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The effects of urine nitrogen application rate on nitrogen transformations in grassland soils

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Abstract

Urine is a critical nitrogen (N) input in temperate grazed grasslands and can drive substantial nitrous oxide (N_2O) production in soils. However, it remains unclear how differences in the N input rate affect N_2O fluxes and vary between different grassland soils. The effect of increasing urine N application on ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations and N_2O production was tested in two grassland soils, a free-draining loam and an imperfectly drained sandy-loam. It was hypothesized that high-urine N application rates would lead to ammonia/ammonium (NH_3/NH_4^+) accumulation influencing N transformation rates and N_2O production which differ between grassland soils. Fresh cattle urine was applied at rates equivalent to 300 and 1000 kg N/ha in an aerobic incubation experiment. Soils were destructively sampled over 80 days to measure changes in inorganic-N and pH. The higher N addition rate was associated with elevated NH_3 concentrations up to day 35 in soils, probably inhibiting NO_2^- to NO_3^- reduction. In contrast, there was no inhibition of nitrification in the 300 kg N/ha treatment. Cumulative N_2O fluxes were greatest from the 300 kg N/ha treatment for the loam soil, but were greater for the sandy-loam under the 1000 kg N/ha treatment. The results also show that differences in soil properties, in particular carbon availability, can be important in regulating N transformation and N_2O production. Collectively, these results demonstrate the proposed mechanism of nitrification inhibition at high-N input rates, driven by either high NH_3/NH_4 and/or increased levels of NH_4HCO_3 from urea hydrolysis.

Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) with a global warming potential 265–298 times greater than carbon dioxide (CO_2) (Wrage *et al.*, 2001). Atmospheric concentrations of N_2O have increased substantially since 1750 and now exceed pre-industrial levels by 0.20 (IPCC, 2013). Globally, agriculture is one of the most significant sources of N_2O production, accounting for ~0.6 of N_2O emissions in 2005 (Reay *et al.*, 2012). In the context of increased demand for animal products and the intensification of agricultural ecosystems, there is an urgent need to understand the N_2O mitigation potential of common agricultural practices.

In 2010, permanent grassland and meadows accounted for 0.92 of agricultural land use in Ireland, with the majority used as grazing for cows (CSO, 2016). The temperate climate supports a long grazing season for livestock (mainly cows, beef and sheep) which can run from February to November, with urine deposited throughout this period. Consequently, N_2O emissions account for 0.36 of Irish agricultural emissions, which in turn comprise one-third of national GHG emissions (Duffy *et al.*, 2014). Under the EU Climate and Energy Package, Ireland must reduce GHG emissions by 20% from 2013–2020, posing a considerable challenge to the agriculture sector (EPA, 2017).

The frequency of urine excretion for dairy cows is 8–12 times per day (Lantinga *et al.*, 1987), with an average urination volume of 1.5–3.5 litres, resulting in a total production of 12–42 litres of urine per day, with an estimated nitrogen (N) concentration of 6–15 g N/litre (Holmes, 1989). Cow urine can affect an area of 0.2–0.5 m^2 (Hayes and Williams, 1993), with deposition potentially affecting herbage growth beyond the immediate cover area due to lateral uptake by roots (Whitehead, 1986). The amount of urine deposited also depends on the stocking rate and grazing rotation, as overlapping can occur (Dennis *et al.*, 2011). Depending on the diet and grazing regime of the cow, up to 0.70 of N in urine will be present as urea, with the remainder consisting of peptides and amino acids (Haynes and Williams, 1993). Intensively farmed dairy cows excrete 0.75–0.90 of N consumed in their diet in the form of dung and urine (van Vuuren and Meijs, 1987; Whitehead, 1995). An increase in soil N of between 20–80 g N/m^2 can occur in urine patches (Oenema *et al.*, 1997).

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Following urine deposition in moist soils, the enzyme urease hydrolyses urea to produce ammonium (NH_4^+) (Jarvis and Pain, 1990). Under aerobic conditions, NH_4^+ is subsequently converted to nitrate (NO_3^-) via nitrite (NO_2^-). Through denitrification, NO_3^- can be canonically reduced to N_2O or N_2, a process that generally occurs in water saturated soils where oxygen is depleted and bacteria use NO_3^- as an electron acceptor (Bolan *et al.*, 2004). Hydrolysis of urea to NH_4^+ also results in a rapid increase in pH of up to 3 units per day (van Groenigen *et al.*, 2005) and subsequent nitrification of NH_4^+ to NO_3^- results in a decrease of pH over a period of ~2 weeks (Doak, 1952). In general, between 0.001–0.038 of urine N is emitted as N_2O (Oenema *et al.*, 1997). In addition to N loss through N_2O or N_2 emissions, 0.04–0.44 of applied urine N may be lost due to ammonia (NH_3) volatilization, which occurs at higher soil pH (Bussink and Oenema, 1998).

The balance of nitrification and denitrification is influenced by changes in N availability. Clough *et al.* (2003) found that a urine N concentration applied to soil at a rate of 1000 kg N/ha suppressed nitrification and proposed that inhibition was due to the accumulation of NH_3/NH_4^+ (Malhi and McGill, 1982). Inhibition has subsequently been reported in other studies of soil N dynamics (van Groenigen *et al.*, 2005), but the length of inhibition is variable (Baral *et al.*, 2014). Many studies do not observe inhibition of N_2O fluxes or N transformation under high N input rates as they investigate changes over longer time periods with less intensive sampling following the initial N input (de Klein *et al.*, 2014). The extent of inhibition is also likely to vary between soil types due to a combination of contrasting soil textures, moisture, pH, temperature, oxygen and nutrient availability driving different N transformation rates (Parker and Schimel, 2011).

The objectives of the current study were to explicitly test the extent to which high urine N application inhibits nitrification/denitrification over the medium term (up to 80 days) and assess if nitrification to NO_3^- is inhibited by high concentrations of NH_3/NH_4^+ and whether N losses are via NO_2^-. It was hypothesized that (i) high-urine N application rates would inhibit nitrification, resulting in reduced N_2O production, (ii) high-urine N application rates would be associated with high-accumulated NH_3/NH_4^+ and (iii) N transformation rates and N_2O production would differ between different grassland soils.

Methods

Study sites

The current study was conducted using two soils collected from grassland sites in Johnstown Castle (JC), Co. Wexford, Ireland (52°18′01.7″N 6°30′10.8″W), and Moorepark (MP), Fermoy, Co. Cork, Ireland (52°09′27.4″N 8°14′40.4″W) in April 2014 (Table 1). Two grassland top soils (0–10 cm) were collected using a spade cleaned with ethanol. Soils were stored overnight in sealed bags. The soils were air dried for 1 week, sieved to 2 mm and mixed separately to form two homogeneous samples. Subsequent references to soil mass are expressed on a dry mass equivalent basis.

Experimental design

To investigate the effects of urine N application rates on nitrification and denitrification, fresh cow urine was collected from a dairy herd at the Johnstown Castle farm and analysed for N content. Urine N concentration was subsequently adjusted by adding urea or diluting urine accordingly to produce two urine concentrations for application rates equivalent to 300 and 1000 kg N/ha. These concentrations represent low-moderate and high-urine N application rates (Krol *et al.*, 2016; Minet *et al.*, 2018). Deionized water was used as a control (0 kg N/ha).

Table 1. Initial site and soil properties of JC and MP soils for soils 0–10 cm

Site	JC	MP
Soil type	Loam	Sandy-loam to loam
Drainage	Imperfect	Free-draining
pH	5.02	5.33
Water holding capacity (cm^3/cm^3)	0.55	0.63
NH_4^+ (mg N/kg)	47.62	29.50
NO_2^- (mg N/kg)	0.01	0.01
NO_3^- (mg N/kg)	19.85	66.07
Organic matter content (g/kg)[a]	70.2	79.0
C (g/kg)[a]	28.3	30.2
N (g/kg)[a]	2.8	3.2
C:N[a]	10.11	9.44
30-year mean rainfall (mm)[a]	1060	1026

NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate; C, calcium; N, nitrogen.
[a]Additional site data from Harty *et al.* (2016).

Sieved soil (350 g) was placed into 24 replicate 1-litre acid-washed Kilner jars and packed to a depth of 4.5 cm. Soil was moistened with 59.68 and 89.88 ml of deionized water for JC and MP, respectively, and left for 5 days for acclimation. Then the cow urine treatments (47 ml for 1-litre jars and 9.54 ml for 0.1-litre jars) were pipetted across the soil surface ensuring equal distribution of urine which brought the moisture content to 0.7 cm^3/cm^3 water-filled pore space (WFPS). The urine application rate was equivalent to a moderate urine application volume of 6 litres/m^2. The final weight of each jar was recorded and for the duration of the experiment, soils were maintained at 70 cm^3/cm^3 WFPS through weighing the jars and adding deionized water to maintain the moisture content. Water-filled pore space was calculated using the approach of Haney and Haney (2010) and following Eqn (1):

$$\text{WFPS} = \frac{(\text{Soil water content} \times \text{Bulk denisty})}{(1 - (\text{Bulk denisty}/\text{Particle density}))} \quad (1)$$

Concurrently, to assess the effects of addition on soil NH_4^+, NO_2^- and NO_3^- concentrations, 35 g soil was placed into 226 replicate 0.1-litre jars for destructive sampling at each time point. Soils were maintained at the same WFPS as the 1-litre jars.

Jars were arranged in a randomized block design in a temperature-controlled growth chamber maintained at 15 °C and 70% relative humidity for 80 days. Jars were left open when not being sampled to maintain aerobic conditions. Soils were monitored regularly and maintained at the initial soil water content by spraying deionized water onto the surface, returning to initial weight. Water spraying was carried out after N_2O gas sampling to prevent rewetting affecting measured N_2O fluxes.

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Soil and gas sampling

Destructive soil sampling was carried out 1, 4, 7, 12, 15, 18, 21, 25, 28, 32, 35, 55, 63 and 80 days following urine addition to the 0.1-litre jars. Ammonium, total organic nitrogen (TON) and NO_2^- concentrations were determined following extraction with 185 ml 2 M potassium chloride (KCl; Mulvaney, 1996) via colorimetric analysis (Aquakem 600A). Nitrate was calculated as TON minus NO_2^- concentration. A sub-sample of the KCl extract was allowed to return to room temperature (20 °C) and analysed for pH (1:5 ratio). Ammonium concentrations and pH were subsequently used to calculate concentrations of free NH_3 (Watson *et al.*, 1994; Smith *et al.*, 1997):

$$[NH_3] = \frac{[NH_4^+]}{1 + 10^{(10{\cdot}068 - 0{\cdot}033T - pH)}}$$

where $[NH_3]$ is the free NH_3 concentration (mg N/kg), $[NH_4^+]$ is the NH_4^+ concentration (mg N/kg), T is temperature (15 °C) and pH was measured using KCl extracts.

Headspace N_2O concentrations were measured from the Kilner jars immediately after treatment and 3 h post-treatment on day 1, followed by daily sampling up to day 21 and thereafter ~5 days per week. On each sampling day, headspace gases were sampled between 14.00 and 17.00 h using a 20 ml syringe. The jars had a rubber seal on the lid ensuring an air tight seal. Headspace samples were collected immediately following lid closure and after 20 and 40 min through rubber septa. Two samples of air were measured to assess background N_2O concentrations. Headspace gases were mixed twice before collection of a 10 ml sample, which was injected at overpressure into helium flushed and pre-evacuated 7 ml glass vials (Labco Ltd, UK). N_2O concentration was analysed within 3 days using a Varian CP 3800 gas chromatograph (Agilent Inc., UK) equipped with a ^{63}Ni electron capture detector and Combi-Pal auto-sampler (CTC Analysis, Switzerland). Daily atmospheric pressure was also recorded for N_2O flux calculation. N_2O fluxes were calculated assuming the linear accumulation of headspace gases and according to the ideal gas law (Hogg *et al.*, 1992).

Statistical analysis

A two-way analysis of variance (ANOVA) was used to assess differences between the two grassland soils and the three treatments. Ammonium, NO_2^- and NO_3^- concentrations, and cumulative N_2O fluxes were $\log_{10}$ transformed to meet test assumptions. A *post-hoc* Bonferroni test was used to test differences between treatments for cumulative N_2O fluxes. Full ANOVA tables are presented in Tables 2 and 3. All statistical tests were carried out using Genstat v17.01. Figures were produced using GraphPad Prism v7.

Results

Soil properties

The JC soil was a loam of imperfect drainage with a pH of 5.02, a water holding capacity of 0.55 cm^3/cm^3 and an initial NH_4^+ concentration of 47.62 mg N/kg (Table 1). The MP soil was a free draining sandy-loam to loam texture with a pH of 5.33, a water holding capacity of 0.63 cm^3/cm^3 and an NH_4^+

Table 2. Results of two-way ANOVAs for pH, ammonium (NH_4^+), ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations

	n.d.f.	d.d.f.	*F* statistic	*P* value
pH				
Treatment	2	247	9713.23	<0.001
Soil type	1	247	120.21	<0.001
Time	13	247	1217.25	<0.001
Treatment × soil type	2	247	23.25	<0.001
Treatment × time	26	247	194.16	<0.001
Soil type × time	13	247	7.05	<0.001
Treatment × soil type × time	26	247	13.54	<0.001
log[NH_4^+]				
Treatment	2	247	3203.72	<0.001
Soil type	1	247	394.59	<0.001
Time	13	247	1091.75	<0.001
Treatment × soil type	2	247	140.54	<0.001
Treatment × time	26	247	77.29	<0.001
Soil type × time	13	247	20.62	<0.001
Treatment × soil type × time	26	247	29.64	<0.001
log[NH_3]				
Treatment	2	241	5030.07	<0.001
Soil type	1	241	296.49	<0.001
Time	13	241	1362.18	<0.001
Treatment × soil type	2	241	141.65	<0.001
Treatment × time	26	241	75.22	<0.001
Soil type × time	13	241	21.87	<0.001
Treatment × soil type × time	26	241	32.43	<0.001
log[NO_2^-]				
Treatment	2	247	985.73	<0.001
Soil type	1	247	2.1	0.198
Time	13	247	88.14	<0.001
Treatment × soil type	2	247	129.71	<0.001
Treatment × time	26	247	61.58	<0.001
Soil type × time	13	247	29.55	<0.001
Treatment × soil type × time	26	247	17.09	<0.001
log[NO_3^-]				
Treatment	2	247	3566.33	<0.001
Soil type	1	247	305.49	<0.001
Time	13	247	479.39	<0.001
Treatment × soil type	2	247	0.4	0.672
Treatment × time	26	247	183.93	<0.001
Soil type × time	13	247	7.92	<0.001
Treatment × soil type × time	26	247	6.93	<0.001

Significance was assessed at $P < 0.05$.

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Table 3. Results of two-way ANOVAs for cumulative N_2O production

Log[Cumulative N_2O flux]	n.d.f.	d.d.f.	*F* statistic	*P* value
Treatment	2	11	7.39	0.009
Soil type	2	6	30.12	0.002
Treatment × soil type	2	11	6.59	0.013

Significance was assessed at $P < 0.05$.

concentration of 29.5 mg N/kg. Both soils had low-nitrite concentrations ⩽0.01 mg N/kg. NO_3^- concentrations were greater in the MP soil compared to the JC soil.

Soil pH

Urine addition increased soil pH, with the magnitude of the change dependent on N concentration ($P < 0.001$, Fig. 1) and the 1000 kg N/ha addition associated with greatest increase. The pH varied significantly ($P < 0.001$) between soils, and was greater for MP soil (6.7) compared to JC soils (6.5). In addition, pH varied significantly over time ($P < 0.001$) and there was a significant ($P < 0.001$) difference in the interaction between treatments and time and between treatments, soils and time. On day 1, soil pH for both urine treatments and soils was similar with a mean pH of 7.5 and 7.6 for JC and MP urine treatments, respectively. For all treatments, pH peaked between day 4 and 7, followed by a steady and significant decrease over time. Between 55 and 80 days, differences in pH between treatments were minimal.

Soil inorganic nitrogen

Ammonium concentrations varied significantly over time following urine application ($P < 0.001$, Figs 2(*a*) and (*b*)), and there were significant differences between treatments ($P < 0.001$), soils ($P < 0.001$) and for all levels of interaction ($P < 0.001$). Ammonium concentrations peaked on day 4 for both soils and treatments, with 753 and 1568 mg/kg for JC soils subject to 300 and 1000 kg N/ha treatments, whilst MP concentrations were 554 and 1565 mg/kg for the 300 and 1000 kg N/ha treatments, respectively. For both soils, NH_4^+ concentrations increased from day 4 to 12 and then decreased gradually up to day 80. Ammonium concentrations for JC soils were consistently higher than the MP soil throughout the experiment.

Free NH_3 concentrations (Figs 2(*c*) and (*d*)) calculated from NH_4^+ concentrations and pH exhibited similar temporal trends, with significant changes over time ($P < 0.001$), between treatments ($P < 0.001$), soils ($P < 0.001$) and for all levels of interaction ($P < 0.001$).

For both grassland soils, NO_2^- concentrations varied significantly over time following urine application ($P < 0.001$, Figs 2(*e*) and (*f*)) and varied significantly between treatments ($P < 0.001$) and for all levels of interaction ($P < 0.001$) except for between soils ($P = 0.198$). In general, NO_2^- concentrations were greatest for the 300 kg N/ha treatment and in MP soils compared to JC soils. Nitrite concentrations increased rapidly for both soils following 300 kg N/ha treatment from day 4 to 21, with gradual declines up to day 32. For the JC soil 1000 kg N/ha treatment, NO_2^- concentrations remained low up to day 35 of the experiment compared to the control, but began to increase from day 35, reaching a maximum of 1.33 mg/kg. For the MP soil, concentrations initially peaked at 2.00 mg/kg on day 4, returning to 0.38 mg/kg on day 7.

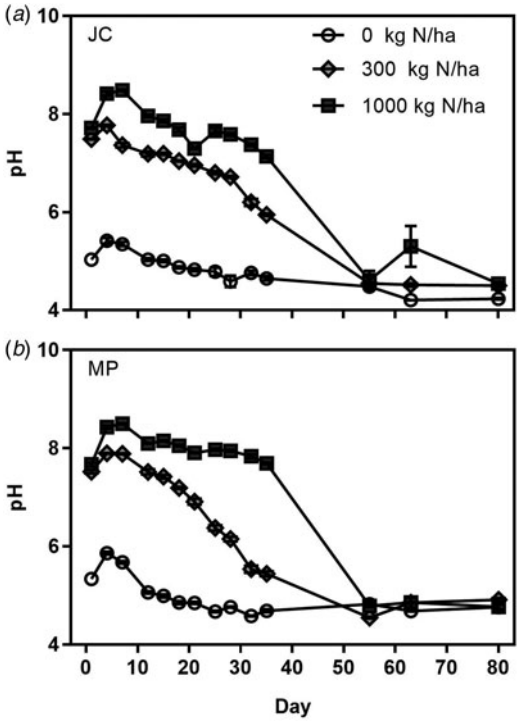

Fig. 1. The temporal effect of urine treatment on pH for (*a*) JC and (*b*) MP soils. Day 1 was sampled 1 h after urine treatment application. Means ± one s.e.. Note: error bars are included, but most are smaller than the symbols and therefore difficult to see.

Concentrations again peaked at 11.98 mg/kg on day 35 but declined to background concentrations by day 80.

For both grassland soils, NO_3^- concentrations also varied significantly over time ($P < 0.001$, Figs 2(*g*) and (*h*)), between treatments ($P < 0.001$), soils ($P < 0.001$) and for all levels of interaction ($P < 0.001$) except for between treatments and soils ($P = 0.672$). On day 1, NO_3^- concentrations in the controls were 19.85 and 66.07 mg/kg for JC and MP respectively, higher than both the 300 and 1000 kg N/ha treatments which were <13 mg/kg. The 1000 kg N/ha treatment remained low for the JC soil (<2.5 mg/kg) but increased to a peak of 324.35 mg/kg on day 55 before gradually decreasing. Nitrate concentrations for the MP soil 1000 kg N/ha treatment remained low (<1.75 mg/kg) until day 25 and then increased rapidly to a maximum of 467.80 mg/kg by day 55. The 300 kg N/ha treatment followed a similar pattern, with increases and peaks intermediate between the control and 1000 kg N/ha treatment. Nitrate concentrations were generally greater in MP soils compared to JC soils.

Nitrous oxide fluxes

N_2O fluxes increased for all treatments following urine addition, with an initial peak in fluxes occurring on day 2 (Fig. 3). This peak was most pronounced for 300 kg N/ha treatment, particularly for the MP soil. Fluxes remained low until day 16 when they reached a maximum of 300 kg N/ha for the JC soil. Fluxes from 1000 kg N/ha started to increase from day 16 for both treatments, with the most rapid change occurring in the MP soil, which was followed by a gradual decline. In the JC soil, fluxes continued to increase until day 35. Cumulative fluxes, calculated as the net N_2O emitted during 35 days incubation, varied significantly between treatments ($P = 0.002$, Fig. 4), soils ($P = 0.009$) and in the interaction term between

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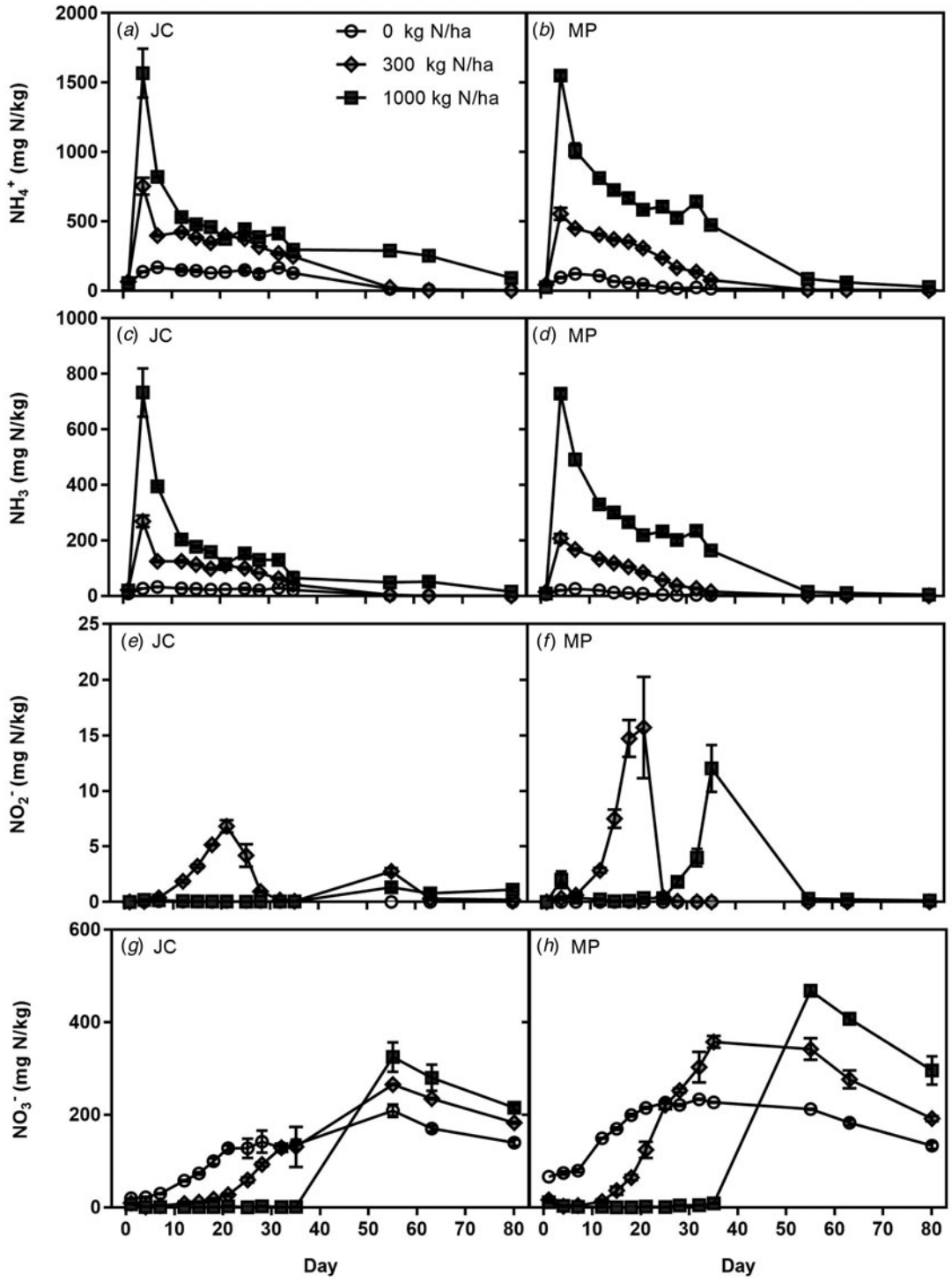

Fig. 2. Temporal trends in (*a, b*) ammonium (NH_4^+), (*c, d*) calculated ammonia (NH_3), (*e, f*) nitrite (NO_2^-) and (*g, h*) nitrate (NO_3^-) concentrations for JC and MP soils following 0, 300 and 1000 kg N/ha urine addition. Day 1 was sampled 1 h after urine treatment application. Means ± one S.E..

treatments and soils ($P = 0.013$). Mean cumulative emissions were greatest from the 300 kg N/ha treatment, with the lowest fluxes (equivalent to 0.20–1.30 g N/ha) consistently occurring in the control treatments (0 kg N/ha). MP soils had higher cumulative fluxes across all treatments, with increasing N_2O production with increasing urine N application rates, with greatest mean flux following the 1000 kg N/ha treatment. In contrast, JC soils had greatest N_2O production during 35 day incubation under the 300 kg N/ha treatment.

Discussion

Soil nitrogen transformations

Following urea hydrolysis, pH in both soils increased, driving increased nitrification. In turn, changes in inorganic-N concentrations resulted in a subsequent decrease in pH (Doak, 1952). While nitrification occurs within a narrow range of pH (6.6–8.0), with a significant decline below pH 5.5, the optimum pH

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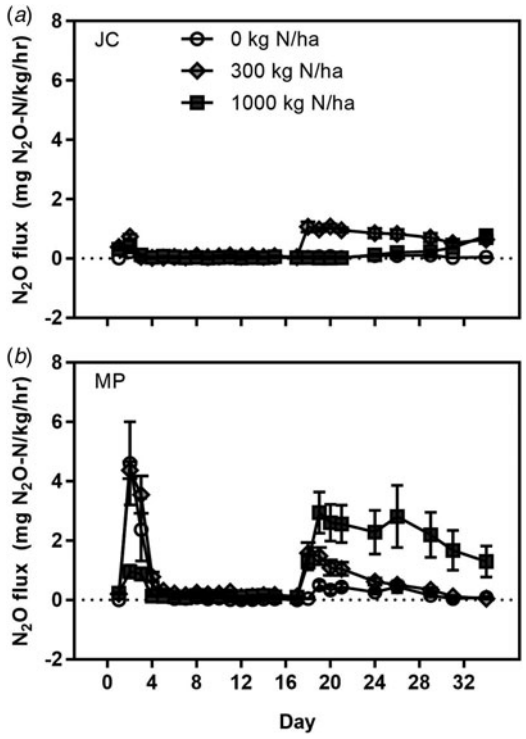

Fig. 3. N_2O fluxes over time following urine-N application for (*a*) JC and (*b*) MP soils. Day 1 was sampled 1 h after urine treatment application. Means ± one S.E..

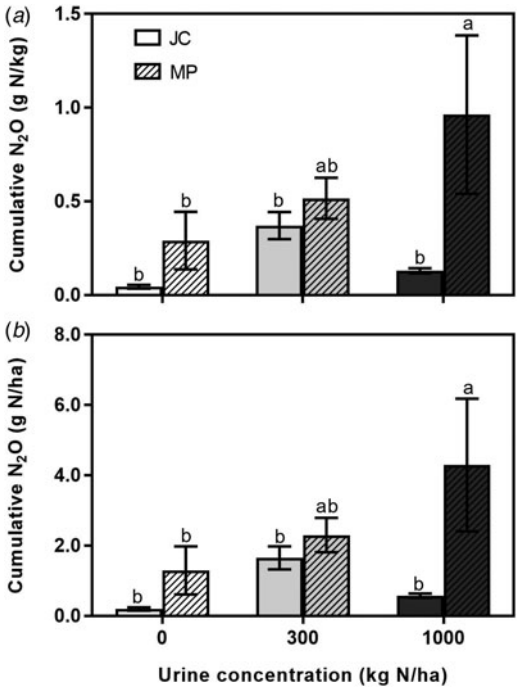

Fig. 4. Cumulative N_2O flux (*a*) per kg and (*b*) per/ha for JC and MP soils following 0, 300 and 1000 kg N/ha urine addition. Means ± one S.E.. Letters indicate significant differences from a *post-hoc* Bonferroni test.

for denitrification is 7–8 but can still occur at more acidic pH (Yamulki *et al.*, 1997).

In both soils, the 1000 kg N/ha treatment resulted in high NH_4^+ concentrations (1566 mg N/kg) on day 4, which subsequently stabilized at >400 mg N/kg. However, NO_3^- concentrations peaked at 325 and 468 mg N/kg, indicating a substantial missing pool of ~1100 mg N/kg. This pool was not accounted for by N_2O emissions (130–960 mg N/kg). Part of this missing N was probably immobilized as soil microbial biomass (Silva *et al.*, 1999) but may also have been stored as urea, emitted as NH_3 (Bussink and Oenema, 1998) or as N_2. Previously, codenitrification, the production of a hybrid N_2O/N_2 molecule from nitric oxide (NO) or NO_2^- and a second from a co-substrate (including N_3, NH_3/NH_4^+) (Rex *et al.*, 2018), has been proposed as the dominant driver of N_2O/N_2 fluxes in grassland soils (Selbie *et al.*, 2015) and may account for the missing fraction. Chemodenitrification (abiotic nitrosation) is less likely, as this process is generally restricted to low pH (<5.2) (Clough *et al.*, 2001).

The formation of NO_3^- in the 1000 kg N/ha treatments for both soils was inhibited until after day 35. This either may have been caused by toxic levels of NH_3/NH_4^+ (Clough *et al.*, 2003) and/or increased levels of ammonium bicarbonate (NH_4HCO_3) driven by urea hydrolysis and nitrification (Malhi and McGill, 1982). The former mechanism is supported by calculated NH_3 concentrations of ~730 mg N/kg across both soils for the 1000 kg N/ha treatment (Watson *et al.*, 1994; Smith *et al.*, 1997). Clough *et al.* (2003) found nitrification to be inhibited by a 1000 kg N/ha treatment, resulting in NO_2^- accumulation and subsequent N losses occurring as NO_2^-, rather than denitrification via NO_3^-. The gradual increase in NO_2^- by day 55 in the loam (JC) soil and the rapid increase in the sandy-loam (MP) soil would indicate the recovery of *Nitrobacter* and a resulting increase in nitrification rates. In contrast, the 300 kg N/ha treatment in both soils showed no inhibition as nitrification of NH_4^+ to NO_3^- occurred readily. Significantly higher concentrations of NO_2^- and NO_3^- accumulated in the sandy-loam in comparison with the loam soil for the 300 kg N/ha treatment, suggesting N loss or immobilization in the loam.

The initial N_2O production between day 1 and 4 observed for all treatments, and in line with field observations by Krol *et al.* (2016), was most likely driven by utilization of native inorganic-N in the soil prior to urine application. N_2O production at this point is likely to have been due to denitrification because of the addition of aqueous treatments, somewhat increasing soil WFPS (Linn and Doran, 1984) and resulting in the formation of transient anaerobic microsites (Clough *et al.*, 2003). Under the 300 kg N/ha treatment, nitrification to NO_3^- proceeded rapidly, with N_2O production occurring as soon as NO_3^- became available. Although for the 1000 kg N/ha treatment NO_3^- levels in both soils remained very low up to day 55, NO_3^- concentrations for the sandy-loam (MP) soil began to increase from day 30 due to increasing concentrations of NO_2^-, albeit it at an impeded rate.

For the loam (JC) soil, the highest cumulative N_2O emissions occurred for the 300 kg N/ha treatment over the 34-day experiment, which closely matched the lower end of annual N_2O emissions from urine patches at the same application rate on the same soils (2.86 kg N/ha) (Krol *et al.*, 2016). At the end of the experiment, cumulative N_2O fluxes from the 1000 kg N/ha treatment were increasing steadily and may have resulted in a greater flux than the 300 kg N/ha if the sampling had continued for 80 days. The 1000 kg N/ha treatment for the MP soil had a higher flux from day 20 than the 300 kg N/ha treatment resulting in significantly higher cumulative N_2O emissions, suggesting nitrification and subsequent denitrification occurred at a faster rate in the sandy-loam (MP) soil. While NO_2^- concentrations increased

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by day 25, NO_3^- did not accumulate likely because it was denitrified as soon as it was produced (Davidson *et al.*, 1990).

Differences in nitrogen dynamics between grassland soils

N_2O emissions from soils subjected to 0 kg N/ha were substantially higher for the sandy-loam (JC) soils, probably due to greater starting NO_3^- concentrations. Both soils demonstrated significant NH_4^+/NH_3 inhibition at high-N addition rates, but subsequent N transformations varied significantly between soils. Differences in the extent of the inhibitory effect may partially be driven by temporary immobilization of N, which could reduce the accumulation of toxic levels of NH_3 (Hansen and Bakken, 1993; van Groenigen *et al.*, 2005). Differences in N transformations between contrasting soil types have been reported extensively and are particularly important in the context of ecosystem scale fluxes of N_2O and understanding the effects of different management practices (Li *et al.*, 2013). Moreover, emission factors for urine for both soils have previously been shown to be significantly different, ranging from 0.0009–0.0091, although these differences were largely ascribed to contrasting weather rather than inherent differences in soil properties (Krol *et al.*, 2016).

The N_2O production potential has previously been found to be greater in loam compared to sandy soils (Maag and Vinther, 1996), with organic soils generally considered as more significant N_2O sources than mineral soils (Duxbury *et al.*, 1982; Pihlatie *et al.*, 2004). Denitrification rates correlate significantly with the availability of labile carbon (McCarty and Bremner, 1989), as denitrifiers are strict heterotrophs and use organic carbon as an electron donor (Groffman and Tiedje, 1989). In the current study, the sandy-loam (MP) had higher organic matter content and greater cumulative N_2O fluxes than the loam (JC). Urine application can increase pH due to hydrolysis of organic matter, increasing both carbon and N turnover (Monaghan and Barraclough, 1993; Selbie *et al.*, 2015). Other differences between soils, such as P availability (Mehnaz and Dijkstra, 2016) and soil structure (Li *et al.*, 2014) have been suggested previously as significantly influencing N dynamics, particularly for *in situ* studies.

Conclusion

At higher rates of urine N addition, NH_3/NH_4^+ toxicity or NH_4HCO_3 accumulation in the soil-inhibited nitrification and thereby reducing N_2O emissions in the short term, although the extent varied between soil types. Prolonged inhibition of nitrification in the 1000 kg N/ha treatment may have resulted in increased NH_3 volatilization, resulting in increased N loss. The significant differences in changes in N transformation between soil types may have been driven by differences in soil chemical properties, including carbon availability which can limit rates of denitrification. These findings are particularly important in understanding how N input rates can result in differences in N transformation in soils, and changes in net N_2O production.

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