# Non-target impacts of the nitrification inhibitor dicyandiamide on soil biota

**Maureen O' Callaghan**[A], Tracey Nelson[A], Richard Lardner[B], Philip Carter[B], Emily Gerard[A] and Mike Brownbridge[A]

[A]AgResearch Ltd., Canterbury Agricultural Research Centre, Christchurch, New Zealand, Email maureen.ocallaghan@agresearch.co.nz
[B]ESR, Kenepuru Research Centre, Porirua, Wellington, New Zealand.

## Abstract

Intensively grazed dairy pastures in New Zealand routinely receive concentrated inputs of nitrogen (N) both through application of fertiliser and deposition of urine by grazing animals. Leaching of nitrate from soil into aquifers, rivers and lakes is a source of increasing environmental and public health concern. Nitrification inhibitors have been shown to decrease leaching and denitrification from urea- and ammonium-based fertilisers and from urine patches in pastures. To date there have been few studies on effects of nitrification inhibitors on non-target soil biota. Three laboratory experiments were carried out to measure the short term effects of a nitrification inhibitor, dicyandiamide (DCD), on diversity of soil bacterial populations, earthworms and Collembola. Molecular analysis of the soil bacterial community indicated that application of DCD to soil did not affect the composition of the predominant bacterial phyla present in soil, unlike the addition of bovine urine which caused rapid changes in bacterial diversity. Survival and growth of the earthworm *Aporrectodea caliginosa* was unaffected by application of urine or DCD to soil. Collembola populations were not inhibited by DCD, while the addition of urine appeared to increase numbers. Results confirm the view that DCD can be considered one of the more environmentally benign nitrification inhibitors, making it an important tool in countering the environmental impacts resulting from ongoing land use intensification.

## Key Words

Environmental impact,mitigation,SARST, soil quality, soil bacterial communities.

## Introduction

Intensification of New Zealand's farming systems has resulted in an increase in fertiliser inputs, nutrient recycling rates and stocking densities. Intensively grazed dairy pastures in New Zealand routinely receive large inputs of nitrogen (N) both through application of fertiliser and deposition of urine by grazing animals. Several studies have indicated that, at least in New Zealand, animal urine patches are the major source of N leached from grazed pastures (Ledgard *et al.* 1999). Leaching of nitrate from soil into aquifers, rivers and lakes is causing severe environmental impacts (apparent in eutrophication of some of New Zealand's iconic lakes, such as Lake Taupo) and public health concerns.

Nitrification inhibitors are being used increasingly in New Zealand to reduce N loss from soil and increase N use efficiency. The inhibitors have been shown to decrease leaching from urea- and ammonium-based fertilisers and from urine patches in grazed pastures (e.g. Di *et al.* 2007). However, there have been few independent studies on the non-target effects of these now widely used agricultural compounds. Cuttle (2008) concluded that there appeared to be no evidence of wider environmental impacts arising from the use of nitrification inhibitors but after reviewing the available literature, Edmedes (2004) stated that research was needed to quantify the effects (both long and short term) of these chemicals, and their repeated use, on soil quality. Given the importance of maintaining soil quality and function for ongoing productivity of pastoral systems, it is essential that soil treatments such as nitrification inhibitors do not impact on activity of non-target soil microbes and fauna.

This contribution reports on results of studies carried out to determine the short term effects of the nitrification inhibitor dicyandiamide (DCD, $C_2H_4N_4$), which is used in New Zealand, on soil bacterial diversity and representative soil biota. The Collembolan species *Folsomia candida* has frequently been used as an indicator species in ecotoxicological testing and is highly sensitive to intensive use of pesticides. The earthworm species chosen for experiments, *Aporrectodea caliginosa*, is commonly found in New Zealand pastures and is likely to be exposed to DCD through standard agricultural practice.

## Methods

### *Pot trials*

Three laboratory experiments were carried out to examine impacts of DCD on 1) soil bacterial community; 2) earthworms; 3) Collembola. For assessment of impacts of DCD on the soil bacterial community, a pot trial was established. Pots containing 1 kg of field moist soil (Horotiu sandy loam) were sown with ryegrass seedlings (*Lolium perenne*) and maintained in a controlled-environment (CE) room (12°C day/8°C night) following application of treatments. For assessment of impacts of DCD on Collembola and earthworms, planter bags containing 500g of field moist soil (Wakanui loam) were sown with ryegrass. The planter bags were maintained in a shade house and the ryegrass was allowed to grow for six weeks before treatments were applied to the soil surface and soil was removed to containers in a CE room, as detailed below.

### *Treatments*

Cow urine was collected from a dairy farm near Hamilton, New Zealand. Pots/planter bags were treated with equivalent of 600 kg/ha of urine-N (within the range of N concentration typically found under urine patches) and equivalent of 30 kg of DCD/ha, a rate typical of the amount applied to pasture in New Zealand. Treatments applied were: urine; DCD, urine+DCD and an untreated control.

### *Molecular analysis of soil bacterial community*

Microbial community nucleic acids were extracted from soil samples collected 2 and 56 days after application of treatments as described previously (Griffiths *et al.* 2000). Genetic diversity of the soil bacterial community was profiled by serial analysis of ribosomal sequence tags (SARST) as described by Yu *et al.* (2006). The taxonomic identity of aligned 16S sequences from each treatment was determined using the classify tool within greengenes (DeSantis *et al.* 2006). Because of the relatively short sequences obtained using SARST, classifications were made to the level of phylum only.

### *Earthworms*

After treatments were applied, the ryegrass was cut to approximately 1cm and the trimmings discarded. The contents of the bags were then mixed to incorporate the remaining grass and roots into the soil, with a subsample of 500g from each planter bag transferred into lidded plastic containers. *A caliginosa* were collected from the field and held in field soil at 15°C before use. Earthworms were individually weighed and then grouped in sets of three, so that the average weight of the three worms was similar for all groupings. Three earthworms were added to each container and the containers were maintained in a CE room at 12°C. Earthworm survival, development (weight) and sexual maturity (presence of a clitellum) was assessed every 2 weeks for 10 weeks. After each assessment the worms were returned to the soil, the soil moistened as necessary and food (dried, powdered cow dung and grass) was added. Weight data was analysed in Genstat (ANOVA), while sexual maturity was analysed using a generalised linear mixed model.

### *Collembola*

As for the earthworms, the treated soil in the bags was mixed and subsamples of 150g were transferred to a lidded plastic containers with two replicates prepared from each planter bag. Ten adult Collembola from a laboratory culture were added to each container and containers were maintained in an incubator at 15°C. Collembola populations were assessed after 28 and 56 days by destructively sampling two replicate containers per treatment, and analysis of variance was performed to compare standard error of the means for each assessment time.

## Results

### *Soil bacterial diversity*

Analysis of sequence data showed that bacterial diversity in soil treated with DCD did not differ significantly from untreated control soil at either 2 or 56 days after soil treatment (Figure 1). The proportions of the predominant phyla present in untreated soil, Proteobacteria, Acidobacteria, Actinoacteria and Firmicutes, were unchanged following application of DCD to soil. In contrast, there was rapid shift in the microbial community in response to urine and urine+DCD treatment. Shifts in community structure 2 days after application of treatments could mainly be attributed to increased members of the phylum Firmicutes, which comprised approximately 10% of the total phyla in control and DCD treated soils, in comparison with approximately 50% in the urine and urine+DCD treated soils.

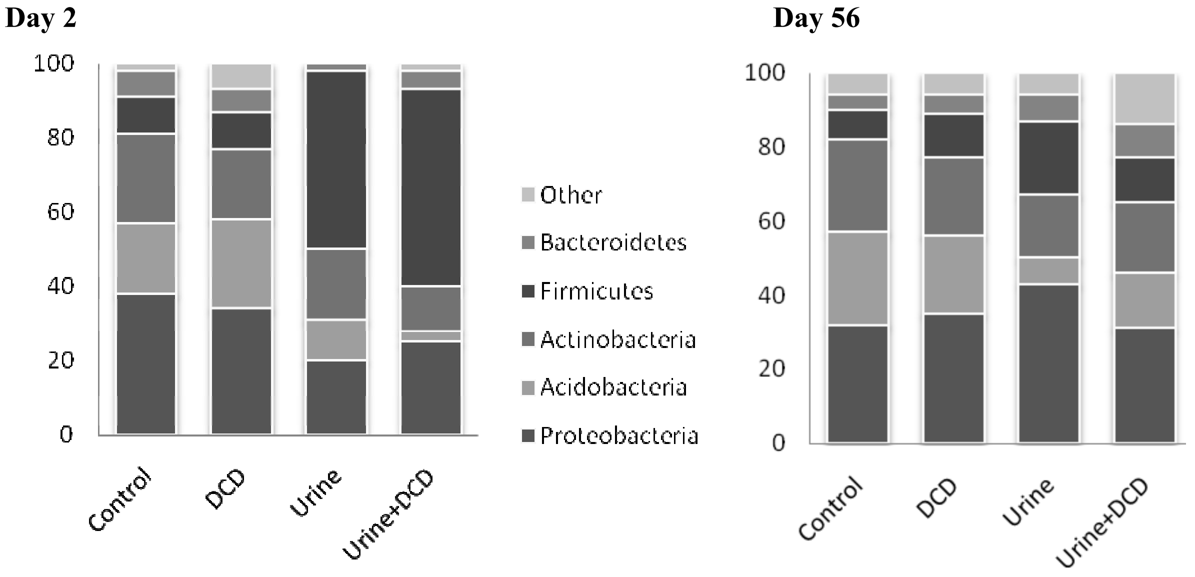

**Figure 1. Dominant taxa at a phyla level based on SARST analysis of 16S rRNA sequences, in untreated soil and in soils treated with urine, DCD, and urine+ DCD, 2 and 56 days after the application of treatments (based on an average of 560 OTUs from each sample).**

*Earthworms*

Survival and development of the earthworms was unaffected by any of the treatments. Earthworms increased in weight during the 10 weeks of the experiment (Figure 2), apart from a slight decrease at week four across all treatments, when they received a smaller amount of food. Treatment had no effect on the maturation rate of the earthworms (data not shown).

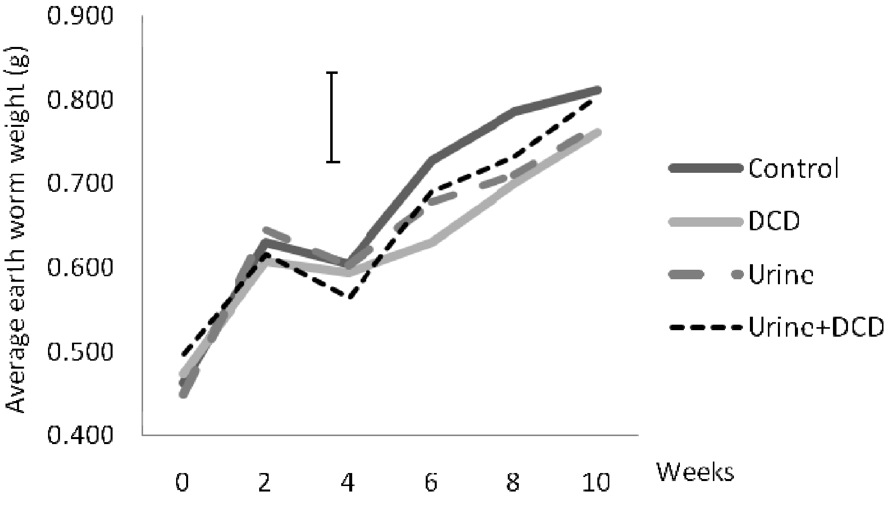

**Figure 2. Weight of earthworms incubated in soil treated with urine, DCD and urine+ DCD. Error bar is SEM.**

*Collembola*

Presence of DCD in soil had no significant effect on populations of *F. candida*, with populations similar to those in untreated soil at 28 and 56 days after treatment. Addition of urine to the soil appeared to have a positive effect on Collembola populations, with larger populations recovered from urine and urine+DCD treatments than the control after 28 days, although this difference was only significant ($P<0.05$) for the urine+DCD treatment. There were no significant differences between treatments after 56 days.

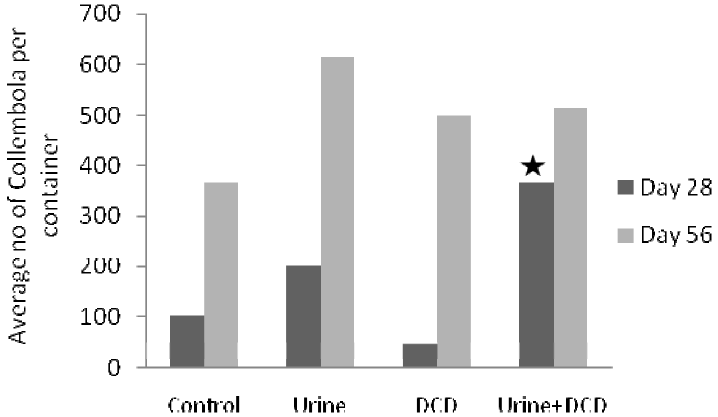

**Figure 3**. **Numbers of the Collembolan *Folsomia candida* in soils at 28 and 56 days after application of urine, DCD, and urine+ DCD. Star indicates significant difference between Control and Urine+DCD treatments at Day 28.**

## Discussion

DCD is considered one of the more environmentally benign nitrification inhibitors. Certainly SARST analysis of bacteria diversity at days 2 and 56 indicated that there was no short term effect on the predominant phyla present in soil. The current analysis was limited to the level of phylum, and it is possible that changes in the bacterial community were occurring beneath this level. However, given that the inhibitor is targeted at activity of a specific functional group of bacteria, the ammonia-oxidisers, these results add further weight to the conclusion that DCD has little impact on non-target microflora. Similarly, DCD had no impact on earthworm growth rate and survival and fecundity of a Collembola population. Both Collembola and earthworms are regarded as key “ecosystem service providers”, as they play important roles in maintaining the soil ecosystem, with Collembola in particular being regarded as key indicators of soil fertility and health. The use of chemical inhibitors to disrupt natural soil processes seems at first glance to be contrary to ecological concepts of soil quality and conservation of biodiversity. However, given its low level of non-target impact on soil microflora and fauna, application of DCD to pasture is a relatively benign intervention that has an important role to play in mitigating the environmental hazards imposed by ongoing land use intensification.

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