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Effect of soil moisture status and a nitrification inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier growth and nitrous oxide emissions in a grassland soil

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ABSTRACT

Nitrous oxide (N_2O) is a potent greenhouse gas and is produced in the soil by nitrification and denitrification processes. These processes are catalysed by a number of key enzymes from microbial communities in the soil. In grazed pasture soils, most of the N_2O is emitted from nitrogen (N) returned in animal excreta deposited during outdoor grazing, particularly in the urine. The nitrification inhibitor, dicyandiamide (DCD), has been used to mitigate N_2O emissions from grazed pasture soils. However, how the nitrifying and denitrifying communities and N_2O emissions are affected by the urine and DCD applications under contrasting soil moisture conditions in grazed pasture soils is poorly understood. Here we report a laboratory incubation study to determine the impact of soil moisture status in combination with animal urine and DCD applications on the abundance of ammonia oxidizer (AO) and denitrifier functional genes, and N_2O emissions from a grazed pasture soil. The soil used was a Horotiu silt loam (Typic Udivitrand) developed from volcanic ash. The treatments included three soil moisture contents: 60%, 100% and 130% field capacity (FC), each with Control, Urine and Urine + DCD treatments. The incubation temperature was 12 °C to simulate New Zealand autumn/winter soil temperatures (when DCD is used). Results showed that soil moisture significantly increased N_2O emissions from the urine treated soil, with total N_2O emissions at 100% and 130% FC being 9 and 400 times that at 60% FC, respectively. Soil moisture content also significantly affected the growth of ammonia oxidiser and denitrifier communities, with the functional genes increasing with increased soil moisture content. The application of urine significantly increased the abundance of AOB *amoA*, *nirK* and *nosZ* (clades I and II) genes, but decreased the abundance of AOA *amoA* and *narG* genes at 130% FC. DCD was highly effective in inhibiting the growth of AOB communities, and reducing N_2O emissions at 100% and 130% FC. The abundance of *nirK* gene was also reduced by DCD at 130% FC, but this was, most likely, a reflection of changes in AOB populations, bearing the majority of the *nirK* gene detected in this study. DCD did not affect the other denitrifier genes studied. Total N_2O emissions were significantly related to the abundance of AOB ($R^2 = 0.94$, $p < 0.001$) and *nirK* genes ($R^2 = 0.57$, $p < 0.05$), but not to the other genes studied. These results suggest that soil moisture has a major influence on ammonia oxidizing and denitrifying microbial communities in urine treated soils and that this affects the N_2O emissions. Ammonia oxidizers are able to grow under very wet soil conditions, probably in part due to the growth of AOB communities that also carry the *nirK* gene.

1. Introduction

Nitrous oxide (N_2O) is a major greenhouse gas with a long-term global warming potential 298 times that of carbon dioxide (CO_2) (IPCC, 2007). N_2O is produced as a by-product of the nitrification and denitrification processes. Under aerobic conditions, nitrifying microbes convert ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-) (Fig. 1), and can produce N_2O under certain soil conditions (Wrage et al., 2001). N_2O is produced through denitrification where denitrifying microbes reduce NO_3^- to NO_2^-, nitric oxide (NO), N_2O, and N_2 under anaerobic conditions (Fig. 1) (Firestone and Davidson, 1989; Mosier et al., 1998).

Soil moisture content can affect N_2O emissions from soil and the contribution of nitrification and denitrification to N_2O emissions. N_2O emissions usually increase in wetter soils as the soils become more anaerobic (Linn and Doran, 1984; Smith et al., 1998; Dobbie

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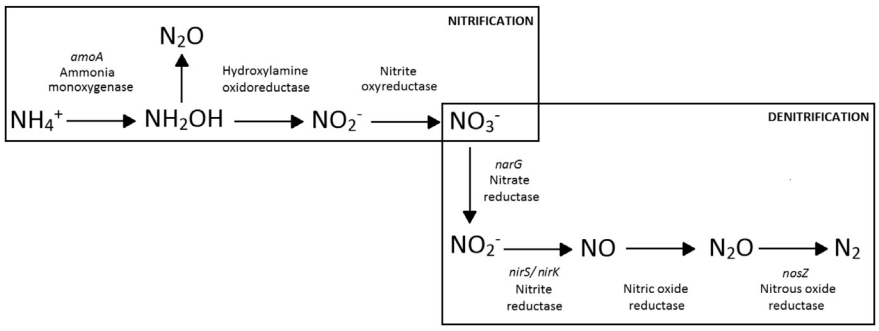

Fig. 1. Nitrification and denitrification processes with associated enzymes and functional genes measured in this study (adapted from Wrage et al., 2001).

et al., 1999; Dobbie and Smith, 2001). However, N_2O emissions are usually higher when the soil is only partially anaerobic than when it is completely saturated, as N_2O is converted to N_2 under the latter conditions (Smith et al., 1998). It is generally thought that N_2O emitted from soil with a high moisture content is mainly by denitrification (Bateman and Baggs, 2005; Diba et al., 2011). However, other studies (Maag and Vinther, 1996; Zhu et al., 2013) found that N_2O emissions from nitrification increased with increasing soil moisture content or decreasing oxygen concentration. Further research is required to improve our understanding of the effect of soil moisture content on the growth of nitrifying and denitrifying microbes in the soil and how they affect N_2O emissions.

In New Zealand, the predominant land use is grazed pastures, where animals graze outdoors. In such production systems, most of the N_2O emissions are from nitrogen (N) returned to the pasture soil in the excreta of the grazing animal, particularly the animal urine (MFE, 2011). Urine deposition creates unique areas of soil, with very high N concentration, far exceeding the utilisation capacity of the plants, with the surplus N contributing to N_2O emissions and nitrate (NO_3^-) leaching (Haynes and Williams, 1993; Jarvis et al., 1995; Di and Cameron, 2002). The losses of N_2O from animal urine are particularly high during the winter when the soils are wet (de Klein et al., 2006). The addition of animal urine to the soil has a major effect on soil microbial communities involved in N cycling and thus on N dynamics. Di et al. (2010) found that the addition of urine to grazed pasture soil with a moisture content around field capacity significantly increased the population abundance of ammonia oxidizing bacteria (AOB), but did not affect, or inhibit, the population abundance of ammonia oxidizing archaea (AOA). However, our understanding is poor on the effect of animal urine addition on ammonia oxidizer and denitrifier communities, N dynamics and N_2O emissions under different soil moisture conditions.

Major efforts have been made to mitigate N_2O emissions from grazed pasture soils. One of the mitigation technologies that have been shown to be effective in decreasing N_2O emissions is the use of a nitrification inhibitor, dicyandiamide (DCD), to treat grazed pasture soils (Amberger, 1989). It has been shown that N_2O emissions from animal urine patches can be decreased by 60–80% by the use of DCD (Di and Cameron, 2002, 2006; Smith et al., 2008). DCD inhibits ammonia oxidizer growth and activity by deactivating the ammonia monoxygenase enzyme (Di et al., 2009, 2010). However, it is not known if DCD also affects the growth of denitrifiers in the soil, particularly under wet soil conditions. It is hypothesized that denitrifier growth might also be indirectly affected by DCD as a result of DCD effect on the nitrification process.

The objectives of this study were to determine the effect of contrasting soil moisture conditions and the nitrification inhibitor DCD on ammonia oxidizer and denitrifier growth and N_2O emissions from animal urine treated soils under controlled incubation conditions. The ammonia oxidizer and denitrifier community abundance was studied by determining the copy numbers of key functional genes encoding the key enzymes involved in the ammonia oxidation and denitrification processes as shown in Fig. 1. DCD was included in this study not with the aim of validating its efficacy in nitrification inhibition, but mainly to determine its impact on denitrifier communities, an area that is not well understood.

2. Materials and methods

2.1. Soil

A Horotiu silt loam volcanic soil (74% silt, 20% sand and 6% clay) (NZ Soil Classification: Typic Orthic Allophanic Soil (Hewitt, 1992); USDA classification: Typic Udivitrand (Soil Survey Staff, 1998)) was used for the study. Surface soil samples (0–10 cm) were collected from a dairy farm near Hamilton in Central North Island of New Zealand (38°46′38″ S; 175°18′26″ E). The samples were bulked into a single sample, sieved through a 6 mm screen and stored at 4 °C until use. The soil properties were: pH (H_2O): 5.9; organic C: 40.4 g kg^{-1}; total N: 3.5 g kg^{-1}; Olsen P: 16.9 mg kg^{-1}; CEC: 13.0 $cmol_c$ kg^{-1}; exchangeable Ca^{2+}: 7.1 $cmol_c$ kg^{-1}; exchangeable Mg^{2+}: 0.19 $cmol_c$ kg^{-1}; exchangeable K^+: 0.19 $cmol_c$ kg^{-1}; and base saturation: 60.6%.

2.2. Incubation experiment

Two corresponding studies were set up, one set for measuring N_2O emissions, and another set for soil sampling to measure N dynamics and functional gene abundance. Three soil moisture conditions were assessed in the study: 60%, 100% and 130% field capacity (FC). These are equivalent to about 20%, 35% and 45% gravimetric soil water content, respectively. The following treatments were applied at each soil moisture content: Control; Urine (700 mg urine-N kg^{-1} dry soil, equivalent to 700 kg N ha^{-1}); Urine (700 kg N ha^{-1}) + DCD (10 mg DCD kg^{-1} dry soil, equivalent to 10 kg DCD ha^{-1}). Each treatment had four replicates.

For N_2O measurement, 275 g of sieved soil samples (dry weight basis) were weighed and packed into 575 mL glass jars (glass jar diameter 7.5 cm; packed to 1.0 g cm^{-3} bulk density), leaving the rest of the space in the jar for N_2O sampling. Freshly collected dairy cow urine was analysed for N concentration and the correct volume was applied to the surface of the soil to achieve the desired application rate. DCD was dissolved in water and sprayed on to the soil surface. The jars were closed with lids that had two 1 cm holes to allow air flow during incubation. The incubation vessels were placed in an incubator in a randomized design. The temperature was set at 12 °C to simulate autumn/winter soil temperatures in