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Influence of soil bulk density and matric potential on microbial dynamics, inorganic N transformations, N_2O and N_2 fluxes following urea deposition

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ABSTRACT

Transformation of ruminant urine-nitrogen (N) can contribute to negative environmental outcomes such as nitrate leaching which leads to eutrophication of waterways and production of nitrous oxide (N_2O), a greenhouse gas. Although abiotic factors influencing urine-N processing have been well studied, detailed studies of the soil microbial community dynamics following urine application are required to improve mitigation strategies for reducing harmful N fluxes from urine deposition. A factorial laboratory experiment using packed silt-loam soil cores with two levels each of urea (±), soil matric potential (ψ −6.0 or −0.2 kPa) and soil bulk density (ρ_b 1.1 or 1.5 g cm^{-3}) was performed to study the interaction of urea and soil physical conditions on both soil inorganic N transformations and the abundance of ammonia-oxidizers and denitrifiers. Soil ψ and ρ_b treatments had an immediate impact on soil pH, dissolved organic carbon, inorganic N pools and emissions of N_2O and N_2 following urea deposition, and microorganisms carrying the *nosZ* gene were present in lower numbers in the most aerobic soil (−6.0 kPa and 1.1 g cm^{-3}) from day 7. In all treatments, both bacterial *amoA* and denitrifier *nirS*, *nirK* and *nosZ* gene copy numbers increased within 1 day following urea application. Dynamics in the NH_4^+ concentrations were significantly correlated with cumulative changes in the abundance of the ammonia-oxidizers, but no relation was found between cumulative changes in the denitrifier *nirS*, *nirK* and *nosZ* gene copy numbers and the dynamics in soil inorganic N, N_2O or N_2 emissions. Throughout most of the study period the specific soil conditions, induced by the ψ and ρ_b treatments, determined nitrifier and denitrifier activity rather than the size of the microbial communities involved. However, by day 35 soil ψ and ρ_b treatments exerted large treatment effects on bacterial *amoA*, *nirS* and *nirK* gene copy numbers. Thus, although nitrate concentrations and N_2O emissions following urea deposition were determined by the soil ψ and ρ_b conditions in the short-term, our results indicate that changes in the population sizes of denitrifiers and AOB in ruminant urine patches may influence environmental N fluxes in the long-term.

1. Introduction

Intensification of pastoral agriculture has resulted in increased stocking rates, fertiliser inputs and irrigation. Intensively grazed pastures typically receive large nitrogen (N) inputs through deposition of urine by grazing animals and application of N fertilizer. Up to 70% of the N consumed by grazing animals is excreted as urine (Haynes and Williams, 1993) with bovine urine-N deposition rates ranging from 600 to 1200 kg N ha^{-1} (Haynes and Williams, 1993). This deposited N greatly exceeds the level that can be assimilated by pasture plants. Therefore, a significant fraction of the urine-N is lost from pasture soil, via nitrate (NO_3^-) leaching and the emissions of gaseous N compounds (ammonia, NH_3; nitric oxide, NO; nitrous oxide, N_2O or dinitrogen, N_2), resulting in economic and environmental issues (e.g. Ledgard et al., 1999).

Typically over 70% of the N in ruminant urine is present as urea (Doak, 1952). When deposited on soil, urea is rapidly hydrolysed to form ammonium (NH_4^+), bicarbonate and hydroxide ions,

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resulting in an increase in the soil pH of up to 3 units within one day of urine application (Haynes and Williams, 1993). After urine deposition the elevated soil pH drives the equilibrium between NH_4^+ and NH_3 towards the formation of NH_3, and between 4 and 44% of the applied urine-N may be lost due to volatilization of NH_3 (Bussink and Oenema, 1998). Ammonia is an atmospheric pollutant that leads to the formation of harmful NH_4^+ containing particulates and aerosols (Forster et al., 2007). In addition, emitted NH_3 is ultimately re-deposited onto land or water and therefore contributes to indirect N_2O emissions (Mosier et al., 1998), acidification of water and biodiversity loss (Beusen et al., 2008). The loss of NH_3 and nitrification of the remaining NH_4^+ in the soil to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), and further to NO_3^- by nitrite-oxidizing bacteria leads to a consecutive decrease in pH over a period of approximately 2–4 weeks (Haynes and Williams, 1993). Subsequently, NO_3^- may be assimilated by pasture plants, leached from the soil into the groundwater, potentially causing eutrophication of rivers and lakes and contamination of drinking water (Galloway et al., 2003), or under anoxic soil conditions it may be reduced by denitrifying bacteria to NO_2^-, NO, N_2O and N_2 (Zumft, 1997). Nitrous oxide is a potent greenhouse gas (Forster et al., 2007) and is recognized as the most significant anthropogenic ozone-depleting emission (Ravishankara et al., 2009). Denitrifiers produce N_2O as an obligate intermediate in the production of N_2 (Zumft, 1997), whereas nitrifying bacteria produce N_2O as a by-product of NH_3 oxidation or as an intermediate during the reduction of NO_2^- to N_2 during nitrifier denitrification (Wrage et al., 2001).

Rates of nitrification and denitrification in ruminant urine patches depend on soil and environmental conditions (Haynes and Williams, 1993). Previous studies examining the transformations of inorganic N and related N_2O emissions following urine deposition have mainly focused on soil chemical and physical effects. For example, urine-N processing has been shown to be affected by soil moisture (e.g. Clough et al., 2004; van Groenigen et al., 2005a), compaction (e.g. van Groenigen et al., 2005a; van Groenigen et al., 2005b; Uchida et al., 2008), soil pH (e.g. Clough et al., 2004), aggregate size (e.g. Uchida et al., 2008) and temperature (e.g. Uchida et al., 2011). However, information relating to the associated changes in the soil microbial community during ruminant urine-N transformation in pasture soils remains sparse. Changes in the community composition of both AOB and denitrifying bacteria have been reported following urine application (Mahmood and Prosser, 2006; Orwin et al., 2010). Furthermore, increases in the population size of AOB, but not AOA, have been observed following urine deposition (Di et al., 2009, 2010; O'Callaghan et al., 2010). However, the response of denitrifying bacteria to urine deposition is relatively unknown. Wakelin et al. (2013) found that denitrifier *nirS* gene copies were largely unaffected by urine application, but increased in abundance with soil temperature and are therefore likely to be generally related to elevated soil N mineralisation processes. The copies of denitrifier *nosZ* genes, however, were highly responsive to addition of urine, and thus the overall ratio of *nir*S to *nos*Z copies was affected (Wakelin et al., 2013). Given the different physiological requirements of nitrifying and denitrifying organisms, their response to urine deposition may differ. In order to develop and fully understand the implications of mitigation options for reducing environmentally harmful N fluxes from urine deposition, detailed studies of the soil microbial community dynamics following urine application under various soil conditions are required.

Thus, the objectives of this study were (i) to monitor both the inorganic N transformations and the abundance of AOB, AOA and denitrifying bacteria for 35 days following urea application to soils maintained at two levels of soil matric potential and two compaction levels, and (ii) to relate changes in the size of the microbial communities to the observed dynamics in soil inorganic N pools, N_2O and N_2 fluxes. The AOB and AOA populations were monitored using their respective *amoA* genes encoding a subunit of the ammonia monooxygenase gene as molecular markers. For the denitrifying bacteria, *nirS*-, *nirK*- and *nosZ*-type genes encoding the cytochrome cd_1 heme nitrite reductase, copper-nitrite-reductase, and N_2O reductase, respectively, were used as molecular markers.

2. Materials and methods

2.1. Experimental design and set-up

A Templeton silt-loam soil (Immature Pallic Soil; Hewitt, 1998), under pasture, was collected from Lincoln University, Canterbury, New Zealand to a depth of 15 cm. The soil was air-dried prior to sieving to ≤2 mm and packed into stainless steel cylinders (7.3 cm inner diameter by 4.1 cm depth). A factorial experiment was performed with two levels of N (±urea), two levels of soil water tension (ψ; −0.2 and −6.0 kPa), and two levels of soil bulk density (ρ_b; 1.1 and 1.5 g cm^{-3}), across 5 destructive soil sampling times (1, 7, 14, 24, and 35 days). Four replicate cores per treatment were set up for each of the sampling days, resulting in 160 soil cores. Minus urea cores received deionised water (DIW) while plus urea cores received a urea solution (see below). Urea was used in preference to collecting urine so that the urea-N could be highly enriched in ^{15}N, thus enabling N_2 flux determinations.

To obtain a uniform bulk density during packing, soil was compressed uniaxially into the cores. The bottom of the soil cores was covered with a nylon mesh (0.1 mm) to prevent soil loss. Cores were packed with soil that was wetted up with DIW to a moisture content that allowed for the subsequent addition of 30 ml of the urea solution (or DIW for the control cores), without drainage occurring, to bring the soil moisture to the required water-filled pore space (WFPS; Table 1) when the cores were placed on tension tables. The required WFPS was calculated assuming a particle density of 2.65 g cm^{-3}. Wetting up of soil occurred 5 h prior to urea solution addition and was performed at room temperature. The N rate of the urea solution simulated a typical ruminant urine-N deposition rate onto pasture and was applied at the conservative rate of 700 kg N ha^{-1} by pipetting the solution (10 g N l^{-1}) onto the soil surface. Only the soil cores to be destructively sampled at day 35 received the ^{15}N enriched urea solution (40 atom % ^{15}N, Cambridge Isotope laboratories, Inc. MA., USA), thus enabling the contribution of urea-^{15}N to the N_2O and N_2 fluxes (see below) to be determined over the 35 day experimental period. The room temperature ranged from 23 to 25 °C during the experiment.

Soil cores were thoroughly homogenized before destructive sampling. The homogenized soil was subsampled for inorganic N content (10 g), dissolved organic carbon (DOC; 5 g) and gravimetric water content (θ_g; 10 g). On each of the five sampling days, two

Table 1
Physical characteristics of the soil matric potential (ψ) and bulk density (ρ_b) treatments following the addition of urea or DIW, where θ_v, ε, and Φ are soil volumetric moisture, air-filled porosity, and total porosity, respectively. Values are the means and standard deviations of 4 replicates per treatment.

Treatment	WFPS (%)	θ_v (cm^{-3} cm^{-3})	ε (cm^{-3} cm^{-3})	Φ (cm^{-3} cm^{-3})
ψ −0.2 kPa ρ_b 1.1 g cm^{-3}	98 ± 1	0.57 ± 0.08	0.001 ± 0.000	0.58 ± 0.00
ψ −0.2 kPa ρ_b 1.5 g cm^{-3}	99 ± 1	0.43 ± 0.01	0.000 ± 0.000	0.43 ± 0.00
ψ −6.0 kPa ρ_b 1.1 g cm^{-3}	54 ± 1	0.32 ± 0.05	0.270 ± 0.050	0.58 ± 0.00
ψ −6.0 kPa ρ_b 1.5 g cm^{-3}	90 ± 2	0.38 ± 0.02	0.060 ± 0.020	0.43 ± 0.00