Agriculture, Ecosystems and Environment 167 (2013) 60–70

Contents lists available at SciVerse ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Impact of short-interval, repeat application of dicyandiamide on soil N transformation in urine patches

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ARTICLE INFO

Article history:
Received 20 August 2012
Received in revised form
29 December 2012
Accepted 5 January 2013
Available online 1 March 2013

Keywords:
DCD
Nitrification inhibitor
Urine patch
Ammonia oxidizing bacteria

ABSTRACT

In pastoral farming systems, bovine urine patches are 'hot-spots' of elevated soil nitrogen (N). Nitrification in urine patches is linked to deleterious environmental outcomes, such as formation of the greenhouse gas nitrous oxide (N_2O). The nitrification inhibitor dicyandiamide (DCD) reduces the rate of nitrification in soils subsequently limiting N-losses. Dicyandiamide's bacteriostatic mode of inhibiting ammonia oxidising bacteria (AOB), has raised concerns about the efficacy of frequent DCD use. For example, frequent use could result in selection for a DCD-tolerant AOB community. Furthermore, the impacts of DCD on other aspects of microbial N transformation in soil are largely unknown. To test the influence of short-term repeat application of DCD on soil N cycling, we established a replicated field-trial in which ±urine and ±DCD were added to pasture soils (fully crossed, 2 × 2 factorial design). After 57 d, treatments were re-applied. Mineral N pools, pH, DCD concentration, moisture and temperature were measured, along with N_2O fluxes. Microbial communities involved in ammonia oxidation (bacteria and archaea), nitrite reduction, and N_2O reduction were quantified using real-time PCR targeting functional genes (*amoA*, *nirS*, and *nosZ*). Overall, the addition of DCD significantly ($P < 0.05$) reduced both the formation of nitrate (~64%), and N_2O loss from urine-treated soils (~44%). The effect of repeating the urine and DCD applications produced similar results with no decline in DCD efficacy. This was despite a doubling in the size of the AOB community, stimulated by the initial urine application. The application of urine + DCD had a significant but minor impact on denitrifying bacteria (*nirS*), rather the population size of these bacteria correlated with increasing soil temperature over time (rho = 0.7; $P = 0.002$). In contrast, copies of *nosZ* increased with urine addition ($P < 0.05$) but were unaffected by sampling date (increasing soil temperature). Our results demonstrate that an initial DCD application does not affect its efficacy to inhibit nitrification and reduce N_2O emissions following a subsequent DCD application 57 days later.

1. Introduction

Pastoral farmland is the single largest human land use in New Zealand, comprising 37% of the total land area (Ministry for the Environment, 2007). Over the last two decades, the conversion of farms from low-input beef- or sheep-based enterprises to dairy production systems has resulted in intensification of pastoral agriculture. Thus, while numbers of beef cows and sheep have declined, the national dairy herd nearly doubled from 2.9 million cows in 1981 to nearly 6 million in 2010 (Ministry for the Environment, 2007, 2010). This has resulted in enhanced use of nitrogen (N) fertiliser, irrigation and increases in stocking rates (Ministry for Primary Industries, 2012)

Intensification of New Zealand's pastoral sector has not been without environmental cost (Ledgard et al., 2009), particularly with respect to water quality and greenhouse gas emissions. Central to the environmental issues is the soil N enrichment that occurs following a bovine urine event (Scholefield et al., 1993; Ledgard et al., 1999). A single urination event from a dairy cow can increase N loading in soil to between 600 and 1000 kg N ha^{-1} (Haynes and Williams, 1993), well in excess of levels that can be readily assimilated by pasture plants.

The major N-component of urine is urea, accounting for over 70% of the total N present (Doak, 1952). In the urine patch, the urea rapidly undergoes hydrolysis to ammonium-N (NH_4^+), generating ammonia-N (existing in soil solution equilibrium with NH_4^+) (Haynes and Williams, 1992). Nitrification, i.e. the combined activities of ammonia oxidising bacteria (AOB) and archaea (AOA) coupled with nitrifying bacteria, then drives the formation of nitrate (NO_3^-; Fig. S1). As the process is driven by microorganisms, factors that regulate their activity, such as soil temperature and moisture, thereby influence nitrification rate in the field (Sherlock and Goh, 1984).

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0167-8809/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.agee.2013.01.007

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2013.01.007.

The biogeochemical cycling of N in urine patches results in elevated levels of nitrous oxide (N_2O) and NO_3^-, both of which are harmful to the environment. Nitrous oxide is a potent greenhouse gas (GHG) and a precursor to compounds involved in stratospheric ozone depletion (Forster et al., 2007; Ravishankara et al., 2009). Agriculture is the largest anthropogenic source of N_2O accounting for about 60% of the total global anthropogenic N_2O emissions in 2005 (Smith et al., 2007). Production of N_2O occurs predominately via microbial processes, including nitrification, nitrifier–denitrification, and denitrification (Fig. S1; Webster and Hopkins, 1996; Wrage et al., 2001). The largest driver of agricultural N_2O emissions result from biogeochemical cycling of soil N under livestock urine patches. Similarly, NO_3^- leaching from agricultural production systems is the major anthropomorphic source of reactive N in groundwater and results in eutrophication of wetlands, streams, lakes, and coastal systems globally (Galloway et al., 2003) and in New Zealand (Ministry for the Environment, 2007). The presence of NO_3^- in ground and surface waters also leads to indirect losses of N_2O (Galloway et al., 2003). Overall, the accumulation of high levels of NO_3^- in soil is undesirable; combined, N_2O emissions and NO_3^- leaching represent some of the most significant impacts of agriculture on environmental quality.

An important tool, with the potential to reduce these environmental impacts, is the use of nitrification inhibitors, such as dicyandiamide (DCD; Clough et al., 2007; Wilcock et al., 2008). The DCD compound can reduce the rate of conversion of NH_4^+ to NO_3^- by suppressing the activity of AOB (Hauck, 1980; Fig. S1). Numerous studies have shown that reducing the urine-N derived NH_4^+ oxidation rate significantly reduces the potential for NO_3^- leaching and retains N in the soil for plant assimilation (e.g. Di and Cameron, 2004a, 2005, 2007; Menneer et al., 2008; Monaghan et al., 2009). Furthermore, as a nitrification inhibitor, the use of DCD can significantly reduce N_2O emissions (Smith et al., 2008a,b; de Klein et al., 2011) from both the nitrification and denitrification pathways since it delays the formation of NO_2^-, which is the gate way for N_2O formation mechanisms (Fig. S1; Stevens and Laughlin, 1998). The measured efficacy of DCD at reducing the nitrification rate can vary considerably (Cookson and Cornforth, 2002; Menneer et al., 2008; Singh et al., 2009), and is under strong influence of soil temperature and moisture conditions (Di and Cameron, 2004b; Kelliher et al., 2008).

In addition to the direct environmental and agronomic benefits, regulatory pressure to reduce NO_3^- leaching and GHG emissions may result in greater use of DCD both in New Zealand and in many other countries where it is being promoted as a potential solution/mitigation tool. This could occur through expansion of areas treated (number of farms), and also through more frequent application per farm. Furthermore, since DCD can be rapidly metabolised by the soil microbial community (Kelliher et al., 2008; Rajbanshi et al., 1992; Singh et al., 2008), and can be lost through leaching (Shepherd et al., 2012), management strategies based on repeat DCD applications over the growing season will be progressively adopted. Currently advised best practice for one commercial DCD product, available in New Zealand, prescribes two applications of DCD, with the first applied in late autumn (May) within 7 days of grazing when soil temperatures are <15 °C, followed by a second approximately 60 days later in early winter (July). When using the recommended DCD rate (10 kg ha^{-1}), the average reduction in N_2O emissions from urine affected soils is 57% (de Klein et al., 2011) while an average 59% reduction in NO_3^- leaching was observed on three differing soil types (Di et al., 2009a).

However, most of the results to-date have been derived from studies where urine and DCD were applied simultaneously. Yet, other studies have shown that repeat applications of a chemical inhibitor can potentially result in adaptation and selection within the nitrifying microbial community (Rodgers, 1986). For example, Mertens et al. (2009) showed that application of zinc (Zn) completely inhibited soil nitrification, but within 2 years a Zn-tolerant AOB community had developed and nitrification was completely restored (Mertens et al., 2009). Such community-level adaptation of AOB communities to metal and chemical stress factors has been shown to be wide-spread in soils (e.g. Díaz-Raviña and Bååth, 1996; Mertens et al., 2010). As such, emergence of DCD-tolerance is possible, but has yet to demonstrated (Rodgers, 1986).

While considerable effort has been applied to studying the agronomic and environmental impacts of DCD, in terms of pasture production (e.g. Moir et al., 2007), N_2O emissions, and NO_3^- leaching, relatively few studies have been performed to assess the effects of DCD on pasture soil microbial communities and their functions (e.g. Di et al., 2010; O'Callaghan et al., 2010). A recent study has shown that annual applications of DCD did not alter its effectiveness (de Klein et al., 2011), suggesting no longer-term priming effect occurred. No studies have examined the short-term effects of repeated application of DCD on soil microbiology and function in the field.

Reduction in the rate of accumulation of soil NO_3^- formation may also have consequences for denitrification, and also the ratio of N_2O to N_2 following incomplete N-reduction. DNA-based tools are available to measure the abundances of denitrification-associated genes in soils; the numbers of these may be linked to process rates (Wallenstein et al., 2006). A number of functional gene targets are available for assessing denitrification; these include *nap*A, *nar*G, *nir*K, *nir*S, *cnor*B, and *qnor*B. Recently, however, it has been shown that the abundance of *nir*S-genes, encoding nitrite reductase enzyme ($NO_2^- \rightarrow NO$), can be linked to rates of total denitrification, and that the ratio of *nir*-genes to *nos*Z (nitrous oxide reductase) has been linked to N_2O:N_2 molar ratios (Morales et al., 2010; Regan et al., 2011).

The aims of this study were to determine the effectiveness of repeat applications of DCD within 60 days in slowing NO_3^- formation and N_2O emissions from bovine urine patches. Several research questions are addressed. Firstly, does DCD degrade more rapidly following repeat application to soil, potentially reducing the efficacy of repeat application? Secondly, does DCD-application to soil result in selection of a DCD-tolerant ammonia oxidising community which, on repeat urine + DCD application, restore the nitrification rate? Thirdly, given DCD interrupts a 'gate way' within the soil N cycle (Fig. S1), what are the impacts on microbial communities in coupled processes such as denitrification?

2. Materials and methods

2.1. Experimental design and trial establishment

A trial was established on pasture at the Lincoln University research farm, Canterbury, New Zealand. The soil is a Templeton sand-loam soil (immature Pallic soil; NZ Soil Bureau, 1968) with the pasture composed of a mixed ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) sward which had been grazed by sheep. A section of the field was isolated from the stock using electric fencing for 2 months before establishment of experimental treatments, minimising potential legacy effects of previous urine patches. Pasture at the trial site was mown to 5 cm before application of the treatments.

The field trial was set up in a fully crossed factorial design, with ±urine addition and ±DCD for each urine level. Each treatment had four field replicated plots (described below) which were repeatedly sampled over time. Initial applications of urine and DCD were applied to soil on 30th June 2010 and re-application of treatments