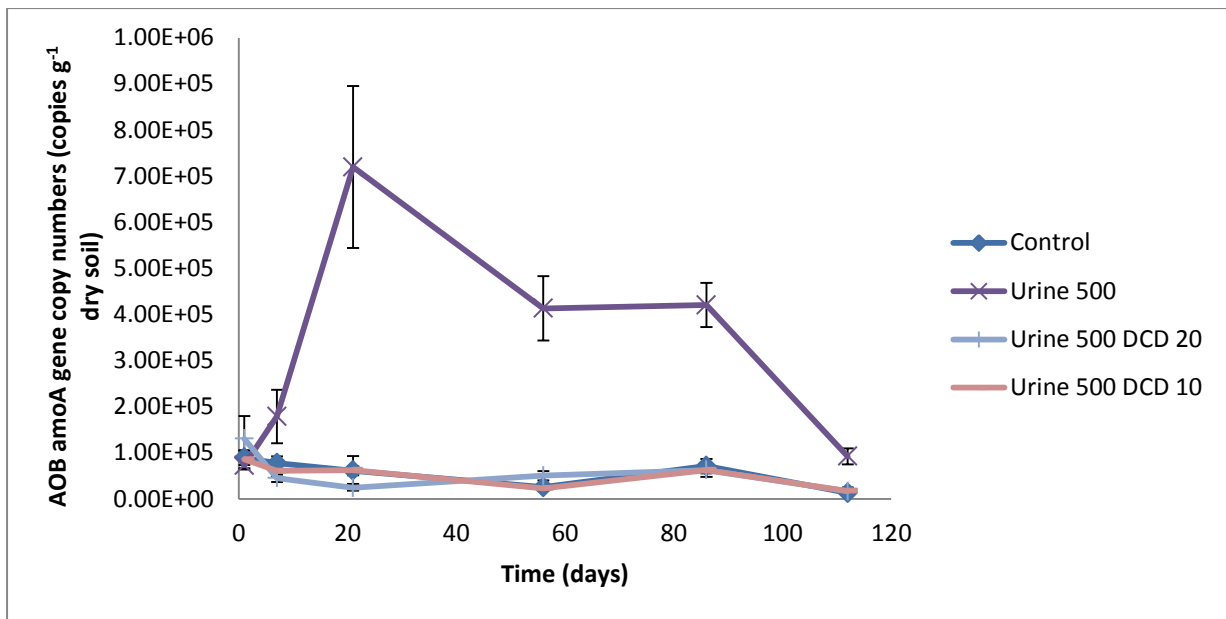


Figure 2 AOB gene copy numbers within the soil in a soil that has been treated with animal urine and DCD

Through the application of the NH<sub>3</sub> substrate animal urine, AOB growth was stimulated in the urine only treatment (applied at 500 kg N ha<sup>-1</sup> (Figure 2). Peak *amoA* gene copy numbers occurred on day 21 and were 11.7 times the control for the urine only treatment. The treatments that had DCD present showed no such peak. These treatments showed similar results to that of the control, with little differences in the *amoA* gene copy numbers and values remained low across the study period. By day 112, the end of trial, all *amoA* gene copy numbers were close to background levels.

There was no indication of any differences in *amoA* gene copy numbers between the treatments across the study period.

## Discussion

The results from this study illustrate that using the nitrification inhibitor DCD is a highly effective way to reduce the concentration of NO<sub>3</sub><sup>-</sup> in soil. The study indicated that, even though DCD reduced soil nitrate concentrations, the different concentrations of DCD had little to no effect, on AOB gene copy numbers, indicating that the 10 kg DCD ha<sup>-1</sup> application rate was sufficient to effectively inhibit the AOB population under the conditions. Any differences were due to the presence of urine and its concentration. The results also showed that nitrification is driven by AOB not AOA in the nitrogen-rich soils even under the wet soil conditions of the present study. These findings are in support of those of Di *et al.* (2009).

## Conclusions

Results from this study show that although AOA are present within the soil, it is the presence of AOB that mediates nitrification. Mitigation to reduce nitrification rates within a soil is an important factor in reducing the NO<sub>3</sub><sup>-</sup> leaching potential. This trial shows that DCD is a suitable option for inhibiting nitrification within the soil under winter forage grazing conditions. There was no difference between the two different DCD application rates.

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