Geoderma 406 (2022) 115516

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Greenhouse gas emissions from cattle dung depositions in two *Urochloa* forage fields with contrasting biological nitrification inhibition (BNI) capacity

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ARTICLE INFO

Handling Editor: Jan Willem Van Groenigen

Keywords:
Cattle dung
Tropical forages
Static chamber technique
Methane
Nitrous oxide
Emission factor

ABSTRACT

Grazing-based production systems are a source of soil greenhouse gas (GHG) emissions triggered by excreta depositions. The adoption of *Urochloa* forages (formerly known as *Brachiaria*) with biological nitrification inhibition (BNI) capacity is a promising alternative to reduce nitrous oxide (N_2O) emissions from excreta patches. However, how this forage affects methane (CH_4) or carbon dioxide (CO_2) emissions from excreta patches remains unclear. This study investigated the potential effect of soils under two *Urochloa* forages with contrasting BNI capacity on GHG emissions from cattle dung deposits. Additionally, the N_2O and CH_4 emission factors (EF) for cattle dung under tropical conditions were determined. Dung from cattle grazing star grass (without BNI) was deposited on both forage plots: *Urochloa hybrid* cv. Mulato and *Urochloa humidicola* cv. Tully, with a respectively low and high BNI capacity. Two trials were conducted for GHG monitoring using the static chamber technique. Soil and dung properties and GHG emissions were monitored in trial 1. In trial 2, water was added to simulate rainfall and evaluate GHG emissions under wetter conditions. Our results showed that beneath dung patches, the forage genotype influenced daily CO_2 and cumulative CH_4 emissions during the driest conditions. However, no significant effect of the forage genotype was found on mitigating N_2O emissions from dung. We attribute the absence of a significant BNI effect on N_2O emissions to the limited incorporation of dung-N into the soil and rhizosphere where the BNI effect occurs. The average N_2O EFs was 0.14%, close to the IPCC 2019 uncertainty range (0.01–0.13% at 95% confidence level). Moreover, CH_4 EFs per unit of volatile solid (VS) averaged 0.31 g CH_4 $kgVS^{-1}$, slightly lower than the 0.6 g CH_4 $kgVS^{-1}$ developed by the IPCC. This implies the need to invest in studies to develop more region-specific Tier 2 EFs, including farm-level studies with animals consuming *Urochloa* forages to consider the complete implications of forage selection on animal excreta based GHG emissions.

1. Introduction

Agriculture, forestry and other land use (AFOLU) management systems are significant sources of anthropogenic greenhouse gas (GHG) emissions, responsible for up to 24% of global GHG emissions (Smith et al., 2014). About half of the GHG emissions from AFOLU are associated with the livestock sector; cattle production being the largest GHG source responsible for two-thirds of total livestock sector emissions (Gerber et al., 2013). The estimated 1.5 billion global cattle herd produces a tremendous amount of excreta, 50% of which is deposited on grazed pastures (FAOSTAT, 2018; Oenema et al., 2008). In the tropics, 70% of agricultural land is under livestock grazing, and open-grazing is the most common management system for cattle production (Rao et al., 2011). A large portion of the nutrients consumed by grazing animals return to the grassland soils in the form of urine and dung (Vendramini et al., 2014), providing substrate for soil-borne microbes that are responsible for the production of three GHGs: carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Lin et al., 2009; Rivera et al.,

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https://doi.org/10.1016/j.geoderma.2021.115516
Received 24 March 2021; Received in revised form 30 September 2021; Accepted 1 October 2021

2019; Wu et al., 2020).

Cattle dung deposited on grassland soils drives GHG production through microbial transformations of the carbon (C) and nitrogen (N) contained in the dung (Wu et al., 2020). The microbial mineralization of organic matter in fresh dung and the soil beneath drives CO_2 production (Cai et al., 2017; Zhu et al., 2020). Moreover, fresh dung contains abundant methanogens from the rumen that potentially convert the soluble C into CH_4 under anaerobic conditions (Cai et al., 2017; Hahn et al., 2018). Organic matter decomposition can also deplete oxygen within the dung or in the soil beneath, and that may create a favourable environment for the activity of methanogenic bacteria responsible for CH_4 production (Saggar et al., 2004). On the other hand, the organic N in dung is mineralized into ammonium (NH_4^+) and later transformed into nitrate (NO_3^-) through the activity of nitrifiers (nitrification process). Subsequently, NO_3^- can be converted into dinitrogen (N_2) by denitrifying bacteria (denitrification process) (Butterbach-Bahl et al., 2013; Oenema et al., 2008). The N_2O gas is produced as a by-product during nitrification and an intermediate product in the denitrification process (Cai et al., 2017). Several times, high CH_4 production from dung has been correlated with lower N_2O fluxes and vice versa, related to the high moisture content of dung and the redox potential that originates a further reduction of N_2O into dinitrogen (N_2) and more CH_4 production (Mazzetto et al., 2014; Pelster et al., 2016; Saggar et al., 2004). Thus, the amount of GHG produced depends on several factors such as nutrient input and abiotic factors like aeration, temperature, and soil water content (Cai et al., 2017) which also are regulated by climatic factors (Cardenas et al., 2016; Mazzetto et al., 2014).

As N_2O is a potent GHG linked to animal production, multiple strategies have been explored to reduce N_2O emissions from excreta deposited on grazed pastures, including the application of nitrification or urease inhibitors, biochar, lime, or changing cattle diets (Cai et al., 2017; Cardenas et al., 2016; Lombardi et al., 2021; Mazzetto et al., 2015; Simon et al., 2018). However, how these practices affect CH_4 or CO_2 emissions from excreta patches is still poorly understood (Cai et al., 2017). The use of nitrification inhibitors is a reasonable strategy to maintain the excreta N in the NH_4^+ form, blocking the nitrification pathway and reducing the NO_3^- and N_2O losses (Simon et al., 2018). Again, this reduction in N_2O emissions was related to an increase in CH_4 emissions (or a decrease in CH_4 oxidation), presumably due to the effect of accumulating NH_4^+ which may directly affect CH_4 monooxygenase activity (MMO) (Cai et al., 2017). The N_2O mitigation potentials of various chemical nitrification inhibitors have been proven on cropland and grassland under diverse climates (Misselbrook et al., 2014; Volpi et al., 2017). However, besides the prohibitive costs of these inhibitors, their long-term efficacy under tropical conditions (elevated rainfall and temperatures) remains uncertain (Coskun et al., 2017; Mazzetto et al., 2015). A promising mitigation strategy is the biological nitrification inhibition (BNI), a phenomenon through which certain plant species have the natural characteristic of inhibiting the activity of soil microbial nitrifiers by releasing exudates through their roots (Subbarao et al., 2013). Several genotypes from the genus *Urochloa* (formerly known as *Brachiaria)*, a tropical forage grass extensively used for cattle grazing, have been identified as high BNI plants (Nuñez et al., 2018). The N_2O reduction effect of BNI forages in urine patches is well documented (Bowatte et al., 2018; Byrnes et al., 2017; Gardiner et al., 2018; Simon et al., 2020; Simon et al., 2019). However, the potential effect of BNI on soil-animal-dung interactions and GHG emissions remains unexplored.

In addition, the diverse environmental and livestock management conditions alter both nutrient budgets and GHG fluxes; therefore, the Intergovernmental Panel on Climate Change (IPCC) encourages the development of country-specific GHG emission factors (EFs) that better reflect GHG emissions from excreta under existing production systems (Pelster et al., 2016; Zhu et al., 2018). Studies on the effects of diverse tropical conditions such as climate and soil properties and livestock management (livestock species, feed supply and quality, production system) on N_2O and CH_4 emissions from dung deposited on grasslands remain limited (Pelster et al., 2016; Tully et al., 2017; Zhu et al., 2018). Considering that animal excreta is a significant source of GHG emissions, there is a need to quantify the magnitude of grazing systems-based GHG emissions and then identify and promote actions to curb them (Cai et al., 2017).

As a follow-up to the study by Byrnes et al. (2017), who reported a 60% reduction in urine-based N_2O cumulative emissions in a high BNI forage, this study aimed to (i) investigate the potential effect of soil under two *Urochloa* forages with contrasting BNI capacity on GHG emissions from dung depositions, particularly focused on N_2O emissions; and (ii) determine the regionally N_2O and CH_4 EFs for cattle dung deposited on tropical rangelands. To achieve these objectives, this study was performed twice, applying the same dung on both *Urochloa* forage fields. The first trial was developed to measure GHG emissions and some chemical properties of dung and the soil beneath. The second trial evaluated only the GHG emissions under wetter conditions through a simulated daily rainfall applied after dung deposition. We hypothesized that (i) the high BNI *Urochloa* forage can reduce N_2O emissions from dung patches and therefore increase CH_4 emissions, and ii) the N_2O and CH_4 EFs are consistent with updated EF values established by IPCC 2019.

2. Materials and methods

2.1. Field experiment

The present study was performed at the International Center for Tropical Agriculture (CIAT) in Palmira, Colombia (3°30′7″ N, 76°21′22″ W and an elevation of 965 m.a.s.l.). The selected area was part of a long-term field experiment comparing several forage genotypes for their BNI capacity over time (Subbarao et al., 2009). In this study, evaluations were performed on two contrasting *Urochloa* forages selected based on previous findings: *Urochloa hybrid* cv. Mulato (low BNI capacity) and *Urochloa humidicola* cv. Tully (CIAT 679, high BNI capacity) (Byrnes et al., 2017; Subbarao et al., 2009). The soil was classified as a Vertisol (Typic Pellustert) with a silty clay texture. Soil properties (upper 10 cm) under low-BNI Mulato forage were: bulk density of 1.52 g cm^{-3}, pH of 6.3, EC of 263 μS cm^{-1}, organic C of 2.4%, and total N content of 0.17%, and under high BNI Tully forages were: bulk density of 1.39 g cm^{-3}, pH of 5.5, EC of 249 μS cm^{-1}, organic C of 3.5%, and total N content of 0.19%. Soil properties were measured and reported by Teutscherova et al. (2019).

Two trials were performed from 16 July to 5 September 2018 (Trial 1 – 51 days) and from 9 October to 13 November 2018 (Trial 2 – 35 days). Experimental plots (10 × 10 m) with the selected forages (Tully and Mulato; n = 3) were distributed in a randomized block design with three replicates. Two treatments (with dung and without dung as control) were evaluated in plots with the two selected forages. Dung deposition was realized in duplicates within each plot and then averaged for data analysis (n = 3). On the other hand, a single area was demarcated to act as the control (no dung application) within each forage plot (n = 3). Before the current study, the selected plots remained unfertilized for a year, and grazing was simulated by cutting the grass to approximately 5 cm sward height before dung deposition. The site was located in a tropical dry climate (IPCC, 2019) with a mean annual air temperature of 23.8 °C and total precipitation of 932 mm (1979–2019). Rainfall and air temperature data were recorded in CIAT's meteorological station located 900 m away from the field site. During the monitoring period, the average air temperature and total precipitation were correspondingly 24.7 °C and 135 mm (Trial 1); 24.2 °C and 58 mm (Trial 2).

2.2. Dung handling

Dung was collected fresh from zebu crossbred (with either Holstein or Brown Swiss) cows of approximately 500 kg weight belonging to a dairy farm. The cows were allowed to graze star grass (*Cynodon*

nlemfuensis – no BNI activity reported) with free water access. Alternatively, dung used in the experiment could have been collected from animals fed Tully or Mulato forages exclusively; however, we used a single type of dung to reduce variability and avoid differences associated with diets, as others reported (Lombardi et al., 2021; Simon et al. 2019). Thus, the samples represented the typical water and nutrient content of dung in the region. Before morning milking on the day of dung application on each trial, recently excreted dung used to simulate the dung patches was carefully collected (60 kg) from a concrete floor where animals were kept overnight. The dung was immediately transported to the experimental site and thoroughly mixed to form a composite sample. Aliquots were taken for subsequent analysis of dry matter (DM by oven drying at 60 °C until constant weight), total N content (by the Kjeldahl procedure as Simon et al. (2018)) and volatile solids (VS) content (through loss-on-ignition in a muffle furnace at 550 °C for 4 h). Applied C and N were calculated by multiplying the applied fresh dung weight by the total C or N content.

In each plot, two fresh dung patches (1.5 kg) were surface applied, kept 50 cm apart to prevent cross-contamination, an amount equivalent to a natural deposition for tropical conditions reported by Rivera et al. (2019). The patches were placed inside cylindrical PVC bases (20.3 cm internal diameter), inserted 5 cm into the soil, and used as the bottom of the static chamber for GHG measurement. Another chamber base that did not receive dung was included on each plot to act as a control. The procedures for obtaining and depositing dung were the same in both trials, although the position of chambers within each plot was changed, so there was no overlap of dung applied between trials. In addition, during the second trial, a simulated daily rainfall (20 mm) was applied four times during the first week after dung deposition to evaluate wetter conditions.

2.3. Dung and soil analyses

During trial 1, mirror-bases with the same size and treatments as those used for quantification of GHG emissions (dung and control) were installed within each plot to characterize the condition of the dung patch, the soil beneath dung (0–5 cm), and control soil (0–5 cm). Dung and soil samples were taken at 3, 8, 18, 28 and 51 days after dung application. Moisture content in the dung was estimated as the percentage of water content by subtracting the amount of DM measured. The water-filled pore space (WFPS) of soils was calculated as described by (Paul, 2015). Soil inorganic N in the form of NH_4^+ and NO_3^- concentrations were determined through the 1 M KCl (1:10 w/v) extraction method on freshly sieved soil right after sampling (Teutscherova et al., 2019). Potential nitrification rates (PNR) were determined by aerobic soil incubation following the procedure described by Byrnes et al. (2017). The PNR assay has been used to test the BNI activity in soils under different *Urochloa* genotypes (Byrnes et al., 2017; Nuñez et al., 2018).

2.4. GHG flux measurements and emission factors

Manual closed static chambers, similar to those described in Byrnes et al. (2017), were used for GHG monitoring. In brief, the chamber consisted of a cylindrical PVC base (10 cm height and 20.3 cm diameter) and a lid of the same dimensions fitted to the base during gas sampling with an airtight rubber band. The lid had a sampling port and a digital thermometer to record the internal air temperature during gas collection for gas flux correction. The chamber bases were uncovered between sampling periods, exposing the soil to natural incident rainfall and solar radiation.

Sampling occurred in the morning between 8:00 and 11:00 a.m. The air samples were collected at 0, 15, 30, and 45 min after chamber closure by using 20 mL propylene syringes and immediately transferred to pre-evacuated 10 mL Labco Exetainer vials. However, during the first ten days of each experiment, air samples from dung chambers were collected at 0, 6, 12, and 18 min after closure to avoid chamber saturation. While the scientific community seems to lean towards vented chambers (Clough et al., 2020), chamber vents were previously an evolving issue, and, in this study, we did not use vented chambers, as we had not tested them on the chambers we used. If not adequately designed, vents can cause errors (Bain et al., 2005). Once sampling was completed, the chamber tops were removed. Gas sampling started the day before dung application and daily during the first week following dung application, then two to three times per week for the subsequent three weeks and once per week during the last period of the trials. Gas samples were analysed for CO_2, CH_4, and N_2O using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and an electron capture detector. Each day two certified calibration gas standards were analysed by triplicate alongside the samples (2000 ppm CO_2, 10 ppm CH_4, and 1 ppm N_2O; and 5000 ppm CO_2, 2000 ppm CH_4, and 5 ppm N_2O; Air Liquide) used to calculate the concentration of the samples assuming a linear response.

Gas fluxes were estimated by calculating the rate of change in concentration over time using a linear approach corrected with the chamber volume, internal temperature, and air pressure at the site using the ideal gas law (Parkin and Venterea, 2010), following the equations detailed in Lombardi et al. (2021). All flux data were checked for linearity and validated by examining the CO_2 concentrations. Chamber replicates were excluded from the dataset when chamber fluxes had an $R^2 < 0.8$. Besides, we assumed the absence of flux (i.e., flux = 0) when the rate of change in gas concentration within each chamber was below the analytical precision limit of the GC, determined by analysing several standards near ambient concentration and then calculating its coefficient of variation (i.e., minimum change of 2.1 % for CH_4 and 3.8% for N_2O) as calculated Parkin et al. (2012). The hourly fluxes were multiplied by 24 to obtain the daily fluxes. Cumulative fluxes were calculated from mean GHG emissions by interpolation between measurement days (Chirinda et al., 2010). Nitrous oxide emission factor (EF) was estimated as the fraction of dung-N emitted as N_2O. The CH_4 EFs were calculated from the fraction of volatile solids (VS) emitted as CH_4 after subtraction of the emissions from the control plots (without dung) (IPCC, 2019).

Table 1
Cattle dung characteristics used in trial 1 and 2.

	Trial 1	Trial 2
DM (g kg^{-1})	246.2	188.5
VS (g kg^{-1} DM)	644.4	711.8
N (g kg^{-1} DM)	17.0	15.6
C (g kg^{-1} DM)	352.1	387.1
N rate (g m^{-2})	177.4	124.9
C rate (g m^{-2})	3683.7	3100.7
C:N	20.8	24.8

DM: dry matter. VS: volatile solids.

2.5. Statistical analyses

All analyses were performed using InfoStat (v. 2020), statistical software linked to the R programming environment (Di Rienzo et al., 2020). Daily fluxes and chemical parameters were analysed for each trial separately using a generalized linear mixed model (*lmer* package) with the forage genotype, excreta treatment, and sampling days as fixed factors, while each field plot was considered as a random factor. Several models were carried out, and the most appropriate fit was selected according to the lowest Akaike's information criterion. Cumulative emissions were analysed for each trial, comparing forages and excreta treatments with a two-way ANOVA. Differences were determined using the LSD Fisher test at $p < 0.05$ level. EFs were compared among forages using a *t*-test for each trial separately. The data were tested for normality using Shapiro-Wilk and for homogeneity of variance using Levene's test, and data were not transformed as they were normally distributed. Correlations between soil and dung chemical parameters and fluxes were

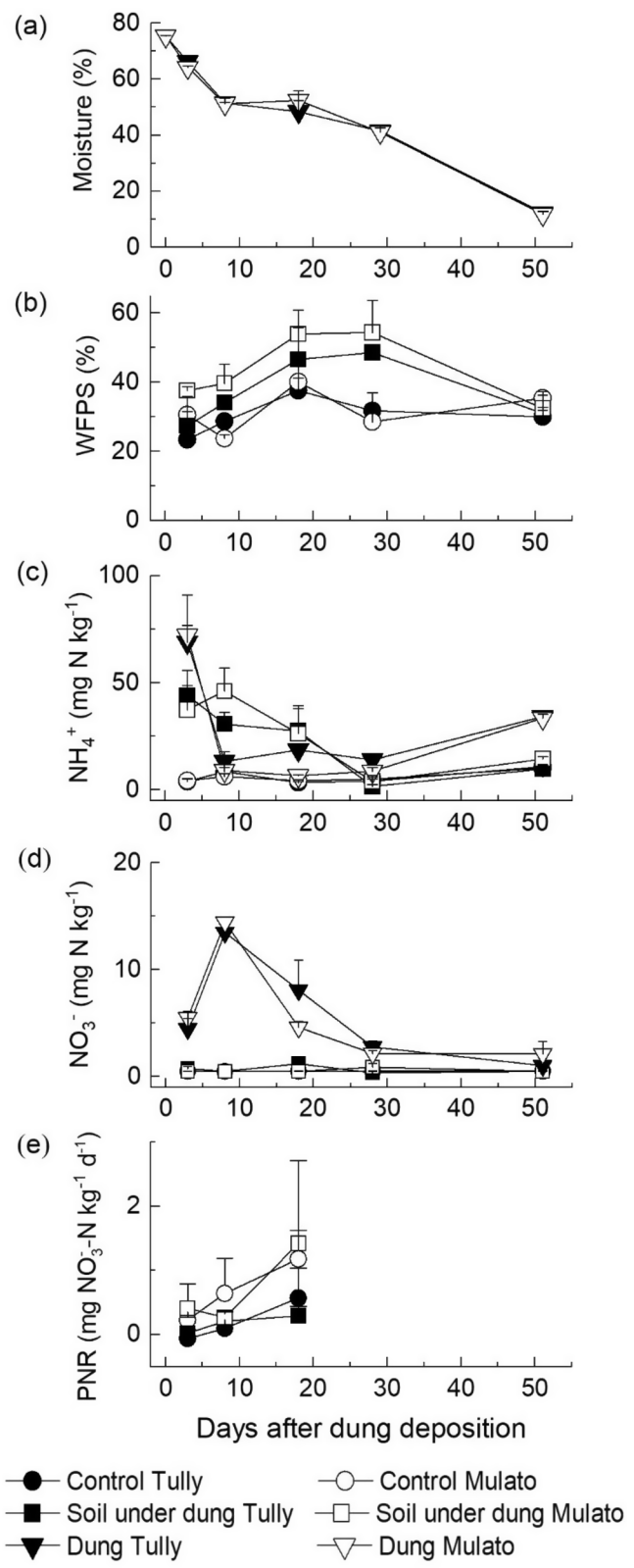

Fig. 1. Parameters on dung (triangle), soil beneath dung (square) and control soil (circle) after cattle dung deposition on either high-BNI Tully (black) or low-BNI Mulato (white) forages in Colombia: dung moisture (a), soil water full pore space (b), ammonium (c), nitrate (d) and potential nitrification rate (e) during trial 1. Bars represent SE of the mean (n = 3).

determined using Spearman's coefficient.

3. Results

The VS content in dungs were 64 and 71% for trials 1 and 2, respectively; and the amount of total N was 1.70 and 1.56% (Table 1). Dung collected in trial 1 had >31 % of DM content compared to dung used in trial 2; thus, dung patches in trial 1 had higher N and C contents. During trial 1, dung moisture decreased over time until a final moisture content of 12% without being affected by the forage type (Fig. 1a). As dung patches were drying, they began to exhibit a crust formation from the 3rd and 5th day in trials 1 and 2, respectively. The deposition of fresh dung increased the %WFPS in the soil beneath the dung patches compared to the control soils and showed significant effects of the excreta treatment ($P = 0.006$), but did not differ statistically between forages ($P = 0.26$, Fig. 1b). The WFPS in the soil beneath dung patches averaged 40.5%, ranging from 21.2 to 72.4% throughout the experimental period, with higher values for WFPS in the soil beneath dung patches in the Mulato forage plots ($P < 0.05$).

The main mineral N form in dung and the soil beneath dung was exchangeable NH_4^+, while NO_3^- contents remained low throughout the entire study period, with no significant differences among genotypes ($P = 0.96$ and $P = 0.77$, for NH_4^+ and NO_3^-; respectively). Dung NH_4^+-N concentrations were high following application and then decreased sharply with a significant variation among sampling days, which increased NH_4^+-N content in soils beneath the dung patches compared to control soils ($P < 0.001$, Fig. 1c). The soil beneath the dung patches and control plots contained 2 and 20 times less initial NH_4^+-N content than dung patches, respectively. Dung NO_3^--N concentrations increased during the first eight days after application, followed by a decrease to near-background levels at the end of trial 1 with a significant variation among sampling days (Fig. 1d). Unlike NH_4^+-N, the application of dung did not significantly increase the NO_3^--N content in the soil beneath the dung patch compared to soil in the control plots. Potential nitrification rates (PNR) were measured in soils during the first 18 days, and values seemed to be lower in soils under high-BNI Tully forage than under the low-BNI Mulato, although there were no significant differences, except for the 3rd day after deposition in control soil (Fig. 1e). The highest PNR was observed for Mulato 18 days after dung deposition in the soil beneath the dung patch.

The prevailing pattern across both trials was that the dung application increased daily GHG fluxes compared to control chambers, regardless of the GHG considered (all $P < 0.01$, Fig. 2). The CO_2 fluxes from dung patches increased immediately after deposition and then decreased and had similar values to the control chambers two weeks after dung deposition (Fig. 2b), revealing a distinct pattern affected by the sampling days in both trials ($P < 0.001$). The forage genotype had a significant effect on daily CO_2 fluxes during the first trial ($P < 0.001$), with higher values from dung on Tully forage, a difference which was not significant during the second trial ($P = 0.62$). Similarly, cumulative CO_2 emissions from dung patches were higher in comparison with control soil (Table 2), revealing a significant effect of excreta treatment application for both trials ($P = 0.002$ and $P < 0.001$, for trials 1 and 2, respectively) being affected by forage genotype only during the first trial ($P = 0.03$ and $P = 0.52$; respectively).

Dung deposition significantly increased N_2O emissions compared to the control chambers in both trials ($P < 0.001$ and $P = 0.008$, for trials 1 and 2, respectively). One month after dung application (35 d), the N_2O fluxes from dung were statistically similar to those from control chambers, where several negative fluxes were observed. The sampling day significantly affected N_2O daily fluxes during the first trial ($P = 0.001$), which was not significant during the second trial ($P = 0.27$). There was a three-day period between dung deposition and observing an increase in N_2O fluxes from dung patches. Peak N_2O emissions were observed around the 6th day after dung deposition (Fig. 2c). The maximum N_2O fluxes reached by dung depositions ranged between 0.10 and 1.31 mg

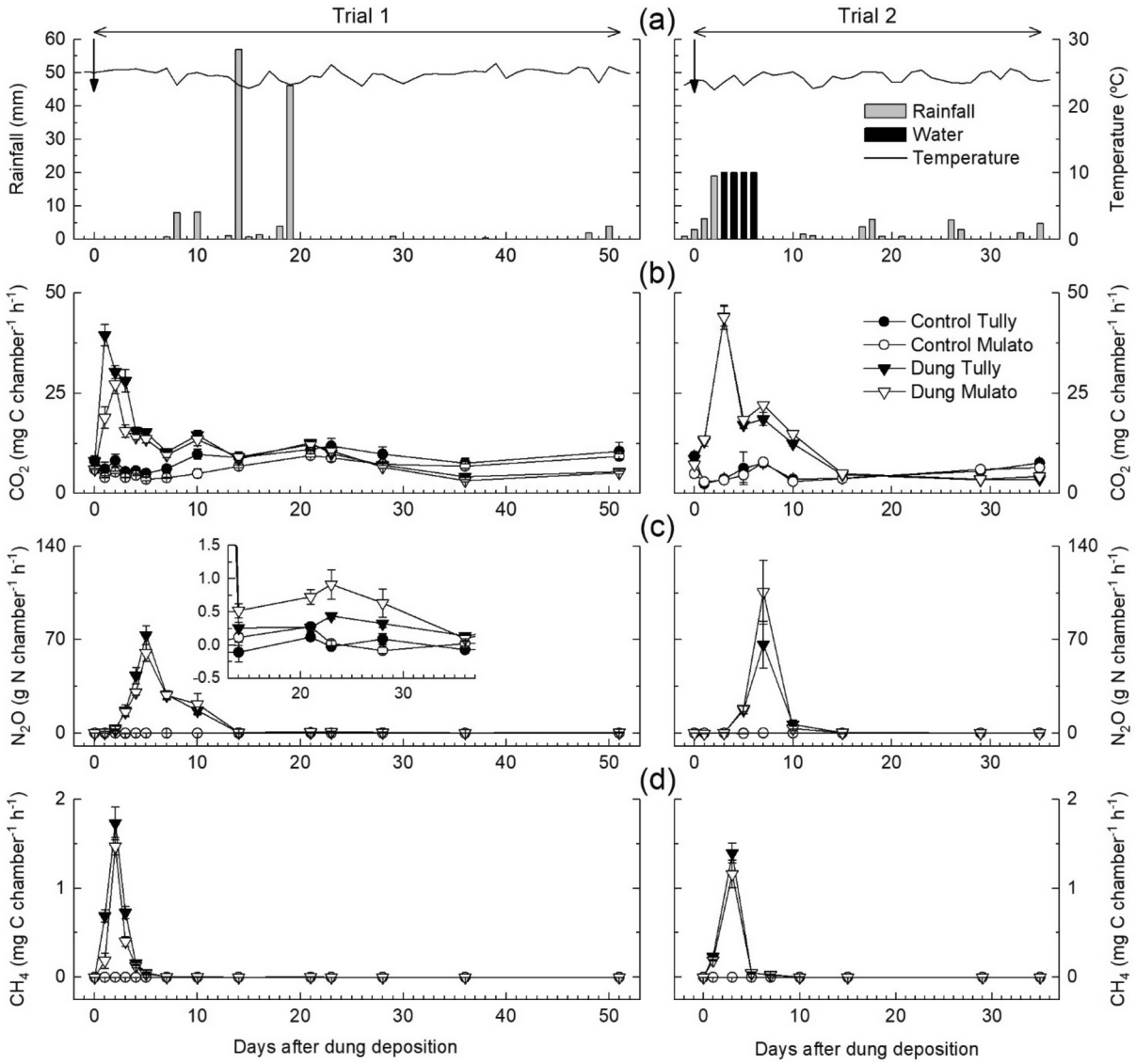

Fig. 2. Dynamics of (a) rainfall, simulated precipitation with water addition, and mean daily air temperature for trial 1 (left) and trial 2 (right) in Colombia. Evolution of CO_2 (b), N_2O (c), and CH_4 (d) fluxes over days after cattle dung deposition (arrows) on high-BNI Tully and low-BNI Mulato forages. Bars represent SE of the mean (n = 3).

Table 2
Cumulative CO_2, N_2O and CH_4 emissions and N_2O and CH_4 EF over the monitoring period in Trial 1 and 2 as affected by the addition of 1.5 kg cattle dung on different *Urochloa* forages (low-BNI Mulato and high-BNI Tully).

			Cumulative emissions			Emission factor	
Trial	Treatment	Forage	CO_2 (g C chamber^{-1})	N_2O (mg N chamber^{-1})	CH_4 (mg C chamber^{-1})	N_2O (%)	CH_4 (g CH_4 kgVS^{-1})
Trial 1	Control	Mulato	5.65 a	0.02 a	−0.19 a		
		Tully	7.58 ab	−0.02 a	−0.18 a		
	Dung	Mulato	8.63 bc	7.80 b	54.69 b	0.13 a	0.20 a
		Tully	9.85 c	7.93 b	81.82 c	0.13 a	0.31 a
		SE (±)	0.60	0.96	5.25	0.02	0.04
Trial 2	Control	Mulato	4.03 A	−0.06 A	−0.20 A		
		Tully	4.21 A	−0.01 A	−0.15 A		
	Dung	Mulato	8.56 B	7.94 B	63.65 B	0.13 A	0.33 A
		Tully	7.94 B	5.65 B	75.55 B	0.19 A	0.39 A
		SE (±)	0.33	1.46	7.20	0.05	0.07

Values are the mean and SE standard error (n = 3). Different letters indicate significant differences between excreta treatment and forage genotype ($p < 0.05$). Trials were analysed separately: lowercase and uppercase letters are for trial 1 and 2, respectively.

N_2O-N kg DM^{-1} d^{-1}. For trial 1, following a heavy rain event on day 19, there was a slight reduction in mean N_2O emissions from patches on Tully (on day 14, there was no rain effect on GHG emissions as sampling was done before the observed rainfall). However, as 95% of the total N_2O emission occurred around 10–15 days' time window after dung deposition, the rainfall event did not significantly reduce total N_2O emissions. Net cumulative N_2O emissions from dung were between 1.79 and 12.27 mg N_2O-N $chamber^{-1}$, whereas control soil ranged from −0.09 to 0.04 mg N_2O-N $chamber^{-1}$ (Table 2). Although average N_2O cumulative emissions from dung seemed to be reduced by 29% on high-BNI Tully forage when water was added in trial 2, the differences between forages were not statistically significant ($P = 0.46$). Similarly, although N_2O EF from dung on Tully forage appeared to be 28% lower in comparison with dung applied on Mulato (Table 2), there was no significant difference in the N_2O EFs for either of the forages evaluated in both trials ($P = 0.93$ and $P = 0.47$, for trials 1 and 2, respectively). Dung moisture and dung NO_3^- content strongly correlated with N_2O fluxes ($r = 0.83$ and $r = 0.88$, respectively).

The application of fresh dung also resulted in an initial increase in CH_4 fluxes affected by the excreta application, the sampling day, and the interaction, revealing a distinct pattern in both trials ($P < 0.05$) that was not affected by forage genotype ($P > 0.37$). Peak CH_4 fluxes were observed around the 2nd day of GHG monitoring (5.6 to 15.5 mg CH_4-C kg DM^{-1} d^{-1}) and then decreased sharply (Fig. 2d). About 95% of the net cumulative CH_4 emissions from dung patches occurred during the first five days after application (from 12.9 to 29.0 mg CH_4-C kg DM^{-1}). From day 28 onwards in both trials, CH_4 fluxes from dung patches were statistically similar to those from control chambers, with fluxes near zero or even negative. Similarly, net cumulative CH_4 emissions from dung patches differed from those observed in the control chambers (Table 2, $P < 0.001$ for both trials). Specifically, the soils without dung deposition acted as CH_4 sinks; meanwhile, the dung patches acted as a localized CH_4 source. The total CH_4 cumulative emissions were significantly influenced by excreta application ($P < 0.001$ for both trials), but in the first trial, those emissions were also affected by the forage genotype ($P = 0.03$), which, conversely was not significant during the second trial ($P = 0.43$). On the other hand, CH_4 EFs were not affected by the forage genotype ($P = 0.013$; ranged from 0.20 to 0.48 g CH_4 kg VS^{-1}, Table 2). Dung moisture was positively correlated with daily CH_4 fluxes indicating a strong and direct relationship ($r = 0.79$), which was also observed in a trend towards an increase in CH_4 EF with wetter conditions ($P = 0.078$, not significant at 5% level of significance).

4. Discussion

4.1. Dung-based GHG emissions and water addition

The amounts of nutrients in dung deposited in trials 1 and 2 varied slightly (Table 1) even though dungs were generated by the same animals with the same pasture; this corroborates with findings by Zhu et al. (2018), who reported that the quality of the excreted dung varies by season. The variations in dung nutrient and moisture content across trials could affect the magnitude of GHG emissions from dung patches, which were elevated several days after the excreta application and then returned to baseline levels in one month.

Cattle dung deposition on the forage fields stimulated CO_2 fluxes, which may have been due to high microbial activity within the fresh dung itself, microbial activity in the soil and autotrophic respiration from the forage, as pointed out by Cai et al. (2017). Further research is needed to test the relative contribution of each part under tropical conditions. The observed surge in CO_2 fluxes following dung deposition was consistent with Zhu et al. (2020), who also reported CO_2 flux peaks after dung application onto different tropical soils. In the present study, daily CO_2 fluxes were significantly affected by the different forage genotypes during the dry conditions but were not influenced by genotype during wetter conditions, suggesting that the dung moisture content may have influenced the CO_2 emission patterns.

Nitrous oxide fluxes increased three days after dung application; a similar delay in flux increases was reported in other studies on dung patches performed in tropical rangeland soils (Cardoso et al., 2016; Tully et al., 2017; Zhu et al., 2020; Zhu et al., 2018). Cumulative N_2O emissions from dung patches (55 to 371 mg N_2O-N m^{-2}) were similar to those observed by Mazzetto et al. (2014) after cattle dung deposition to a Brazilian grassland (between 1 and 218 mg N_2O-N m^{-2}) and higher in comparison to other studies reported in Kenya (2 and 90 mg N_2O-N m^{-2}; Tully et al. 2017; Zhu et al. 2018; Zhu et al. 2020). These high values may be related to the higher quality and quantity of the dung applied in our study since the N application rates were higher than other East African studies with poor-quality diet (Tully et al., 2017; Zhu et al., 2018) and similar to those under Brazilian conditions (Cardoso et al., 2019; Lessa et al., 2014; Mazzetto et al., 2014). Negative N_2O fluxes were observed in dung chambers when they returned to background values in corroboration with the findings reported by Mazzetto et al. (2014). When low NO_3^- is available in the soil, denitrifying bacteria may consume N_2O as the electron acceptor resulting in net N_2O uptake. Results during the wetter conditions of the second trial suggest that low mineral N and high moisture content enhanced N_2O consumption. A review study by Chapuis-Lardy et al. (2007) cited numerous studies that reported net negative N_2O fluxes under similar conditions in both tropical and temperate regions.

During the first five days after dung deposition, dung patches were localized hotspots of CH_4, likely due to high methanogenic activity, as Hahn et al. (2018) described. Several previous studies conducted under tropical conditions reported that 80% of total CH_4 emissions occurred during the first week after dung deposition (Cardoso et al., 2016; Tully et al., 2017; Zhu et al., 2018). This emission pattern contrasts with studies conducted under temperate conditions where high positive CH_4 emissions were observed over 40 days (Lombardi et al., 2021; Priano et al., 2014; Saggar et al., 2004). Cumulative CH_4 emissions (38 and 55 mg CH_4-C kg^{-1} dung) were consistent with other studies performed in Brazil (10 and 60 mg CH_4–C kg^{-1} dung; Mazzetto et al. 2014) and Kenya (11 to 75 mg CH_4-C kg^{-1} dung; Pelster et al. 2016). In the present study, forage genotype influenced the cumulative CH_4 emissions from dung during the dry conditions, but it did not affect CH_4 emissions during wetter conditions. To our knowledge, no other study reported similar results on CH_4 emission. On the other hand, net CH_4 fluxes from control chambers (i.e., without dung) were negative and similar among forage treatments and trials, suggesting that the soils at this site acted as a CH_4 sink. Although grasslands in tropical zones have low CH_4 negative fluxes compared to other zones, they represent a large portion of the earth's surface and thus a potentially large CH_4 sink (Dutaur and Verchot, 2007). Taking this sink potential into consideration when conducting national GHG inventories may reduce net GHG emissions associated with the livestock sector in the Tropics.

Water addition during trial 2 affected the drying of the dung patch and, consequently, GHG emissions. The weather immediately after dung deposition and dung consistency affect its degradation (Haynes and Williams, 1993). Dry weather conditions early after dung deposition produce a hard layer of dry dung-crust over the dung surface, which commonly reduces air permeability and hydraulic conductivity within dung patches (Evans et al., 2019). Such crust formation partially protects the dung patch from the eroding effect of raindrop impact, and it also inhibits rain from penetrating and rewetting the patch (Haynes and Williams, 1993). Therefore, at the beginning of trial 1, weather conditions (high temperature without rain) may have resulted in the rapid formation of a dung-crust, thus, maintaining anaerobicity and resulting in the observed high peak CH_4 fluxes. On the other hand, the combined effect of the wet dung and water addition of trial 2 resulted in a delayed dung-crust formation, which may increase gas exchange (particularly oxygen) and inhibit CH_4 production (Haynes and Williams, 1993; Saggar et al., 2004). In addition, these conditions of more water infiltration could also promote further N mobilization and specific microbial

activity, resulting in higher CO2 and N2O fluxes, as found in other tropical studies (Mazzetto et al., 2014; Zhu et al., 2020; Zhu et al., 2018). However, in corroboration with Zhu et al. (2018), rainfall events following crust formation did not increase CH_4 emissions, suggesting the formation of a crust is an important factor affecting dung degradation and GHG emissions.

4.2. Influence of forage genotype on dung-based GHG emissions

The forage genotype influenced the dynamics of the daily CO_2 fluxes and the cumulative emissions of CH_4 and CO_2, especially during the dry conditions of the present study. Dung deposited in plots with Tully forage had the highest daily CO_2 fluxes and cumulative emissions. However, the difference of daily CO_2 fluxes observed in plots with Tully and Mulato did not result in significant differences in total CO_2 emissions. In contrast, although the forage genotype did not influence the dynamics of daily CH_4 fluxes, the total CH_4 emissions showed a measurable effect of the forage genotype during the dry conditions. As we hypothesized, the highest cumulative CH_4 emissions were produced from the dung patch deposited on the high-BNI *Urochloa* forage (Tully). Other studies (Mazzetto et al., 2014; Saggar et al., 2004) reported an increase in CH_4 emissions following the application of nitrification inhibitors and attributed this to the effect of accumulating NH_4^+ on the CH_4 oxidation pathway. Specifically, similarities in the size and structure of CH_4 and NH_4^+ has been observed to result in the inhibition of CH_4 consumption (Gulledge and Schimel, 1998). However, in the present study, there was no measurable difference in NH_4^+ content due to forage genotype; thus, other drivers that we did not measure, or characteristics of the forages, could have influenced the environmental conditions of the dung patch and the subsequent CH_4 emissions. For instance, Horrocks et al. (2019) found that soil characteristics under Tully had greater aggregate stability, friability and soluble organic carbon concentrations compared to the soil under Mulato. However, considering that there were no differences in total CH_4 emissions from control soils under the different forages, we can assume that CH_4 emissions mainly originated from the dung patch; this suggests that the soil properties might not have influenced the differences in dung-based CH_4 emissions. However, the different soil cover structure of the two forage types could have modified the dung crusting and influenced the CH_4 emissions. Specifically, whilst the Tully plots were entirely covered by grass, the tufted growth habit of Mulato resulted in only half of the ground being covered by grass, also observed by Horrocks et al. (2019). The distance between tussocks in Mulato could modify the distribution of the patch, allowing more aerobic conditions in some parts of the patch, resulting in higher CH_4 oxidation (lower CH_4 emissions in Mulato). During the second trial, the addition of water likely hampered the effect created by the structure of the forages; thus, no differences in total CH_4 emissions were observed between the forages. Further research is needed under a wide range of conditions since uncertainties about the differential effect of dung deposition on CH_4 emission are large.

Contrary to our expectations, we did not find a measurable forage genotype effect on N_2O emissions from dung patches deposited on the *Urochloa* forage fields under the current study conditions. Although average N_2O cumulative emissions and EF values from dung patches deposited on high-BNI Tully seemed to be about 28% lower than those deposited on Mulato during wetter conditions, this difference was not statistically significant. The lack of effect of the underlying soil properties on N_2O emission after dung deposition was consistent with previous observations in tropical and temperate regions, where no significant effects of soil type on N_2O emissions derived from dung patches were observed (van der Weerden et al., 2011; Zhu et al., 2020). Those studies related the low incorporation of dung-derived N into the soil because of dung crusting. In contrast to urine or N fertilizers that easily infiltrate soils, the nutrients in cattle dung deposited on grazed pastures either remain on the soil surface or are partially mixed into the soil by animal trampling, the activity of macrofauna or water movement (Wu et al., 2020). The lower spatial distribution and slow entrance into the soil of dung-derived N, compared to urine, could hamper BNI expression, which depends on the interaction between N and the rhizosphere; only the dung-derived N that reach the rhizosphere may be affected by root exudates and, consequently, BNI. Therefore, the low N mobility may be a plausible explanation of why the high-BNI *Urochloa* was ineffective in reducing N_2O emissions in our study.

Besides, N present in dung (complex organic compounds (Wu et al., 2020; Zhu et al., 2020)) may limit the potential effect of BNI because the dung-derived N must undergo mineralization before nitrification and denitrification processes. In our study, most organic N of dung was probably kept in the patch for an extended period due to crusting. It was probably then slowly mineralized and converted to the relatively immobile NH_4^+ and was vulnerable to volatilization as NH_3 before nitrification. Part of the mineralized NH_4^+ can be nitrified/denitrified within the patch leading to the observed peak of N_2O emissions after dung deposition (that could be supported by the strong positive correlation observed between N_2O emissions and dung NO_3^- content). High NH_4^+ concentrations observed in the soil beneath the dung patches compared to the control support the hypothesis that a portion of dung-derived NH_4^+ was probably leached into the rhizosphere, likely in the form of dissolved NH_4^+, or possibly a small portion of the organic N in dung leached into the soil and then was mineralized. However, by day 30, most NH_4^+-N content in the soil beneath the patch was gone. There was no nitrification apparent in soils (NO_3^--N in the soil beneath dung was insignificant), indicating that most of the NH_4^+-N was immobilized, probably by the microbial community and by the *Urochloa* grasses, which are well adapted to take up NH_4^+ from soils (Vázquez et al. 2020). Therefore, even if PNR results would have confirmed higher BNI potential of Tully compared to Mulato grass forage as other studies did (Byrnes et al., 2017; Nuñez et al., 2018; Teutscherova et al., 2019), no forage effect on N_2O emissions would have been seen since neither soil was close to its potential nitrification rate. Nevertheless, a recent paper (Vázquez et al. 2020) has shown that the PNR is not a reliable measurement to demonstrate the BNI effect, suggesting determining relative gross N transformation rates by the ^{15}N dilution technique. Vázquez et al. (2020) described that a high microbial N immobilization rather than simple gross nitrification inhibition might explain the low NO_3^- concentrations and N_2O emissions commonly observed in high-BNI forages. In addition, other authors found that another reliable method for evaluating the BNI effect, besides the bioassay with luminescent bacteria, was determining the nitrifiers bacteria abundance (amoA gene) in soil (Byrnes et al., 2017; Nuñez et al., 2018).

To sum up, our findings suggest that in the case of dung patches, both the spatial distribution of dung-derived N in the soil and environmental conditions (i.e., rainfall) are essential regulators of the microbial processes and GHG emissions, as others have suggested (Cai et al., 2017; Wu et al., 2020). Also, the diet consumed by cattle is known to influence dung-based GHG emissions caused by various dung chemical properties, such as nutrient and water composition (Lombardi et al., 2021; Simon et al., 2019). Therefore, the absence of cattle diet manipulation enabled us to study the direct effect of soil under *Urochloa* forages on dung-based GHG emissions regardless of the type of dung deposited above. Further studies may need to consider quantifying GHG emissions from *Urochloa* fields using dung derived from each specific *Urochloa* forage grazed by the cattle. Such studies should include the simultaneous assessment of the BNI effect within the rhizosphere and the impacts on GHG emissions caused by changes in the dung and urine composition. On the other hand, based on our results, high-BNI *Urochloa* forages, known for their capacity to reduce N_2O emissions from urine patches, did not mitigate N_2O emissions from dung patches, presumable due to the lack of contact between the dung-N and the rhizosphere. Several methodology limitations may have influenced the results obtained. More frequent gas sampling, especially during the first two weeks after dung deposition, would be more suitable for determining a measurable effect on N_2O

Table 3
Comparison of the GHG monitoring period, fresh dung applied, % DM, and N_2O and CH_4 emission factors from dung under tropical conditions for the current study and other published studies.

Reference	Location	Observation period (days)	Fresh dung applied (kg)	DM (%)	CH_4 EF (g $kgVS^{-1}$)	N_2O EF(%)
This study	Colombia	36–51	1.5	19–25	0.20–0.39	0.13–0.19
Bretas et al. (2020)	Brazil	80–90	1.6	–	0.01–0.03	0.0–0.06
Zhu et al. (2018)	Kenya	25–29	0.5–1.0	15–29	–	−0.01–0.01
Tully et al. (2017)*	Kenya	60–63	0.5	18	–	0.0–0.21
Pelster et al. (2016)*	Kenya	28	1	20–25	–	0.1–0.2
Cardoso et al. (2016)*	Brazil	14–16	1.2–2.4	15	–	0.15–0.21
Lessa et al. (2014)*	Brazil	40–60	1.6	–	–	0.11–0.16

DM: dry matter, VS: volatile solids.
* Used for estimation of default emission factor EF_{3PRP} by IPCC (2019)

emissions since the second trial's statical analysis showed no effect of sampling days on N_2O daily fluxes. The sampling methodology must be adjusted to each specific research objective when conducting similar types of GHG studies. Also, a greater number of plots per treatment ($n > 3$) might have been helpful. Finally, additional studies may be required to verify our findings under different weather conditions and combining dung and urine depositions. Those studies are highly relevant for Latin American countries keen to evaluate and adopt GHG mitigation practices in the livestock sector, enabling them to achieve their Nationally Determined Contributions (NDCs) and meet their GHG emissions reduction targets under the Paris agreement (Arango et al., 2020).

4.3. Emission factors

The EFs found in this study were calculated during a relatively short period (five to seven weeks) in comparison to the time recommended for monitoring GHG emissions to develop robust EFs from dung depositions (IPCC, 2019). Although one month after dung deposition, the N_2O and CH_4 fluxes measured from dung patches were statistically similar to those from control soils, the potential for more GHG emissions remained. Whereas longer GHG monitoring campaigns may enable capturing the remainder of dung-based GHG emissions, short-term studies provide important insights on the major GHG emissions after cattle dung depositions. On the other hand, the length of the GHG monitoring in this study was within the time range (two to nine weeks) used by other available studies conducted under tropical conditions and used by the IPCC (2019) to estimate the current default EFs for national GHG inventories (Table 3). Monitoring of GHG emissions from dung patches performed under tropical conditions during up to nine weeks was generally sufficient to capture the major dung-based GHG emissions from soils under forages; yet, studies that were conducted under temperate conditions generally lasted longer (Cai et al., 2017; Cardenas et al., 2016; Lombardi et al., 2021; van der Weerden et al., 2011). Other studies conducted in tropical regions (i.e., in Brazil: Lessa et al. 2014; Mazzetto et al. 2014; or in Kenya: Pelster et al. 2016; Tully et al. 2017; Zhu et al. 2018) showed that the effect of dung deposition on CH_4 and N_2O emissions last only 2 to 3 weeks.

The N_2O EFs obtained in the current study ranged from 0.04 to 0.30%, corroborating other studies performed under tropical conditions (Cardoso et al., 2019; Cardoso et al., 2016; Lessa et al., 2014). We found an average N_2O EF of 0.14 ± 0.07% from dung patches, beyond the 0.07% default value (with a percentile range between 0.01 and 0.13%) for cattle dung depositions in dry tropical zones established by the refinement of the 2006 national GHG inventory guidelines (IPCC, 2019). However, as the percentile range reflects the 95% confidence interval for the mean, the measured N_2O EFs in this study were not too different from the IPCC range (2019). These updated default N_2O EFs disaggregate emission by the excreta and climate type, improving the previous N_2O EFs established by the IPCC (2006) that overestimated the N_2O emissions from cattle dung patches. On the other hand, the CH_4 EFs from fresh dung deposition in tropical forages resulting from this study averaged 0.31 ± 0.09 g CH_4 $kgVS^{-1}$, with values ranging from 0.20 to 0.48 g CH_4 $kgVS^{-1}$ for the different trials (Trials 1 and 2 of this study). However, these values were higher than those reported by Bretas et al. (2020) under Brazilian conditions (0.01 to 0.03 g CH_4 $kgVS^{-1}$). This difference could be explained by different nutrients and water contents of dungs and by distinct methodological procedures since we filled the chamber bases with dung. This set-up was unlike Bretas et al. (2020), who used chamber bases eight times larger than the area occupied by the dung patch. This meant their bases included soil without excreta deposition, which could consume CH_4 since grassland soils are typically a large sink for atmospheric CH_4. Our findings were lower than the IPCC 2019 default CH_4 EFs of 0.6 ± 0.2 g CH_4 $kgVS^{-1}$, estimated for dung deposition into pasture range and paddocks without differentiating between livestock species and productivity class. These findings suggest a need to reduce dependence on default Tier 1 EFs and invest in studies that result in more region-specific Tier 2 emission factors.

5. Conclusions

Cattle dung was a direct source of GHG emissions, which occurred during a relatively short period of one month after the deposition of the same dung on two plots of tropical forages with contrasting BNI capacity. The forage genotype beneath the dung patch influenced daily CO_2 and total CH_4 emissions only during periods characterized by dry conditions. However, no significant effect of forage genotype was found on mitigating N_2O emissions from dung. Probably, the limited incorporation of dung-derived N into the soil rhizosphere (where BNI occurs) hampered the potential effect of BNI to mitigate the N_2O emissions. Additional studies conducted under different weather conditions with animals consuming *Urochloa* forages with contrasting BNI capacity are required to obtain more representative results at the farm level to consider the complete implications of forage selection on excreta and animal-based GHG emission.

On the other hand, the mean N_2O EFs from dung deposition found here was 0.14%, close to the default value developed by the 2019 refinement of the IPCC for cattle dung on dry tropical zones (between 0.01 and 0.13% with a 95% confidence interval). Moreover, the CH_4 EFs averaged 0.31 g CH_4 $kgVS^{-1}$, slightly lower than the 0.6 g CH_4 $kgVS^{-1}$ developed by the IPCC (2019). These findings suggest that, in this region, the use of the updated N_2O EFs of the IPCC 2019 may improve certainty in emission estimations of GHG inventories compared to the IPCC (2006) EFs. However, further studies are needed to reduce dependence on default EFs and develop more region-specific emission factors.

Declaration of Competing Interest

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.

Acknowledgements

Thanks to the CLIFF-GRADS programme, the International Center for Tropical Agriculture for hosting the grant recipient and to the Government of New Zealand for providing financial support. We would also like to thank Gabriel Gatica from CIFICEN (Argentina) for the valuable advice on statistical analyses. Thanks to the Environment and Health Applied Sciences Doctoral Program (DCAAS) at UNICEN, Argentina, where Banira Lombardi performs her doctoral studies.

Funding

This research was supported by the CGIAR Research Program on Climate Change (CRP), Agriculture and Food Security (CCAFS) and the Global Research Alliance on Agricultural Greenhouse Gases (GRA) through their CLIFF-GRADS programme. CCAFS is carried out with support from CGIAR Fund Donors and through bilateral funding agreements. For details, please visit https://ccafs.cgiar.org/donors. Also, this work was conducted as part of the Livestock CRP and with funding from the Biotechnology and Biological Sciences Research Council project grants RCUK-CIAT Newton Fund - *Towards climate-smart forage-based diets for Colombian livestock* (BB/R021856/1) and *Advancing sustainable forage-based livestock production systems in Colombia* (*CoForLife*) (BB/S01893X/1).

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