

Influence of soil properties on N₂O and CO₂ emissions from excreta deposited on tropical pastures in Kenya

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ABSTRACT

Urine and dung patches deposited by grazing cattle on grassland are an important source of nitrous oxide (N₂O). While a number of studies have investigated the effects of excreta on soil N₂O fluxes in developed economies and in China, observations in sub-Saharan Africa (SSA) are scarce. Moreover, the effects of soil properties (e.g. pH or texture) on N₂O emissions from excreta patches have hardly been studied. In this study we investigated the importance of soil properties on N₂O and carbon dioxide (CO₂) emissions from cattle excreta (dung, urine, and manure [dung + urine]) for five typical tropical soils in Kenya. For this, intact soil cores were translocated from Western Kenya (Nandi county) to Nairobi, where N₂O and CO₂ fluxes were measured over four individual periods (two during dry seasons and two during wet seasons). Fluxes were measured for between 25 and 73 days following surface application of excreta, depending on how quickly emissions returned to baseline. Both dung and manure applications led to increased CO₂ and N₂O fluxes during both dry and wet seasons. On average, the N₂O emission factor (EF) for manure was higher than for dung. The EFs during the wet season were higher for both the dung (0.12%) and urine (0.50%) compared to the dry season EFs (0.01% and 0.07% for dung and urine respectively). Soil type had no measurable effect on N₂O and CO₂ emissions for either dung or manure application. In contrast, soil clay content was negatively ($P < 0.05$) and pH positively ($P < 0.05$) correlated with N₂O emissions after urine application. Assuming an excreta-N ratio of dung to urine of 66:34, as evidenced in earlier studies for SSA, and averaging across all treatments and soils, we calculated a cattle excreta N₂O EF of 0.14%, which is one magnitude lower than the IPCC default N₂O EF of 2%. Our results call for a revision of the IPCC guidelines for calculating N₂O emissions from excreta deposition on tropical rangelands.

1. Introduction

Nitrous oxide (N₂O) is a potent greenhouse gas with a global warming potential 265 times greater than that of carbon dioxide (CO₂) on a per mass basis over 100-years (IPCC, 2014), and it is estimated to account for 6% of total anthropogenic global warming (Davidson, 2009). Current atmospheric concentrations of N₂O are approximately 18% higher than pre-industrial levels and concentrations were projected to further increase by 35–60% between 2007 and 2030 (Smith et al., 2007). More specifically, annual N₂O emissions from excreta (i.e. dung

and urine) deposited by grazing livestock were estimated to be 1.5 Tg N₂O–N yr^{−1}, which is equivalent to 41% of all N₂O emissions derived from global livestock production systems and 22% of total anthropogenic N₂O emissions (Oenema et al., 2005; Taghizadeh-Toosi et al., 2011). Smallholder mixed-crop and pastoral livestock systems, which rely on native pasture grazing are widespread across sub-Saharan Africa (SSA), and support an estimated 123 million cattle (Thornton and Herrero, 2014). Due to the free grazing in daytime, it is estimated that in SSA more than 40% of total cattle excreta are deposited on rangelands (Rufino et al., 2006). As a result, direct N₂O emissions from urine and

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dung excreta on SSA pastures were estimated to cause 62% of total annual N_2O emissions in the region (Tully et al., 2017).

Up to 75–90% of nitrogen (N) ingested by grass-fed animals is returned to the soil through dung and urine excretion (Saggar et al., 2013; Bell et al., 2015). As adult cattle can excrete as much as 23 kg dung and 21 L urine over 13 dung and ten urine patches per day (Haynes and Williams, 1993), the amount of N contained in individual urine or dung patches by far exceeds plant N demands in the deposited area (Di and Cameron, 2007; Chadwick et al., 2018). In fact, the amount of N in a dung patch was calculated to be equivalent to up to 1130 kg N ha⁻¹ (Saarijärvi et al., 2006), while N loading rates for urine patches were found to be equivalent to 613 kg N ha⁻¹ for dairy cattle and 345 kg N ha⁻¹ for beef cattle (Selbie et al., 2015). Thus, high environmental N losses due to nitrate (NO_3^-) leaching, ammonia (NH_3) volatilization, or other gaseous emissions (N_2O , nitric oxide [NO] and dinitrogen [N_2]) are often associated with N excretion on rangeland (Chadwick et al., 2018).

Although there are many studies focusing on N_2O emissions from excreta patches, most of these have focused either on timing (Lessa et al., 2014; Rochette et al., 2014; Bell et al., 2015) or application rate (Van Groenigen et al., 2005a; Sordi et al., 2014; Zhu et al., 2018) of excreta, while neglecting the influence of soil properties on N_2O emissions. However, the magnitude and temporal dynamics of soil N_2O emissions are determined not only by soil N availability but also by key soil properties (Butterbach-Bahl et al., 2013; Neira et al., 2015; Samad et al., 2016; Wang et al., 2018a,b; Ghezzehei et al., 2019). For example, soil texture and soil organic carbon content (SOC) affect the water holding capacity and therefore the gas diffusivity of soils. Both factors are crucial parameters controlling the availability of oxygen (O_2) in soils and consequently soil microbial processes, thus N_2O production (Davidson et al., 2000; Butterbach-Bahl et al., 2013; Balaine et al., 2016). Consistent with this, two other previous studies suggested that certain soil properties such as soil texture, pH and bulk density may affect denitrification rates and soil N_2O emissions from livestock excreta applied to pasture soils (Cai and Akiyama, 2016; Wang et al., 2018a,b). However, to our knowledge, no previous study has systematically explored how soil properties may affect N_2O emissions from excreta patches.

In general, N_2O emissions from animal excreta on grasslands have been found to scale linearly with the dung mass (Zhu et al., 2018) or urine volume (Sordi et al., 2014), with higher N_2O emissions from cattle urine compared to cattle dung due to higher N availability in urine and greater interactions of urine with the soil microbial communities as it infiltrates into the soil (Cai et al., 2017).

Depending on diet, N partitioning between cattle dung and urine is thought to range from 50:50 to 25:75 (Valk, 1994; Webb and Missetbrook, 2004; Van der Weerden et al., 2011; Chadwick et al., 2018). The split depends on the crude protein (CP) intake and concentration in feedstuffs. In Western Kenya, CP of feed ranges from 3.2 to 14% (Onyango et al., 2019), which is much less than that in intensive production systems such as in the USA where CP concentrations range from 17 to 23% (Council, 2015; Korir et al., 2016). The low CP concentration of feeds in SSA therefore results in an average N partitioning between cattle dung and urine of 66:34 (Rufino et al., 2006).

Given the differences in N_2O emission factors (EF) between dung and urine (Cai et al., 2017), disregarding excreta-N partitioning might cause large uncertainties when estimating regional N_2O emission inventories. However, the Intergovernmental Panel on Climate Change (IPCC) guidelines do not disaggregate the EF for urine and dung, rather they propose a default N_2O EF of 2% excreta-N (IPCC, 2006). As such, many recent studies have suggested that disentangling N_2O emissions from urine and dung is critical to improve our understanding of N_2O emissions from grazed pastures, better assess this key source for atmospheric N_2O and identify potential mitigation options (Van der Weerden et al., 2011; Krol et al., 2016; Chadwick et al., 2018). Despite differences in climate and soils, most countries in SSA, due to the scarcity of local and regional studies, still use the IPCC default value to estimate their

country-level greenhouse gas (GHG) inventories, even though this may not accurately reflect the specific conditions of SSA (Ogle et al., 2014). The objectives of our study were to: a) examine the influence of soil type on the CO_2 and N_2O emissions from single dung, urine or manure patches after deposition on tropical pasture; b) quantify the cumulative CO_2 and N_2O emissions from urine and dung applications to the five soil types; and c) examine whether CO_2 and N_2O emission magnitudes from dung, urine or manure patches would differ between dry and wet seasons. We hypothesized that: a) soil properties such as pH or texture have minimal effects on N_2O emissions from dung but significant effects on N_2O emissions from urine deposited onto pasture; b) soils with a high SOC would have higher N_2O emissions from urine deposited onto pasture than soils with low SOC; c) N_2O emissions would be higher and effects of soil properties would be stronger during the wet than during the dry season; and d) soil CO_2 fluxes would be stimulated by all additions of excreta.

2. Materials and methods

2.1. Soil and site description

For our study, five soil types including (i) poorly-drained Gleysols, (ii) well-drained Nitisols, (iii) well-drained Acrisols, (iv) well-drained Cambisols, and (v) well-drained Ferralsols (IUSS Working Group WRB, 2014) differing in SOC (34–45 g C kg⁻¹ dry matter), clay content (29–53%) and pH (5.3–6.4) were selected in Nandi County, Western Kenya (Table 1). Sampling locations were selected based on a soil map for Kenya (Jaetzold et al., 2010) and the willingness of smallholder farmers to participate in the study. In total, 14 farms with grazing pastures or rangelands were chosen. There were four farms each for Nitisols and Gleysols, while two farms each were allocated for the other soil types. Before taking the cores, grass was cut down to 2 cm above the soil surface, after which intact soil cores with a diameter of 26 cm and a depth of 12 cm were collected with spades. In the area immediately adjacent to the soil cores, we took soil samples using 100 cm³ soil cylinders to measure soil bulk density (BD) and with a 4.5 cm diameter soil auger to 5 cm depth to measure soil carbon (C), N and pH (for a detailed description see Saiz and Albrecht, 2016).

The intact soil cores were carefully wrapped in plastic bags and put into 50 L-buckets. Small holes were made to allow for gas exchange, and the cores were immediately transported to the Mazingira Centre of the International Livestock Research Institute (ILRI) in Nairobi, Kenya (S 1°16'13"; E 36°43'23"; altitude 1809 m asl). At the study site, soil cores were embedded into a flat grassland dominated by a mixture of Kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov.) and Rhodes grass (*Chloris gayana* Kunth), i.e. grass species that were also found in the swards of the sampled rangelands. In addition to the five different soil types, dried and sieved (mesh width 2 mm) sand was included in this study as a control. Unfortunately, the sand contained high amount of soil, which still supported microbial activities. The soil cores were placed into holes (30 cm diameter, 15 cm deep) in an existing grassland immediately adjacent to the Mazingira Centre that had been lined with the same sand at the bottom. The sand was also used to fill the gaps on the sides of the cores in order to separate the soil cores from the adjacent soil. This procedure did not likely significantly affect N movement in the soil as the main rooting depth of the sward was generally less than 15 cm and as vertical N transport with the soil water movement was not influenced. After installation, soil cores were left to settle for three weeks to allow for equilibration with environmental conditions at the ILRI site. Thereafter, the grass was cut down to 2 cm again and excreta applied in the following days. Note that before the start of each trial, new soil cores were obtained from the field sites in Nandi County, transferred to ILRI, Nairobi, and placed in newly opened holes to avoid any legacy effects from the previous trial(s). A meteorological station was installed to record precipitation (tipping rain gauge, ECRN-100 high-resolution, Decagon, Pullman, WA; USA) and air temperature

Table 1

Soil pH, bulk density, carbon and nitrogen concentration, C/N ratio, soil sand and clay content of the different soils used in the experiment.

Soil type	pH	Bulk density (g cm ⁻³)	C content (g kg ⁻¹ dry matter)	N content (g kg ⁻¹ dry matter)	CEC (cmol kg ⁻¹)	C/N ratio	Sand (%)	Clay (%)
Gleysols	6.0 ± 0.3 ^{bc}	0.94 ± 0.13 ^b	45.6 ± 9.3 ^a	3.91 ± 0.92 ^a	151 ± 16 ^a	12 ± 0.5 ^{bc}	27.2 ± 14.4 ^{bc}	46.6 ± 2.9 ^a
Nitisols	5.9 ± 0.5 ^c	0.95 ± 0.11 ^b	42.9 ± 8.7 ^a	3.63 ± 0.93 ^{ab}	147 ± 62 ^{ab}	12 ± 0.9 ^b	25.2 ± 5.0 ^c	50.1 ± 5.3 ^a
Acrisols	6.4 ± 0.6 ^b	1.17 ± 0.14 ^a	35.2 ± 6.1 ^b	3.12 ± 0.64 ^{bc}	137 ± 37 ^{ab}	11 ± 0.6 ^c	52.7 ± 1.4 ^a	28.6 ± 21.4 ^b
Cambisols	5.6 ± 0.4 ^d	1.10 ± 0.13 ^a	34.7 ± 6.5 ^b	2.89 ± 0.69 ^c	85 ± 43 ^{bc}	12 ± 0.8 ^b	37.7 ± 2.8 ^b	42.6 ± 4.2 ^a
Ferralsols	5.3 ± 0.5 ^e	0.98 ± 0.08 ^b	37.0 ± 5.1 ^b	2.80 ± 0.61 ^c	67 ± 7 ^c	14 ± 1.7 ^a	24.5 ± 1.4 ^c	52.6 ± 4.3 ^a
Sand	8.4 ± 0.0 ^a	1.07 ± 0.11 ^{ab}	0.3 ± 0.1 ^c	0.00 ± 0.00 ^d	–	–	–	–

Values are mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences within columns (P < 0.05).

and humidity (ATMOS 14, Decagon, Pullman, WA; USA) at a 5 min resolution. Soil moisture and temperature at 0.05 m depth were also measured during gas sampling (Decagon 5TM sensors, Decagon, Pullman, WA; USA).

2.2. Experimental design

In this experiment, four separate trials were conducted; two during the wet season and two during the dry season. Trial 1 was conducted from 26 July 2016 to 25 August 2016 (dry season) with eight cores of six different soils, resulting in a total of 48 soil cores. We applied 0.5 kg of fresh dung to the soil surface of four cores of each soil type, while the other four served as controls (i.e. no application, Fig. 1). For the following three trials, 12 new soil cores of the same six soils were installed, giving a total of 72 soil cores for each of the remaining three trials. Trial 2 was conducted from 16 October 2017 to 01 December 2017 (wet season). The 12 soil cores of each soil type were divided evenly into three groups that received either no application, or a surface application of either 0.5 kg fresh dung or 0.5 kg fresh manure (dung + urine) (Fig. 2). Trial 3 was conducted from 25 March 2018 to 14 May 2018 (wet season, Fig. 3), while the fourth trial was conducted from 02 July 2018 to 04 October 2018 (dry season, Fig. 4). In these two trials, 12 soil cores of each soil type were divided evenly into four groups with either no application, or with 0.5 L distilled water, 0.5 L urine or a surface application of 0.5 kg fresh dung. Excreta application was done following a few days of background measurements (i.e. prior to excreta application) at the beginning of each trial (see Figs. 1–4). Excreta application rates were scaled to the chamber area (0.04 m²) based on previous studies that found that emissions scale linearly with mass/volume of applied excreta (Sordi et al., 2014; Zhu et al., 2018). Due to an

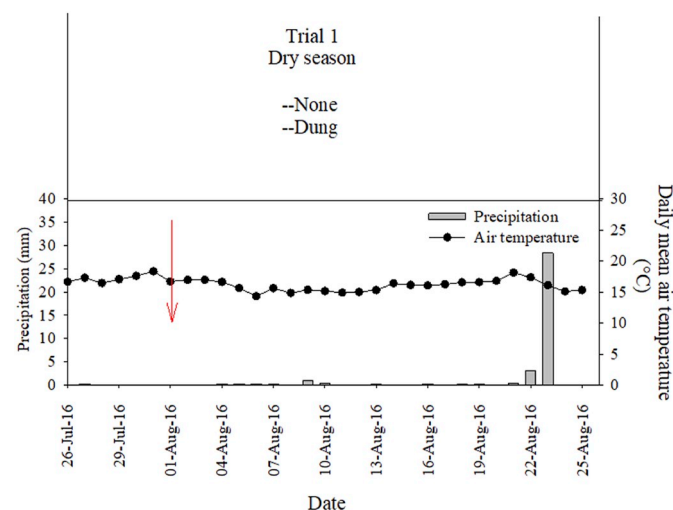


Fig. 1. Observation time and treatments in Trial 1. The lower panel shows air temperature (°C) and precipitation (mm). Red arrows indicate application dates for dung. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

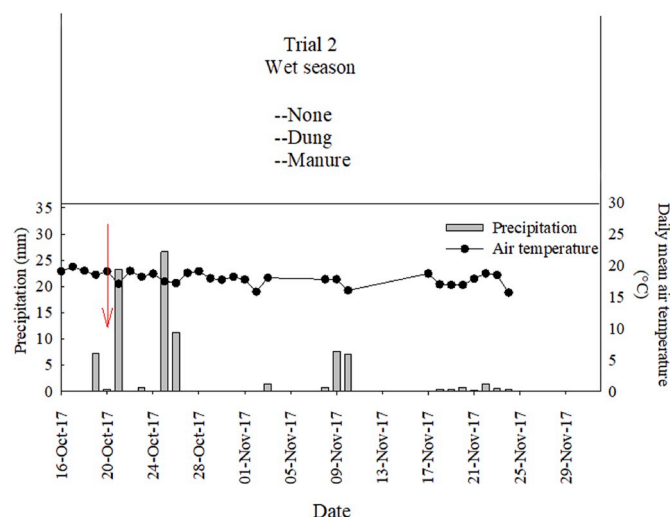


Fig. 2. Observation time and treatments in Trial 2. The lower panel shows air temperature (°C) and precipitation (mm). Red arrows indicate application dates for dung or manure. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

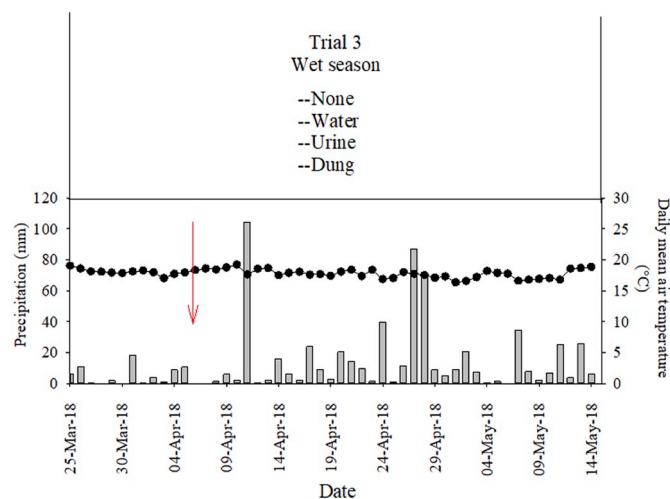


Fig. 3. Observation time and treatments in Trial 3. The lower panel shows air temperature (°C) and precipitation (mm). Red arrows indicate application dates for excreta (or water). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

initial lack of urine-collection harnesses, urine collection was only possible in 2018. Therefore, urine and soil interaction effects on soil N₂O fluxes could only be studied in trials 3 and 4.

Annual rainfall distribution at Nairobi, Kenya (mean 1982–2012: 869 mm) is bimodal with a long rainy season from the end of March to

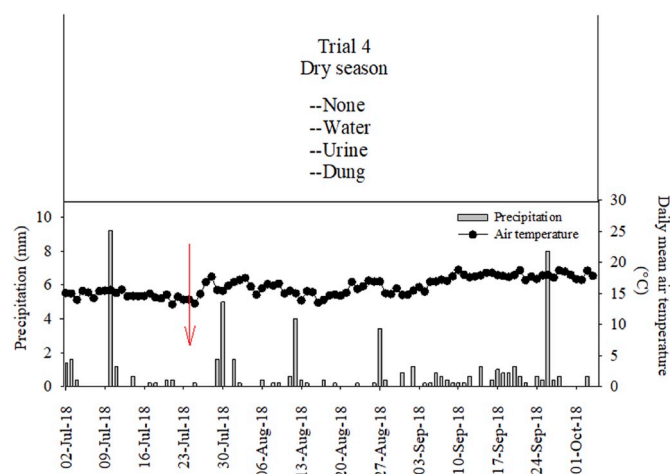


Fig. 4. Observation time and treatments in Trial 4. The lower panel shows air temperature (°C) and precipitation (mm). Red arrows indicate application dates for excreta (or water). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the end of June and a short rainy season from October to December. Trials 1 and 4 were conducted during the dry season, with 34.8 mm precipitation in 25 days (trial 1) and 41.6 mm precipitation in 73 days (trial 4); trial 2 was conducted during the short rainy season with 83.2 mm precipitation in 43 days and trial 3 was conducted during the long rainy season with 596.4 mm precipitation in 39 days. Across our four observation periods, the mean temperatures were 16.0, 17.8, 17.8 and 16.5 °C during trials 1, 2, 3 and 4, respectively (Figs. 1–4).

Fresh dung and manure were collected at the ILRI farm from a cattle herd that was grazed on pasture dominated by a mixture of Kikuyu grass and Rhodes grass during the day and then housed in single-animal pens at night, where they had access to hay from Kikuyu and Rhodes grass. Excreta were collected from the concrete floors of each pen in the mornings, after the animals had been taken outside for grazing. To avoid contamination of dung with urine, only the upper half of dung cakes was collected from the floor. To collect manure (dung + urine), a waterproof plastic sheet was placed on the concrete floor, and the edges were raised upward to capture all the urine and dung from individual animals overnight, which was then homogenized in a bucket. Dung and manure were collected immediately before application to prevent nutrient losses during storage. Fresh urine was collected from three steers, each fitted with collection harnesses. The three urine samples were pooled after collection and the pH was measured immediately. Urine was acidified with 50 ml 20% HCl to a pH ≤ 3 to minimize N loss during collection and storage and then frozen at -20°C until application. Before application, urine was thawed and the pH re-adjusted to the original pH with 2 M NaOH.

collected via a sampling port in the centre of the lid at times 0, 10, 20, and 30 min. The airtight lid was removed between samplings. Gas samples were immediately analyzed for CO_2 and N_2O concentrations using a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, CA, USA) equipped with a flame ionization detector (FID) for CO_2 and an electron capture detector (ECD) for N_2O . The oven was operated at 70°C , and the ECD and FID were heated to 340 and 350°C . The carrier gas (pure N_2) had a flow rate of 25 mL min^{-1} . CO_2 (5%) in 95% N_2 was used as purge gas (3 mL min^{-1}) for the ECD to avoid cross-interferences of the N_2O signal with CO_2 (Zheng et al., 2004). Greenhouse gas fluxes were calculated on basis of temporal changes of CO_2 and N_2O concentrations in the headspace of closed chambers. Slopes were calculated either using linear or non-linear regression analysis, using R^2 values as decision criteria (Yao et al., 2015). Corrections for air pressure and headspace temperature were applied as described by Wolf et al. (2010). The flux detection limits were $2.3\text{ }\mu\text{g N}_2\text{O-N m}^{-2}\text{ h}^{-1}$ and $1.3\text{ mg CO}_2\text{-C m}^{-2}\text{ h}^{-1}$ (Parkin et al., 2012). The fluxes were calculated by the following formula:

$$F = (b \times M_W \times V_{Ch} \times 60 \times 10^6) / (A_{Ch} \times V_m \times 10^9)$$

Where F is the flux rate ($\text{mg CO}_2\text{-C m}^{-2}\text{ h}^{-1}$ or $\mu\text{g N}_2\text{O-N m}^{-2}\text{ h}^{-1}$), b is the slope of concentration change (ppm min^{-1} or ppb min^{-1}), M_W is the molecular weight of component ($12\text{ g CO}_2\text{-C mol}^{-1}$ or $28\text{ g N}_2\text{O-N mol}^{-1}$), V_{Ch} is the chamber volume (m^3), A_{Ch} is the chamber area (m^2), and V_m is the corrected standard gaseous molar volume ($\text{m}^3\text{ mol}^{-1}$).

The sampling scheme was as follows: Before excreta application CO_2 and N_2O fluxes were measured for at least three individual days. Following excreta application for the first trial, gas fluxes were measured twice per day for three days, then every two days for the next two weeks, and every three days for the last eight days. For the other three trials, fluxes were measured after excreta application daily for the first week, then every two days for the next two weeks, and three or four days per time for the remaining days. To capture the entire CO_2 and N_2O emissions of the applied excreta, samples were collected for a minimum of one additional week (sometimes longer) after CO_2 and N_2O fluxes had returned to background in all treatments, i.e. to levels as observed for the unamended soil cores. In the majority of cases fluxes returned to background levels within two or three weeks after application of dung or manure. Still, it needs to be noted that during the third trial (wet season) fluxes in the urine treatment only returned to background levels after five weeks. Accordingly, we extended the observation time for the fourth trial to make sure that the entire period with elevated fluxes was captured. In total, CO_2 and N_2O fluxes were measured on 17 occasions/14 days (trial 1), 19 days (trial 2), 18 days (trial 3), and 30 days (trial 4).

Cumulative emissions were calculated using trapezoidal integration. Net cumulative emissions were calculated by subtracting cumulative emissions from control plots, i.e. plots not receiving excreta, from cumulative emissions of plots with excreta. The N_2O EF (i.e. the proportion of applied N emitted as N_2O) was calculated by dividing the net cumulative emissions by the amount of added excreta-N according to the equation:

$$\text{N}_2\text{O EF (\%)} = \frac{\text{Cumulative N}_2\text{O emission (g N}_2\text{O - N) from excreta application} - \text{Cumulative N}_2\text{O emission (g N}_2\text{O - N) from control}}{\text{Nitrogen content applied as excreta (g N)}} \times 100$$

2.3. Measurements of CO_2 and N_2O fluxes

For CO_2 and N_2O fluxes measurements, we used closed static chambers (Norman et al., 1997). Polyvinyl chloride collars with 22.5 cm inner diameter and 13 cm height were inserted 8 cm into the soil on the plots with the soil cores. During chamber deployment, the collars were covered with an opaque, airtight lid and four 20 ml gas samples were

2.4. Soil and excreta analysis

For soil BD determination soil was sampled with a 100 cm^3 ring, the oven-dried at 105°C for 24 h, and weighed. Soil pH was measured using

a glass electrode in a water:soil suspension of 2.5:1 (weight:weight). Soil CEC was measured by ETH Zurich using BaCl₂-TEA method (Hendershot et al., 2007). Water content of dung and manure was determined by drying fresh samples in the oven at 105 °C until constant weight. For total C and N, soil, dung or manure samples were dried at 50 °C for three days, ground and analyzed with an elemental combustion system (Costech International S. p.A., Milano, Italy). Total urine N concentration was analyzed by the Kjeldahl method (Kirk, 1950). Soil texture was estimated based on the soil particle size distribution as analyzed by the hydrometer technique (van Reeuwijk, 2002; Scrimgeour, 2008).

2.5. Data analysis

Two-way ANOVA with Tukey's HSD test was used to test for differences of cumulative emissions from unamended control plots and excreta amended plots and for testing differences between soils during the same trial. The differences of N₂O EF for urine, dung and manure among soils in the same trial were also tested using two-way ANOVA with Tukey's HSD test. All statistical calculations were done in R v3.5.3 (R core team, 2019). The linear regression between urine EF and soil clay content or pH was conducted by Sigmaplot 12.5 (Systat Software, Inc. SigmaPlot for Windows).

3. Results

3.1. Soil properties

The pH of the different soils sampled from individual farms ranged from 5.3 ± 0.5 (Ferralsols) to 8.4 ± 0.0 (Sand) (Table 1). Soil BD varied from 0.94 ± 0.13 g cm⁻³ (Gleysols) to 1.17 ± 0.14 g cm⁻³ (Acrisols). The Gleysols had the highest C and N concentrations (45.6 g ± 9.3 C kg⁻¹ dry matter [DM] and 3.91 ± 0.92 g N kg⁻¹ DM), while the Sand had the lowest C and N concentrations (0.3 ± 0.1 g C kg⁻¹ DM and 0.0 ± 0.0 g N kg⁻¹ DM). There were large differences in the proportion of sand and clay in the different soils, with the sand content ranging from 24.5% (Ferralsols) to 52.7% (Acrisols) and clay content ranging from 28.6% (Acrisols) to 52.6% (Ferralsols) (Table 1).

3.2. Excreta properties

Dung properties used in the different trials varied substantially (Table 2) because the quality of the grasses in the pasture differed between dry and wet season. As a result, the dung used in trial 1 (dry season) had the highest N content (18.10 ± 0.12 g N kg⁻¹ DM) and lowest C content (397.2 ± 0.2 g C kg⁻¹ DM), while the lowest N concentration and highest C concentration was measured in the dung used for the third trial (wet season), which had 11.75 ± 0.09 g N kg⁻¹ DM and 422.7 ± 0.3 g C kg⁻¹ DM (Table 2). Consequently, the dung C/N ratio also varied significantly from 22 (trial 1, dry season) to 36 (trial 3, wet season). Compared to dung, manure had a higher water content, and lower C and N concentrations. Urine N concentration also varied between the two trials and ranged from 6.42 ± 0.23 g N L⁻¹ (trial 3, wet season) to 5.69 ± 0.14 g N L⁻¹ (trial 4, dry season) (Table 2).

Table 2

Water content, carbon and nitrogen concentrations and C/N ratio of dung and nitrogen concentrations of urine applied to soil cores during the four trials.

Period	Season	Excreta type	Water content (%)	C concentration (g C kg ⁻¹ DM)	N concentration (g N kg ⁻¹ DM or g N L ⁻¹)	C/N ratio
26-Jul — 25-Aug-16	Dry season	dung	82.7 ± 0.1 ^a	397.2 ± 0.2 ^d	18.10 ± 0.12 ^a	22 ± 0.1 ^e
16-Oct — 01-Dec-17	Wet season	dung	80.6 ± 0.2 ^c	412.0 ± 0.2 ^b	13.60 ± 0.04 ^c	30 ± 0.1 ^c
		manure	81.8 ± 0.1 ^b	399.9 ± 0.5 ^c	11.93 ± 0.03 ^d	34 ± 0.1 ^b
25-Mar — 14-May-18	Wet season	dung	81.7 ± 0.2 ^b	422.7 ± 0.3 ^a	11.75 ± 0.09 ^d	36 ± 0.3 ^a
		urine	—	—	6.42 ± 0.23 ^A	—
02-Jul — 04-Oct-18	Dry season	dung	78.9 ± 0.4 ^d	410.3 ± 1.5 ^b	14.19 ± 0.17 ^b	29 ± 0.4 ^d
		urine	—	—	5.69 ± 0.14 ^B	—

Values are mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences among dung and manure property and the uppercase letters indicate the significant difference of urine N concentration (P < 0.05).

3.3. Background N₂O and CO₂ emissions from different soils

Cumulative CO₂ emissions from the unamended soils only differed significantly between soil types in trial 3 (wet season, 2018) with the lowest emissions from the Sand (1186 ± 235 kg CO₂-C ha⁻¹ 39 days⁻¹) and the highest from the Acrisols (3234 ± 968 kg CO₂-C ha⁻¹ 39 days⁻¹) (P < 0.05). Cumulative N₂O emissions from the different unamended soils were similar in all of the trials (Tables 3–6). Soil N₂O fluxes were largely similar or below the detection limit (2.3 µg N₂O-N m⁻² h⁻¹) during the dry season (trial 1 and 4; Figs. 5 and 8) and the only notable fluxes were following a re-wetting by a 28 mm rainfall at the end of trial 1. As expected, rainfall did not cause elevated N₂O fluxes for the sand plots (Fig. 5). During the wet season, soil CO₂ and N₂O fluxes were higher than during the dry season (see Figs. 5–8).

3.4. Influence of soil type and type of excreta on CO₂ and N₂O emissions

In all trials, dung and manure application increased CO₂ fluxes from all soils, with higher fluxes observed during wet seasons than during dry seasons (Figs. 5–8). The highest CO₂ flux of 890 ± 70 mg CO₂-C m⁻² h⁻¹ was measured in Acrisols amended with manure in trial 2 (Fig. 6). Urine application increased CO₂ fluxes more than either dung or water application within 1–2 days in all soils. However, the stimulative effect of urine additions differed between soils, ranging from 456 ± 35 (Gleysols) to 1464 ± 175 mg CO₂-C m⁻² h⁻¹ (Ferralsols) in trial 3 (Fig. 7), and from 71 ± 3 (Acrisols) to 1026 ± 81 mg CO₂-C m⁻² h⁻¹ (Gleysols) in trial 4 (Fig. 8).

The N₂O fluxes also increased following either dung or manure application. Among dung and manure applications, the highest N₂O flux of 461 ± 136 µg N₂O-N m⁻² h⁻¹ was measured for manure amended Gleysols in trial 2 (Fig. 6). Compared to unamended control soils, cumulative N₂O emissions from dung application were similar across all soils during all trials (Tables 3–6), while during trial 2 the manure application resulted in higher cumulative N₂O emissions compared to the unamended controls only in the Gleysols and Ferralsols (Table 4; P < 0.05).

Neither the water nor the dung application significantly increased cumulative N₂O emissions relative to the unamended control across all soil types and all trials. However, the response to urine additions was highly variable across soils (Tables 5 and 6), with fluxes ranging from 33 ± 38 (Ferralsols – trial 4; Fig. 8) to 8760 ± 1322 µg N₂O-N m⁻² h⁻¹ (Acrisols – trial 3; Fig. 7). Soil N₂O fluxes were not only larger but also lasted for longer (several weeks) for urine as compared to dung additions (Figs. 7 and 8). As a result, cumulative N₂O emissions were higher for urine application compared to dung application in the Acrisols (Trials 3 and 4) and Sand (only trial 4) (Tables 5 and 6). The urine EF was negatively correlated with soil clay content in both dry (EF = 0.5936–0.0118 × soil clay content, n = 15, R² = 0.53, P < 0.05) and wet season (EF = 2.5526–0.0466 × soil clay content, n = 15, R² = 0.51, P < 0.05), while positively correlated with soil pH in both dry (EF = –1.3850 + 0.2367 × soil pH, n = 15, R² = 0.36, P < 0.05) and wet season (EF = –6.5575 + 1.2290 × soil pH, n = 15, R² = 0.60, P < 0.05).

Table 3

Cumulative CO₂ and N₂O emission and dung N₂O EF (% applied excreta-N) over 25 days as affected by addition of 0.5 kg cattle dung to different soil cores in Trial 1 (01–25 August 2016, dry season).

Soil type	Cumulative CO ₂ emissions (kg CO ₂ -C ha ⁻¹)		Cumulative N ₂ O emissions (g N ₂ O-N ha ⁻¹)		N ₂ O EF (%)
	None	Dung	None	Dung	Dung
Gleysols	472 ± 100 ^{Aa}	754 ± 148 ^{Aa}	22.3 ± 11.0 ^{Aa}	49.7 ± 44.1 ^{Aab}	0.01 ± 0.01 ^a
Nitisols	353 ± 117 ^{Ba}	684 ± 75 ^{Aa}	11.6 ± 9.3 ^{Aa}	31.3 ± 17.4 ^{Aab}	0.00 ± 0.00 ^a
Acrisols	518 ± 149 ^{Aa}	743 ± 113 ^{Aa}	71.8 ± 71.4 ^{Aa}	108.5 ± 77.6 ^{Aa}	0.01 ± 0.02 ^a
Cambisols	441 ± 69 ^{Ba}	753 ± 125 ^{Aa}	8.9 ± 5.4 ^{Aa}	29.3 ± 7.3 ^{Aab}	0.00 ± 0.00 ^a
Ferralsols	386 ± 82 ^{Ba}	747 ± 132 ^{Aa}	12.7 ± 13.3 ^{Aa}	21.4 ± 15.1 ^{Ab}	0.00 ± 0.00 ^a
Sand	505 ± 140 ^{Aa}	748 ± 121 ^{Aa}	15.5 ± 15.4 ^{Aa}	20.9 ± 21.2 ^{Ab}	0.00 ± 0.00 ^a

Values are mean ± standard deviation (n = 4); different lowercase letters indicate significant differences among soil types within the same treatment and different uppercase letters indicate significant differences among treatment within the same soil type (P < 0.05).

Table 4

Cumulative CO₂ and N₂O emission and N₂O EF (% applied excreta-N) over 43 days as affected by addition of 0.5 kg cattle dung or 0.5 kg cattle manure to different soil cores in Trial 2 (20 October — 01 December 2017, wet season).

Soil type	Cumulative CO ₂ emissions (kg CO ₂ -C ha ⁻¹)			Cumulative N ₂ O emissions (g N ₂ O-N ha ⁻¹)			N ₂ O EF (%)	
	None	Dung	Manure	None	Dung	Manure	Dung	Manure
Gleysols	2028 ± 226 ^{Aa}	2635 ± 520 ^{Aa}	2951 ± 573 ^{Aa}	127 ± 37 ^{Ba}	579 ± 296 ^{ABa}	916 ± 86 ^{Aa}	0.13 ± 0.08 ^{Aa}	0.28 ± 0.03 ^{Aa}
Nitisols	1890 ± 455 ^{Aa}	2555 ± 101 ^{Aa}	2705 ± 232 ^{Aa}	150 ± 38 ^{Aa}	644 ± 448 ^{Aa}	765 ± 56 ^{Aa}	0.14 ± 0.13 ^{Aa}	0.22 ± 0.03 ^{Aa}
Acrisols	1997 ± 134 ^{Aa}	3034 ± 661 ^{Aa}	3024 ± 526 ^{Aa}	218 ± 47 ^{Aa}	571 ± 253 ^{Aa}	709 ± 479 ^{Aa}	0.10 ± 0.06 ^{Aa}	0.17 ± 0.18 ^{Aa}
Cambisols	1945 ± 405 ^{Aa}	3093 ± 544 ^{Aa}	3163 ± 1041 ^{Aa}	110 ± 30 ^{Aa}	818 ± 616 ^{Aa}	814 ± 538 ^{Aa}	0.20 ± 0.17 ^{Aa}	0.25 ± 0.18 ^{Aa}
Ferralsols	1540 ± 286 ^{Ba}	3092 ± 833 ^{Aa}	2949 ± 711 ^{ABa}	158 ± 148 ^{Ba}	620 ± 154 ^{ABa}	916 ± 403 ^{Aa}	0.13 ± 0.07 ^{Aa}	0.27 ± 0.09 ^{Aa}
Sand	1959 ± 1273 ^{Aa}	2273 ± 328 ^{Aa}	2501 ± 729 ^{Aa}	179 ± 112 ^{Aa}	335 ± 203 ^{Aa}	264 ± 96 ^{Aa}	0.05 ± 0.07 ^{Aa}	0.03 ± 0.05 ^{Aa}

Values are mean ± standard deviation (n = 4); there was no significant differences among soil types within the same treatment; different uppercase letters indicate significant differences among treatment within the same soil type (P < 0.05).

3.5. N₂O emission factors for dung, manure and urine

The N₂O EF for dung in trial 1 (dry season) was negligible and similar among all soil types, varying from 0.00 to 0.01% (Table 3), while the N₂O EF for dung was in the range of 0.00–0.20% in all other trial periods (Tables 4–6). Despite a lower dung N concentration, the dung EF was higher during the wet seasons (Trials 2 and 3) than during the dry seasons (Trials 1 and 4; P < 0.05). However, the N₂O EF for dung did not differ across soils. Manure was only applied in the second trial (wet season), and during this period the manure N₂O EF ranged from 0.03 to 0.28% with no differences between the soils tested (Table 4).

In contrast to dung and manure, the urine N₂O EF varied largely among the different soils during both wet and dry seasons. The highest urine N₂O EF was observed for Acrisols both in the wet season (1.36%) and dry season (0.29%), while the lowest urine N₂O EF was measured in the Ferralsols (wet season: 0.12%; dry season: 0.01%). Furthermore, in both wet and dry seasons, the urine N₂O EF was markedly higher for Acrisols than EFs for all other soils (Tables 5 and 6; P < 0.05).

4. Discussion

4.1. Influence of soil properties on CO₂ and N₂O emissions after excreta application

In our study, the CO₂ fluxes after dung or manure applications were similar and no soil type effect was detected indicating that the majority of CO₂ releases was from the fresh dung or manure itself. This was similar to observations by Lin et al. (2009) after yak dung application under laboratory conditions. The short-lived CO₂ fluxes after urine application were likely associated with the rapid hydrolysis of urine urea (Ambus et al., 2007; Cai et al., 2017), during which CO₂ is released, and did not cause any significant difference in cumulative CO₂ emissions among soils in any of the trials.

Consistent with our original hypothesis, the N₂O EF from both manure and dung application to pasture was not affected by the underlying soil properties such as e.g. soil C/N ratio, SOC or pH. This was likely because the low dung-N concentrations limited N availability for

N₂O formation in the dung itself, and because the rapid crusting of the dung hampered incorporation of excreta-N into the soil (Zhu et al., 2018). However, the presence of termites in some of the soil cores was noted shortly after application, which might have altered soil aeration and thus influenced N₂O production and emission. Also, the termites may have transferred some of the dung/manure away from the area covered by the chambers. Therefore, it is possible that the presence of the termites may have contributed to our observation that soil texture and pH did not have measurable effects on soil N₂O production after dung application. The lack of measurable soil effects was consistent with earlier findings by Van der Weerden et al. (2011) who also did not find differences in the N₂O EF for dung in three contrasting regions with differing soil types.

In line with a recent global meta-analysis (Wang et al., 2018a,b), the N₂O EF for urine application to soils was negatively correlated with clay content. Clay particles hold water tightly in soil aggregates. Moreover, clay soils mostly have a low gas diffusivity due to small mean pore sizes (Weitz et al., 2001; Gu et al., 2013). Both factors favor anaerobic conditions in soils and, thus, might promote complete denitrification towards the end product N₂, causing lower N₂O emissions from clay soils (Weitz et al., 2001). In addition, high clay content generally correlates with a high cation exchange capacity. As a result, the mineralized NH₄⁺ ion can be adsorbed due to the high CEC or even fixed to the clay minerals (Chantigny et al., 2004), which decreases soil NH₄⁺ ion availability for nitrification and, thus, NO₃⁻ ion production. Thus, the soil ammonium sorption capacity has been found to affect N₂O production in soils which otherwise showed largely similar physical and chemical properties (Venterea et al., 2015). Consequently, a number of studies have found limited N₂O production following urine application to clay-rich soils (Jarecki et al., 2008; Zhou et al., 2017). Contradicting our findings, a study in Canada reported higher urine EFs in a clay-rich soil in comparison with a sandy loam soil and argued that the good soil aeration in the sandy loam soil limited the N₂O production due to denitrification (Rochette et al., 2014).

In addition to soil clay content, soil pH is also an important factor controlling the magnitude of N₂O emissions after N additions (Wang et al., 2018a,b). Wang et al., 2018a,b found in a meta-data analysis on

Table 5
Cumulative CO₂ and N₂O emission and N₂O EF over 39 days as affected by addition of 0.5 L cattle dung to different soil cores in Trial 3 (06 April – 14 May 2018, wet season).

Soil type	Cumulative CO ₂ emissions (kg CO ₂ -C ha ⁻¹)				Cumulative N ₂ O emissions (g N ₂ O-N ha ⁻¹)				N ₂ O EF (%)	
	None	Water	Urine	Dung	None	Water	Urine	Dung	Urine	Dung
Gleysols	2890 ± 737 ^{Aa}	2610 ± 136 ^{Aa}	2974 ± 296 ^{Aab}	3881 ± 1125 ^{Aa}	219 ± 69 ^{Aa}	177 ± 73 ^{Aa}	2044 ± 963 ^{Ab}	640 ± 332 ^{Aa}	0.22 ± 0.12 ^{Ab}	0.15 ± 0.13 ^{Aa}
Nitisols	2540 ± 939 ^{Aab}	2499 ± 526 ^{Aa}	2815 ± 57 ^{Aab}	3168 ± 498 ^{Aa}	186 ± 120 ^{Aa}	448 ± 440 ^{Aa}	5022 ± 4552 ^{Ab}	533 ± 365 ^{Aa}	0.54 ± 0.49 ^{Ab}	0.12 ± 0.09 ^{Aa}
Acrisols	3234 ± 968 ^{Aa}	2158 ± 327 ^{Aa}	3167 ± 305 ^{Aab}	3244 ± 958 ^{Aa}	186 ± 210 ^{Ba}	194 ± 187 ^{Ba}	11684 ± 5920 ^{Aa}	241 ± 273 ^{Ba}	1.36 ± 0.70 ^{Aa}	0.02 ± 0.02 ^{Ba}
Cambisols	2457 ± 123 ^{Aab}	2398 ± 370 ^{Aa}	3591 ± 230 ^{Aa}	3578 ± 32 ^{Aa}	133 ± 119 ^{Aa}	101 ± 93 ^{Aa}	2212 ± 659 ^{Ab}	377 ± 153 ^{Aa}	0.25 ± 0.08 ^{Ab}	0.09 ± 0.06 ^{Aa}
Ferralsols	2078 ± 172 ^{Aab}	2503 ± 671 ^{Aa}	2519 ± 126 ^{Aab}	3300 ± 572 ^{Aa}	87 ± 35 ^{Aa}	240 ± 67 ^{Aa}	1254 ± 960 ^{Ab}	218 ± 267 ^{Aa}	0.12 ± 0.12 ^{Ab}	0.05 ± 0.10 ^{Aa}
Sand	1186 ± 235 ^{Bb}	1397 ± 140 ^{Ba}	1862 ± 347 ^{ABb}	3484 ± 145 ^{Aa}	52 ± 18 ^{Aa}	88 ± 39 ^{Aa}	1359 ± 196 ^{Ab}	178 ± 110 ^{Aa}	0.15 ± 0.03 ^{Ab}	0.04 ± 0.04 ^{Aa}

Values are mean ± standard deviation (n = 3); different lowercase letters indicate significant differences among soil types within the same treatment and different uppercase letters indicate significant differences among treatment within the same soil type (P < 0.05).

Table 6
Cumulative CO₂ and N₂O emission and N₂O EF over 73 days as affected by addition of 0.5 L cattle dung to different soil cores in Trial 4 (24 July – 04 October 2018, dry season).

Soil type	Cumulative CO ₂ emissions (kg CO ₂ -C ha ⁻¹)				Cumulative N ₂ O emissions (g N ₂ O-N ha ⁻¹)				N ₂ O EF (%)	
	None	Water	Urine	Dung	None	Water	Urine	Dung	Urine	Dung
Gleysols	1589 ± 136 ^{Aa}	1989 ± 564 ^{Aa}	1892 ± 482 ^{Aa}	1716 ± 530 ^{Aa}	18 ± 25 ^{Aa}	75 ± 63 ^{Aa}	180 ± 43 ^{Abc}	60 ± 52 ^{Aa}	0.01 ± 0.01 ^{Ab}	0.01 ± 0.02 ^{Aa}
Nitisols	1937 ± 435 ^{Aa}	1635 ± 201 ^{Aa}	1918 ± 280 ^{Aa}	2286 ± 629 ^{Aa}	65 ± 60 ^{Aa}	77 ± 14 ^{Aa}	325 ± 255 ^{Aabbc}	210 ± 81 ^{Aa}	0.03 ± 0.03 ^{Ab}	0.04 ± 0.02 ^{Aa}
Acrisols	2430 ± 370 ^{Aa}	2256 ± 307 ^{Aa}	2215 ± 256 ^{Aa}	1826 ± 133 ^{Aa}	84 ± 57 ^{Ba}	107 ± 202 ^{Ba}	2285 ± 1729 ^{Aa}	100 ± 37 ^{Ba}	0.29 ± 0.23 ^{Aa}	0.00 ± 0.02 ^{Ba}
Cambisols	1939 ± 438 ^{Aa}	2403 ± 119 ^{Aa}	2163 ± 598 ^{Aa}	2399 ± 1040 ^{Aa}	41 ± 42 ^{Aa}	82 ± 33 ^{Aa}	216 ± 49 ^{Abc}	117 ± 64 ^{Aa}	0.02 ± 0.00 ^{Ab}	0.02 ± 0.01 ^{Aa}
Ferralsols	1922 ± 398 ^{Aa}	1965 ± 285 ^{Aa}	2460 ± 380 ^{Aa}	2144 ± 485 ^{Aa}	36 ± 20 ^{Aa}	40 ± 0 ^{Aa}	120 ± 86 ^{Ac}	162 ± 156 ^{Aa}	0.01 ± 0.01 ^{Ab}	0.03 ± 0.04 ^{Aa}
Sand	1927 ± 568 ^{Aa}	1887 ± 264 ^{Aa}	2075 ± 209 ^{Aa}	2208 ± 476 ^{Aa}	77 ± 27 ^{ABa}	98 ± 57 ^{ABa}	1211 ± 17 ^{Abb}	31 ± 36 ^{Ba}	0.15 ± 0.01 ^{Ab}	-0.01 ± 0.01 ^{Aa}

Values are mean ± standard deviation (n = 3); different lowercase letters indicate significant differences among soil types within the same treatment and different uppercase letters indicate significant differences among treatment within the same soil type (P < 0.05).

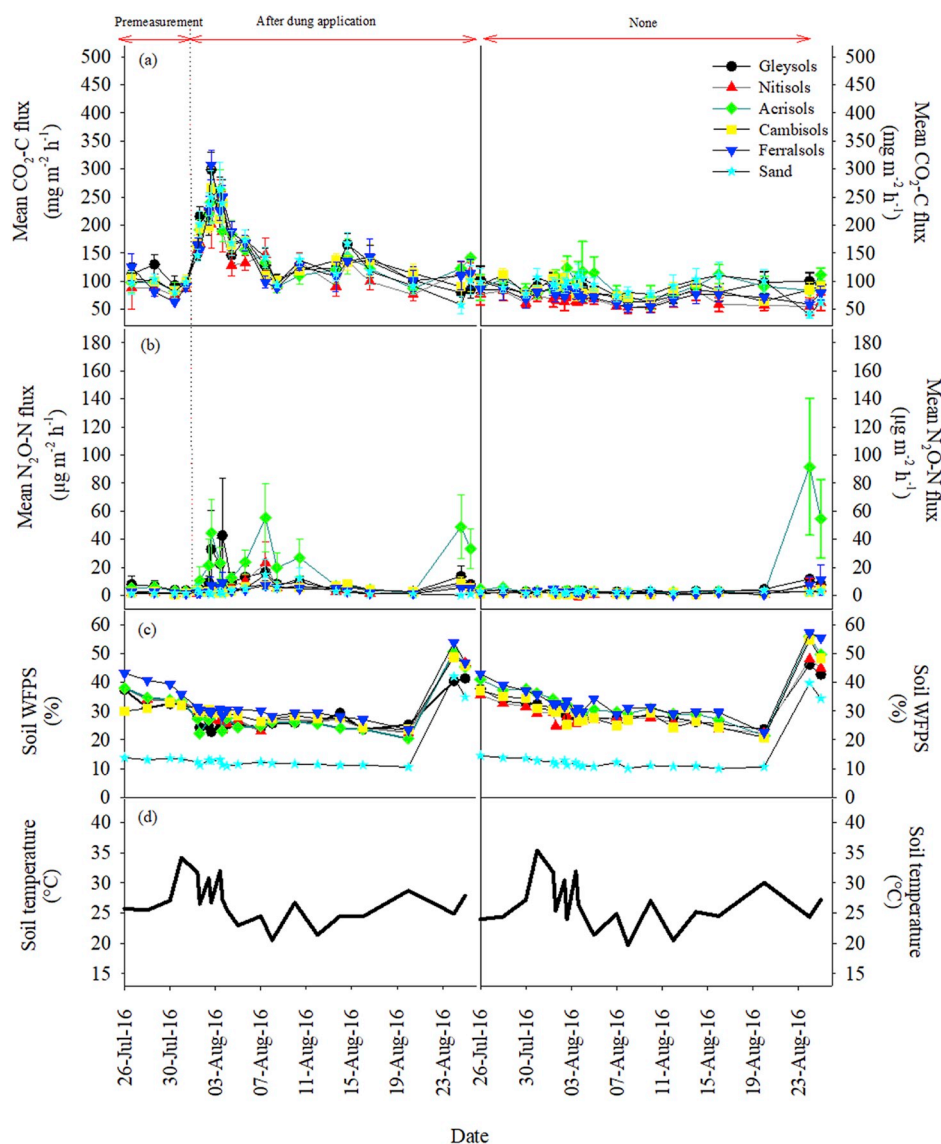


Fig. 5. Dynamics of (a) CO_2 and (b) N_2O daily fluxes from different soil cores to which dung was added (left panel) compared to the same soils without excreta application (right panel) (Trial 1). The lower panels show the observed temporal dynamics of (c) mean daily soil moisture (0.05 m depth), (d) soil temperature (0.05 m depth) measured during gas sampling. Each flux value represents the mean of three chambers (\pm SE).

N_2O emissions from excreta applied to grasslands, that the magnitude of N_2O emissions is negatively correlated with pH, i.e. that emissions increase at lower soil pHs. However, Khan et al., (2011) reported higher cumulative N_2O fluxes from bovine urine applied onto limed acid soils than on non-limed acid soils. The fact that all the soils except for the sand used in our study (pH 8.4, not included in the analysis) were slightly acidic and fairly similar in pH (pH range: 5.3–6.4) might explain why we found a positive correlation between urine EF and soil pH in both dry and wet season.

Contrary to our expectations, soil C and soil C/N ratio did not have any effect on urine N_2O EFs in our study, contrary to Pelster et al. (2012) who reported a potential C-limitation of soil N_2O emissions after N fertilizer application to temperate soils in Canada. However, because of the high C content in our soils (35 g kg^{-1} compared to 19 g kg^{-1} in the previous study), soil N_2O production was likely N- and not C-limited. This would also explain why urine, but not dung or manure additions significantly stimulated N_2O emissions as urine N is quickly available as urease is ubiquitous in all soils (Van Groenigen et al., 2005b). Moreover, not only N but also significant amounts of water was added with urine applications, which increases soil moisture and soil microbial activity

(Marsden et al., 2016). It is noteworthy that in our study water-only additions did not stimulate N_2O emissions while additions of the same amounts of urine did (Figs. 7 and 8), indicating that although significant changes in soil moisture were observed after water application, the effect of the water was minor compared to the urine-N addition effect. As the soil moisture was generally below 60% water filled pore space (WFPS), it is likely that at least in the lighter textured soils nitrification was the dominant process of N_2O production after excreta application (Bell et al., 2015). Generally, high soil moisture promotes N_2O production, as shown in our study when comparing dry and wet season fluxes, but soil moisture can also be related to soil properties causing differences in N_2O fluxes between different soil types. For instance, De Klein et al. (2003) in a study in New Zealand grasslands reported an urine EF of 0.5% for a well-drained stony silt loam soil and 3.7% for a moderately-drained silt loam soil. However, Van der Weerden et al. (2011) reported that N_2O emissions did not always relate to drainage class, when summarizing experimental findings obtained for well- and poor-drained silt loam soils at three sites in New Zealand. Balaine et al. (2013) highlighted that the relative soil gas diffusivity is a key indicator to rank soil N_2O emission potentials.

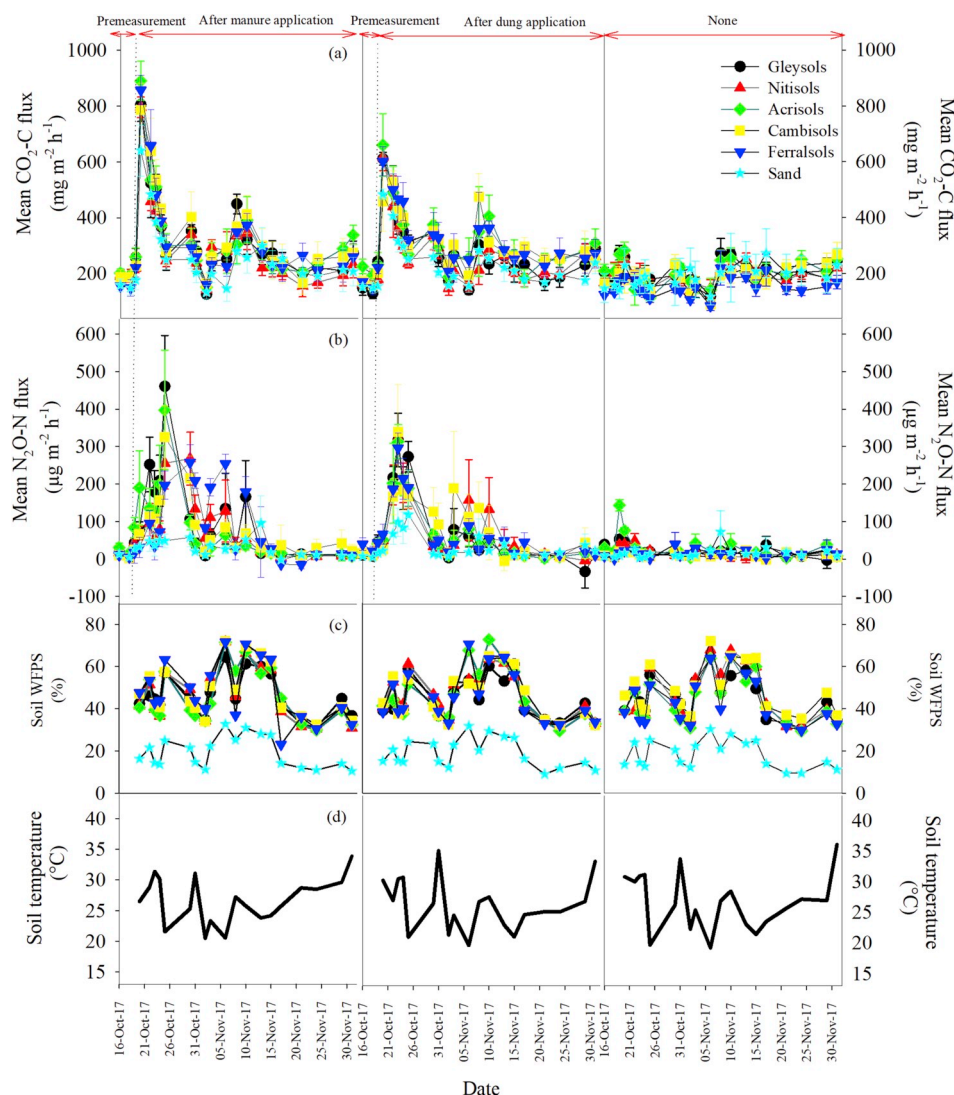


Fig. 6. Dynamics of (a) CO₂-C and (b) N₂O-N daily fluxes from different soil cores to which manure (left panel) or dung (middle panel) was added compared to soils without excreta application (right panel) (Trial 2). The lower panels show temporal dynamics of (c) mean daily soil moisture (0.05 m depth) and (d) soil temperature (0.05 m depth) measured during gas sampling. Each flux value represents the mean of three chambers (\pm SE).

4.2. Influence of season (wet vs dry) and time elapsed since excreta application on CO₂ and N₂O emissions

The higher soil CO₂ fluxes after excreta application in the wet season compared to the dry season was likely caused by the higher soil moisture, since temperature was similar during all four trials, as both soil moisture and temperature are controlling factors for CO₂ production (Rochette and Gregorich, 1998; Ginting et al., 2003). Similar results had been observed by Zhu et al. (2018) after cattle dung application to an East African grassland.

A one year field observation in Canada showed that most of the emissions occur within a few weeks after excreta application, although smaller peaks may occur later (Rochette et al., 2014). In our study, the flux patterns after dung application were consistent with a previous study for tropical rangeland soils amended with dung by Zhu et al. (2018). They reported that N₂O fluxes stayed elevated for around 14 days after dung application. The rather restricted period of elevated N₂O emissions after dung application can likely be attributed to the relatively low mineral N content of the dung, fast crust formation and the wide dung C/N ratio (22–36, Table 2), which likely promoted N immobilization during C decomposition (Van der Weerden et al., 2011; Zhu et al., 2018).

In the present study, N₂O fluxes returned to background levels within 39–73 observation days after urine application, which is consistent with Sordi et al. (2014) who reported 41 ± 10 days of elevated emissions following cattle urine application to a subtropical pasture of Brazil during three different seasons, after which the N₂O fluxes rapidly diminished and returned to background levels. This was also consistent with another study in New Zealand, which showed that soil NH₄⁺-N concentrations returned to background concentrations after 27 days, while elevated soil NO₃⁻ concentrations were observed for around 40 days after cattle urine application (Clough et al., 2009). Similarly, a study carried out in the UK reported that the majority of N₂O emissions occurred during the first 20 days after sheep urine application onto Eutric Cambisol mesocosms (Marsden et al., 2016). Thus, the frequent gas sampling and the length of the observation period in our study should have been sufficient to capture most of the N₂O emissions caused by excreta application.

The higher N concentration and lower C/N ratio of the dung used during the dry seasons (C/N ratio: 22–29, Table 2) compared to the dung used during the rainy seasons (C/N ratio: 30–36, Table 2) should have translated into more substrate for denitrification and generally greater N₂O production during the dry season; however, the dung N₂O EFs were in fact lower during the dry seasons (range 0.00–0.04%) than during the

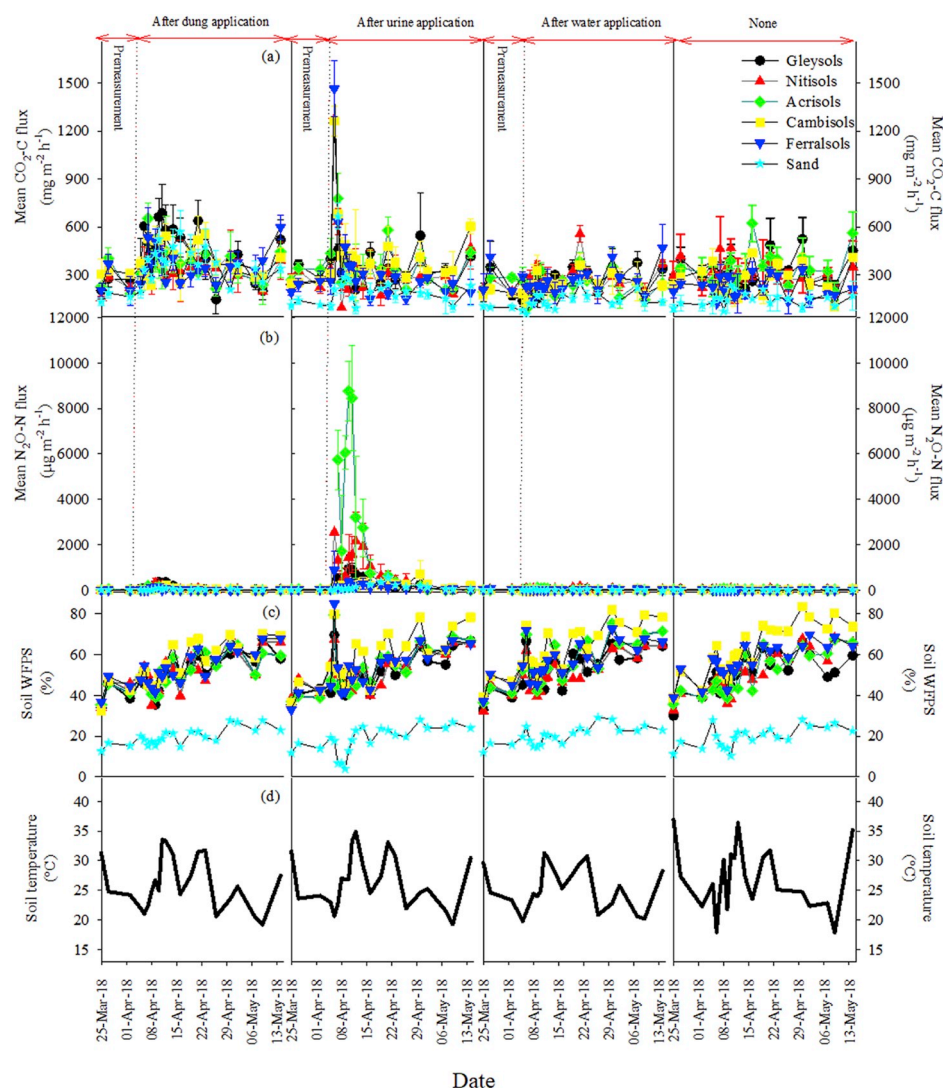


Fig. 7. Dynamics of (a) CO₂-C and (b) N₂O-N daily fluxes from different soil cores to which dung (left panel), urine (second-left panel), water (second-right panel), or nothing (right panel) was added (Trial 3). The lower panels show temporal dynamics of (c) mean daily soil moisture (0.05 m depth) and (d) soil temperature (0.05 m depth) measured during gas sampling. Each flux value represents the mean of three chambers (\pm SE).

wet seasons (0.02–0.20%). This was likely due to delayed dung crust formation due to rainfall (Mazzetto et al., 2014) and higher dung mineralization activity during the wet seasons with more favorable soil environmental conditions for N₂O production (see soil moisture levels in Figs. 5–8), which is in agreement with our hypothesis. The magnitude of total rainfall may cause differences in the amount of anaerobic microsites in the soil/dung, which could provide suitable conditions for further reduction of the microbially-produced N₂O to N₂ (Sordi et al., 2014; Zhu et al., 2018). This may explain why the dung N₂O EFs during trial 3 (range: 0.02–0.15%), which had 596 mm rain, were lower than for the other wet season period (trial 2; range: 0.10–0.20%), during which only 83 mm of rainfall. Furthermore, this lower EF might also have been related to increased N leaching as a consequence of the heavy rainfalls, although this remains speculation as we did not measure N leaching.

The higher urine N₂O EF in wet season than in dry season was similar to other studies in Brazil and Kenya (Sordi et al., 2014; Tully et al., 2017). Seasonal variations for urine N₂O EFs was also measured under a temperate climate (Van Groenigen et al., 2005b), although the authors attributed this seasonality to the seasonality of background N₂O emissions. Nevertheless, it should be noted that temperate climates show large intra-annual changes in temperature with less seasonality in

precipitation rates. In Kenya, air temperature only changes minimally during the year, whereas rainfalls have a distinct seasonality (i.e. a clear separation between dry and wet season). Soil moisture, which exerts significant effects on soil N₂O emissions through modulating soil O₂ concentration and nutrient availability (Butterbach-Bahl et al., 2013), typically mirrors rainfall distribution. Therefore, our observation that excreta effects on soil N₂O emissions were generally stronger in the wet season is not surprising, as soil moisture content was generally higher during the wet season soils favouring coupled nitrification denitrification processes, i.e. the main N₂O production pathway after urine application (Monaghan and Barraclough, 1993). During the dry season, N mineralization and nitrification may be moisture limited, while denitrification would be unlikely as it is limited by the low soil moisture values (Linn and Doran, 1984).

4.3. Influence of excreta type on CO₂ and N₂O emissions

We confirmed our fourth hypothesis that both cattle urine and dung applications would stimulate CO₂ fluxes. The cumulative CO₂ emissions after dung application were similar to or slightly higher than after urine application in our study depending on the amount of C applied (Bertora et al., 2008), which is consistent with Lin et al. (2009) who also reported

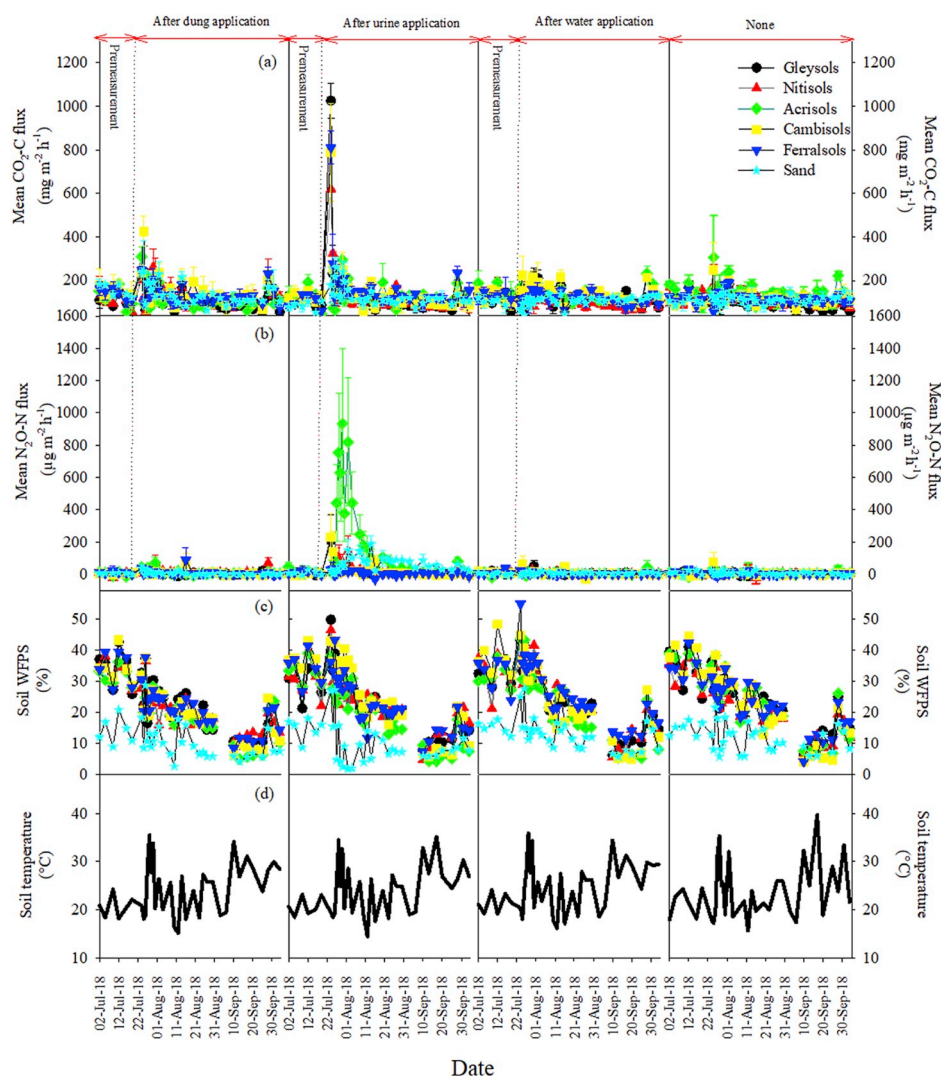


Fig. 8. Dynamics of (a) $\text{CO}_2\text{-C}$ and (b) $\text{N}_2\text{O-N}$ daily fluxes from different soil cores to which water, urine or dung was added (Trial 4). The lower panels show the observed temporal dynamics of (c) mean daily soil moisture (0.05 m depth), (d) soil temperature (0.05 m depth) measured during gas sampling. Each flux value represents the mean of three chambers (\pm SE).

higher cumulative CO_2 emissions from yak dung than from yak urine.

The N_2O EF for dung application (range: 0.00–0.20%, mean = 0.06%) was consistent with another study in Kenya that reported dung N_2O EF ranged from 0.00 to 0.04% (Tully et al., 2017). However, our mean EF was lower than the value of 0.28% synthesized for cattle dung patches through a global meta-analysis by Cai and Akiyama (2016). We suggest that the lower N_2O EF for cattle dung in Kenya in the present study compared to the global mean was caused by low quality feeds (i.e. low protein content) (Wassie et al., 2019) that resulted in lower N excretion and a higher C/N ratio in dung patches. In our study the dung and manure C/N ratios ranged from 22 to 36, much higher than the C/N ratios reported for dung/manure from cattle in temperate regions (<20, see Zhu et al., 2018). Moreover, the soils have low N concentrations (<0.39 g N kg^{-1} DM, Table 1), which could result in rapid immobilization of the applied N causing low NO_3^- availability for denitrification. Furthermore, higher solar radiation and high vapor pressure deficits in the Kenyan highlands leads to fast crust formation on the manure and dung and thus less incorporation of excreta N into the soil (Zhu et al., 2018).

The manure (i.e. a mixture of dung and urine) EF of 0.23% was also similar to a previous study in Kenya that reported a manure EF for the wet season of 0.15% (Tully et al., 2017). The higher manure EF in the present study compared to the dung EF might be explained by

synergistic effects of labile C from the dung and inorganic N from the urine in manure, which combined with higher water contents likely promoted N_2O formation in soils and the manure itself (Hyde et al., 2016).

The observed range of urine EFs in our study (0.01–1.36%, with a mean of 0.29%) were within the range of EFs determined by studies carried out in Brazil (0.19–0.33%) (Barneze et al., 2014; Sordi et al., 2014) and Kenya (0.05–0.21%) (Tully et al., 2017). Cattle urine EFs of more than 3% were reported for an incubation experiment conducted at 25 $^{\circ}\text{C}$ and 80% relative humidity in Brazil (Cardoso et al., 2017). However, this previous study might be misleading, as the high temperature and humidity conditions likely favored N_2O production while the incubation system used by Cardoso et al. (2017) did not allow for N leaching; a major N loss pathway of urine N. In their review, Cai and Akiyama (2016) estimated that at least 17.8% of applied urine N is leached under field conditions. Another recent study from Kenya that simulated a 20 mm rainfall event after application, reported a cattle urine EF of 1.2%, which can be ascribed to more favorable soil condition to N_2O production after rainfall (Pelster et al., 2016). As synthesized by Cai and Akiyama (2016) the average N_2O EF for cattle urine was 0.76%, i.e. approx. three times higher than in our study. Although the N application rate of 845 and 749 kg N ha^{-1} applied as urine in our study was in the range of most studies analyzed in the global meta-analysis

(Cai and Akiyama, 2016), the soil properties and climate conditions in our study differed from most of the studies in the Cai and Akiyama review, which were predominantly data from temperate regions.

As the IPCC default value does not disaggregate the EF for urine and dung, we cannot compare the separate EFs for urine and dung in our study with the default value of 2%. However, the N partitioning between dung and urine for cattle excreta in SSA has been reported to be around 2:1 (Rufino et al., 2006), which is consistent with a recent study in Kenya by Wassie et al. (2019) who found that the N partitioning between dung and urine varied from ~1:1 to ~2:1. Those ratios differ significantly from estimates for western European countries (40:60) (Chadwick et al., 2018). If we apply the split as determined by Rufino et al. (2006) (i.e. an excreta-N ratio of dung to urine of 66:34), the overall N₂O EF for cattle excreta on grassland ranges from 0.06 to 0.30% depending on the soil type, with an average of 0.14%. Thus, the EFs found here are approximately one order of magnitude lower than the IPCC default value or the EF determined for livestock excreta in the tropics and subtropics by Albanito et al. (2017) of 1.2%.

5. Conclusion

Here we showed that while soil type had no influence on CO₂ emissions after excreta application it did affect N₂O emissions after urine, but not manure or dung, application. The differences between soils in N₂O emissions following manure application were likely related to soil properties such as texture and soil pH. Environmental conditions (wet and dry season), and the N content of excreta did affect N₂O losses, with a higher N₂O EF (0.29%) from urine application versus application of cattle dung (EF = 0.06%). Based on the excreta N split estimated by existing literature in SSA, the overall mean N₂O EF for cattle excreta deposited onto grassland was 0.14%, less than 10% of the IPCC default value (2%). Our findings suggests that current IPCC methodology provides a substantial overestimation of the N₂O EF for cattle excreta on tropical rangeland. In order to improve estimates and to identify adequate, region specific mitigation options to reduce emissions from livestock excreta, environmental conditions should be taken into account when estimating N₂O losses.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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