



## Effect of different land use and land use change on ammonia oxidiser abundance and N<sub>2</sub>O emissions



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### ABSTRACT

In recent years, there has been a major expansion of dairy farming in New Zealand, largely as a result of land use change from forestry and sheep farming to dairy farming. Possible impacts of different land uses and land use change on ammonia oxidising bacteria (AOB) and archaea (AOA) and N<sub>2</sub>O emissions are not well understood. A field study was carried out to determine the effects on AOB and AOA abundance by three long-term different land uses, pine tree plantation (30+ years), sheep farming (30+ years) and dairy farming (12+ years), located in close proximity on the same soil type. A laboratory incubation study was carried out to determine the impact of dairy cow urine application (simulating the deposition of dairy cattle urine after the conversion of tree plantation and sheep farming to dairy farming) on AOB and AOA growth and N<sub>2</sub>O emissions. The results showed that AOB abundance was higher in the dairy and sheep farm soils ( $P < 0.05$ ) than in the pine tree soil but that the AOA abundance was higher in the sheep farm soil than in the dairy and pine tree soils ( $P < 0.05$ ). When dairy cow urine was applied in the incubation study, the AOB growth was initially faster in the dairy, followed by sheep and then followed by the pine tree soil, but the growth continued for an extended period in the pine tree soil with the *amoA* gene copy numbers eventually exceeding those in the sheep and dairy pasture soils. AOA grew following urine application in the sheep soil but did not change in the other soils. Total N<sub>2</sub>O emissions in the pine tree soil was more than twice those from the dairy and sheep farm soils. These results demonstrate the significant impact of land use and land use change on ammonia oxidiser communities and subsequent impacts on nitrogen transformations and N<sub>2</sub>O emissions. Further research is needed to verify these results in the field.

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### 1. Introduction

In New Zealand, agriculture is the largest source of greenhouse gas emissions, accounting for 48.4% of total national emissions in 2013 (MFE, 2015). Of the total agricultural emissions, 73% was methane (CH<sub>4</sub>) from enteric fermentation, and 22% was nitrous oxide (N<sub>2</sub>O) from the soil. From 1990 to 2013, New Zealand's greenhouse gas emissions increased by more than 14%, with N<sub>2</sub>O increasing by more than 23%. The main contributor to increased agricultural emissions was an increase of 88.4% dairy cattle population since 1990, largely from the conversion of sheep farming and plantation forestry to dairy farming (MFE, 2015). In grazed pasture

soils, such as dairy farming, the majority of N<sub>2</sub>O is emitted from the nitrogen (N) returned to the soil in animal excreta, particularly animal urine, where the N loading rate under a dairy cattle urine patch may vary between about 700 and 1200 kg N ha<sup>-1</sup> (Haynes and Williams, 1993; Di and Cameron, 2002). In the soil, N<sub>2</sub>O is released from the processes of nitrification and denitrification (Firestone and Davidson, 1989; Mosier et al., 1998; Di et al., 2014). Nitrification not only produces N<sub>2</sub>O as a by-product, it provides the substrate, NO<sub>3</sub><sup>-</sup>, for denitrification to occur which also produces N<sub>2</sub>O as a product of incomplete denitrification. The first and rate-limiting step of the nitrification process, the oxidation of ammonia is therefore a critical step in the soil that is directly related to N<sub>2</sub>O emissions. Ammonia oxidation is carried out by ammonia oxidising bacteria (AOB) and/or ammonia oxidising archaea (AOA), depending on soil properties. It has been shown that AOB may play a dominant role in soils with high N status, such as in the soil under a dairy cattle urine patch in dairy pasture soils (Di

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et al., 2009), whereas AOA may be more abundant and probably play an important role in soils with low pH and low fertility (Nicol et al., 2008; Di et al., 2010b; Zhang et al., 2011). Soils under different land uses are likely to differ in major soil properties, such as pH, soil N status and other soil characteristics. These differences in soil conditions may affect the population abundance and growth of AOB and AOA in the soil under different land uses. In addition, when land use changes, such as when plantation forestry and sheep farming are converted to dairy farming, as has been common practice in the past decade in New Zealand, a number of conditions will change, including vegetation, fertiliser use, irrigation and, importantly, deposition of animal urine. This is also likely to lead to changes in AOB and AOA population abundance and N<sub>2</sub>O emissions. However, our understanding of how different land uses and land use change may affect the population abundance and N<sub>2</sub>O emissions is limited and there is an urgent need for further research to bridge this knowledge gap so that sound management strategies may be developed to mitigate N<sub>2</sub>O emissions. The objectives of this study were to: (1) determine the AOB and AOA population abundance under three different land uses (pine tree, sheep farming and dairy farming), located in close proximity on the same soil type; and (2) determine the impact on ammonia oxidiser population growth and N<sub>2</sub>O emissions from the three soils when they are treated with dairy cow urine. It was hypothesised that the dairy farm soil would have a higher AOB population abundance whereas the pine tree soil would have a higher AOA abundance compared with the other soils, and that N<sub>2</sub>O emissions would be the highest from the dairy farm soil than from the sheep farm and the pine tree soils when urine was applied.

## 2. Materials and methods

### 2.1. Field sampling

Soil samples were collected from three adjacent sites within one km of each other under three different land uses: 1) pine tree plantation (*Pinus radiata*); 2) sheep farming; and 3) dairy farming. The soil type at all three sites was a Templeton silt loam (NZ classification: Immature Pallic soil (Hewitt, 1998); USDA: Udic Haplustepts (Soil Survey Staff, 1998)). The three sites were located to the northwest of Lincoln University about 20 km south of Christchurch, in Canterbury, on the South Island of New Zealand. The coordinates of the selected sites were: dairy farm site (43°38'26.95"S, 172°26'37.85"E), sheep farm site (43°38'38.01"S, 172°27'25.36"E) and pine tree plantation (43°38'38.02"S, 172°27'29.01"E). The mean annual maximum and minimum temperatures in the area vary between 32 °C and 4 °C, respectively, with an annual rainfall of 666 mm. The sheep farm site and the pine tree site had been under the respective land use for more than 30 years. The dairy farm was converted from sheep farming about 12 years ago. The pastures on the sheep and dairy farms were a mixture of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). The clover in the pasture can fix about 100–200 kg N per annum from the atmosphere. In addition, the dairy farm also received around 200 kg N ha<sup>-1</sup> per annum in the form of urea. Both the dairy farm and sheep farm also received N returns in the forms of dung and urine from the grazing dairy cows or sheep. No N fertiliser was applied to the pine tree soil. Other nutrients (mainly P, S, Mg and lime) were also applied from time to time to the dairy and sheep farms as required based on soil tests, but no fertilisers were applied to the pine plantation. The dairy farm was irrigated with a central pivot irrigator during the summer when there was a water deficit. No irrigation was applied to the sheep farm or the pine plantation. The basic properties of the soils are shown in Table 1.

**Table 1**

Soil characteristics of the different land uses used in the study.

Soil properties	Pine tree soil	Sheep farm soil	Dairy farm soil
Soil pH	5.3	6.2	6.1
Organic matter (g kg <sup>-1</sup> )	70.0	49.0	64.0
Total N (g kg <sup>-1</sup> )	2.2	2.5	3.3
Total C (g kg <sup>-1</sup> )	40.4	28.4	36.9
C:N ratio	18	11	11
Ammonium-N (mg kg <sup>-1</sup> )	2.29	3.54	1.23
Nitrate-N (mg kg <sup>-1</sup> )	0	0.79	4.89
Olsen P (µg g <sup>-1</sup> )	17.0	15.0	29.0
Sulphate-S (µg g <sup>-1</sup> )	32.0	2.0	5.0
CEC (cmol <sub>c</sub> kg <sup>-1</sup> )	19.0	13.0	18.0
Exch. K <sup>+</sup> (cmol <sub>c</sub> kg <sup>-1</sup> )	0.37	0.56	0.36
Exch. Ca <sup>2+</sup> (cmol <sub>c</sub> kg <sup>-1</sup> )	5.9	8.1	9.7
Exch. Mg <sup>2+</sup> (cmol <sub>c</sub> kg <sup>-1</sup> )	3.29	0.77	1.69
Exch. Na <sup>+</sup> (cmol <sub>c</sub> kg <sup>-1</sup> )	1.43	0.11	0.19

To determine the AOB and AOA abundance in the soils of the three land use types, five soil samples were collected from 0 to 10 cm depth from each land use type. Each sample consisted of approximately 10 soil cores randomly sampled from one paddock and bulked together. Urine patches were avoided when collecting soil samples from the dairy and sheep sites. Soil samples from the tree area were collected in close proximity (3–5 m) to the pine trees. Subsamples were taken for molecular-biology analysis (see Section 2.5), while the rest of the soil samples were kept refrigerated (4–6 °C) overnight for other soil analyses. In addition, approximately 30 kg of composite soil samples were collected from the same locations as the five samples from each site and were sieved, mixed (5 mm screen) and used for an incubation study as described below.

### 2.2. Incubation study

An incubation study was carried out to determine the growth of AOB and AOA and N<sub>2</sub>O emissions when the pine tree soil, sheep farm soil and dairy farm soil was treated with dairy cow urine.

The treatments included three soils (pine tree plantation, dairy farm and sheep farm), two urine rates (zero urine (Control) and Urine at 700 kg urine-N ha<sup>-1</sup>). Each treatment had four replicates. For each replicate, there were two sets of samples, one set for sampling and analysis of N<sub>2</sub>O emissions, and the other set for subsampling to determine ammonia oxidiser populations and concentrations of ammonium and nitrate in the soil. The soil in the jars (for gas sampling) and pots (for soil sampling) was packed to a bulk density of 1.0 g cm<sup>-3</sup>. A 700 g soil sample was packed to the half way height in gas sampling jars for N<sub>2</sub>O sampling and 600 g of soil was packed into soil sampling pots for subsequent subsampling. The volume of air above the soil surface in each gas jar was 350 mL. Each incubation jar or pot was sealed with a lid with two ventilation holes of 1 cm diameter. Both sets of vessels were randomly placed inside an incubator set at 20 °C. Over the duration of the experiment the soil moisture was maintained at field capacity (about 40% WFPS) by adding additional water to the incubation vessels twice a week based on the weight changes.

Urine was collected from dairy cows from the Lincoln University Research Dairy Farm (LURDF), analysed for N concentration and the right volume (70 mL for the gas sampling jars and 60 mL for the soil sampling pots) was applied to the surface of gas sampling jars and pots. The same volume of water was applied to the Control.

### 2.3. Nitrous oxide measurement

The method for N<sub>2</sub>O sampling is similar to that of Hutchinson and Mosier (1981) as adapted by Di et al. (2014).

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