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Effects of normal and altered cattle urine on short-term greenhouse gas flux from mixed-grass prairie in the Northern Great Plains☆

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Abstract

Use of dietary amendments to reduce nitrogen (N) in excreta represents a possible strategy to decrease greenhouse gas (GHG) emissions from livestock. In this regard, ingestion of small amounts of condensed quebracho tannin has been found to reduce N concentration in livestock urine. In this study, we sought to quantify the effects of tannin-affected cattle urine, normal cattle urine, and NH_4NO_3 in solution on greenhouse gas flux. Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) flux was measured using static chamber methodology from the three N treatments and a no application control over a 6-week period in a mixed grass prairie in west-central North Dakota, USA. Over the course of the study, average CO_2 emission was greatest from normal urine (335 ± 8 mg C m^{-2} h^{-1}) and least from the control (229 ± 19 mg C m^{-2} h^{-1}), with intermediate fluxes for the tannin urine and NH_4NO_3 treatments (290 ± 27 and 286 ± 54 mg C m^{-2} h^{-1}, respectively). Methane uptake was prevalent throughout the study, as soil conditions were predominantly warm and dry. Uptake of CH_4 was greatest within the control (-30 ± 2 μg C m^{-2} h^{-1}) and least in the tannin urine treatment (-12 ± 4 μg C m^{-2} h^{-1}). Uptake of CH_4 was over 40% less within the tannin urine treatment as compared to normal urine, and may have been repressed by the capacity of tannin to bind monooxygenases responsible for CH_4 oxidation. Average N_2O emission from NH_4NO_3 solution was more than twice that of all other treatments. Though the tannin urine treatment possessed 34% less N than normal cattle urine, cumulative N_2O emission between the treatments did not differ. Results from this study suggest the use of condensed quebracho tannin as a dietary amendment for livestock does not yield GHG mitigation benefits in the short-term.
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Keywords: Greenhouse gas emission; Urine patches; Tannin

1. Introduction

Globally, the livestock sector produces approximately 18% of total greenhouse gas (GHG) emissions (Steinfeld et al., 2006), of which the most important sources are nitrous oxide (N_2O) and methane (CH_4) from animals and their excreta (Monteny et al., 2006). Urine patches in pastures and grazing lands, in particular, represent a significant potential source of GHGs due to carbon (C) and nitrogen (N) contained in urine. Levels of N in urine patches can exceed 80 g N m^{-2} (Oenema et al., 2005), and at such levels can affect emission of GHGs for decades (Mosier et al., 1998).

Processes contributing to the release of GHGs from urine patches are highly complex owing to a myriad of interactions between biological and physical factors in soil. Nitrous oxide can be emitted from urine patches via nitrification or denitrification, and its release to the atmosphere can account for up to 3.8% of the N in urine

Abbreviations: GHG, greenhouse gas; CO_2, carbon dioxide; CH_4, methane; N_2O, nitrous oxide; NO_3–N, nitrate–nitrogen; NH_4–N, ammonium–nitrogen; NH_4NO_3, ammonium nitrate; DOY, day of year.

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(Oenema et al., 1997). Large amounts of water and soluble C in urine may be expected to increase the potential for CH_4 emission from urine patches, yet both CH_4 production and uptake have been observed under field conditions (Yamulki et al., 1999). Carbon dioxide (CO_2) emission from urine patches has been observed to increase with increasing N concentration in urea (Petersen et al., 2004), and is most detectable immediately after urine application (Bol et al., 2004).

Management strategies to mitigate GHG emissions from urine patches are needed to reduce the impact of livestock on global climate change. Strategies to reduce GHG emissions from urine patches have focused primarily on modifying diet to alter the amount and composition of N compounds in urine (Whitehead et al., 1989; Van Groenigen et al., 2005; Kool et al., 2006). In this regard, ingestion of small amounts of naturally-occurring tannins by livestock has been found to reduce N concentration in urine without negatively affecting animal performance (Puchala et al., 2005; MacAdam et al., 2006).

Tannins are a group of secondary compounds in plants that possess a polyphenolic structure capable of precipitating proteins (Haslam, 1989). Tannins are present in numerous plants throughout the world, and are used extensively in the conversion of hides and skins to leather. Given their capacity to reduce N concentration in livestock urine, and their ubiquitous presence naturally and within the tanning industry, tannins may be a useful dietary amendment for reducing GHG emissions from livestock.

Given this context, we sought to quantify the effects of cattle urine and urine from cattle that had ingested water containing condensed tannin on GHG flux under field conditions. For purposes of comparison, GHG flux was also evaluated from treatments including ammonium nitrate (NH_4NO_3) in solution and a no application control. Among the three treatments receiving N, tannin urine was hypothesized to result in significant GHG mitigation benefits over the course of the study.

2. Materials and methods

2.1. Site description

The experimental site was located within the Missouri Plateau approximately 6 km south of Mandan, North Dakota, USA (46° 46′ 12″N, 100° 54′ 57″W). The site is on gently rolling uplands (0–3% slope) with a silty loess mantle overlying Wisconsin age till. Soil at the site is a Temvik-Wilton silt loam (FAO: Calcic Siltic Chernozems; USDA: Fine-silty, mixed, superactive, frigid Typic and Pachic Haplustolls). Climate at the site is semiarid continental, with evaporation exceeding precipitation in most years. From 1914 to 2005, annual precipitation averaged 407 mm, with over 75% of the total received during the growing season from April through September. Average annual temperature is 4 °C, though daily averages range from 21 °C in the summer to −11 °C in the winter.

2.2. Experimental setup

The study was conducted in 2005 over a period of approximately 6 weeks, from 13th July (DOY = 194) to 25th August (DOY = 237) within an exclosure (0.2 ha) of a native vegetation pasture. Vegetation composition in the exclosure at the time of the study included a mixture of needle-and-thread (*Stipa Comata* Trin. and Rupr.), Kentucky bluegrass (*Poa pratensis* L.), smooth bromegrass (*Bromus inermis* L.), and carex (*Carex filifolia* Nutt. and *Carex heliophila* Mack.). The pasture was originally established in 1916, and with the exception of 2 years in the mid-1990s, the exclosure was not grazed by cattle (Al Frank, personal communication). The purpose of allowing grazing in the exclosure was to help control smooth bromegrass.

Preparations for the study included the placement of 16 polyvinyl chloride (PVC) pipe anchors (19.6-cm i.d.; 15.2-cm height) in the exclosure on 20th May 2005. Anchors were arranged in four rows of four anchors per row, and were placed approximately 15 cm apart edge-to-edge within and between rows. Anchors were inserted into the soil to a depth of approximately 10 cm using a Giddings probe (Giddings Machine Co., Windsor, CO). A carpenter's level was used during collar insertion to ensure each anchor was level on north–south and east–west axes. After insertion, headspace within each anchor was determined by lining the space within an anchor with plastic wrap and filling it with a known volume of water until the water level was flush with the upper edge of the anchor.

Four treatments were evaluated for their effect on GHG emission: (a) urine from a mature Hereford cow given tap water, (b) urine from the same cow given a mixture of tap water and quebracho tannin (Tannin Corporation, Peabody, MA) at a concentration to provide for tannin intake of 1% of the cow's daily dry-matter consumption, (c) a solution of NH_4NO_3, and (d) a no application control. For both urine treatments, urine was collected in June 2005 from the cow after the cow had drunk either water or the tannin–water mixture for 1 week (in sequence). Urine samples were stored in sealed plastic containers and kept cool at 5 °C until applied in the field. The four treatments were randomly assigned within a row of four anchors for three of the rows, which served as replicates. The fourth row of four anchors was used to monitor surface soil temperature and volumetric water content at time of gas sampling.

Total N concentration of the normal and tannin urine was 12.7 and 8.4 g N L^{-1}, respectively, as determined by the Dumas method using a Vario MAX CN analyzer (Elementar Americas, Inc., Mt. Laural, NJ). The N application rate for the urine and NH_4NO_3 treatments was 33.6 g N m^{-2}. All treatments – with the exception of the control – were applied using a total solution volume of 384 mL per anchor, which

was equivalent to a 1.27 cm solution depth within the PVC anchors. To attain the target N level for urine, 80 mL of urine was mixed with 304 mL of deionized water. Eighty milliliters of tannin urine was also mixed with deionized water, resulting in an N application rate of 22.1 g N m^{-2}. For the NH_4NO_3 treatment, 384 mL of a 0.076 M NH_4NO_3 solution was used.

Treatments were applied at 10:00 on 13th July by pouring the solutions to the appropriately assigned anchor through a perforated PVC tray (19.6-cm i.d.; 5-cm height) that set directly atop each anchor. An inner-tube (19.8-cm i.d.; 7.5-cm height) was wrapped around the outside of the tray and outer rim of each anchor during application to eliminate lateral movement of solution. The tray was gently rotated on the anchor rim during application to distribute solution within the anchor. To eliminate the potential for contamination, the tray was washed with deionized water between each application. No water was applied to the control.

2.3. Gas and soil analyses

Carbon dioxide, CH_4, and N_2O fluxes were estimated from each anchor using static chamber methodology as outlined by Hutchinson and Mosier (1981). Gas samples were collected using a two-part chamber including an anchor (previously described) and a PVC cap (20.3-cm i.d.; 10.0-cm height) with a vent tube and sampling port that set on top of each anchor. Samples were collected beginning 13th July (DOY = 194) 1 h prior to the application of treatments, and then at 14:00, 18:00, and 22:00 on the first day, every day thereafter at 10:00 during the first week, and then every 3 or 4 days for the subsequent 5 weeks for a total of 20 samplings. During each sampling, gas from inside the chambers was collected with a 20 mL syringe at 0, 15, and 30 min after installation. After collection, gas samples were injected into 12 mL evacuated Exetainer glass vials sealed with butyl rubber septa (Labco Limited, Buckinghamshire, UK).

The CO_2, CH_4, and N_2O concentration inside each vial was measured by gas chromatography within 1 day after collection using a Shimadzu GC-17A gas chromatograph (Shimadzu Scientific Instruments, Kyoto, Japan) attached to an ISCO Retriever IV autosampler (Teledyne Isco, Inc., Lincoln, NE). Using this system, each sample was auto-injected and split into two sample loops, with 1 mL directed to a thermal conductivity detector (TCD) in series with a flame ionization detector (FID) using ultra-pure He carrier gas. Ultra-pure He and hydrocarbon-free air were used for combustion in the FID. The second sample loop directed 0.5 mL to a ^{63}Ni electron capture detector (ECD) with ultra-pure N_2 as carrier gas. Prior to reaching each detector, samples passed through a 4-m HayeSep D column (Hayes Separations, Inc., Bandera, TX) for the TCD and FID, and 2-m Porapak Q (Waters Corp., Milford, MA) and 4-m HayeSep D columns for the ECD. The gas chromatograph was calibrated with a commercial blend of CO_2 (350, 400, 1998.7 ppm), CH_4 (1.00, 2.09, 10.1 ppm), and N_2O (0.100, 0.401, 1.99 ppm) balanced in N_2 from Scott Specialty Gases (Scott Specialty Gases, Plumsteadville, PA). Gas flux was calculated from the change in concentration in the chamber headspace over time (Hutchinson and Mosier, 1981). Cumulative CO_2 and N_2O emission and CH_4 uptake was calculated for each chamber within a treatment by linearly interpolating data points and integrating the underlying area (Gilbert, 1987).

At each gas sampling, soil temperature was measured within the fourth row of anchors at a 6 cm depth with an Omega HH81A handheld digital thermometer attached to a heavy-duty T type thermocouple probe (Omega, Inc., Stamford, CT). Volumetric water content was estimated in the surface 10 cm of soil using a time-domain reflectometry technique with a Campbell CS620 HydroSense System (Campbell Scientific, Inc., Logan, UT). One measurement of soil temperature and volumetric water content was made within each anchor, for a total of four measurements. Volumetric water content was converted to percent water-filled pore space (WFPS) as outlined by Linn and Doran (1984) using field measured soil bulk density for the 0–10 cm depth (Blake and Hartge, 1986).

After the final gas sampling, soil samples were collected from each anchor for extractable N determination using a 3.5-cm (i.d.) step-down probe at depths of 0–5, 5–10, and 10–20 cm. Three cores from each depth were carefully extracted from each anchor and composited. Upon collection, all soil samples were saved in double-lined plastic bags and stored at 5 °C until processing. Samples were processed by drying at 35 °C for 3–4 days and then ground by hand to pass a 2.0 mm sieve. Identifiable root material was removed during sieving. Soil NO_3–N and NH_4–N were estimated from 1:10 soil–KCl (2 M) extracts using cadmium reduction followed by a modified Griess–Ilosvay method and indophenol blue reaction (Mulvaney, 1996). Gravimetric data were converted to a volumetric basis for each sampling depth using field measured soil bulk density. All data were expressed on an oven-dry basis.

2.4. Statistical analyses

Gas flux and extractable N data were analyzed using PROC MIXED in SAS (Littell et al., 1996). Application treatment and replicate were considered fixed and random effects, respectively. *P*-values for comparisons among treatments for gas flux at individual gas samplings, cumulative gas flux, and extractable N after completion of the study were computed. Least square means of the fixed effect were calculated, and the PDIFF option of the LSMEANS statement was used to document differences among means using a significance criteria of $P < 0.1$. Where appropriate, associations between measured parameters were identified using Pearson correlation analysis.

3. Results

3.1. Percent water-filled pore space and soil temperature

Measurements of soil moisture and temperature during gas sampling times indicated the soil to be relatively dry and warm over the course of the study. Mean soil water status was 48 ± 13% WFPS, and ranged from 31 to 80% (Fig. 1). During the 20 samplings, WFPS exceeded 60% only three times (DOY 206, 223, 230), and was associated with precipitation events preceding each sampling. Soil temperature averaged 19 °C over the course of the study, with a range of 16–24 °C.

3.2. Carbon dioxide emission

Significant treatment effects on CO_2 emission were observed in 12 of 20 sampling times (Table 1), and were strongest within 24 h after application where added C from the urine and tannin urine treatments contributed to a large and immediate release of CO_2 (Table 2). Over the course of the study, emission of CO_2 tended to be greatest from urine (mean = 335 ± 8 mg C m^{-2} h^{-1}; Cum. = 129.1 ± 3.0 g C m^{-2}) and least from the control (mean = 229 ± 19 mg m^{-2} h^{-1}; Cum. = 88.3 ± 7.3 g C m^{-2}), with intermediate fluxes for the tannin urine and NH_4NO_3 treatments (mean = 290 ± 27 and 286 ± 54 mg C m^{-2} h^{-1}; Cum. = 111.7 ± 10.2 and 110.1 ± 20.9 g C m^{-2}, respectively) (Table 2; Fig. 2a). Following the initial spike in CO_2 emission, flux rates generally decreased over time, and were associated with trends in soil temperature ($r = 0.54$; $P = 0.0138$) (data not shown). An exception to the decreasing trend in CO_2 emission over time occurred near the middle (DOY 213) and end (DOY = 230) of the study when the soil was warm (19–21 °C) and had near-optimum WFPS (51–61%) for aerobic microbial activity (Table 2). Water-filled pore space was not associated with CO_2 emission within any of the treatments (Table 3).

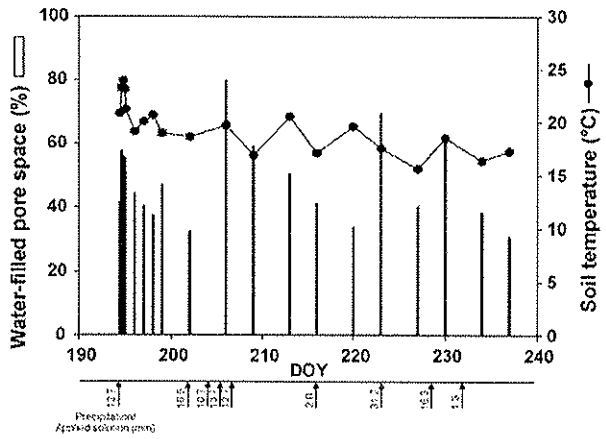

Fig. 1. Percent water-filled pore space (0–10 cm) and soil temperature (at 6 cm) during gas sampling times throughout the course of the experiment. Timing and amount of precipitation is presented below the *x*-axis.

Table 1
Summary of *P*-values from analysis of variance for treatment effects on daily and cumulative CO_2, CH_4, and N_2O flux

DOY/time (h)[a]	*P*-value		
	CO_2	CH_4	N_2O
194, 09:00	0.8636	0.1664	0.9711
194, 14:00	<0.0001	0.0083	0.5119
194, 18:00	0.0044	0.0087	0.7077
194, 22:00	0.0013	0.0181	0.2373
195	0.0180	0.0176	0.1681
196	0.1087	0.0022	0.0562
197	0.1052	0.0825	0.0054
198	0.2448	0.0081	0.0302
199	0.0352	0.0180	0.4036
202	0.0871	0.1127	0.8886
206	0.1984	0.1112	0.8973
209	0.1838	0.0593	0.1583
213	0.0303	0.0681	0.5449
216	0.0619	0.0594	0.4178
220	0.1963	0.0981	0.3947
223	0.1112	0.0248	0.0745
227	0.0760	0.2105	0.5324
230	0.0876	0.5017	0.0981
234	0.0072	0.9032	0.9341
237	0.0089	0.4814	0.5657
Cumulative	0.0233	0.0011	0.0881

[a] DOY, day of year.

3.3. Methane flux

Negative flux of CH_4 (uptake) was the dominant exchange process over the course of the study. Of the 13 sampling times where significant treatment effects on CH_4 flux were observed, CH_4 uptake consistently occurred across all treatments 10 times (Tables 1 and 2). Methane uptake was greatest within the control treatment across all sampling times, with a mean value of −30 ± 2 μg C m^{-2} h^{-1} during the study. Treatments with applied N, regardless of source or composition, decreased CH_4 uptake in the order of normal urine (−22 ± 1 μg C m^{-2} h^{-1}), NH_4NO_3 (−13 ± 6 μg C m^{-2} h^{-1}), and tannin urine (−12 ± 4 μg C m^{-2} h^{-1}). Accordingly, cumulative uptake of CH_4 over the course of the study was −11.6 ± 0.7, −8.5 ± 0.5, −5.1 ± 2.2, and −4.7 ± 1.6 mg C m^{-2} for control, normal urine, NH_4NO_3, and tannin urine treatments, respectively (Fig. 2b). Methane uptake was positively associated with WFPS in all treatments (i.e., greater uptake corresponded to drier soil conditions), with correlation coefficients ranging from 0.63 to 0.81 (Table 3).

3.4. Nitrous oxide emission

Treatment effects on N_2O emission were modest. Of the 20 sampling times, significant treatment effects were observed only on DOY 196–198, 223, and 230 (Table 1). Application of NH_4NO_3 resulted in significantly greater

Table 2
Mean CO_2, CH_4, and N_2O flux rates for days where significant ($P < 0.1$) treatment effects were observed

DOY/time (h)[a]	Control	NH_4NO_3	Urine	Tannin urine
	CO_2 emission (mg C m^{-2} h^{-1})			
194, 14:00	338 c[b]	401 c	1126 a	922 b
194, 18:00	334 b	364 b	685 a	689 a
194, 22:00	241 b	244 b	431 a	423 a
195	236 b	237 b	311 a	342 a
199	188 c	235 bc	300 a	247 ab
202	195 b	227 b	290 a	238 ab
213	285 b	394 a	451 a	382 a
216	215 c	313 ab	336 a	255 bc
227	222 b	340 a	347 a	284 ab
230	286 b	356 ab	417 a	358 ab
234	209 c	315 a	323 a	263 b
237	222 b	281 a	307 a	242 b
	CH_4 flux (μg C m^{-2} h^{-1})			
194, 14:00	−20 c	5 a	−6 b	3 ab
194, 18:00	−28 c	12 a	−8 b	3 ab
194, 22:00	−45 b	−15 a	−14 a	−6 a
195	−28 b	−5 a	−22 b	−2 a
196	−38 b	−9 a	−31 b	−9 a
197	−39 b	−19 a	−20 a	−26 ab
198	−41 b	−11 a	−36 b	−9 a
199	−28 b	−5 a	−20 b	−3 a
209	−31 b	−13 a	−17 a	−19 a
213	−26 c	−14 ab	−22 bc	−11 a
216	−40 b	−27 a	−30 ab	−19 a
220	−35 b	−19 ab	−34 b	−13 a
223	−17 b	1 a	−6 a	0 a
	N_2O emission (μg N m^{-2} h^{-1})			
196	14 b	98 a	0 b	2 b
197	25 b	73 a	26 b	22 b
198	20 b	73 a	10 b	19 b
223	37 a	23 ab	30 a	10 b
230	8 b	28 b	135 a	8 b

[a] DOY, day of year.
[b] Means in a row with unlike letters differ ($P < 0.1$).

N_2O emission during DOY 196–198 as compared to the other treatments (Table 2). On DOY 223, N_2O emission was greatest under the control and normal urine treatments and least under the tannin urine treatment, while on DOY 230, N_2O emission was greatest under the urine treatment compared to the other treatments. Over the course of the study, mean N_2O emission was greatest in the NH_4NO_3 treatment (39 ± 14 μg N m^{-2} h^{-1}), intermediate in the control and normal urine treatments (19 ± 5 and 19 ± 8 μg N m^{-2} h^{-1}, respectively), and least in the tannin urine treatment (18 ± 11 μg N m^{-2} h^{-1}). Water-filled pore space was positively correlated with N_2O emission in the control and normal urine treatments (Table 3).

Differences in cumulative N_2O emission among treatments were established early in the study due to substantially higher initial emissions from the NH_4NO_3 treatment (Fig. 2c). After DOY 199, cumulative N_2O emission followed a similar trend across treatments until the end of the study. Emission of applied N as N_2O from the treatments was relatively small, amounting to 0.021% for normal urine, 0.031% for tannin urine, and 0.045% for NH_4NO_3.

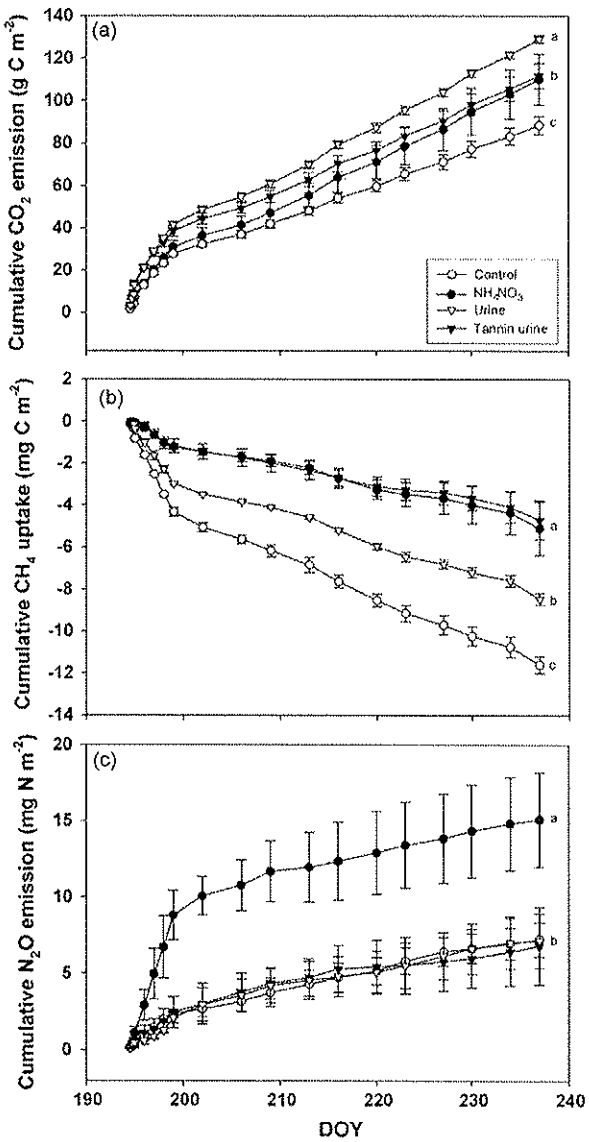

Fig. 2. Cumulative CO_2 (a), CH_4 (b), and N_2O (c) flux for N application treatments and a control from DOY 194–237. Error bars reflect ±1 standard error of the mean. Different letters to the right of the cumulative flux values signify treatment differences at $P < 0.1$.

3.5. Soil nitrate and ammonium

Levels of soil NO_3–N and NH_4–N at the conclusion of the study were greatest in the surface 0–5 cm and generally

Table 3
Pearson correlation coefficients for associations between daily mean gas flux and percent WFPS within each treatment

Treatment	CO_2 emission	CH_4 flux	N_2O emission
Control	0.25	0.74**	0.49*
NH_4NO_3	0.11	0.66**	0.09
Urine	0.22	0.81**	0.45*
Tannin urine	0.27	0.63**	0.39

*, ** Correlation with percent WFPS significant at $P < 0.05$ and 0.01, respectively.

Table 4
Effect of treatments on soil NO_3–N and NH_4–N at the end of the study

Treatment	Depth (cm)		
	0–5	5–10	10–20
Soil NO_3–N (g N m^{-2})			
Control	2.3 b[a]	0.5	0.2
NH_4NO_3	6.6 ab	1.8	0.5
Urine	7.2 ab	2.8	0.6
Tannin urine	11.0 a	2.7	0.7
Soil NH_4–N (g N m^{-2})			
Control	2.2	1.7	2.9
NH_4NO_3	5.6	2.5	2.7
Urine	7.8	4.4	3.4
Tannin urine	4.5	3.6	2.3

[a] Means in a column with unlike letters differ ($P < 0.1$).

decreased with increasing depth (Table 4). Over 0–20 cm, 76% and 44% of the soil NO_3–N and NH_4–N, respectively, was present at 0–5 cm. Treatment effects on soil NO_3–N and NH_4–N were also limited to the near-surface depth, with significantly greater soil NO_3–N under the tannin urine treatment at 0–5 cm than the control. Condensed tannins have been found to inhibit urease activity as well as nitrification (Benoit and Starkey, 1968; Rice and Pancholy, 1973), and may have contributed to a delay in urea conversion to NO_3–N in the tannin urine treatment. Though soil NO_3–N did not differ among treatments at 5–10 and 10–20 cm, it tended to be elevated under the urine treatments and least in the control. Post-study soil NH_4–N levels did not differ among treatments at any depth. Cumulative gas fluxes were weakly associated with soil NO_3–N and NH_4–N levels, with significant correlations found only at 0–5 cm between soil NO_3–N and CH_4 uptake ($r = 0.58$; $P = 0.0477$) and soil NH_4–N and CO_2 emission ($r = 0.77$; $P = 0.0031$) (data not shown).

Of the total amount of N applied to the treatments, approximately 30, 49, and 63% remained as soil NO_3–N and NH_4–N at the conclusion of the study for the NH_4NO_3, urine, and tannin urine treatments, respectively (Table 4). Background levels of soil NO_3–N and NH_4–N in the control treatment were surprisingly high based on knowledge of N dynamics in semiarid grazing lands (Burke et al., 1997). It is likely previous grazing of the study site resulted in substantial N inputs from urine and dung deposition, and as an exclosure, negligible removal of aboveground biomass occurred once cattle were removed. Such a scenario would contribute to persistently high soil N levels in this semiarid grassland.

4. Discussion

This study sought to determine the effects of two types of cattle urine (normal and tannin-affected) and NH_4NO_3 solution on short-term GHG flux under field conditions in a mixed grass prairie. Nitrogen input from the treatments was established to simulate that found in urine patches, and fell within the 20–80 g N m^{-2} range for urine patches reviewed by Oenema et al. (1997). Measurements of WFPS over the course of the study revealed near-surface soil conditions to be predominantly aerobic, but not exceedingly dry. Soil temperature was warm throughout the study, yet not extreme, having fallen within a range of 8 °C during the measurement period. Such soil water and temperature conditions would be conducive for aerobic microbial activity in soil (Linn and Doran, 1984), and would be expected to favor nitrification over denitrification and methanotrophy (CH_4 uptake) more than methanogenesis (CH_4 release) (Paul and Clark, 1996).

Despite the short-term nature of the study, GHG flux rates were comparable to other field evaluations, though there is a general paucity of flux data specific to semiarid grasslands. Nitrous oxide emission rates from all treatments ranged from <1 to 135 μg N m^{-2} h^{-1}, and were generally greatest within the first 5 days after treatment application. The range in N_2O emission was similar to or lower than urine-affected treatments in other studies conducted under field conditions (Bol et al., 2004; Mosier et al., 1998; Yamulki et al., 1998). Methane flux over the course of the study was more variable than that observed under field conditions with greater rainfall (SW England; Yamulki et al., 1999), while average CH_4 uptake from the control treatment was approximately three times greater than that observed for unfertilized grassland in the shortgrass steppe (Mosier et al., 1991). Average CO_2 emission from the control treatment was similar to measurements of growing season soil CO_2 emission from grazed mixed grass prairie (Frank et al., 2006). Rates of CO_2 emission across all treatments were likely near maximum, as previous evaluations have found the highest CO_2 emission rates to coincide with peak aboveground biomass production, which typically occurs between mid-July and early August (Frank, 2002).

Addition of mineral N to grassland soils has been found to increase N_2O emission (Mosier et al., 1991). Among the treatments receiving N in this study, N_2O emission was greatest from the NH_4NO_3 solution, indicating the relative ease by which the microbial community was able to metabolize this synthetic N source. It is likely the rapid solubility of NH_4NO_3 contributed to increased N_2O emission, as there would have been an immediate increase in substrate (NO_3) for denitrification following application. No difference was observed in N_2O emission between urine treatments, indicating the lower N level in the tannin urine treatment did not result in decreased N_2O emission in the short-term. Though the N input to the soil from the tannin urine treatment was 34% lower than normal urine, the applied amount was still quite high (22.1 g N m^{-2}) for a semiarid grassland. Given this application rate, coupled with the relatively dry climate of the region, it is likely residual soil N available for N_2O production would persist for a significant amount of time. These conditions would make it difficult to discriminate between urine treatments with

different N levels in the short-term. Such speculation is supported by research in the shortgrass steppe, where Mosier et al. (1998) found large (45 g N m^{-2}) and smaller (30 g N m^{-2}) N doses simulating cattle urine deposition stimulated N_2O emission from sandy and clay loam soils for 6–15 years after application.

Methane uptake from the applied treatments differed considerably throughout the course of the study. Though the amount of N applied in the normal urine and NH_4NO_3 treatments was the same, CH_4 uptake was approximately 40% less by the NH_4NO_3 treatment. Post-experiment levels of soil NH_4–N did not differ between the normal urine and NH_4NO_3 treatments, thereby ruling out a simple explanation of CH_4 oxidation suppression by inorganic N. It is possible, however, that the NH_4–N from the synthetic source was more readily available in soil solution, thereby effectively competing for active sites on monooxygenases (Knowles, 1993).

The tannin urine treatment repressed CH_4 uptake to the same degree as NH_4NO_3 relative to the other treatments. The mechanism causing this repression, however, may be different than NH_4–N competition, owing to the fact that soil NH_4–N was lowest in the tannin urine treatment when compared to the other two treatments receiving N. It is possible the tannin in the urine bound to monooxygenases responsible for CH_4 oxidation, as tannins are particularly effective at binding proteins to form tannin–protein complexes (Haslam, 1989). Depending on the nature of the complex, the activity of the monooxygenases could be reduced or negated completely. Further investigation is needed to ascertain the relative importance of this possible mechanism of repressed CH_4 uptake.

5. Conclusions

Use of condensed tannins as a dietary amendment for cattle was investigated for its possible effect on mitigating GHG emissions from urine patches. Over the course of a 6-week study conducted in an exclosure of a mixed grass prairie, our data suggest – at least in the short-term – tannin-affected urine does not reduce N_2O emission relative to normal cattle urine. Furthermore, tannin-affected urine appears to repress CH_4 uptake as compared to normal cattle urine, though the mechanism for the repression is unknown. Relative to cattle urine treatments, NH_4NO_3 solution resulted in greater N_2O emission, and similar CH_4 and CO_2 fluxes as tannin-affected urine.

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