Nitrous oxide and methane fluxes from cattle excrement on C3 pasture and C4-dominated shortgrass steppe

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Abstract

Cattle play a major role in nutrient cycling of grassland ecosystems through biomass removal and excrement deposition (urine and feces). We studied the effects of cattle excrement patches (urine at 430 and feces at 940 kg N ha⁻¹) on nitrous oxide (N₂O) and methane (CH₄) fluxes using semi-static chambers on cool-season (C3), Bozoiisky-select (*Psathyrostachys juncea*) pasture, and warm-season (C4)-dominated native rangeland of the shortgrass steppe (SGS) in northeastern Colorado. Nitrous oxide emission factors (EF; i.e., percent of added N emitted as N₂O-N) did not differ between urine and feces on the C4-dominated native rangeland (0.11 and 0.10%) and C3 pasture (0.13 and 0.10%). These EFs are substantially less than the Intergovernmental Panel on Climate Change (IPCC) Tier 1 Default EF (2%) for manure deposited on pasture, indicating that during dry years the IPCC Tier 1 Default EF would result in a significant overestimation of emissions from excrement patches deposited on SGS C4-dominated native rangeland and C3 pasture. Over the first year of the study (19 June 2012 to 18 June 2013), cumulative CH₄ uptake was 38% greater for urine (-1.49 vs. -1.08 kg CH₄-C ha⁻¹) and 28% greater for control plots (-2.09 vs. -1.63 kg CH₄-C ha⁻¹) on C4-dominated native rangeland compared to C3 pasture. In contrast, feces patches were net sources of CH₄ with emissions from the C3 pasture (0.64 kg CH₄-C ha⁻¹) 113% greater than the C4-dominated native rangeland (0.30 kg CH₄-C ha⁻¹). Conversion of C4-dominated native rangeland to C3 pasture

can have long term effects on CH₄ uptake; therefore consideration should be taken before implementing this management practice.

Abbreviations: N₂O, nitrous oxide; CH₄, methane; EF, emission factor; GHG, greenhouse gas; SGS, shortgrass steppe; DOY, day of year; WFPS, water-filled pore space.

Keywords: nitrous oxide, methane, shortgrass steppe, urine, feces, greenhouse gas

1. Introduction

Cattle play a significant role in the nitrogen (N) cycle of grassland ecosystems by redistributing up to 80% of consumed N through their excrement in urine and feces patches (Milchunas et al., 1988; Wachendorf et al., 2008). The high N rate deposited through excrement patches greatly exceeds the demands of semi-arid grassland flora, thereby subjecting excrement-N to losses through nitrification, denitrification, ammonia (NH₃) volatilization, and leaching (Williams et al., 1999; de Klein et al., 2003; Maljanen et al., 2007; Wachendorf et al., 2008). Leaching is minimal in semi-arid grasslands such as the shortgrass steppe (SGS) since potential evapotranspiration (PET) is substantially larger than the amount of precipitation received and hence water movement below the rooting zone rarely occurs (Schimel et al., 1986; Augustine et al., 2013). Direct nitrous oxide (N₂O) emissions on grazing lands range from 0.1-3.8% for urine and 0.05-0.7% for feces patches of total excrement N applied (Milchunas et al., 1988; Oenema et al., 1997; Follett, 2008; Yao et al., 2010; van der Weerden et al., 2011; Hoefft et al., 2012). The Intergovernmental Panel on Climate Change (IPCC) Tier 1 Default Emission Factor (EF; i.e.,

percent of added N emitted as N₂O-N) for manure deposited on pasture is 2%. This method assumes that the applied-N is entirely cycled within one year (IPCC, 2006).

Currently, knowledge on greenhouse gas (GHG) fluxes from feces patches is based on studies conducted over a short time period (< 1 year) (van der Weerden et al., 2011; Lessa et al., 2014; Mori and Hojito, 2015). Short-term studies, encompassing a single growing season, may underestimate cumulative N₂O emissions from feces patches since organic N is the predominant form of feces-N. Depending on environmental conditions, feces composition, and microbial community composition, organic forms of feces-N may take more than a single growing season to mineralize (Wachendorf et al., 2008). Wachendorf et al. (2005) found that a year after cattle feces deposition on a sandy soil in Germany, 70% of the feces-N remained in the soil, accounting for 15% of the soil organic-N. In addition, lysed microbial cells following freeze-thaw cycles may release excrement-derived N previously assimilated in microbial biomass, which can lead to pulses of N₂O emissions (Koponen and Martikainen, 2004; Holst et al., 2008; Wu et al., 2012). Therefore, when studying cumulative GHG fluxes from feces patches, it is important to conduct measurements for > 1 year to allow adequate time for mineralization of feces organic N.

Due to the vast land area that the SGS encompasses, 11% (3.4×10^5 km²) of the central grasslands in North America, land management practices on the SGS can have a significant impact on the North American GHG budget (Lauenroth, 2008). While grazing is the dominant land management practice on the SGS, the impacts are relatively un-documented. Conversion of SGS C₄-dominated native rangeland to cool-season (C₃) pasture species has been found to be economically beneficial for ranchers (Derner and Hart, 2010), by lengthening the growing season and providing more sustained forage for cattle. However, data on the impacts of such conversions on GHG emissions are lacking. Prior research has shown that conversion of C₄-

dominated native rangeland to a winter wheat-fallow production system increased N₂O emissions and decreased CH₄ uptake (Mosier et al., 1997). Mosier et al. (1997) found that three years following a tillage event, CH₄ uptake was 35% less and N₂O emissions 25-50% greater than undisturbed C₄-dominated native rangeland. Once cultivated soils of the SGS are allowed to revert back to grassland, it takes 8-50 years for CH₄ and N₂O soil-atmosphere gas exchange rates to return to that of undisturbed native rangeland (Mosier et al., 1997).

The primary goal of this study was to evaluate effects of cattle excrement patches on CH₄ and N₂O flux rates over a two year period on a site representative of typical SGS C₄-dominated native rangeland and C₃, Bozoi-sky-select, pasture. We tested the following hypotheses for each plant community: (1) a greater proportion of the urine-N will be emitted as N₂O compared to feces-N, (2) CH₄ uptake rates will be less for urine and feces compared to control plots, and (3) N₂O emissions will be greater from feces compared to the urine and control plots following the spring freeze-thaw cycle.

2. Materials and methods

2.1. Study site and experimental design

The study was conducted at the USDA– Agricultural Research Service Central Plains Experimental Range (CPER), located about 12 km northeast of Nunn, (40.841801,-104.70621; 1,650 m above sea level) on the western portion of the Pawnee National Grasslands in north-central Colorado. The soil is a Zigweid (Fine-loamy, mixed, superactive, mesic Ustic Haplocambids). Mean annual precipitation (1939-2012) was 341 mm yr⁻¹, with 80% occurring between May-September. Mean annual temperature was 8.6 °C, with lowest temperatures in January (-1.5 °C) and highest in July (22.2 °C).

This project focused on two plant communities, C3 pasture and C4-dominated native rangeland, which were directly adjacent to one another. The native rangeland site was characteristic of SGS native rangeland, dominated by the C4 grass, blue grama (*Bouteloua gracilis*). Other common plants were fringed sagebrush (*Artemisia frigida*), buffalo grass (*B. dactyloides*), and plains prickly pear (*Opuntia polyacantha*). The C3 pasture was plowed and seeded to Bozoisky-select in 1994, after having been ‘go-back’, or abandoned cropland that was allowed to naturally revegetate following prior cultivation in the 1930s and 1950s with winter wheat. Bozoisky-select, a C3 bunch grass adapted to semi-arid grasslands, is a cultivar of *Psathyrostachys juncea*, selected for improved seedling vigor, winter hardiness, and drought-resistance. Bozoisky-select soils were significantly sandier and contained less C and N than C4-dominated native rangeland soils for the top 10 cm (Table 1). Soil organic C accounted for the majority ($\geq 89\%$) of the total soil C (0-10 cm) for C4-dominated native rangeland and C3 pasture soils, with carbonate-C making up $< 11\%$ of total C (data not shown). The C4-dominated native rangeland was typically grazed from mid-May to early-October, while the C3 pasture was grazed in both the spring (mid-April to mid-May) and fall (late-October to early-December). Both plant communities had been grazed annually leading up to the experiment, with the exception of 2007 and 2008 on the C3 pasture.

In the spring of 2012, we established a randomized complete block design on each plant community with four blocks, or replicates. Exclosures (7.3 m²) were constructed around each block using panels to exclude cattle. Four treatments 1) urine (U), 2) feces (F), 3) control water (Cw), and 4) control blank (Cb), were randomly assigned to plots within each block. Treatment plots were 3 m² in area and were separated by a 0.5 m buffer. To simulate grazing, vegetation within the exclosures was periodically clipped to five cm, removed from the study area, and kept

for C and N analysis. Due to minimal aboveground biomass production in 2012, vegetation was clipped just once in the C4-dominated native rangeland, and no clipping occurred in the C3 pasture.

Excrement was collected in May 2012 at Colorado State University's (CSU) Agricultural Research, Development and Education Center (ARDEC). All sampling techniques, animal use, and handling were pre-approved by the CSU Animal Care and Use Committee. Excrement was collected over a 24 hour period from eight, 360 kg weight cross-bred commercial steers. Steers were fed a mixed ration that consisted of whole corn, silage, and hay. Homogenized samples of the mixed ration feed were oven dried at 55 °C for C and N analysis. Urine was collected from each steer using a urine collection harness and aspirated into a 45 L polypropylene carboy under vacuum. Fifty mL of six *N* hydrochloric acid (HCl) was added to each carboy prior to urine collection to prevent NH₃ volatilization. Upon completion of excrement collection, urine was homogenized and consolidated into two 22.5 L carboys. A one L subsample of urine was collected to analyze the C and N content. Feces were collected and stored in sealed, 18.9 L buckets, and weighed. Urine and feces were stored at -4 °C.

One week prior to treatment application, excrement was transferred to a walk-in cooler at 10 °C for gradual thawing. Once thawed, feces was homogenized, and partitioned by wet weight (two kg) into 3.78 L sealable plastic bags. Subsamples were oven dried at 55 °C to calculate gravimetric moisture content and ground to two mm using a Wretch grinder for C and N determinations (see below). Urine was homogenized and pH adjusted to eight the morning of treatment application by adding 300 mL of six *N* Sodium Hydroxide (NaOH) to approximately 30 L of urine. Subsamples (1 L) were taken to analyze for C and N on a LECO Tru-SPEC elemental analyzer (Leco Corp., St. Joseph, MI). During analysis, liquid urine subsamples were

added to Com-Aid, an inorganic compound, to dehydrate the samples for dry combustion. Mixed ration, feces, and C4-dominated native rangeland and C3 vegetation clipping samples were analyzed on a Europa Scientific automated N and C analyzer (ANCA/NT) with a Solid/Liquid Preparation Module (Dumas combustion sample preparation system) coupled to a Europa 20-20 Stable isotope analyzer continuous flow isotope ratio mass spectrometer (Europa Scientific Ltd., Crewe, England).

Semi-permanent rectangular aluminum anchors (80.5 cm x 43 cm, 0.312 m²) were installed to a depth of 10 cm over representative areas within each treatment plot on 15 May (DOY 135) 2012. Excrement applications were conducted on the morning of 19 June (DOY 170) 2012. Liquid treatments, U and Cw, were applied (1.7 L per treatment anchor) using separate watering pitchers. Homogenous coverage of the entire surface area within treatment anchors was achieved by slowly pouring liquids from an approximate height of 30 cm above the soil surface in effort to allow infiltration with minimal pooling. Each F treatment anchor received an addition of six kg (19.2 kg m⁻²) of wet feces (76% water). Feces were evenly spread across the soil surface within the treatment anchor's area to an approximate thickness of 2.5 cm using a trowel. In terms of the mass and volume applied per area, each F plot was equivalent to 4-6 actual feces patches and each U plot was equivalent to approximately one urine patch (Yamulki et al., 1998). Nitrogen application rates were 430 kg N ha⁻¹ for U and 940 kg N ha⁻¹ for F. Application rates (for the excrement deposition area) in this study represented the average range for grazing cattle, 200-800 kg N ha⁻¹ for urine and 500-2000 kg N ha⁻¹ for feces (Oenema et al., 1997; Wachendorf et al., 2008; van der Weerden et al., 2011).

2.2. Gas and soil analyses

Soil-atmosphere CH₄ and N₂O gas exchange was measured using the static chamber methodology outlined in Mosier et al. (2006). Baseline GHG measurements began on 22 May 2012 and were taken 1-3 times a week for a month prior to treatment application to establish baseline. Following treatment application on 19 June 2012, sampling frequency intensified. Sampling took place one, four, and eight hours following treatment application and then once per day for the next three days. Sampling frequency for the first year of the study was three times a week during the growing season (May to September), two times a week during the fall (October to mid-November), two to four times a month during the winter (mid-November to March), and one time a week during the spring (March-June). During the second year of the study (2013), sampling was reduced to one time per week during the growing season, two times per month during the winter (November to March), and one time per week during the spring of 2014 until the termination of the project in late May. Due to the importance of soil moisture on GHG emissions, sampling frequency was increased following large precipitation (> 10 mm) and freeze-thaw events (as soil temperatures increased from < 0 °C to > 0° C) in order to capture the resulting GHG dynamics.

Decagon Devices EC-TM soil moisture and temperature probes (Decagon Devices Inc., Pullman, WA) were installed to a depth of 10 cm in two of the four replicates for all treatments on each plant community to measure soil moisture and temperature during each trace gas sampling occasion. The raw dielectric permittivity values from the probes were converted to volumetric water content (VWC) using Decagon Devices ProCheck reader with the mineral soil calibration option, which implements the Topp equation (Topp et al., 1980). Volumetric water content values measured by the probes were cross-checked with gravimetric water content values

($n = 5$) determined by the soil core method for the 0 – 10 cm depth. For comparison purposes, the gravimetric water content values were converted to VWC by multiplying by the bulk density. While the probes accurately measured soil water content trends, they consistently overestimated absolute values by $0.05 - 0.10 \text{ m}^3 \text{ m}^{-3}$. Water-filled pore space (WFPS) was then calculated by dividing the VWC by the soil porosity and multiplying by 100 to convert value to a percent.

Gas samples were collected in the morning, between 9:00 - 12:00 h, to approximate an average daily flux and avoid diurnal variation (Mosier et al., 1981; van der Weerden et al., 2013). On the day of treatment application, gas samples were also taken in the afternoon and evening. Van der Weerden et al. (2013) found that gas sampling urine patches three times a week between 10:00 - 12:00 hours resulted in minimal bias when compared to sampling every two hours over 28 days. During trace gas sampling, chambers were seated onto anchors to create an airtight seal while limiting soil disturbance. Chambers were deployed for 30 minutes with gas samples taken at 15 minute intervals (0, 15, and 30 minutes). Gas samples were collected using 35 mL polypropylene syringes. The average air temperature at approximately 10 cm above the soil surface was recorded during each trace gas sampling occasion using a Taylor digital thermometer (Taylor Precision Products, Oak Brook, IL).

Upon completion of sample collection, 25 mL of each sample was immediately transferred to a corresponding 12 mL evacuated-glass exetainer fitted with a screw cap and rubber butyl septum (Exetainer vial from Labco Limited, High Wycombe, Buckinghamshire, UK) for storage until analysis within a month from the collection date (Laughlin and Stevens, 2003). Five mL of each sample was analyzed on an automated gas chromatograph (Varian model 3800, Varian Inc., Palo Alto, CA) equipped with an electron capture detector and a flame ionization detector for N_2O and CH_4 analysis, respectively (Mosier et al., 2006).

Due to the dry soil conditions in 2012, baseline soil samples were not taken until after significant rainfall was received in July. Duplicate cores (3.5 cm core diameter) to 30 cm were sampled in Cb plots, outside of the trace gas anchors, in late July 2012 for both the C3 pasture and C4-dominated native rangeland plant communities. Soil cores were separated into increments according to GRACEnet (2010) protocol (0-5, 5-10, 10-20, 20-30 cm). Soils were air-dried and passed through a two mm sieve to remove the roots and rocks ≥ 2 mm. Roots ≤ 2 mm were removed using an electrostatic wand and remaining soils were analyzed for total soil C and N. Soil inorganic C (IC) concentrations were determined by acidifying soil with 1.0 *N* phosphoric acid and analyzing the C content for acid-treated (SOC only) and non-acid-treated (SOC + IC) (Follett et al., 1997). Soil C and N content analyses were conducted on the same instrument used for plant and feces analyses (see above). Soil bulk densities were determined by using the soil core method and a particle density of 2.65 g cm⁻³. Soil textures were determined using the hydrometer method developed by Bouyoucos (1962).

2.3. Statistical Analysis

Because N₂O and CH₄ concentrations were typically low from our field site, the linear equation method was used in calculating fluxes to avoid over estimation. Parkin et al. (2012) found that linear regression had the lowest detection limit, and was least sensitive to analytical precision and chamber deployment time when compared to the Hutchinson/Mosier, revised Hutchinson/Mosier, and quadratic methods (Hutchinson and Mosier, 1981). Treatment flux rates for each sampling occasion used the average of four replicates. Flux estimates for non-sampling days were calculated by linear interpolation. Cumulative N₂O emissions were calculated by taking the sum of measured and interpolated values (Hoeft et al., 2012). Volume of the chamber

headspace for feces plots was adjusted by subtracting 1.9 cm from the chamber height to account for the thickness of the feces layer.

Treatment effects on the total cumulative CH₄ and N₂O fluxes were determined using the MIXED procedure of SAS (SAS Institute, 2013). Nitrous oxide and CH₄ flux data were non-normal so the data were normalized by log transformation prior to analysis. Means were compared using LSMEANS with Tukey's HSD test for multiple comparisons ($P < 0.10$). Spearman's correlation analyses were used to determine significant relationships ($\alpha = 0.10$) between soil water-filled pore space (WFPS) and temperature and CH₄ and N₂O flux from each treatment. Data for years one and two were analyzed independently. Because the plant communities, C4-dominated native rangeland and C3 pasture, were not randomized and replicated, inferences comparing soil-atmosphere gas exchange between the two plant communities are limited.

The EFs for U and F treatments were calculated by subtracting the cumulative emissions of the Cb treatment (M_C) from the cumulative emissions of the excreta treatment (M_T), U or F, from the first year (19 June 2012 to 18 June 2013), dividing by the rate of urine-N or feces-N applied, and multiplying by 100 (van der Weerden et al., 2011). The Eq. (1) follows:

$$EF (\%) = \frac{M_T - M_C}{\text{Treatment N Applied}} \times 100 \quad (1)$$

3. Results

3.1. Environmental conditions

Drought conditions occurred at the onset of this experiment. The spring (April-June) of 2012 was the second driest in the previous 74 years, receiving 27% (40 mm) of the seasonal average (146 mm). Total precipitation for 2012 (206 mm) was well below (60%) the 74 year annual

average (340 mm) and was dominated by a few, large rain events in July and September. For example, 25% (51 mm) of the annual precipitation in 2012 occurred on July 7th and 8th. In addition, air temperatures were also high (>30 °C) during the early part of the experiment. Overall trends in precipitation during 2013 and the first half of 2014 were close to average, with the exception of a monsoonal rain event in September 2013.

3.2. Plant and Soil Characteristics

Dry conditions during 2012 inhibited vegetative growth. The C4-dominated native rangeland biomass production in 2012 (358 kg d.m. ha⁻¹) was approximately 39% of the 20 year (1992-2011) average 892 kg d.m. ha⁻¹. Aboveground biomass sampling was not conducted on the C3 pasture during 2012 due to the lack of growth. Nitrogen concentrations of the aboveground vegetation from the U and F plots were 15% and 21% greater, respectively, than in the control plots on C4-dominated native rangeland 29 July 2012 (41 days after treatment application), while in spring 2013 (7 June 2013; 354 days after treatment application) N concentrations of the aboveground vegetation from the same plots were 62% and 57% greater than control plots (data not shown). Nitrogen concentrations of the aboveground vegetation from U and F plots in C3 pasture were 22% greater than the control plots in May 2013 (data not shown). Soil temperature was relatively warm and WFPS extremely low at the time of treatment application (Figure 1).

3.3. N₂O emissions

Cumulative N₂O emissions from the excrement plots, U and F, were significantly greater ($P < 0.0001$) than those from the control plots, Cw and Cb, over the first year of the study (19 June 2012 to 18 June 2013) (Table 2). Emissions were also greater from the F plots (1.26 and

1.27 kg N₂O-N ha⁻¹ for C4-dominated native rangeland and C3 pasture, respectively) compared to the U plots (0.80 and 0.94 kg N₂O-N ha⁻¹ for C4-dominated native rangeland and C3 pasture, respectively) for both plant communities. However, after accounting for the greater N application rate for the F treatment (940 kg N ha⁻¹) relative to the U treatment (430 kg N ha⁻¹) the EFs for U and F were not significantly different on C4-dominated native rangeland (0.11% vs. 0.10%, $P = 0.46$) and C3 pasture (0.13% vs. 0.10%, $P = 0.17$)(Table 2).

During the second year (19 June 2013 to 27 May 2014) of the study, significant differences in cumulative N₂O emissions between the excrement and control plots were still present (Table 2). On the C4-dominated native rangeland, N₂O emissions from the F plots were significantly greater than the control plots, Cw ($P = 0.0033$) and Cb ($P = 0.0059$), while emissions from the U plots were greater than the Cw plots ($P = 0.08$). The only significant difference in N₂O emissions observed on the C3 pasture during the second year was between F and Cw ($P = 0.08$).

Large N₂O fluxes from the F plots, 74 and 262 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$ on C4-dominated native rangeland and C3 pasture, respectively, occurred 3 days following a substantial rain event (53 mm) on 7-8 July, 2012. Elevated N₂O emissions were also observed from the U treatment following this precipitation event for the C4-dominated native rangeland (35 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$) and C3 pasture (49 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$) communities, but the response was delayed 10 days, July 18 (Figure 2). Nitrous oxide emissions from excrement plots remained above baseline levels until the end of July 2012, when the soil WFPS dropped below 30%. Substantial N₂O emissions were observed consistently from excrement plots following large precipitation events (>10 mm) until the fall of 2013, about 15 months post application (Figures 2 and 3). By the spring of 2014,

N₂O fluxes from the excrement patches were similar to the control plots except for two instances on the C4-dominated native rangeland (Figure 3).

Soil WFPS and temperature were significant drivers of N₂O emissions from the excrement plots. Positive correlations between WFPS and N₂O flux from the U and F plots were highly significant ($P < .0001$) for soils of both C4-dominated native rangeland and C3 pasture during the first year of the study (19 June 2012 to 18 June 2013). During the second year of the study, lower positive correlations were observed between WFPS and N₂O flux for the excrement plots, all of which were significant except the U plots on the C3 pasture (Table 3). The majority of N₂O emissions occurred when WFPS was between 25-55% (data not shown). Correlations between soil temperature and N₂O flux were negative for all treatments during the first year of the study, with the exception of the F treatment on the C3 pasture. In the second year, significant positive correlations between soil temperature and N₂O flux were observed from the F plots on both plant communities and the U plots on C3 pasture (Table 3).

3.4. CH₄ Uptake/Emissions

Cumulative CH₄ net production (kg CH₄-C ha⁻¹) was observed from the F plots on both plant communities over the first year (19 June 2012 to 18 June 2013), while all other treatment plots (U, Cw, and Cb) exhibited a net uptake of CH₄. However, during this period CH₄ uptake was significantly less ($P < 0.10$) from the U plots compared to the control plots, Cw and Cb, on both plant communities (Table 2). Over the second year (19 June 2013 to 27 May 2014) of the study, cumulative CH₄ net uptake occurred from all treatment plots, but was significantly less ($P < 0.10$) from the F compared to the control plots, with the exception of C4-dominated native rangeland – Cw (C3 pasture - Cw $P = 0.02$, C3 pasture - Cb $P = 0.07$, C4-dominated native

rangeland – Cw $P = 0.22$, and C4-dominated native rangeland – Cb $P = 0.06$). In addition, CH₄ uptake from U plots was significantly less ($P < 0.10$) than that of the Cw plots ($P = 0.08$) on the C3 pasture (Table 2).

Methane production from the F treatment was observed for approximately six days following treatment application (Figure 4). Cumulative CH₄ emissions from F plots during this period (19 June 2012 to 24 June 2012) were 1.58 and 1.52 kg CH₄-C ha⁻¹ on C4-dominated native rangeland and C3 pasture, respectively. Maximum CH₄ emissions were observed four and eight hours following treatment application for C3 pasture and C4-dominated native rangeland, respectively. Approximately a week after treatment application, net CH₄ uptake resumed from the F plots (with the exceptions of 18 and 25 July 2012 on both plant communities), but at a significantly lesser rate than the other treatments. Methane uptake rates were still significantly less from the F plots compared to the Cb plots during the second year of the study (Figure 5). During the entire study, net CH₄ production from U plots occurred only once on each system (0.47 ± 1.28 on 15 August 2012 for C4-dominated native rangeland and 0.97 ± 3.33 $\mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$ on 4 January 2013 for C3 pasture) (Figure 4).

Relationships between CH₄ uptake and WFPS were curvilinear, with the greatest CH₄ uptake rates occurring when the WFPS was between 20-40% (Figure 6). Immediately following the large precipitation event on 7-8 July 2012, CH₄ uptake rates were near 0 $\mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$ from all treatments. As soils dried, CH₄ uptake rates increased until the third week of July when rates peaked from the control plots at 47 and 32 $\mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$ on the C4-dominated native rangeland and C3 pasture, respectively. Methane uptake rates decreased over the next couple months as soils dried out to WFPS levels below 20% (Figure 6). Rewetting of soils from mid-September precipitation provided sufficient soil moisture levels to support methanotrophic

activity (Figures 1 and 4). Substantial CH₄ uptake occurred during the winter, accounting for 18-20% of the cumulative uptake for the control plots in both plant communities. Trends in CH₄ flux were similar between years and plant communities; however cumulative CH₄ uptake was substantially less on the C3 pasture compared to C4-dominated native rangeland for each treatment (Table 2).

4. Discussion

4.1. N₂O

Low N₂O EFs for cattle excrement observed in this study, 0.10-0.13%, are at the low end of the range reported in the literature; 0.1 – 3.8% and 0.02 – 0.7% for urine and feces, respectively (Oenema et al., 1997; van der Weerden et al., 2011; Hoefft et al., 2012; Lessa et al., 2014; Rochette et al., 2014; Sordi et al., 2014; Mori and Hojito, 2015). Lessa et al. (2014) determined that during the dry season the N₂O EFs for cattle urine and feces on Brazil pastures were near zero. A laboratory study found that soil texture was an important factor controlling N₂O EFs from urine-treated soils, with greater EFs for fine-textured relative to coarse-textured sands (Singurindy et al., 2006). Thus coarse-textured soils in combination with the dry soil conditions likely contributed to the extremely low EFs reported in the present study. The IPCC uses a default EF of 2% to calculate emissions from manure deposited on pasture, which implicitly assumes that the applied-N is entirely cycled within one year (IPCC, 2006). In accordance to previous findings, our results suggest that calculating N₂O emissions for urine and feces deposited on SGS native rangeland and cool-season pasture using the IPCC Tier 1 Default EF (2%) would result in a significant overestimation of emissions (IPCC, 2006; van der Weerden et al., 2011; Lessa et al., 2014; Rochette et al., 2014; Sordi et al., 2014).

The coarse-textured soils in the present study theoretically provided a highly aerobic environment in the upper soil profile, ideal for the nitrification pathway (Parton et al., 1996; Rochette et al., 2008). Baral et al. (2014) determined that nitrification was the primary pathway for N₂O emissions from urine patches on a sandy soil in Denmark. However, conditions for denitrification may have occurred within excrement patches following a substantial rain event (51 mm) on 7-8 July, 2012, when the WFPS was near 60% and the availability of labile C and mineral N was presumably high. Furthermore, CH₄ production from the F treatment plots was observed on both plant communities during July, which implies anaerobic conditions were present. The two-year study period, was a sufficient amount of time to capture the majority of the N₂O emissions from the U and F patches on C3 pasture and C4-dominated native rangeland as fluxes from the excrement plots remained near baseline in the spring of 2014 even when the soil moisture was relatively high.

While not uncommon, drought conditions were experienced at the time of treatment application, which may have led to a lesser proportion of excrement-N emitted as N₂O and a greater proportion emitted as NH₃ and NO_x. Drought at the SGS is anticipated to occur more frequently as a result of climate change (Hartmann and Niklaus, 2012). Ball and Ryden (1984) found that on average 28% of urine-N was volatilized as NH₃, but during warm, dry conditions NH₃ volatilization accounted for 66% of applied urine-N on a New Zealand pasture. Furthermore, dry conditions likely accelerated the formation of the surface crust on feces patches, limiting gas diffusion and nutrient transport into the soil profile and thus reducing N₂O emissions (van der Weerden et al., 2011). In addition, a significant proportion of the urine-N emitted during nitrification may have been lost as nitric oxide (NO) from the sandy soils studied in this experiment. Mosier et al. (1998) found that NO_x emissions were 10-20 times greater than

N₂O emissions from a coarse textured N amended soil at the SGS. Further research is needed to determine whether the IPCC Tier 1 Default EF is representative of N₂O emissions from urine and feces patches deposited on SGS native rangeland and cool-season pasture during years of average and above average precipitation.

Mosier et al. (1998) determined that 0.5-1% of a synthetic urine solution (45 g N m⁻²) was emitted as N₂O from SGS soil. While the EF for cattle urine patches on SGS native rangeland in the present study was considerably less than that reported by Mosier et al. (1998) (0.5-1%), dry conditions and the use of real urine opposed to synthetic may have contributed to the lesser EF. According to Kool et al. (2006) synthetic urine solutions that do not contain hippuric acid may over estimate N₂O emissions by up to 50%. Benzoic acid, a by-product of hippuric acid, has been shown to inhibit enzymatic and microbial activity (Fenner et al., 2005), which alters N turnover and hence N₂O emissions.

Even though cumulative N₂O emissions were similar between plant communities, the magnitude of seasonal fluxes varied. This phenomenon was likely due to phenology differences between the two plant communities. Lower N₂O emissions following the large rain event in July from the excrement plots on the C4-dominated native rangeland compared to the C3 pasture could be due to greater plant N uptake from the C4-dominated perennial grasses during the hot summer months compared to the C3 pasture. In addition, there is more bare ground interspace on the C3 pasture compared to the C4-dominated native rangeland, which could have contributed to greater N₂O emissions shortly after treatment application. Aboveground biomass production for the C4-dominated native rangeland in the 2012 growing season was less than half of the 20 year average. While we did not conduct an aboveground biomass sampling on the C3 pasture in 2012, the production of these systems is highly dependent on spring precipitation, which was

only 27% of the 74 year seasonal average. Thus in order to better understand trace gas dynamics from these plant communities, additional research is needed during average precipitation years, when plant-microbial competition for N is greater.

4.2. CH_4

Our hypothesis that the addition of urine and feces would reduce the rate of CH_4 uptake was supported from the U and F plots on both plant communities. This observation was likely the result of high concentrations of mineral N and the competition of NH_3 and CH_4 for the active binding site of the active enzyme (Epstein et al., 1998; Sylvia, 2005). Reduced CH_4 uptake from F patches may also have resulted from inhibited gas diffusion due to a surface crust that forms as the patch dries. Due to the drought conditions at the time of treatment application, desiccation of the F patch likely occurred faster than it would have during an average precipitation year, thus reducing the period of time when anaerobic conditions were present within the patch and an earlier onset of the surface crust resulting in less CH_4 production (Yamulki et al., 1999). Cumulative CH_4 uptake was less for all treatments on the C3 pasture compared to the C4-dominated native rangeland during the first year of the study, while CH_4 uptake between plant communities was similar during the second year of the study. This phenomenon may have been due to greater sand content in the soil surface layer on the C3 pasture relative to C4-dominated native rangeland, leading to greater evaporation rates at the soil surface exacerbating biological limitation on CH_4 uptake during dry conditions (Table 1) (Mosier et al. 1997).

Methane production from the F plots initially following treatment application was likely due to a large microbial population, high concentration of C, and high water content of the added feces (75% water content). Yamulki et al. (1999) found 80% of CH_4 production occurred within

the first week of feces patch establishment and CH₄ uptake rates from fecal patches returned to baseline levels 15 days after treatment application on a perennial ryegrass pasture. In contrast, significant differences in CH₄ uptake between feces and control plots were still observed during the second year of the present study. Thus, a study period greater than two years is needed in order to determine the cumulative effects of excrement patches on CH₄ uptake on C3 pasture and C4-dominated native rangeland.

The isolated CH₄ production events on 18 and 25 July 2012 were accompanied by warm, moist soil conditions increasing the likelihood of soil anaerobic microsites. In addition, noticeable dung beetle activity was documented on F plots during the first half of July, which could have affected the CH₄ flux dynamics. Dung beetle activity can reduce the rate of CH₄ production and increase N₂O emissions through the aeration of fecal patches (Penttilä et al., 2013). Additional research is needed to better understand the influence that dung beetles have on GHG emissions under various environmental and fecal patch conditions.

The highest rates of CH₄ uptake for all the treatments occurred when WFPS was between 23-35% and 25-40% on C4-dominated native rangeland and C3 pasture, respectively. These results do not correspond to earlier findings of Mosier et al. (1996) and Chen et al. (2010) who found that maximum CH₄ uptake rates occurred with 13-23% WFPS levels. Differences in soil moisture sampling methodologies are likely responsible for this discrepancy. In the present study WFPS was calculated using soil volumetric water content values from time-domain reflectrometry (TDR) probes for the 5-10 cm depth, Mosier et al. (1996) conducted gravimetric analysis of soil water content for the 0-15 cm depth, and Chen et al. (2010) used TDR probes for the 0-6 cm depth. We found that the TDR probes used in the present study accurately captured trends in soil moisture, but overestimated absolute values.

5. Conclusion

Our results show that various land management and environmental factors interact to control N₂O and CH₄ fluxes. This suggests that models need to account for the legacy effects of disturbance, plant growth patterns, moisture, temperature, and heterogeneity of C and N resources across the landscape to accurately predict GHG fluxes. The potential to mitigate GHG emissions is limited as management options for semi-arid rangeland primarily consist of modifying the stocking rate and the duration of the grazing season (Liebig et al. 2005). Future trace gas research on semi-arid grasslands should focus on pasture areas where cattle tend to congregate, such as pasture corners and areas near water tanks. These highly disturbed areas sustain high rates of C and N deposition (Augustine et al., 2013), soil compaction, and reduced vegetative cover, which may lead to greater N₂O emissions and reduced CH₄ uptake.

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Figure 1. Mean (n=2) water-filled pore space (WFPS; %) and soil temperature (°C) for the 5-10 cm depth from soils of both plant communities, a.) C4-dominated native rangeland and b.) C3 pasture, for days that trace gas sampling occurred between 5/22/12 – 5/27/14.

*Trends in WFPS are accurately represented while absolute values are not.

Figure 2. Average nitrous oxide (N_2O ; $\mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$) fluxes $\pm$ standard errors (n = 4) for each treatment, urine (U), feces (F), and control blank (Cb) from a.) C4-dominated native rangeland and b.) C3 pasture soils and precipitation (mm) from 22 May 2012 to 21 May 2013. The arrow in the diagram indicates when the treatments were applied.

Figure 3. Average nitrous oxide (N_2O ; $\mu\text{g N}_2\text{O-N m}^{-2} \text{ hr}^{-1}$) fluxes $\pm$ standard errors (n = 4) for each treatment, urine (U), feces (F), and control blank (Cb) from a.) C4-dominated native rangeland and b.) C3 pasture soils and precipitation (mm) from 31 May 2013 to 27 May 2014.

Figure 4. Average methane (CH_4 ; $\mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$) fluxes $\pm$ standard errors (n = 4) for each treatment, urine (U), feces (F), and control blank (Cb) on a) C4-dominated native rangeland and b) C3 pasture from 22 May 2012 to 21 May 2013. The arrow in the diagram indicates when the treatments were applied.

Figure 5. Average methane (CH_4 ; $\mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$) fluxes $\pm$ standard errors (n = 4) for each treatment, urine (U), feces (F), and control blank (Cb) on a) C4-dominated native rangeland and b) C3 pasture from 31 May 2013 to 27 May 2014.

Figure 6. Average methane (CH_4 ; $\mu\text{g CH}_4\text{-C m}^{-2} \text{ hr}^{-1}$) fluxes (n = 4) for each treatment, urine (U), feces (F), and control blank (Cb) as a function of water-filled pore space (WFPS; %) on a.) C4-dominated native rangeland and b.) C3 pasture.

*Trends in WFPS are accurately represented while absolute values are not.

Figure 1

a.)

b.)

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Tables

Table 1. Soil properties (texture n=2; bulk density and total N and C n=16) for the 0-10 cm depth of plant communities, C4-dominated native rangeland and C3 pasture.

Site	Depth Increment (cm)	Sand (% $\pm$ SE)	Clay (% $\pm$ SE)	Bulk Density (g cm ⁻³ $\pm$ SE)	Total N (Avg. % $\pm$ SE)	Total C (Avg. % $\pm$ SE)
C4-dominated Native Rangeland						
	0-5	63 $\pm$ 7.1	9 $\pm$ 1.0	1.16 $\pm$ 0.03	0.12 $\pm$ 0.008	1.32 $\pm$ 0.13
	5-10	72 $\pm$ 2.0	10 $\pm$ 0.0	1.37 $\pm$ 0.03	0.07 $\pm$ 0.002	0.66 $\pm$ 0.02
C3 Pasture						
	0-5	83 $\pm$ 0.6	5 $\pm$ 0.8	1.46 $\pm$ 0.04	0.08 $\pm$ 0.011	0.76 $\pm$ 0.14
	5-10	83 $\pm$ 0.8	5 $\pm$ 1.0	1.45 $\pm$ 0.03	0.06 $\pm$ 0.002	0.53 $\pm$ 0.02

Table 2. Nitrous oxide (N₂O) and methane (CH₄) cumulative fluxes (kg ha⁻¹) and standard error for year one (19 June 2012 to 18 June 2013; 365 days) and year two (19 June 2013 to 27 May 2014; 343 days) for treatments urine (U), feces (F), water (Cw), and blank (Cb) for soils of each plant community, C4-dominated native rangeland and C3 pasture. The N₂O emission factors are provided for U and F on each plant community, C4-dominated native rangeland and C3 pasture.

		<i>Emission Factor</i>	<i>Cumulative Flux</i>	
			Year 1	Year 2
<i>N₂O</i>		% of Excrement N	<i>kg N₂O-N ha⁻¹</i>	
	C4-dominated native rangeland			
	U	0.11	0.80 ± 0.14 ^a	0.37 ± 0.03 ^{a,b}
	F	0.10	1.26 ± 0.30 ^b	0.56 ± 0.09 ^b
	Cw		0.31 ± 0.03 ^c	0.24 ± 0.02 ^c
	Cb		0.33 ± 0.04 ^c	0.28 ± 0.06 ^{a,c}
	C3 pasture			
	U	0.13	0.94 ± 0.09 ^a	0.31 ± 0.07 ^{a,b}
	F	0.10	1.27 ± 0.14 ^b	0.39 ± 0.05 ^a
	Cw		0.37 ± 0.03 ^c	0.24 ± 0.06 ^b
	Cb		0.37 ± 0.03 ^c	0.28 ± 0.04 ^{a,b}
<i>CH₄</i>			<i>kg CH₄-C ha⁻¹</i>	
	C4-dominated native rangeland			
	U		-1.49 ± 0.06 ^a	-1.24 ± 0.10 ^{a,b}
	F		0.30 ± 0.10 ^b	-1.17 ± 0.08 ^a
	Cw		-1.99 ± 0.12 ^c	-1.34 ± 0.12 ^{a,b}
	Cb		-2.09 ± 0.09 ^c	-1.50 ± 0.04 ^b
	C3 pasture			
	U		-1.08 ± 0.07 ^a	-1.08 ± 0.13 ^{a,b}
	F		0.64 ± 0.12 ^b	-0.94 ± 0.15 ^a
	Cw		-1.56 ± 0.07 ^c	-1.39 ± 0.11 ^c
	Cb		-1.63 ± 0.21 ^c	-1.52 ± 0.17 ^{b,c}

Cumulative values are an average of four replicates for each treatment. Cumulative values with different letters indicate a significant difference (ANOVA with Tukey's HSD adjustment, $\alpha=0.10$).

Table 3. Spearman's correlation coefficients (r) with p-values for the relationship between soil water-filled pore space (WFPS) and soil temperature (°C) to N₂O flux for each treatment, urine (U), feces (F), water (Cw), and blank (Cb), and year (Year 1, 19 June 2012 to 18 June 2013; Year 2, 19 June 2013 to 27 May 2014) from C4-dominated native rangeland and C3 pasture.

Plant Community / Treatment	WFPS (%)	Soil Temperature (°C)			
		-----r (p-value)-----			
		<i>Year 1</i>	<i>Year 2</i>	<i>Year 1</i>	<i>Year 2</i>
C4-dominated Native Rangeland					
U	0.43 (<.0001)*	0.31 (0.006)*	-0.23 (0.008)*	0.11 (0.33)	
F	0.65 (<.0001)*	0.25 (0.03)*	-0.023 (0.79)	0.24 (0.04)*	
Cw	0.028 (0.7)	0.022 (0.87)	-0.29 (0.0005)*	0.35 (0.008)*	
Cb	0.017 (0.8)	-0.17 (0.16)	-0.16 (0.05)*	0.12 (0.3)	
C3 Pasture					
U	0.57 (<.0001)*	0.14 (0.2)	-0.009 (0.9)	0.20 (0.07)*	
F	0.64 (<.0001)*	0.31 (0.005)*	0.22 (0.009)*	0.35 (0.001)*	
Cw	0.11 (0.18)	0.30 (0.007)*	-0.19 (0.02)*	-0.27 (0.02)*	
Cb	0.23 (0.006)*	-0.00903 (0.9)	-0.25 (0.003)*	0.10 (0.4)	

*Indicates a significant difference ($\alpha = 0.10$).