Increased hippuric acid content of urine can reduce soil N_2O fluxes

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Abstract

Urine patches in grazed pastures are a major source of nitrous oxide (N_2O) emission. It is well-documented that the relative concentration of the various nitrogenous urine constituents varies significantly with diet. The effect of these variations on N_2O emissions from urine patches, however, has never been reported. The aim of this study was to test whether variations in urine composition, consistent with different diets, lead to significant differences in N_2O emission. Four varieties of artificial urine, all with similar total N concentrations, but varying in the relative contribution of the nitrogenous constituents, were applied to undisturbed cores from a sandy pasture soil. N_2O fluxes were monitored for 65 days at two moisture treatments; 92% WFPS for the entire incubation, and 70% WFPS up to day 41 and 92% afterwards. Extra replicates were included for destructive analysis on mineral N concentrations and pH. Urine composition was a significant ($P < 0.001$) factor determining N_2O emissions. An increase in the relative hippuric acid concentration from 3 to 9% of total N resulted in a significant decline in average N_2O fluxes, from 16.4 to 8.7 µg N_2O–N h^{-1} kg^{-1} soil (averaged over all treatments). Cumulative emission decreased from 8.4 to 4.4% of the applied urine-N ($P < 0.01$). Soil mineral N showed a modest but significant decrease with an increase of hippuric acid content. pH did not show any significant relationship with urine composition. Increasing the urea concentration with 12% of applied urinary N did not significantly affect N_2O emissions. Moisture content significantly affected N_2O emissions ($P < 0.001$), but no interaction between moisture and urine composition was found. As the inhibitory effect of hippuric acid could not be linked directly to mineral N concentrations in the soil, we hypothesize that the breakdown product benzoic acid either inhibits denitrification or decreases the N_2O/N_2 ratio. We conclude that hippuric acid concentration in urine is an important factor influencing N_2O emission, with a potential for reducing emissions with 50%. We suggest alternative rationing leading to higher hippuric acid concentrations in urine as a possible strategy to mitigate N_2O emission from grazed pastures.

1. Introduction

Nitrous oxide (N_2O) contributes to global warming and can break down stratospheric ozone (Crutzen, 1981; Duxbury et al., 1993), thus anthropogenic N_2O emissions are of environmental concern. Grasslands are important and dominant ecosystems in the world, covering 17% of the world land surface (Steinfeld et al., 1997). Global N_2O emissions from pastures are estimated to be 635 Gg N_2O–N per year (Oenema et al., 2005). Within pastures, animal excreta can account for local increases in nitrogen of 20–80 g N m^{-2} in urine patches and 50–200 g N m^{-2} in dung patches (Oenema et al., 1997). This causes N_2O emissions from grazed grassland generally to be higher than from ungrazed grasslands (Smith et al., 1998).

Urine patches are a source of N_2O emission due to this local increase in nitrogen. When voided on the soil, urea, the major component of urine, is hydrolyzed to NH_4^+ within a few days, which may partly volatilize as NH_3 due to the concomitant increase in pH. Nitrification (i.e. oxidation of NH_4^+ to NO_3^-), and subsequent nitrifier denitrification and heterotrophic denitrification (i.e. stepwise reduction of NO_3^- to N_2O or N_2), are the dominant microbial processes responsible for N_2O formation (Wrage et al., 2001).

Generally, most of the nitrogen in urine is present in the form of urea (50–90% of the urine-N), but other significant nitrogenous compounds in cattle urine include allantoin (2.2–22.2%), hippuric acid (1.9–23.7%), uric acid (0.6–1.9%), creatine (0–6.3%) and creatinine (0–8.1%) (Doak, 1952; Bussink and Oenema, 1998).

Urine composition is largely controlled by diet. Van Vuuren and Smits (1997) reported that rations high in rumen protein surplus (1.0 kg RPS d^{-1}) resulted in relatively more urea (from 67 to 79% of total urine-N) and less allantoin (from 14 to

8% of total urine-N) in cattle urine, compared to urine of cattle fed on rations low in RPS (0.1 kg RPS d^{-1}). Reducing crude protein content in cattle diet (from 19 to 14%) resulted in reduced total N excretion and a reduced proportion of urea-N in urine-N (from 62 to 42%) (Misselbrook et al., 2005). Kreula et al. (1978) reported a highly significant variation of hippuric acid in cattle urine, varying between 0.2 and 10.7% due to different rations and additives such as benzoic acid and tyrosine. Martin (1970a, b) found for sheep a negative relationship between maturity of ryegrass in the diet and hippuric acid in urine, due to declining concentrations of aromatic acid precursors in the grass with aging.

Previous studies showed no effect of urine volume and total N content on N_2O emissions within the range of 2–8 L urine m^{-2} and 18–37 g N m^{-2} (Van Groenigen et al., 2005a, b), but it remains to be determined whether variation in nitrogenous composition of urine affects N_2O emissions. The study of Whitehead et al. (1989) provided indirect evidence that urine composition may affect N_2O emissions. They reported differences in NH_3 volatilization from different urine components and compositions. A urea solution resulted in significantly lower NH_3 emission rates than artificial urine containing urea, allantion, creatinine, creatine and hippuric acid. The presence of hippuric acid in addition to urea caused the higher NH_3 volatilization. Urea with hippuric acid (hippuric acid-N = 2.5% of urea-N) gave volatilization rates significantly closer to those of real cattle urine than urea alone. As most of the factors driving NH_3 volatilization also control N_2O formation (i.e. pH, NH_4^+ concentration, soil moisture content), these findings suggest that differences in urine composition may affect N_2O emissions.

A range of urine compositions have been used to examine N_2O emissions from urine patches, despite the previous references indicating that a urine composition effect may occur. Urine types used include both real and artificial urines, with nitrogenous composition of artificial urine (in addition to various salts and buffers) varying from a simple urea-solution (Petersen et al., 2004) or urea plus glycine (Fraser et al., 1994; De Klein et al., 2003; Clough et al., 2004; Muller and Sherlock, 2004) to a more complex composition with multiple nitrogenous compounds (Doak, 1952; De Klein and Van Logtestijn, 1994; Van Groenigen et al., 2005a).

The aim of this study was to demonstrate and quantify possible effects of differences in urine composition on N_2O emissions from soil. We initiated an incubation study on pasture soil, using four artificial urines with differences in nitrogenous compositions that were reflective of differences in diet. We focused on variations in urine composition that may affect the rate of urea hydrolysis, i.e. urea and hippuric acid concentrations (Whitehead et al., 1989). Different soil moisture regimes were used to mimic variable field conditions.

2. Material and methods

2.1. Experimental setup

Four synthetic urine-N treatments, containing 10.5 g N L^{-1}, and differing concentrations of urea, hippuric acid, allantion,

Table 1
Nitrogenous composition (g N L^{-1}) of the artificial urine types

	Reference	High hippuric acid	High urea	High hippuric acid, high urea
Urea	7.98 (76.0)	7.49 (71.3)	8.96 (85.3)	8.40 (80.0)
Hippuric acid	0.32 (3.0)	0.95 (9.0)	0.32 (3.0)	0.95 (9.0)
Allantion	1.68 (16.0)	1.58 (15.0)	0.93 (8.9)	0.88 (8.4)
Uric acid	0.11 (1.0)	0.09 (0.9)	0.07 (0.7)	0.07 (0.7)
Creatinine	0.42 (4.0)	0.40 (3.8)	0.22 (2.1)	0.21 (2.0)

Total urine-N for all urines equaled 10.5 g N L^{-1}. Between brackets is the relative N concentration as a percentage of total N.

uric acid and creatinine were used (Table 1). The composition of these treatments was based on previous work that showed differences in hippuric acid content with variations in diet (Martin, 1970a,b; Kreula et al., 1978) and on the effect of protein and its influence on urea content in urine (Van Vuuren and Smits, 1997). The amount of urine-N was kept constant in order to absolutely exclude the possibility of an N rate effect.

The effect of urine composition on N_2O emissions was measured at two different moisture treatments. The first moisture treatment was an almost saturated soil, with a water-filled pore space (WFPS) of approximately 92% after urine application, in order to optimize denitrification. In the second moisture treatment, urine was applied to a soil that was initially only moderately wet (70% WFPS after urine application), with soil moisture raised to 92% WFPS after 41 days, when ammonium concentrations had declined to background levels (this treatment will hereafter be referred to as 70/92% WFPS).

In addition to the urine treatments, a blank was included receiving neither urine nor water resulting in slightly lower moisture content (62% WFPS), reflecting differences between urinated and not-urinated sites in the field. All urine treatments received a 4 ml urine application (2 mm) containing 10.5 g N L^{-1} amounting to approximately 320 mg N kg^{-1} soil and the equivalent of 214 kg ha^{-1}. To all urine types 14 g L^{-1} KHCO$_3$, 10.5 g L^{-1} KCl, 0.4 g L^{-1} CaCl$_2$·2H$_2$O, 1.2 g L^{-1} MgCl·5H$_2$O and 3.7 g L^{-1} Na$_2$SO$_4$ was added. These salts were added to better mimic real urine resulting in a urine pH more close to that of real urine.

Soil samples were taken from a poor sandy soil under permanent grassland located at the experimental farm De Marke in the east of the Netherlands (3% clay, 12% silt, 85% sand; 5.9% organic matter; 2.0 g N kg^{-1} soil), classified as a cambic podzol (FAO-Unesco, 1988). Ten days before the start of the experiment, undisturbed ring samples (diameter 5 cm, height 5 cm) were taken from just below the sods, where bulk density averaged 1.35 g cm^{-3}. They were pre-incubated for 7 days, until the initial CO$_2$ flux had subsided. The water retention curve was determined, and soil moisture was adjusted to the indicated levels 3 days before application of the urine. The incubations were set up in a conditioned room in which temperature and relative humidity were kept constant at 16 °C and 60%, respectively. The saturated soils were placed in an aluminum tray filled with demineralized water (water level