Hippuric acid effect on N_2O emissions from cow urine patches at a range of soil temperatures

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Abstract
Urine patches deposited by ruminant grazing animals are a source of the greenhouse gas nitrous oxide (N_2O). Reductions in emissions of N_2O have been measured following amendment of cow urine with additional hippuric acid, a natural constituent of the urine. Since the previous studies all used relatively high incubation temperatures, a range of temperatures were used in this study to test the potential effect of hippuric acid under differing conditions. Unlike the earlier studies, there was no inhibition of N_2O emissions due to urinary hippuric acid content during this experiment. The incubation temperature, however, greatly affected N_2O emissions, with fluxes increasing with increasing temperature (13.36 mg N_2O-N at 20°C vs. 0.31 mg N_2O-N at 5°C). Increasing the urinary hippuric acid content also had no effect on the soil pH, nitrogen dynamics, or ammonia (NH_3) emissions. Higher emissions of NH_3 from the 5°C than from the 10°C treatment meant that total gaseous N losses were lowest from the 10°C soil. The lack of effect of hippuric acid in this experiment may have been due to the low soil moisture content allowing aerobic soil bacteria to degrade the acid, or may indicate that hippuric acid does not inhibit nitrification.

Key Words
Greenhouse gas emissions, nitrogen dynamics, ammonia emissions, nitrification, denitrification.

Introduction
Nitrous oxide (N_2O) is a potent greenhouse gas, which is produced in urine patches during the nitrification and denitrification of urinary-N. Globally, 40% of anthropogenic N_2O emissions are agriculturally derived. Hippuric acid (HA), a naturally occurring minor constituent of ruminant urine, was shown in previous studies (Bertram *et al.* 2009; Kool *et al.* 2006; van Groenigen *et al.* 2006) to reduce emissions of N_2O from synthetic and real cow urine applied to soil, when the hippuric acid content of the urine was increased. However, the previous experiments were all carried out at relatively high temperatures (16–21°C) which do not occur throughout the year in the field. Therefore this current experiment was instigated to investigate the inhibitory effect of hippuric acid at a range of temperatures representative of those occurring at the soil collection site at 10 cm soil depth.

Methods
Soil collection and urine amendment
Temuka silt loam soil (Gley soil, NZ Soil Classification (Hewitt 1998)) from 0-10 cm depth was sieved (< 4 mm) and packed in PVC tubes to a depth of 6 cm at a bulk density of 0.79 g/cm^3. Dairy cow urine was amended with urea, and the high hippuric acid treatment (HHA) received additional hippuric acid, while the low hippuric acid treatment (LHA) underwent no further amendment (Table 1). Each soil core had 15.0 mL of urine pipetted onto its soil surface.

Table 1. Concentrations of nitrogen and hippuric acid (HA) and HA content as a percentage of total urinary N in the low hippuric acid (LHA) and high hippuric acid (HHA) urine treatments.

Treatment	N concentration (g/L)	HA concentration (mmol/L)	HA content (% urinary N)	N application rate (kg N/ha)
LHA	10.0	35	4.9	764
HHA	10.6	75	9.9	810

Experimental design
The experiment was a 2 x 4 factorial design, with two urinary hippuric acid treatments (LHA and HHA, as detailed above) and four temperature treatments (5, 10, 15 and 20°C) to give a total of eight treatments. Four replicates of each treatment (a total of 32 cores) were destructively sampled on 5 sampling occasions, and the

set of soil cores used for the final destructive sampling was also used for gas sampling throughout the study. The soil moisture content was initially held at 0.45 g water/g soil to encourage nitrification, as an earlier study had shown evidence of hippuric acid inhibition of nitrification (Bertram *et al.* 2009). From day 17, the soil moisture content was altered to 0.55 g water/g soil to promote denitrification as no effect of hippuric acid had been observed by this time.

Soil, microbial and gas analyses
Sub-samples of soil were analysed for surface and bulk soil pH, and for ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations. The set of 32 cores used for gas sampling were placed into 0.5 L Mason jars on each sampling occasion to allow headspace sampling of soil emissions of N_2O, and ammonia (NH_3) emissions were measured at the same time using acid traps.

Results
In contrast to the results of the previous studies, there was no indication of inhibition of N_2O emissions due to increased levels of urinary HA over the course of the experiment (Figure 1a). There was, however, an increase in N_2O emissions with increasing incubation temperature (Figure 1b), with the highest flux of > 11000 µg N_2O-N/m^2/h measured in the 20°C treatment. These results were reflected in the concentrations of soil inorganic N, with NH_4^+ decreasing more rapidly and NO_3^- increasing more rapidly at higher temperatures, while being unaffected by HA concentration. Since pH in urine patches is linked to N dynamics, the measured pH values remained elevated for longer at the lower temperatures.

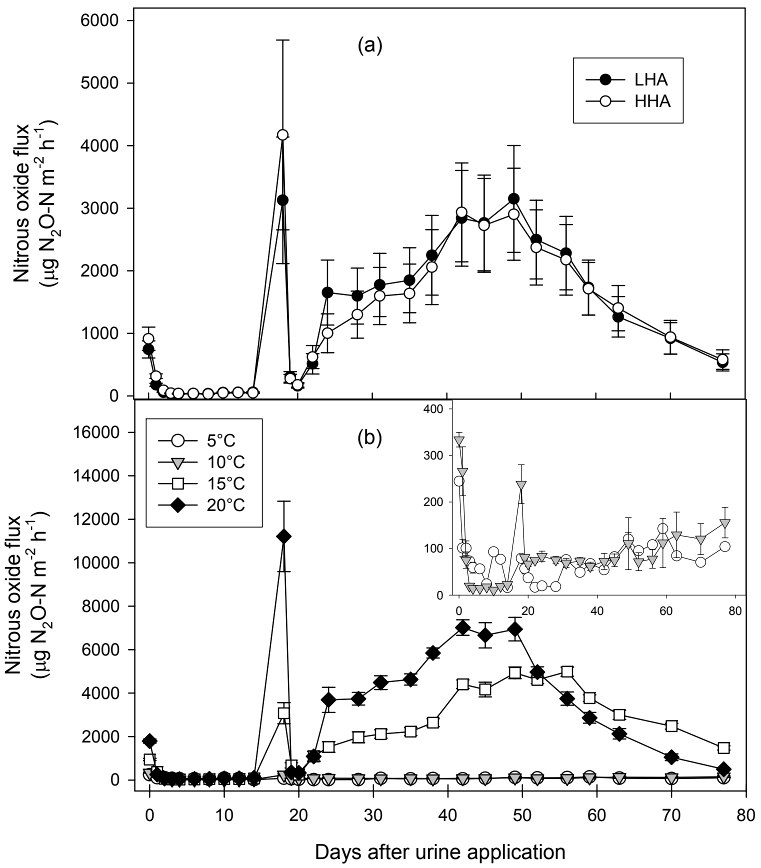

Figure 1. Nitrous oxide emissions measured from (a) hippuric acid treatments and (b) all temperature treatments, with (inset) 5°C and 10°C temperature treatments only (Error bars = SEM, n = 16 (a) or 8 (b)). NOTE: differing scales on y axes.

As seen previously (Bertram *et al.* 2009), there was no effect of HA amendment on NH_3 emissions, but an increase in incubation temperature reduced emissions of NH_3. Thus, overall, HA amendment did not affect gaseous N emissions from the applied urine, but an increase in incubation temperature caused emissions of nitrogenous GHGs to increase from < 12% of the total N applied in the 5 and 10°C treatments to > 17% of the total N applied in the 20°C treatment (Table 2).

Table 2. Cumulative emissions of N_2O-N and NH_3-N as total N emitted (mg) and percent N applied. Means followed by same letter in each column do not differ significantly.

Treatment	Mean cumulative N emitted per core					
	mg N			% total N applied		
Temperature	N_2O-N	NH_3-N	Total[#]	N_2O-N	NH_3-N	Total[#]
5°C	0.31 c	18.18 a	18.48 c	0.20 c	11.76 a	11.96 c
10°C	0.37 c	14.75 b	15.12 d	0.24 c	9.56 b	9.80 d
15°C	9.96 b	14.17 b	24.13 b	6.45 b	9.18 b	15.63 b
20°C	13.36 a	13.67 b	27.03 a	8.67 a	8.84 b	17.51 a
significance	***	***	***	***	***	***
Acid						
LHA	6.07	14.93	21.00	4.05	9.95	14.00
HHA	5.93	15.45	21.38	3.73	9.72	13.45
significance	NS	NS	NS	NS	NS	NS

*** $P < 0.001$, NS = not significant   [#]Sum of N_2O-N + NH_3-N

Conclusion

In this experiment, amendment of real cow urine with additional HA did not result in inhibition of N_2O emissions from soil. This was in contrast to the results of previous studies, which used similar differences between HA concentrations. Since, in this current experiment, the soil moisture content was lower than was the case in the earlier experiments, it is possible that the acid was degraded in the soil by aerobic bacteria capable of utilising the benzoic acid produced by hydrolysis of HA (Gescher *et al.* 2002; Philippe *et al.* 2001; Pumphrey and Madsen 2008). Alternatively, this experiment may indicate that HA does not affect nitrification, despite the indications seen in the previous study (Bertram *et al.* 2009). Increasing the soil temperature resulted in increases in total gaseous N emissions, due to large increases in N_2O emissions as the temperature increased. Nitrous oxide emissions in the 5 and 10°C treatments were very low, and enhanced NH_3 emissions from the 5°C treatment, probably due to the continued elevation of soil NH_4^+concentrations, meant that gaseous N emissions were higher from the 5°C soil than from the 10°C soil.

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