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Combination of cattle urine and dung patches synergically increased nitrous oxide emissions from a temperate grassland under wet conditions

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ABSTRACT

During grazing, some of the nutrients ingested by cattle are returned to grassland as urine and dung patches and can be lost as greenhouse gases. Sites where cattle congregate are more likely to have overlapping excreta patches favouring enhanced nitrous oxide (N_2O) emissions. However, there is no consensus about the magnitude of these or simultaneous methane (CH_4) emissions or potential mitigation options. This study investigated the effect of combined cattle dung and urine depositions on N_2O and CH_4 emissions, compared with emissions from separate depositions, under different weather conditions. Local emission factors (EFs) were then calculated for both gases. A quantitative assessment of published studies was also performed to search for N_2O emissions drivers. Two field experiments were performed during two 98-day trials under dry and wet conditions in Tandil, Argentina. Treatments included fresh excreta patches of urine (0.75 L), dung (2.50 kg), dung + urine (2.50 kg + 0.75 L) from Holstein dairy cows, and a control (without excreta). Soil and excreta properties were analysed, and N_2O and CH_4 fluxes from the patches were measured using the static chamber technique. Patches containing dung were shown to be localised CH_4 hotspots. Urine applied to soil, and the addition of urine to dung patches had a negligible effect on CH_4 fluxes. Urine, dung and combined patches were found to be localised N_2O sources. Adding urine to dung patches under wet weather had a significant synergetic effect (threefold increase) on cumulative N_2O emissions compared with the theoretical sum of separate excreta patches. Adding urine to dung patches under dry conditions gave an additive effect on N_2O. These findings suggest that preventing overlapping excreta patches under wet conditions can help mitigate N_2O emissions from temperate managed grazed pastures. The effect of combining excreta patches was also evident in the EF values obtained. That for CH_4 was consistent with the default IPCC value (0.75 g CH_4 kg^{-1} VS), while N_2O (EF = 0.03–0.39%) was lower than the updated IPCC 2019 value of 0.6%.

1. Introduction

Over 80% of large ruminants are produced in grazing systems which occupy about one-third of the Earth's ice-free land surface and account for 70% of global agricultural land (Steinfeld et al., 2006). In grassland ecosystems, livestock can help transfer plant biomass into highly nutritious animal protein foods while utilising resources that humans cannot otherwise consume (Leip et al., 2019). However, large proportions of the nutrients ingested by livestock are returned to the soil through excreta depositions (urine and dung) (Haynes and Williams, 1993). Although the deposition rate of nutrients in excreta varies greatly due to diet composition and animal characteristics, the amount of nutrients in the relatively small excreta patches generally exceeds immediate plant requirements. Finally, this excess of nutrients is vulnerable to

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losses via leaching and gaseous emissions (Cai et al., 2017).

Fresh excreta deposited by grazing cattle contain energy-rich and chemically reduced carbon (C) and nitrogen (N) (Oenema et al., 2008), which can result in hotspots of greenhouse gas (GHG) emissions from grassland (Cai et al., 2017). Freshly excreted dung has considerable potential to emit methane (CH_4). It contains abundant methanogenic microorganisms from the digestive tract that convert readily available soluble C into CH_4 under the anaerobic conditions prevailing within the fresh dung patch (Hahn et al., 2018). In addition, since excreta contain significant amounts of organic N (Haynes and Williams, 1993), both urine and dung patches have the potential to act as a localised source of nitrous oxide (N_2O), a potent GHG. Following deposition, a major fraction of the organic N in excreta is mineralised into ammonium (NH_4^+) and later transformed into nitrate (NO_3^-) through the activity of nitrifiers under partial aeration. The NO_3^- produced can then be converted into dinitrogen (N_2) by denitrifying bacteria under anoxic conditions. During both nitrification of NH_4^+ and denitrification of NO_3^-, N_2O gas may escape to the atmosphere as a by-product (Luo et al., 2017). The fraction of N emitted from excreta as N_2O is highly influenced by environmental conditions, farm systems and manure management practices (Oenema et al., 2008).

Pasture-based dairy production systems are common in the temperate southern hemisphere. Cows rotationally graze in non-irrigated fenced paddocks with free access to water in stationary troughs (Luo et al., 2017). They frequently also have access to other preferred areas, like feeding bins, shaded areas, gateways and tracks within paddocks. These sites where animals congregate are subjected to more frequent excreta deposition and trampling, resulting in a high N load in compacted soil without vegetation, favouring higher N_2O emissions (hotspots) (Saggar et al., 2022). The highly soluble N concentrations in urine and the available C and microorganisms in dung may have a synergetic effect, leading to higher GHG emissions than the theoretical sum from each separate excreta patch. Previous laboratory incubation studies indicate that combined dung and urine have a synergetic (super-additive) effect on N_2O emissions, raising the level above the theoretical sum (Liao et al., 2018; van Groenigen et al., 2005a; Wu et al., 2020). However, it is unclear whether this synergetic effect also occurs under field conditions since there is no consensus in the literature on whether and to what extent combined cattle excreta patches enhance N_2O emissions (Cardoso et al., 2019; Hyde et al., 2016; Li et al., 2016; Tully et al., 2017; van Groenigen et al., 2005b; Zhu et al., 2021). Studies examining CH_4 emissions found that overlapping excreta patches may have a negligible effect or even decrease CH_4 emissions compared to separate dung patches (Liao et al., 2018; Tully et al., 2017; Zhu et al., 2021). Data are particularly scarce for temperate grasslands, creating a need to identify grassland management practices that increase GHG emissions and formulate appropriate mitigation measures.

To better characterise GHG emissions from excreta on-farm and understand their contribution to total agricultural GHG in national inventories, the Intergovernmental Panel on Climate Change (IPCC) encourages the development of country-specific GHG emission factors (EFs). Estimating GHG emissions from excreta deposited during grazing includes CH_4 emissions from dung and N_2O emissions from urine and dung (IPCC, 2019). It is also possible to develop individual EFs for N_2O, partitioned into dung and urine, to produce disaggregated N_2O EFs, which is widely recommended by multiple studies (Chadwick et al., 2018; Krol et al., 2016; Luo et al., 2019; van der Weerden et al., 2021, 2011). According to van der Weerden et al. (2021), this disaggregation can improve the accuracy of national inventories, but more data from poorly represented regions (e.g., Asia, Africa, South America) are needed.

Against this background, the aims of this study were i) to investigate the effect of combined cattle dung and urine patches on N_2O and CH_4 emissions, compared with emissions from separate depositions, under different weather conditions and ii) to develop local disaggregated EFs for N_2O and CH_4. A quantitative assessment of literature data on the effects of combined excreta patches was also performed to identify N_2O emissions drivers. The starting hypotheses were that 1) separate urine and dung patches are localised N_2O sources, and adding urine to dung patches results in a synergetic, rather than additive, increase in N_2O emissions; 2) separate dung patches are localised CH_4 hotspots and adding urine to dung patches does not increase dung-based CH_4 emissions; and 3) the N_2O EF for cattle urine is higher than for cattle dung patches, supporting disaggregation by excreta type.

2. Materials and methods

2.1. Site description

The study was performed at the campus of the National University of the Center of Buenos Aires Province (UNCPBA), Tandil, Argentina (37°19'07"S, 59°04'42"W, altitude 211 m asl), where the climate is warm temperate moist (following Fig. 10A.1 in IPCC, 2019). The mean annual temperature is 13.5 °C, and total annual precipitation is 880 mm (based on mean climate records 1981–2011). Data on air temperature at 2 m above the soil surface, and soil volumetric water content and soil temperature at 0.2 m depth during the study period were obtained from a meteorological station located 400 m from the experimental site. Precipitation was recorded with a rain gauge directly at the experimental site.

The soil at the experimental site is a Mollisol (typical Argiudoll, considered a fertile soil) (Panigatti, 2010). It comprises 37% sand, 37% silt and 26% clay. The topsoil (to 0.2 m depth) contains 2.7% organic C and 0.26% total N, its bulk density is 1080 kg m^{-3} and the pH is 6.0 (Priano et al., 2017). During the study period, the soil was covered by naturalised pasture dominated by *Festuca arundinacea*, with presence of *Cirsium vulgare*, *Carduus acanthoides, Trifolium repens* and *Taraxacum* sp., which is typical of cattle pasture in the Pampa region. The experimental soil did not receive any fertiliser and had no grazing animals during the 14 years prior to the study (Faramiñán et al., 2021). The grass was cut every month, between 0.1 and 0.25 m with a mowing machine, and removed from the area. Precisely, the pasture was manually cut within the collars containing the treatments when the height reached about 0.15 m to a final 0.05 m for a better measurement deployment.

2.2. Experimental design

Two separate 98-day trials were performed, the first in winter-spring (September 9 to December 17, 2019) and the second in summer-autumn (February 15 to May 25, 2020). As these experimental periods did not coincide precisely with any regular season in the region, they were named according to the predominant weather condition as "dry" and "wet" trials, respectively (since water content differed between the trials, but not mean temperature). More information on weather conditions is shown in the results (Section 3.1).

Treatments and the experimental design were similar in both trials (Fig. 1). Treatments consisted of the application of separate patches of fresh "urine", "dung", or a combination of dung + urine ("D+U"). A "control" treatment (without excreta) was included to determine background values and allow EF calculation for each excreta type. In both trials, the experimental site comprised a total area of 64 m^2 divided into four blocks (replicates), with four plots in each block (one per treatment). Within the respective plots, each treatment was applied in duplicate within circular collars (0.26 m in diameter) to simulate typical excreta patches on pasture. One of these duplicates was used for gas measurements and the other to monitor soil characteristics. The amount of fresh excreta applied to each chamber was based on the mean mass per defecation (2.5 kg) and mean volume per urination (0.75 L) defined during excreta collection. Naturally, urine and dung patches do not have "delimiting edges", and applying the excreta across the whole area of the chamber might affect N_2O production. However, despite the limitations of the methodology, it was considered appropriate to comparatively

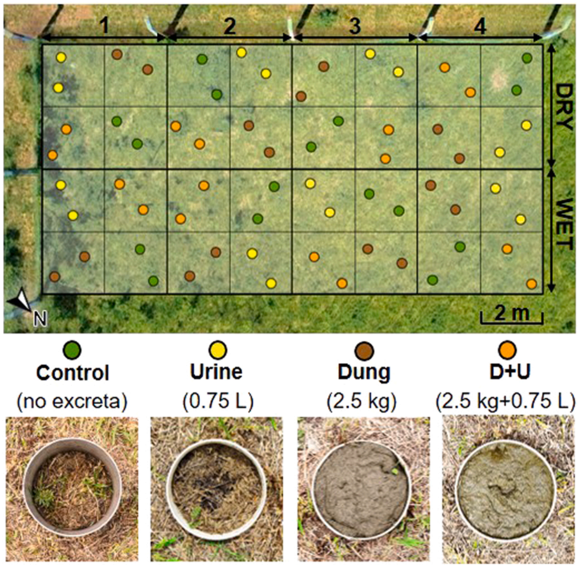

Fig. 1. Diagram of the field area used in the two trials (dry, wet), showing the experimental design with four treatments (control, dung, urine, D+U) and four replicates. Circles represent the location of each chamber (by duplicate within plots), colours indicate the treatment applied.

evaluate the N_2O emissions from overlapping excreta since this methodological approach has been previously used in published studies on excreta N_2O emissions that experts included in the IPCC database (Li et al., 2016; Luo et al., 2013; Thomas et al., 2017).

In the combined patch, 0.75 L of urine were applied overlapping the 2.5 kg of dung after its deposition. Both dung and urine were carefully applied from a height of 1 m directly to the centre of the collars, simulating the height of a natural excretion. The zone of influence included the entire collar as the patch-effective area. Fresh urine and dung were collected from the same lactating dairy cows (Argentinian Holstein, 526 ± 47 kg live weight) between morning and afternoon milking the day before application in each trial (diets described below). Before the dry trial, 30 urinations were collected through manual stimulation under the vulva and poured together into a plastic can. Simultaneously, recently excreted dung was carefully collected from the naked soil surface of the individual feeding pens, weighed separately and then pooled and homogenised. In the wet trial, the same procedure was used for excreta collection (without quantification). The same urine and dung amounts were applied to a nearby field area to avoid the carry-over effect of treatments from the previous trial.

At collection in September (dry trial), the cows were grazing a pasture of *Lolium multiflorum* (ryegrass; intake 7 kg DM animal^{-1} d^{-1}, 21% crude protein (CP) in DM basis), supplemented with a daily mixed ration of 13 kg DM animal^{-1} consisting of corn silage (5 kg DM, 6.6% CP) and energy concentrate (8 kg DM with 20% CP; 39% corn grain, 35% wheat bran, 22% soybean expeller, 4% minerals and vitamins, in DM basis). At collection in February (wet trial), the cows were grazing a pasture of *Medicago sativa* (alfalfa; intake 13 kg DM animal^{-1} d^{-1}, 27% CP), supplemented with a daily concentrate ration of 6 kg DM animal^{-1} (with 27% CP).

2.3. N_2O and CH_4 fluxes and emission factors

Greenhouse gas fluxes were measured using the static chamber technique. Sixteen cylindrical PVC collars (0.26 m internal diameter, 0.1 m height, area 0.05 m^2) were used as the base of the static chambers. The chamber bases were inserted 0.05 m into the ground two days before excreta application to prevent soil disturbance influencing gas emissions and left in place for the entire trial period (98 days). The chamber bases were placed 1 m apart to prevent cross-contamination, and the excreta doses were deposited inside. A lid of the same dimensions and material as the base (0.1 m height) was used during sampling to enclose the internal volume of the chamber. On each sampling occasion, the lid, equipped with a septum for gas sample collection, was fitted to the chamber base and sealed with an airtight rubber belt. Samples were collected using a 25 mL polypropylene syringe immediately after chamber closure (0 min) and after 10, 20 and 30 min to avoid saturation and ensure an adequate linear response in headspace gas concentrations (R^2 >0.8).

Sampling was conducted between 10:00 and 12:00 h when the emission rate resembled the daily average determined by Priano et al. (2014) for the study area. Periodic gas sampling began the day before excreta application to soil and continued for 98 days, when the excreta gas fluxes equalled those of the control, with a total of 20 and 22 sampling occasions for the dry and wet trial, respectively. The sampling frequency varied throughout the 98 days of each trial. The first measurement occurred three hours after treatment application, and the following measurements were performed daily in the first week. The sampling frequency then decreased from three to two times per week during weeks 2–5, once per week during weeks 5–11, and finally once per month until the end of the trial (i.e. week 14). Within two days of collection, all air samples were analysed using a gas chromatograph (GC, Agilent 7890 A) equipped with a flame ionisation detector (FID) for CH_4 and an electron capture detector (ECD) for N_2O (see Lombardi et al., 2021 for details).

Gas concentration was calculated using the ideal gas law, and gas fluxes were estimated by calculating the rate of change in concentration over time using linear regression, as described in Lombardi et al. (2021). Fluxes were corrected for the air temperature at sampling (measured on the soil surface inside the chambers with a data logger i-button DS1921G) and the atmospheric pressure at the site (obtained from the nearby meteorological station). All flux data were checked for linearity by examining the R^2 value of concentration versus time, with replicates excluded from the dataset at R^2 < 0.8. The absence of flux in the chambers (i.e. flux = 0) was assumed when the rate of change in gas concentration was below the analytical precision limit of the GC device. This limit was determined by analysing several ambient air samples and then calculating the coefficient of variation (CV = 2.1% for CH_4, 3.8% for N_2O). Cumulative gas emissions (mg m^{-2}) were estimated by integrating the linear interpolation of the daily fluxes over time (OriginLab 2018 Software). In addition, the theoretical sum of cumulative N_2O emissions from the separate dung and urine patches (named "$D+U_{theoric}$") was calculated to compare with the combined D+U treatment.

Emission factors for CH_4 and N_2O were calculated using estimated cumulative gaseous emissions. The EF for CH_4 (g CH_4 kg^{-1} VS) was calculated only for treatments containing dung as described by IPCC (2019), i.e. as CH_4 emitted directly by the excreta patch minus CH_4 emissions from the corresponding control chamber, divided by units of volatile solids (VS) applied, i.e. organic matter. The EF for N_2O, expressed as a percentage of N emitted per unit of N applied, was calculated by subtracting cumulative N_2O emissions occurring in control from cumulative N_2O emissions lost directly from the excreta patch and then dividing this by the amount of N applied inside the chamber. The units of N_2O and N inputs needed to be the same for this calculation (e.g. cumulative N_2O emissions in g N_2O-N m^{-2} and N applied in g N m^{-2}).

2.4. Soil and excreta parameters

Portions of fresh excreta were taken immediately after homogenisation in each trial and analysed for initial characterisation. Two 100 mL subsamples of urine were stored at − 18 °C with 5 mL sulphuric acid (5%) to immobilise N. Two 100 g subsamples of dung were dried at 65 °C to constant weight to determine DM content. Dung subsamples dried at 105 °C were milled, and a single composite sample was analysed for VS content (through loss-on-ignition in a muffle furnace at 550 °C for four hours; AOAC, 1995, method 942.05). Total N concentrations in urine and dung was determined by the Kjeldahl method (AOAC, 1995, method 991.20). Dung organic C content was calculated from the known VS content using the formula developed by Iglesias Jiménez and Pérez García (1992). Characteristics of the urine and dung used in the trials are presented in Table 1.

Adjacent to each chamber where GHG fluxes were measured, the same treatment was applied using an extra chamber base as a mould. This area was used to evaluate soil water content, water filled pore space (WFPS) and exchangeable NH_4^+ and NO_3^- concentrations in the top 0.1 m of soil. In addition, a 0.03 m diameter mould was used to sample the superficial dung and D+U patch for water content monitoring. Samples were collected on six occasions (days 3, 10, 22, 35, 49 and 98 in the dry trial; days 2, 10, 22, 41, 66 and 98 in the wet trial). Approximately 10 g of these samples were used to determine moisture gravimetrically by drying the soil and excreta samples at 105 °C until constant weight. The WFPS was calculated as Paul (2015) described using the soil water content (g H_2O/g dry soil) of each treatment and sampling day and the bulk density measured at the beginning of each trial. Soil particle density was assumed to be 2650 kg m^{-3}. Separate moist samples, also 10 g, were subjected to extraction with 2 M KCl for determination of NH_4^+ by the sodium salicylate-nitroprusside method and colourimetric reading at 667 nm (Borrero Tamayo et al., 2017) and direct determination of NO_3^- by salicylic acid nitrated in an alkaline solution and reading at 410 nm (Forster, 1995). For moisture correction, the gravimetric moisture content at 105 °C was applied.

2.5. Data analysis

Studies reporting field measurements of N_2O fluxes from excreta patches were compiled using Scopus® from Elsevier B.V. as the source of peer-reviewed papers to analyse the published data. Among the hits obtained, five studies best matching the characteristics of the present study were selected (Hyde et al., 2016; Li et al., 2016; Tully et al., 2017; van Groenigen et al., 2005b; Zhu et al., 2021). Including criteria were: i) field studies with N_2O measurements using the static chamber technique and GC; ii) treatments including separate cattle excreta patches (dung and urine), a combination of both (maintaining the amount of excreta applied in both separate patches) and a background (i.e. no excreta application); and iii) field monitoring for longer than 30 days. From these studies, data on mean cumulative N_2O emissions from each treatment were gathered by extracting from tables or the main text or, when these were not available, from diagrams using Data Thief III (https://datathief.org/). Within each study, each excreta type (control, urine (U), dung (D), and D+U) identified by the authors, divided into different weather conditions (dry and wet), was considered an observational unit. Also, specific study characteristics, such as climate zone, soil type, and amount of excreta applied, were gathered. Data were corrected for soil baseline N_2O emissions (by subtracting the control value from each excreta result) to compare cumulative N_2O emissions across different experimental site characteristics (i.e. background properties). The theoretical sum of each observation ($D+U_{theoric}$) was calculated by adding the separate values for dung and urine and dividing by the amount of N applied to avoid differences due to variations in excreta N input. The proportional increase in cumulative N_2O emissions was defined as $\Delta = (D+U/D+U_{theoric})$, i.e. comparing the combined D+U and $D+U_{theoric}$. With this definition, the effect of combining excreta is additive when the interaction term is equal to one and super-additive (synergetic) if the value shifts toward higher values (>1). This approach helped interpret the available data on response ratios of N_2O emissions (N = 12, including our results). The statistical significance of differences in the proportional increase between dry and wet conditions was tested using ANOVA.

Statistical analyses were performed using the software InfoStat (v. 2020), linked to the R programming environment (Di Rienzo et al., 2020). Daily CH_4 and N_2O fluxes and soil parameters (variables with repeated measures) were analysed for each trial separately using a generalised linear mixed model (glmm, *lmer* package) with excreta treatment and sampling day as fixed effects and field plot as a random effect. Several models were developed with different link functions. The most appropriate fit was selected according to the lowest Akaike's information criterion (AIC) value for models that met the assumptions of residuals (homogeneity and normal distribution). Cumulative emissions and EFs were analysed for each trial separately with a glmm including excreta treatment and plot as fixed and random effect, respectively. Differences were determined using the LSD Fisher test at $P < 0.05$ level. Correlations between soil/dung parameters and gas fluxes were determined using Spearman's correlation coefficient (ρ).

Table 1
Excreta composition and N application rates used in the dry and wet trials.

	Dry	Wet
Excreta composition		
Urine		
N (g L^{-1})	5.0	8.9
Dung		
DM (g kg^{-1})	126.8	123.3
VS (g kg^{-1} DM)	761.0	847.5
N (g kg^{-1} DM)	23.2	24.8
C (g kg^{-1} DM)	410.9	456.4
C:N ratio	17.7	18.4
N application rates		
Urine (g N m^{-2})	72.1	126.9
Dung (g N m^{-2})	140.6	145.1
D+U (g N m^{-2})	212.7	272.0

DM: dry matter, VS: volatile solids, N: nitrogen, C: carbon.

3. Results

3.1. Experiment characteristics

Overall mean air and soil temperature (16 °C and 18 °C) at the meteorological station were similar for the two 98-day trials (Fig. 2a, b). However, the dry trial had lower cumulative rainfall and soil volumetric water content (210 mm and 35%) than the wet trial (360 mm and 42%, respectively). During the initial ten days after excreta application, the air and soil temperature and rainfall were lower in the dry trial (11 °C, 11 °C and 7 mm, respectively) than in the wet trial (19 °C, 22 °C and 35 mm, respectively).

The chemical composition of the excreta across trials is shown in Table 1. Urine N content per volume in the dry trial was about half that in the wet trial. Therefore, the amount of total N applied inside each chamber containing urine, dung, and the D+U in the dry trial was 3.8, 7.4 and 11.1 g, respectively, while in the wet trial, it was 7.6, 6.6, and 14.2 g, respectively. These values converted to rates (g N m^{-2}) are shown in Table 1. The organic C applied in dung patches (chambers) in the dry and wet trials was 130.2 and 139.6 g, respectively.

3.2. N_2O and CH_4 fluxes

The deposition of the different excreta treatments showed differing emissions patterns depending on the type of excreta, producing localised GHG hotspots (Fig. 2). The data included several points on both the ascending and descending parts of the flux curves, suggesting that the gas sampling intervals were sufficient to capture the pulse of GHG emissions after excreta deposition.

Within each trial, the daily N_2O fluxes revealed a distinct pattern depending on the excreta treatment and time since application (Fig. 2c, d), resulting in a significant effect of excreta treatment, sampling day and their interaction ($P < 0.001$ for all, in both trials). Soil N_2O fluxes (from control chambers) were low across both trials and sometimes even negative, also observed and explained in Lombardi et al. (2022). In urine patches, N_2O emissions increased immediately after urine application

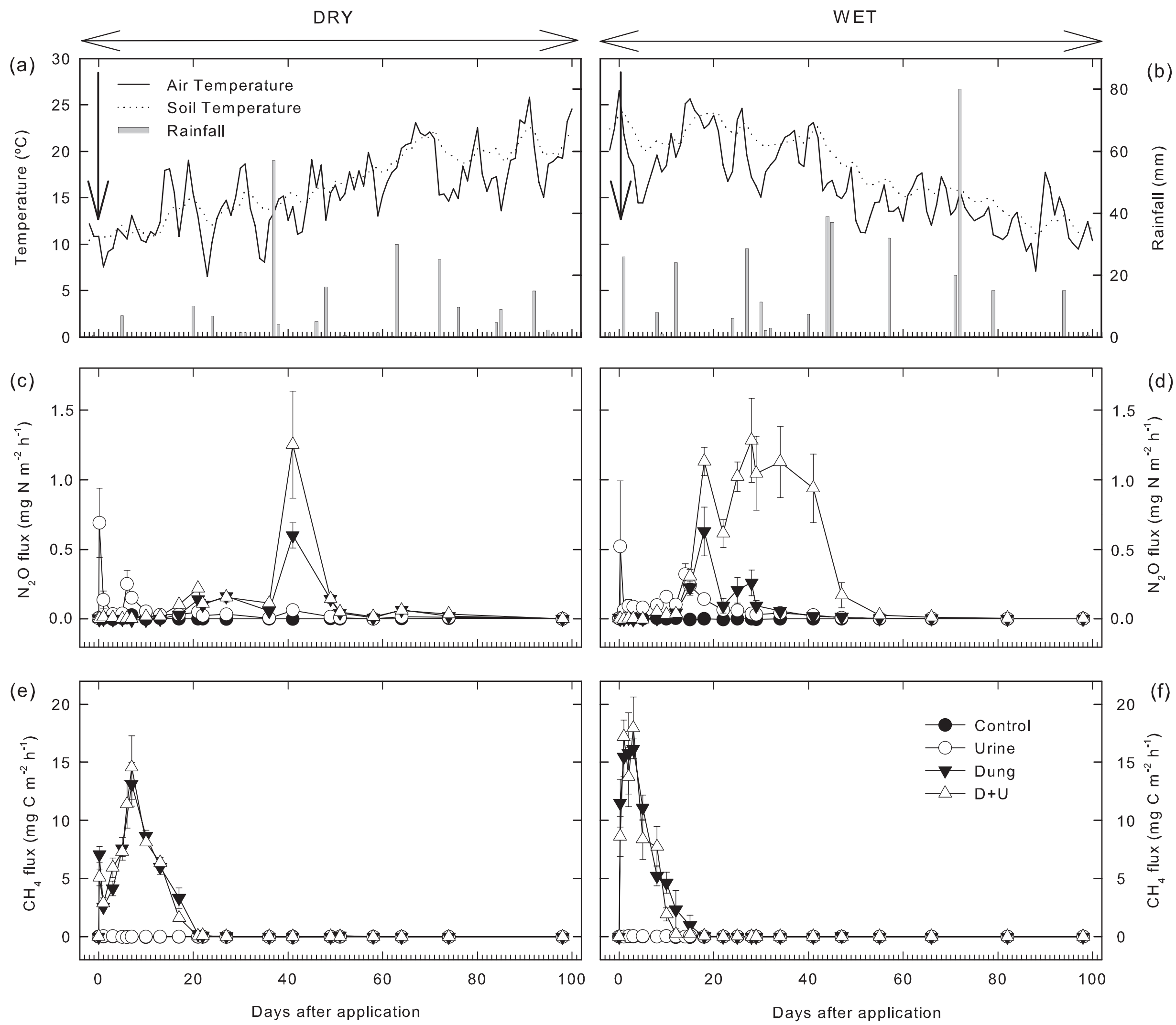

Fig. 2. Dynamics of (a,b) rainfall and mean daily air and soil temperature and changes in (c,d) nitrous oxide (N_2O-N) and (e,f) methane (CH_4-C) fluxes over time (days) after application of cattle excreta (arrows) during the dry trial (left; from Sept 9 to Dec 17, 2019) and wet trial (right; from Feb 15 to May 25, 2020). Bars represent standard error of the mean (n = 4).

and then decreased to reach background levels at around day 49 and day 28 in the dry and wet trials, respectively. In contrast, N_2O emissions from dung and D+U patches increased slowly until they reached a peak and then decreased. The N_2O peak from the D+U patches was double the N_2O peak from the dung only and lasted longer. During the dry trial, the dung and D+U patches took 74 and 98 days to reach similar fluxes as control, respectively, whereas in the wet trial, this took 41 and 55 days, respectively.

Cumulative N_2O emissions were significantly affected by excreta application (Table 2). The combination of D+U gave the highest cumulative N_2O emissions in both trials. Although urine-based cumulative N_2O emissions were at least ten times greater than control values in both trials, there were no statistical differences between those treatments. There were also no statistical differences in cumulative N_2O emissions between dung, urine and control during the wet trial. In addition, cumulative N_2O emissions from the D+U patches were 1.2 and 3.1 times greater than the theoretical sum ($D+U_{theoric}$) of the separate excreta patches, with total $D+U_{theoric}$ emissions of 265.3 and 236.6 mg N_2O-N m^{-2} in the dry and wet trials, respectively. These values were significantly different in the wet trial (P = 0.007) but were not significant in the dry trial (P = 0.357).

The quantitative assessment comparing combined D+U and $D+U_{theoric}$ on N_2O emissions from this and the five available studies (Hyde et al., 2016; Li et al., 2016; Tully et al., 2017; van Groenigen et al., 2005b; Zhu et al., 2021) showed a mean proportional increase of $\Delta = 1.44$ (SEM 0.18). Only three observations (from N = 12) were below the additive effect ($0.94 < \Delta < 1$), while half the values were within the range $0.94 < \Delta < 1.27$, and the rest $\Delta > 1.27$. A trend for an additive effect ($\Delta = 1.15$) was observed under dry weather conditions and for a synergetic effect ($\Delta = 1.73$) under wet conditions (SEM 0.23, significant at 10% level) (Fig. 3). No specific study characteristics, such as climate zone, soil type or amount of excreta applied, coincided with the explanation of any variation in the data.

The EF for N_2O was not affected by the different excreta treatments during the dry trial but under wetter conditions (Table 2). Thus, the combined D+U differed from the separate urine and dung patches during the wet trial, with a significantly greater N_2O EF. Furthermore, there were no significant differences in N_2O EF when analysing by disaggregation among separate excreta types (urine vs dung) in both trials (P = 0.18 and P = 0.68 for dry and wet, respectively). There was also no seasonal variation within separate dung or urine patches (P = 0.16 and P = 0.64, respectively). Thus, the mean N_2O EF values obtained for separate dung and urine patches in this study were 0.11 and 0.09 ± 0.01%, respectively, without significant differences (P = 0.19). Considering all three types of excreta applied, the mean N_2O EF was 0.14 ± 0.08%.

Table 2
Cumulative emissions and emission factors (EFs) for nitrous oxide (N_2O) and methane (CH_4) as affected by the type of excreta deposited on grassland during the dry and wet trials.

	Control	Urine	Dung	D+U	SE	P-value
Dry trial						
Cumulative emissions						
N_2O (mg N m^{-2})	6.9 a	72.6 a	192.7 b	327.7c	30.1	< 0.001
CH_4 (mg C m^{-2})	-56.2 a	-53.3 a	2546.9 b	2670.1 b	218.5	< 0.001
Emission factor, EF						
N_2O (%)	n.d.	0.09 a	0.13 a	0.15 a	0.02	0.194
CH_4 (g kg^{-1} VS)	n.d.	n.d.	0.75 a	0.79 a	0.09	0.785
Wet trial						
Cumulative emissions						
N_2O (mg N m^{-2})	3.4 a	103.2 a	133.4 a	736.9 b	61.9	< 0.001
CH_4 (mg C m^{-2})	-31.9 a	-17.7 a	2538.9 b	2822.7 b	174.4	< 0.001
Emission factor, EF						
N_2O (%)	n.d.	0.08 a	0.09 a	0.27 b	0.03	0.002
CH_4 (g kg^{-1} VS)	n.d.	n.d.	0.77 a	0.69 a	0.07	0.447

N: nitrogen, C: carbon, VS: volatile solids. Values are the mean and SE standard error (n = 4). Different lowercase letters within rows indicate significant differences between excreta treatments ($p < 0.05$).

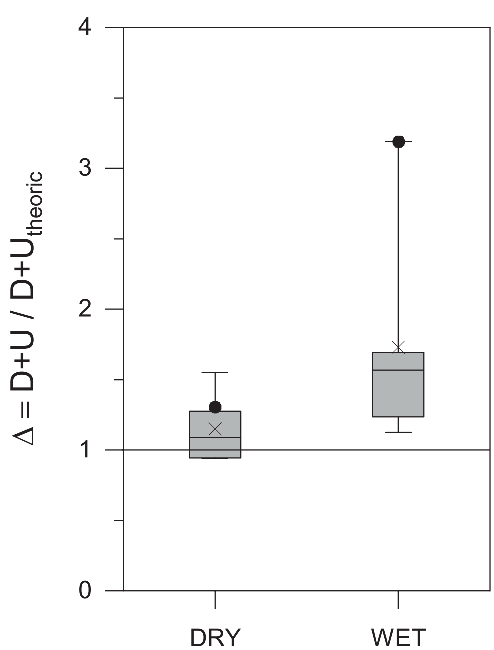

Fig. 3. Proportional increase (Δ) in cumulative N_2O emissions from the combined D+U treatment compared to the theoretical sum $D+U_{theoric}$ of the individual patches, separated by weather conditions (dry and wet). If the relation is $\Delta = 1$, then the effect of combining urine and dung on N_2O emissions is additive. If the $\Delta > 1$, combining urine and dung gives a positive synergetic effect on N_2O production, more than the sum of the individual emissions. Data included the present and previously published studies (N = 12), and calculation details are in "Data analysis" section. Crosses represent the average data values; black circles are this study's result. The range of whiskers is minimum and maximum values.

The daily CH_4 fluxes were significantly affected by sampling day, type of excreta treatment and their interaction ($P < 0.001$ for all, in both trials) (Fig. 2e, f). Control and urine chambers were a sink for atmospheric CH_4 during the two trials (mean flux of −0.02 mg CH_4-C m^{-2} h^{-1}), although transient CH_4 emissions of about 0.03 mg CH_4-C m^{-2} h^{-1} were detected shortly after urine application. The CH_4 fluxes from the dung and D+U patches were observed immediately after excreta application (faster in the wet trial), which decreased to near background levels. From days 21 and 14 onwards in the dry and wet trials, respectively, the CH_4 fluxes from all treatments were statistically similar. Cumulative CH_4 emissions were significantly affected by the excreta treatments applied (Table 2). Those from urine patches were similar to control, whereas patches containing dung acted as a CH_4 source without being significantly affected by urine addition in both trials. The CH_4 EF for dung (with and without urine) averaged 0.75 ± 0.14 g CH_4 kg^{-1} VS (range 0.52–1.01 g CH_4 kg^{-1} VS), with no effect of urine addition.

3.3. Changes in soil and excreta parameters

Dung water content decreased over time after application in both trials, starting from an initial average moisture of 88% (Fig. 4a, b). Dung moisture reached lower values during the dry trial, which was significantly affected by addition of urine, sampling day and their interaction ($P = 0.029$, $P < 0.001$ and $P = 0.013$, respectively). Under wet conditions, dung moisture was not significantly affected by addition of urine ($P = 0.834$). Dung moisture content was significantly and positively correlated with dung-based CH_4 flux in the dry ($\rho = 0.85$ and $\rho = 0.80$ with and without urine addition, respectively) and in the wet trial ($\rho = 0.72$ and $\rho = 0.70$, respectively). The dung patches began to show a thin crust layer of approximately 0.05 m due to drying the first week after deposition in the dry trial and the second week in the wet trial. Large numbers of ants and woodlice were observed under the dung patches 20–30 days after application.

The mean value of WFPS, including all treatments, was slightly lower in the dry trial (49.2 ± 15.3%) than in the wet trial (51.5 ± 12.8%) but without significant differences ($P = 0.27$). Within each trial, the soil WFPS revealed a distinct pattern depending on the excreta treatment and time since application (Fig. 4c, d), with a significant interaction in both trials ($P < 0.001$). For instance, soil with urine addition had the highest WFPS values (59.0–78.0%) during the first ten days after deposition, but it had lower values than soil beneath dung the rest of the days. The soil beneath dung had the highest mean WFPS values over the entire trials (49.6–56.7%). The mean WFPS in the control soil was 48.9% and 49.8% for the dry and wet trials, respectively. WFPS was significantly correlated with N_2O fluxes from the urine patches in the dry trial ($r = 0.43$, $P < 0.04$) and with control soil in the wet trial ($r = 0.52$, $P < 0.02$).

The dominant mineral N form in the soil was exchangeable NH_4^+, while the NO_3^- content remained lower, representing about 10% of the NH_4^+ content within each treatment throughout both trials. The NH_4^+ concentration in the soil beneath excreta patches increased following excreta application, especially in urine. It was affected by the type of excreta applied ($P < 0.001$), with significant variation also between sampling days ($P < 0.001$) (Fig. 4e, f). Higher exchangeable NH_4^+ concentrations were observed in the soil beneath patches containing urine during the wet trial than during the dry trial. Similarly, the NO_3^- content increased following urine application (Fig. 4g, h). The NO_3^- concentration in soil was also affected by excreta treatment ($P < 0.02$) and sampling day ($P < 0.001$). Application of dung without urine did not significantly increase the NH_4^+ and NO_3^- content in the soil beneath patches compared with the control.

During the dry trial, the NO_3^- content in the soil under urine patches was significantly and strongly correlated with N_2O flux ($r = 0.57$, $P < 0.04$). In contrast, during the wet trial, the exchangeable NH_4^+ content in the soil under urine patches was significantly and strongly correlated with N_2O fluxes ($r = 0.77$, $P < 0.001$). The other treatments

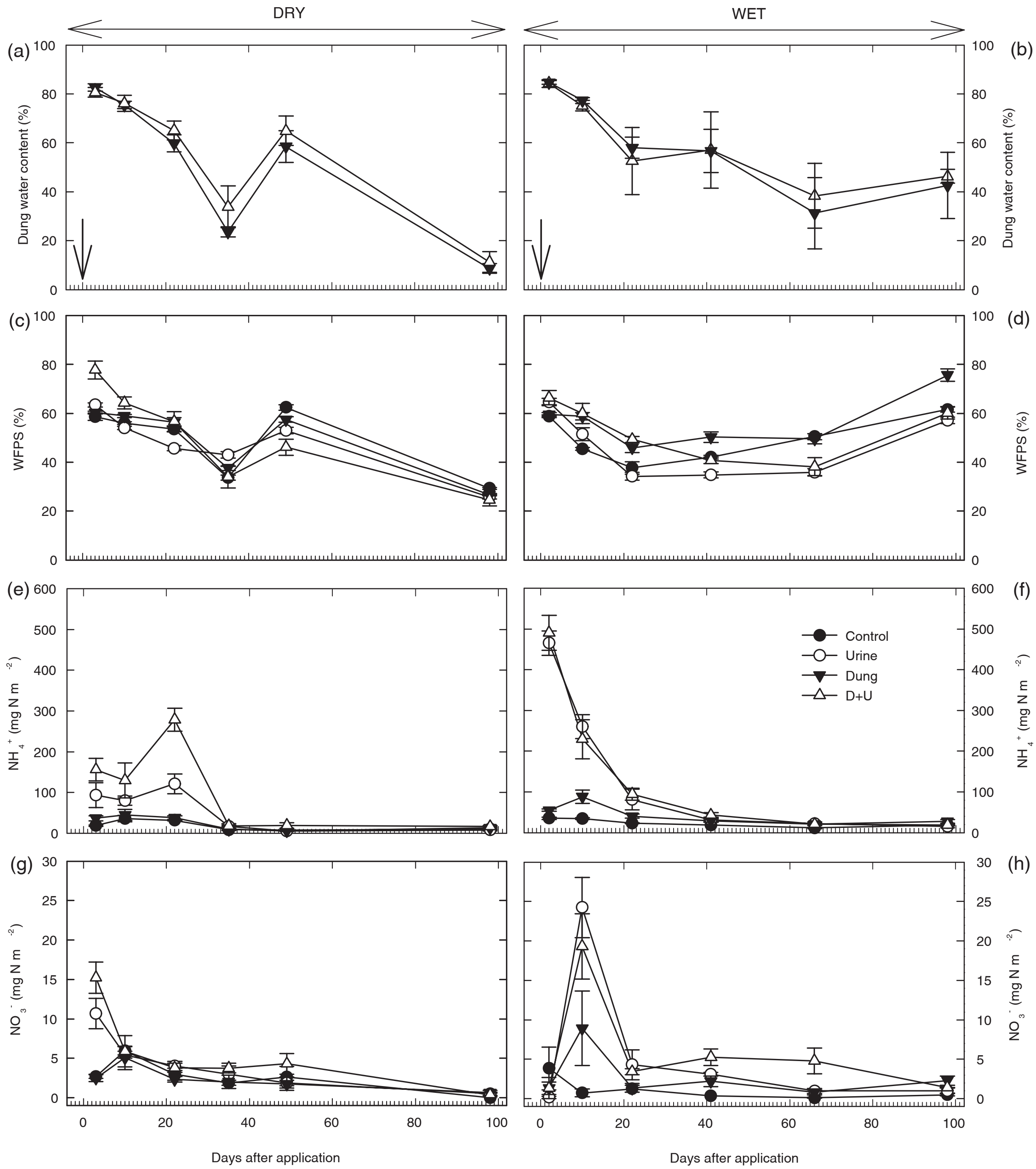

Fig. 4. Change in (a,b) dung water content and (c,d) water full pore space (WFPS), (e,f), ammonia (NH_4^+) and (g,h) nitrate (NO_3^-) content in soil beneath excreta treatments over time (days) after application of cattle excreta (arrows) in the dry trial (left; from Sept 9 to Dec 17, 2019) and wet trial (right; from Feb 15 to May 25, 2020). Bars represent standard error of the mean (n = 4).

showed no significant correlation between NH_4^+ or NO_3^- content in soil and N_2O flux. Probably, with a shallower soil sampling depth, more differences in soil parameters and better correlation with GHG fluxes could be observed. None of the treatments had significant amounts of NH_4^+ or NO_3^- remaining in the soil at the end of the trial periods (day 98).

4. Discussion

4.1. *Effect of combined dung and urine patches on N_2O fluxes*

The key finding in this study was that adding urine to dung patches under wet conditions gave a significant threefold increase (synergetic effect) in cumulative N_2O emissions compared with the theoretical sum $D+U_{theoric}$ of the separate patches. This confirms the starting hypothesis (1). However, under dry conditions, the cumulative N_2O emissions from the combined D+U showed an additive effect compared with the $D+U_{theoric}$ value. Assessment of the proportional increase in N_2O emissions from available data (Fig. 3) also indicated, although with weak evidence, a synergetic effect in wet conditions and an additive effect during dry weather. Three of the studies reviewed provided strong evidence of such an effect in temperate and tropical climates (Hyde et al., 2016; Li et al., 2016; Tully et al., 2017), whereas another study found weak evidence (van Groenigen et al., 2005b). And Zhu et al. (2021) found no significant differences pointing to an additive effect regardless of weather conditions. Thus, there is still a gap in understanding the interaction between dung and urine and the main factors affecting the N_2O emissions.

Direct N_2O emissions from excreta depositions (separate or combined) were related, to some degree, to changes in moisture, soil WFPS, and soil N content. This was confirmed in the present study by the strong correlation between soil WFPS, mineral N and urine-based N_2O emissions. It is well documented that urine deposition unavoidably increases denitrification, at least in the short-term, increasing N_2O production (de Klein et al., 2003). Denitrification activity is also expected to increase after dung depositions, promoted by the anaerobic conditions and high levels of soil NO_3^- and readily available C (Oenema et al., 2008). However, no significant correlation was found between soil properties and N_2O fluxes from patches containing dung. The soil beneath D+U patches initially accumulated NH_4^+ due to the rewetting with urine (Fig. 4e, f) but did not immediately increase N_2O emissions. It began to emit 15 days after application, similar to separate dung. It showed significant N_2O emissions when dung water content was between 50% and 70%, soil WFPS between 40% and 60% and soil mineral N content higher than control. This indicates that N transformation in D+U results from an interrelation of factors controlling excreta degradation, including what occurs within the patch.

Higher soil NH_4^+ and NO_3^- contents were observed beneath excreta patches in the wet trial. It seems that the wetter conditions promoted further N mobilisation. According to Haynes and Williams (1993), the weather immediately after dung depositions and the initial consistency of dung affect the initial release of nutrients. When the wetter conditions followed excreta application in this study, the crust took longer to form and more rain could penetrate and rewet the patch (supported by the higher dung moisture values, Fig. 4a, b). As denitrification is a microbially facilitated process, it is strongly controlled by moisture and temperature (de Klein et al., 2003). Thus, the synergetic increase in D+U under wet conditions might have been due to the formation of more anaerobic microsites for N_2O production. High N_2O emissions were also observed after rainfall events (>10 mm); for instance, after the 57 mm rain event in the dry trial, which probably indicates that low moisture limits denitrification. Similarly, van Groenigen et al. (2005b) observed increased N_2O emissions from combined dung and urine patches during wet summer conditions (mean temperature >20 °C, WFPS ~60%), while Tully et al. (2017) found elevated N_2O emissions coincident with rainfall events. However, Zhu et al. (2021) did not observe such effect, possibly because of the little rain registered during the rainy seasons that did not increase soil moisture enough to promote denitrification (maximum WFPS < 44% in rainy seasons). Therefore, the wetter the weather, the greater the denitrification and N_2O stimulation.

Moreover, mineral N concentrations in the soil beneath D+U were higher during the wet trial, likely due to higher urine N content derived from higher dietary N intake. In ruminants, it is well documented that the dietary N use efficiency decreases as the N intake increases, being the urine the main via of N excretion (Dijkstra et al., 2013; Whitehead, 2000). This increase in urine N content during the wet trial likely reduced the C:N ratio of the combined D+U patch. This ratio was negatively correlated with N mineralisation and N_2O emissions in several studies (Pelster et al., 2016; Simon et al., 2018). The lower C:N ratio of D+U in the wet trial would probably be responsible for the higher N_2O emissions. Thus, when excreta are combined, moisture and C:N ratio can play an important role in N transformations and N_2O emissions.

Results from this study indicate that management practices that reduce the load of N derived from overlapped excreta depositions could mitigate N_2O emissions from grasslands under wet conditions in temperate climates. Rotating livestock accumulation areas in the field could cycle nutrients more efficiently, reduce losses and lower the fertiliser use, resulting in a more sustainable system (Luo et al., 2017; Saggar et al., 2022). Strip grazing and movable troughs for water and supplementation could help make the distribution of excreta patches as uniform as possible (Whitehead, 2000). The success of applying these strategies may also be seasonal, by moving water points when stressing summer conditions are present (White et al., 2001) or by reducing grazing time during wet months of the year (de Klein et al., 2006). However, animal housing periods may create other hotspots, meaning that GHG emissions should be investigated using a life cycle assessment approach.

4.2. Urine addition did not affect CH_4 fluxes

The addition of urine to dung patches had a negligible effect on dung-based CH_4 emissions, agreeing with the hypothesis (2). Similar findings have been made in other studies examining CH_4 emissions from combined excreta patches (Liao et al., 2018; Zhu et al., 2021). However, another study found that urine addition to dung patches can significantly decrease CH_4 emissions compared with the dung patch alone (Tully et al., 2017). They attributed the decrease in CH_4 to the redox potential of the different treatments. In the present study, urine addition most likely simultaneously stimulated and inhibited CH_4 production, giving no overall significant effect. Similarly, soil CH_4 fluxes were not significantly affected by urine addition. Other studies have found similar results for temperate and tropical grasslands (Nichols et al., 2016; Pelster et al., 2016; Zhu et al., 2021), where both control and urine depositions acted as sinks for atmospheric CH_4, regardless of the type of pasture. According to Cai et al. (2017), urine deposition does not influence CH_4 fluxes from different soils, regardless of the type of grazing animal involved.

Patches containing dung acted as localised CH_4 hotspots and depended strongly on the age of the dung (supported by the significant effect of time after deposition). Several studies have observed similar CH_4 emission patterns in temperate climates (Kelly et al., 2016; Nichols et al., 2016; Priano et al., 2014). Dung CH_4 emissions are attributed to entrained enteric CH_4 from freshly voided dung and new CH_4 produced within the dung patch (Cai et al., 2017). The latter is often considered the main origin of CH_4 from dung patches, explained by the simultaneous abundance of methanogens, readily available soluble C and anaerobic conditions (Hahn et al., 2018). In the present study, dung moisture content appeared to explain the dung and D+U based CH_4 emissions since high CH_4 emissions were observed when moisture was > 65%, i.e. when anoxic conditions were present, supported by the strong positive correlation between these parameters.

4.3. Emission factors

Dung-based N_2O EF values in this study ranged from 0.05% to 0.16% and were within the 0.01–0.53% range reported in other studies performed under temperate climates (Table 3). Moreover, the urine-based N_2O EFs showed lower values (range 0.03–0.14%) than those gathered in Table 3 (range 0.05–2.93%). There were no significant differences in N_2O EF between separate urine and dung patches. This contradicts hypothesis (3) and previous findings recommending disaggregation for excreta type with higher EFs from urine patches (Bell et al., 2015; Chadwick et al., 2018; Krol et al., 2016; Luo et al., 2019; van der Weerden et al., 2011, 2020, 2021). The low N_2O emissions from urine could be explained by high N uptake by plants and soil heterotrophs of long-term unfertilised grassland, which are strongly N-limited and can outcompete nitrifiers (Liu et al., 2016). The low soil fertility at our study site (SOC 2.7%, total N 0.26%, pH 6.0) could also mean that, if the N input is below a certain threshold, there might not be enough substrate (e.g. NO_3^-) for N_2O production, as observed by van der Weerden et al. (2020). In line with this, a review by Cai and Akiyama (2016) found that when specific soil conditions were present (SOC <4%, total N < 0.4, pH >5.5), coinciding with the soil characteristics mentioned above, there were no significant differences between dung and urine N_2O EF.

Combined D+U under wet field conditions resulted in threefold higher N_2O EF than the separate depositions, whereas there were no

Table 3
Field studies in temperate climates measuring N_2O from excreta patches are shown. Data included location, measuring period (days), and mean N_2O EF (%) range for urine and dung patches.

Reference	Location	Days	Urine EF (%)	Dung EF (%)
Bell et al. (2015)*	United Kingdom	365	0.20–1.07	0.10–0.20
Cardenas et al. (2016)*	United Kingdom	350	0.11–2.96	0.10–0.39
Chadwick et al. (2018)*	United Kingdom	365	0.05–2.93	0.04–0.53
Hyde et al. (2016) *	Ireland	180	0.11	0.01
Li et al. (2016)*	New Zealand	271	1.72	0.48
Lombardi et al. (2021)	Argentina	125	n.d.	0.06–0.13
Luo et al. (2013)*	New Zealand	120–365	0.07–1.35	0.02–0.40
Luo et al. (2019)	New Zealand	90–180	0.10–0.72	0.05–0.27
Nichols et al. (2016)*	United States	365	0.11–0.13	0.10
Rochette et al. (2014)*	Canada	120–365	0.11–2.63	0.04–0.28
Thomas et al. (2017)*	Canada	365	1.32	0.03
van der Weerden et al. (2011)*	New Zealand	135–180	0.05–0.94	0.00–0.17
This study	Argentina	98	0.03–0.14	0.05–0.16
IPCC (2006)	Global	365	2.00	2.00
IPCC (2019)	Wet climate locations	365	0.77 (0.03–3.82)	0.13 (0.00–0.53)

* Studies used by IPCC (2019) for estimation of default N_2O EF.

significant differences under dry conditions (Table 2). These results were consistent with van Groenigen et al. (2005a), who found that dung addition to urine patches led to an eightfold increase in N_2O EF and pointed out a need for realistic EF to consider the sites where cattle congregate. However, this N_2O EF (0.10–0.39%) were below the updated 0.6% default EF estimated by IPCC for cattle N in wet climates (IPCC, 2019). Besides, when disaggregating for excreta type, mean urine-based N_2O EF (0.09%) was lower than the urine-EF value (0.77%) of IPCC (2019), whilst mean dung-based N_2O EF (0.11%) was closer to the dung-EF value (0.13%) of IPCC (2019). Therefore, the more recent IPCC values seem more accurate for the Pampa region than the previous IPCC default value (2%; IPCC, 2006), which overestimated N_2O emissions from cattle excreta patches.

The mean CH_4 EF for patches containing dung, regardless of urine addition, was 0.75 ± 0.14 g CH_4 kg^{-1} VS. This value was within the range between 0.3 and 1.9 g CH_4 kg^{-1} VS of other studies performed in Argentina, Australia and Colombia (Lombardi et al., 2021, 2022; Kelly et al., 2016). Besides, it was within the range of the updated IPCC value (0.6 ± 0.2 g CH_4 kg^{-1} VS) for dung deposition on rangelands (IPCC, 2019). As few studies have examined CH_4 EF from dung patches and expressed it based on VS, more region-specific studies are necessary.

5. Conclusions

This study showed that cattle urine and dung deposition affect CH_4 and N_2O emissions from grassland soils in different ways. Separate dung patches were found to be a localised CH_4 hotspot while adding urine to dung patches had a negligible effect on CH_4 emissions. In addition, separate and combined urine and dung depositions were also localised N_2O sources. The results indicated a synergetic (super-additive) effect on cumulative N_2O emissions from overlapping dung and urine patches, compared with individual depositions, under wet conditions. This suggests that avoiding combined dung and urine patches under wet conditions, e.g., encouraging a more uniform distribution of grazing animals, could be part of an alternative mitigation strategy for N_2O emissions in temperate managed grazed pastures.

The mean dung-based CH_4 EF (0.75 g CH_4 kg^{-1} VS) found here, representing pasture systems common in the Pampa region of Argentina, was within the range of the updated IPCC (2019) value (0.6 g CH_4 kg^{-1} VS) for dung deposition on pasture. The mean direct N_2O emissions (0.14%) were well below the EF of 2% established by IPCC 2006 for cattle N in excreta. Thus, the revised dung (0.13%) and urine (0.77%) EF for wet climates in IPCC (2019) are much closer to the value found in this study for dung (0.11%), but higher than the value found for urine (0.09%). This suggests a need to reduce dependence on default Tier 1 EF values and invest in studies to develop more region-specific Tier 2 EFs for the IPCC Guidelines for National Greenhouse Gas Inventories. Additional studies are required to verify these findings under different cattle production systems and environmental conditions to develop robust EFs from excreta depositions on pastures.

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.

Data Availability

Research Data from combined an separate cattle dung and urine depositions on N_2O and CH_4 emissions under different weather conditions are included: https://data.mendeley.com/datasets/tmhyptg3xy.

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