



# Can pH amendments in grazed pastures help reduce N<sub>2</sub>O emissions from denitrification? – The effects of liming and urine addition on the completion of denitrification in fluvial and volcanic soils

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## ABSTRACT

Soil pH plays a critical role in determining the overall rate of several important processes in the agricultural nitrogen cycle. During denitrification, the activity of nitrous oxide reductase (N<sub>2</sub>O-R) is reduced at low pH. This effect has led to suggestions that soil pH adjustment via liming to enhance the activity of this enzyme might be a viable agricultural greenhouse gas mitigation strategy by enhancing the reduction of N<sub>2</sub>O in the soil to climatically inert N<sub>2</sub>. We assessed the effect of liming on the apparent activity of N<sub>2</sub>O-R by measuring the denitrification end products, N<sub>2</sub>O and N<sub>2</sub>, in a series of short-term anaerobic incubations. We compared a weakly-buffered fluvial soil and a well-buffered, volcanic soil under different incubation temperatures and in the presence or absence of a ~600 kg ha<sup>-1</sup> cow urine-N amendment. Our results indicated that the liming effect was heavily modulated by soil type, temperature, and urine amendment. Liming (at rates of 1.5 and 3.0 t ha<sup>-1</sup> for the volcanic soil and at rates of 5 and 10 t ha<sup>-1</sup> for the fluvial soil) caused pH increases of between 0.43 and 1.25 pH units. The highest reductions in N<sub>2</sub>O in the fluvial soil occurred when the 1.5 t ha<sup>-1</sup> rate was used in the fluvial soils under urine addition and at the higher temperature. The combined flux of N<sub>2</sub>O + N<sub>2</sub> did not change with liming. However, an interaction of soil type and urine amendment caused large differences in the partitioning of the denitrification end-products between N<sub>2</sub>O and N<sub>2</sub> – an effect that overwhelmed the relatively modest effects of liming. When the soils were amended with urine-N, the resulting denitrification gases from the volcanic soil were mostly in the form of N<sub>2</sub>O (60–77%), whereas in the fluvial soil the denitrification products were mostly in the form of N<sub>2</sub> and a much smaller portion were in the form of N<sub>2</sub>O (11–45%). Nevertheless, we found liming-induced enhancements of N<sub>2</sub>O-R of 15–20% ( $P < 0.05$ ) in urine-amended, fluvial soil. We suggest some possible mechanisms that would explain such large differences in the N<sub>2</sub>O/(N<sub>2</sub>O + N<sub>2</sub>) product ratio.

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## 1. Introduction

In intensively grazed livestock farming animal excreta in the form of dung and urine comprise a major part of soil's nitrogen (N) input (de Klein et al., 2003), in quantities normally in excess of what is assimilated by plants. The resulting surplus N is subject

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to loss via three major mechanisms: volatilisation, leaching, and denitrification (Ball et al., 1979; Bolan et al., 2004; Monaghan et al., 2007). Nitrous oxide (N<sub>2</sub>O) emitted from pastoral soils in New Zealand is most likely to be derived from denitrification, rather than nitrification (Saggar et al., 2009). Research directed towards mitigating N<sub>2</sub>O emissions from pastoral agriculture has mainly focused on inhibiting nitrification (Monaghan et al., 2013), with the joint aim of both reducing emissions of N<sub>2</sub>O from nitrification and minimising the production of nitrate, the initial substrate for denitrification. However, nitrification inhibitors are not 100% effective especially when soil temperatures

are high (Kelliher et al., 2008), and moreover, their commercial use has been discontinued due to export sensitivities associated with the detection of dicyandiamide traces in New Zealand dairy exports (Archer, 2013). It is therefore worthwhile to investigate other approaches for N<sub>2</sub>O mitigation, including methods to minimise the N<sub>2</sub>O emissions from denitrification.

The reduced denitrifying activity of soils under low pH has been understood for several decades, but it is not clear that a universally optimal pH exists for denitrification (Simek and Cooper, 2002). The advent of the acetylene inhibition technique has allowed estimation of the relative production rates of the denitrification end-products, N<sub>2</sub>O and N<sub>2</sub>, and there is now a general acceptance that the product ratio, defined here as N<sub>2</sub>O/(N<sub>2</sub>O + N<sub>2</sub>), is also larger under acidic conditions (Liu et al., 2010; Saggar et al., 2013). In the highly fertilised soils of China, pH has been identified as the master variable controlling the product ratio (Qu et al., 2014). The increased amounts of fertiliser-N usage and animal urine deposition associated with agricultural intensification leads to an acidification of soil and pH levels that are increasingly suboptimal for N<sub>2</sub>O-R activity.

The greater pH sensitivity of N<sub>2</sub>O-R, relative to other denitrifying reductase enzymes, has previously thought to have been because the enzyme is located in the periplasm of the cell – a poorly buffered region in Gram-negative bacteria (Simek et al., 2002). Recent work has indicated that it is the translation or assembly of N<sub>2</sub>O-R, rather than transcriptions rates of the encoding gene, which is sensitive to pH (Liu et al., 2010). However, the effect of pH on soil microbial ecology is multifarious. Fungal communities tend to predominate at lower pH and denitrifying fungi have been reported to be responsible for an increasing portion of the N<sub>2</sub>O flux as soil becomes more acidic (Chen et al., 2014). Since fungi are not known to synthesise N<sub>2</sub>O-R, this may be an additional reason for elevated N<sub>2</sub>O fluxes at low pH.

The possibility that pH-manipulation of soils through liming is a viable N<sub>2</sub>O mitigation strategy has been raised several times previously (Clough et al., 2003; Zaman et al., 2007; Galbally et al., 2010; Bakken et al., 2012), but its effect on the total flux of N<sub>2</sub>O from the soil is not clear. Both nitrification and denitrification can be enhanced at higher pH (Simek and Cooper, 2002) and therefore the mitigation benefit of reducing the N<sub>2</sub>O/(N<sub>2</sub>O + N<sub>2</sub>) product ratio must be weighed against the increased bulk N<sub>2</sub>O emissions that might result from a stimulation of either of these processes. Additionally, since the practice of liming is a known source of carbon dioxide (CO<sub>2</sub>) emissions (De Klein et al., 2006), this must ultimately be incorporated into the cost-benefit of lime as a mitigation strategy.

The influence of liming on N<sub>2</sub>O production and consumption from denitrification is likely to vary according to soil temperature, the level and type of N input, and the buffering capacity of the soil. Temperature affects the various N transformations in a differential manner. Quite different Q<sub>10</sub> values (i.e. the net change in gas production per 10 °C increase in temperature) for N<sub>2</sub>O production compared with N<sub>2</sub>O consumption have been reported recently (Phillips et al., 2014).

The extent to which liming changes N<sub>2</sub>O losses will vary according to soil type. For example, volcanic soils with high allophane content have a high buffering capacity and require more lime to produce a given pH increase compared with poorly buffered, fluvial soils.

In intensively grazed pasture soils, the majority of the N<sub>2</sub>O emitted is derived from the N deposited as animal urine that causes very high local N inputs of typically ~600 kg ha<sup>-1</sup> (Selbie et al., 2015) in patches that cover 2–5% of area after each grazing (Moir et al., 2011). In addition to the marked increase in N content, urine deposition causes large changes to the physical and chemical

properties of the affected soil including a marked but temporary elevation in pH due to urea hydrolysis (of up to 3–5 days), increases in the soil moisture content and increases in the available carbon (C) (Selbie et al., 2015). Therefore, urine presence is likely to heavily modulate a soil's response to lime. We might expect this effect to be particularly pronounced in volcanic soils due to the highly pH-dependent nature of their cation exchange capacity (Holland and During, 1977).

Here, we investigated how liming influences N<sub>2</sub> and N<sub>2</sub>O emissions from a volcanic and a fluvial soil under anaerobic conditions, and compare these effects at different temperatures, with and without an amendment of dairy cow urine. Our hypotheses were:

1. The liming-induced pH increases will lower the N<sub>2</sub>O production;
2. The liming-induced pH increases will increase the overall rate of the denitrification end products, N<sub>2</sub>O and N<sub>2</sub>;
3. The liming-induced pH increases will lower the product ratio (irrespective of the changes in the bulk production rates of N<sub>2</sub>O or N<sub>2</sub>);
4. This liming effect on N<sub>2</sub>O and N<sub>2</sub> production will be modulated by soil type, incubation temperature and the presence of urine.

Thus to test these hypotheses we investigated the response of denitrifiers to interacting effects of temperature, soil and urine-amendment; second, utilizing a methodology that achieves simultaneous, automated and direct N<sub>2</sub>O and N<sub>2</sub> measurements, incorporating an extended pH adjustment period to allow the soils to equilibrate to the liming treatments.

## 2. Methods and materials

### 2.1. Soils

The soils from the permanent pastures of two farms were used in this study. One soil was a weakly-buffered, non-allophanic fluvial sandy loam from a farm near Massey University, Palmerston North (40° 22' 60" S, 175° 36' 37" E) while the other was a strongly buffered, allophanic volcanic silt loam from a farm near Hamilton, New Zealand (40° 46' 40" S, 175° 18' 46" E). The fluvial soil is classified as a weathered fluvial recent soil in the New Zealand Soil Classification System (Hewitt, 1992). It is a well-drained soil derived from slowly accumulating alluvium and has low allophane content (Cowie and Rijkse, 1977), and is hereafter referred to as the fluvial soil. The volcanic soil is classified as a Typic Orthic Allophanic soil. It is a well-drained soil derived from largely volcanic alluvium and has high allophane content (Singleton, 1991), and is hereafter referred to as the volcanic soil. Relevant properties of each of these soils are given in Table 1.

Field fresh soil samples (0–100 mm depth) were collected by taking multiple replicate cores from randomly assigned locations in each farm. A total quantity of 10–12 kg was collected from each farm, and the soil was bulked, sieved to 4 mm, and stored at 4 °C.

**Table 1**  
Relevant properties of soil used in this study.

| Property   | Soil       |                   |
|--|------------|-------------------|
|  | Fluvial    | Volcanic          |
| Soil texture class   | Sandy loam | Silt loam         |
| Cation exchange capacity (cmol <sub>c</sub> kg <sup>-1</sup> ) | 13.0       | 35.6 <sup>a</sup> |
| Organic C content (mg g <sup>-1</sup> )                        | 44.6       | 62.7              |
| Bulk density (g cm <sup>-3</sup> )                             | 1.06       | 1.10              |

<sup>a</sup> Volcanic CEC values from Srinivasan et al. (2013).

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