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Article

Dynamics of Soil CH_4 and CO_2 Fluxes from Cattle Urine with and Without a Nitrification Inhibitor, and Dung Deposited onto a UK Grassland Soil

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Abstract

Food production systems associated with livestock management are significant sources of greenhouse gases (GHGs). Livestock excreta are one of the primary sources of GHG emissions from grazing livestock. Against this context, a field experiment was established in a UK grassland to establish the extent of soil methane (CH_4), carbon dioxide (CO_2), andN_2O fluxes upon the deposition of (i) cattle urine (U), (ii) urine + dicyandiamide (DCD) (U + DCD), (iii) artificial urine (AU), and dung (D), and compared with a (iv) control, where neither urine nor dung was applied. Excreta applications were made at three experimental periods during the grazing season: early-, mid-, and late-season. Soil N_2O emissions data have been published already by co-authors; hence, this paper summarizes the emissions of soil-borne CH_4 and CO_2 emissions, and explores in particular, the effects of the addition of DCD, a nitrification inhibitor used to reduce direct and indirect N_2O emissions from urine patches, on these (carbon) C-GHGs. Soil moisture ($p = 0.47$), soil temperature ($p = 0.51$), and nitrate (NO_3^-) ($p = 0.049$) and ammonium (NH_4^+) ($p = 0.66$) availability, and C ($p = 0.54$) addition were key controls of both soil CH_4 and CO_2 emissions. The dung treatment stimulated the production and subsequent emissions of soil CH_4 and CO_2, a significantly high net CH_4 and CO_2-based global warming potential (GWP). The findings of the current study lay a foundation for an in-depth understanding of the magnitude and dynamics of soil-borne CH_4 and CO_2 upon urine and dung deposition during three different seasons. This study implies that the use of DCD may have the potential to reduce carbon-based GHGs from the urine and dung of grazing animals.

Keywords: carbon-based-greenhouse gases; nutrient deposition; grassland soil; nitrification inhibitors

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1. Introduction

Food production systems associated with livestock management are significant sources of greenhouse gases (GHGs), contributing to about 12% of the annual global anthropogenic emissions [1]. Urine and dung deposition by grazing livestock on pasture soils, manure management, and ruminant enteric fermentation are the primary sources of the GHGs: methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) [2–5]. In grazed pastures, livestock excreta create patches of nutrient-rich soil, stimulating microbial activities and enhancing GHG emissions [6,7].

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The deposition of urine and fresh dung by grazing livestock in intensively grazed pastures may increase soil moisture and subsequently increase anaerobic microsites, for N_2O-producing processes, i.e., denitrification [8–10]. The decomposition and mineralization of deposited dung and the locally added organic carbon (C) may also result in CH_4 and CO_2 production and emission "hotspots" in intensively grazed pastures [11].

Globally, methane from livestock systems comprises 46% of all agricultural GHG emissions [3,12] and has a global warming potential (GWP) ~27 times that of CO_2 over a 100-year horizon [13]. Carbon dioxide is one of the globally important greenhouse gases contributing to the greenhouse effect, and its atmospheric concentrations reached 419.3 ppm in 2022, which is 51% above the pre-industrial levels (278 around 1750) [14]. Increases in atmospheric CH_4 and CO_2 have significant effects on global climate change. On the other hand, research on the dynamics of these C-GHGs has received far less attention than the N_2O emissions from these sources [15] and especially following the application of urine and dung, as well as the use of a nitrification inhibitor. Also, nitrification inhibitors (NIs), including dicyandiamide (DCD), have been well-documented in their role in reducing nitrate (NO_3^-) leaching and N_2O emissions from urine deposition [7,16,17]. However, the role of DCD has seldom been explored regarding its influence on the dynamics of C-GHGs upon urine and dung deposition. This is despite the evidence that DCD controls soil N_2O by delaying nitrification, retaining nitrogen (N) in the more immobile ammonium (NH_4^+) in the soil, and reducing NO_3^- concentrations [18], consequently reducing denitrification rates and N_2O emissions [19]. On the other hand, soil NH_4^+ is known to inhibit CH_4 oxidation [20–22] often resulting in a net increase in CH_4 emitted from soil [23].

Thus, it is imperative to understand the dynamics of these GHGs in livestock-grazed pastures in order to better quantify their potential contribution to total GHGs, and to understand the effects of using DCD, an NI, on the dynamics and extent of soil-borne CH_4 and CO_2 fluxes. Therefore, this paper aims to (i) clarify the soil and environmental controls of soil CH_4 and CO_2 fluxes from cattle urine with and without the nitrification inhibitor, DCD, and dung deposited onto grassland soil.

2. Results

2.1. Rainfall and Temperature

During the three experiments, the most significant rainfall (up to 238 mm) fell in December 2012, which was preceded by the wet months of October and November, having recorded 158.8 and 169.1 mm, respectively. The second largest amount of rainfall (210.4 mm) was received in June 2012 (Figure 1). The months of May 2012, April, June, and July 2013 proved to be the driest, having received rainfall not exceeding 50 mm. Mean air temperature and soil temperature at 5 cm showed a similar pattern during the three experiments. The largest mean air and soil temperatures, up to 18.5 and 19.1 °C, respectively, were recorded in July 2013. The lowest mean air and soil temperature (2.8 and 4.1 °C, respectively) were recorded in March 2013.

2.2. Soil Moisture Dynamics

In the autumn experiment, soil percent water-filled pore spaces (%WFPS) ranged from 33.3 ± 0.8 to 76.0%. During this experimental period, the largest soil %WFPS (76.0%) was recorded in January and March 2013, while the soil was the driest in September 2013 (33.3 ± 0.8%). On the other hand, during the spring experimental period, soil %WFPS ranged from 44.6 ± 0.95 to 69.1 ± 1.3%, with the driest period recorded in May 2012 and the wettest period recorded in November 2012. Soil % WFPS ranged from 51.8 ± 1.6 to 101.8%, with the driest period recorded during November 2013, whilst the wet period was recorded at the beginning of July 2012 (Figure 2).

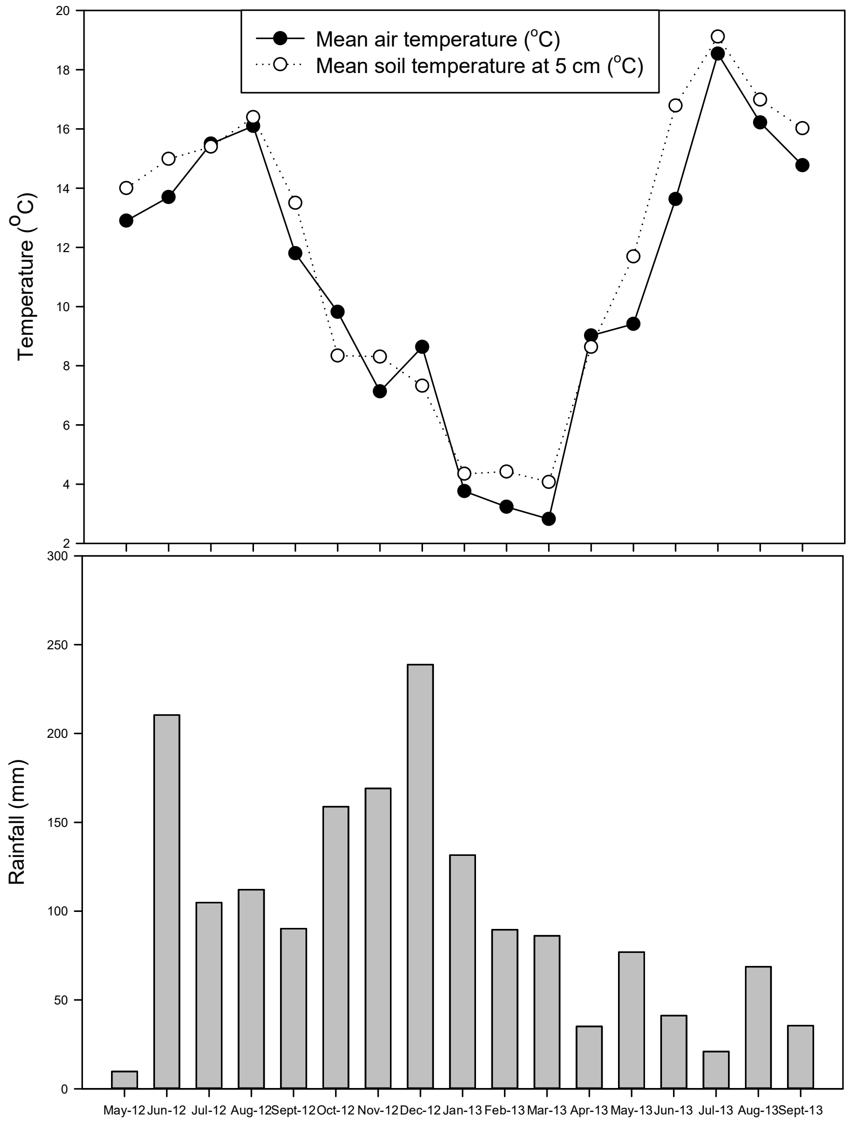

Figure 1. Monthly average rainfall, air temperature, and soil temperature at 5 cm soil surface in the experimental site during the experiment.

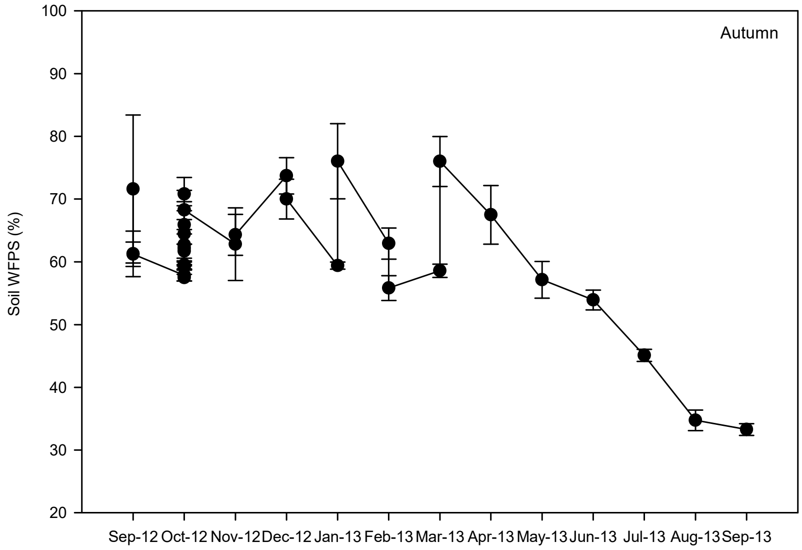

Figure 2. *Cont.*

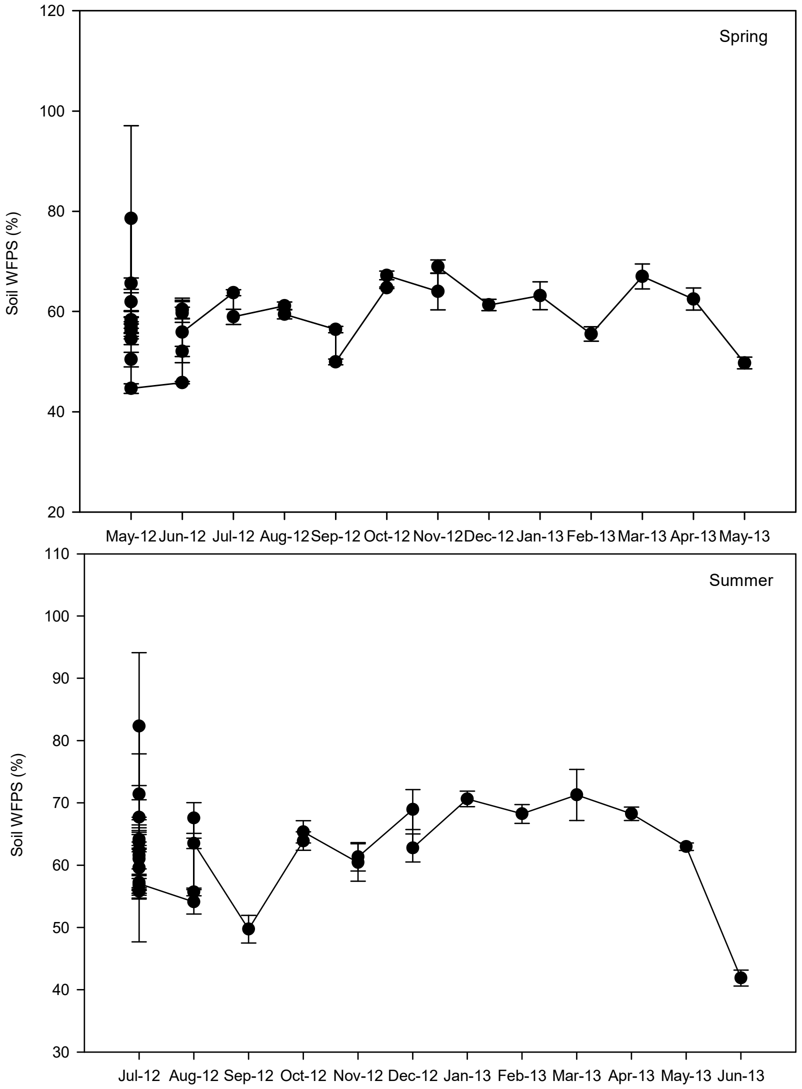

Figure 2. Soil %WFPS variability between autumn, spring, and summer.

2.3. Gas Fluxes

2.3.1. Soil CH_4 Fluxes

The D treatment (1094 ± 67.6 g ha^{-1} day^{-1}) showed the largest CH_4 fluxes immediately after the treatment application in the autumn experiment, whilst other treatments exhibited fluxes < 200 g ha^{-1} day^{-1} during this sampling event. The extensive soil CH_4 fluxes observed in the D treatment immediately after treatment application lasted until April 2013. Thereafter, all treatments had low (below 3 g ha^{-1} day^{-1}) soil CH_4 fluxes until the end of the experiment. Also, in the spring experiment, the D treatment emerged with the largest flux of 629.8 ± 79.9 g ha^{-1} day^{-1}, whilst the remainder had fluxes below 1.5 g ha^{-1} day^{-1}. However, immediately after this peak, soil CH_4 fluxes in the D treatments dropped to below 10 g ha^{-1} day^{-1} and remained low like the rest of the treatments until the end. Similar to the autumn and spring experiments, in the summer experiment, the D treatment (658.8 ± 118.4 g ha^{-1} day^{-1}) had high CH_4 fluxes, while the remainder of the treatments had soil CH_4 fluxes < 10 g ha^{-1} day^{-1}. From the D treatment (1144.8 ± 377.8 g ha^{-1} day^{-1}), the largest soil CH_4 emerged again in mid-July 2012, while the remainder of the treatments had fluxes of <450 g ha^{-1} day^{-1}. After the second peak, the CH_4 fluxes declined to around ~20 g ha^{-1} day^{-1} and remained low until the end of the experiment (Figure 3).

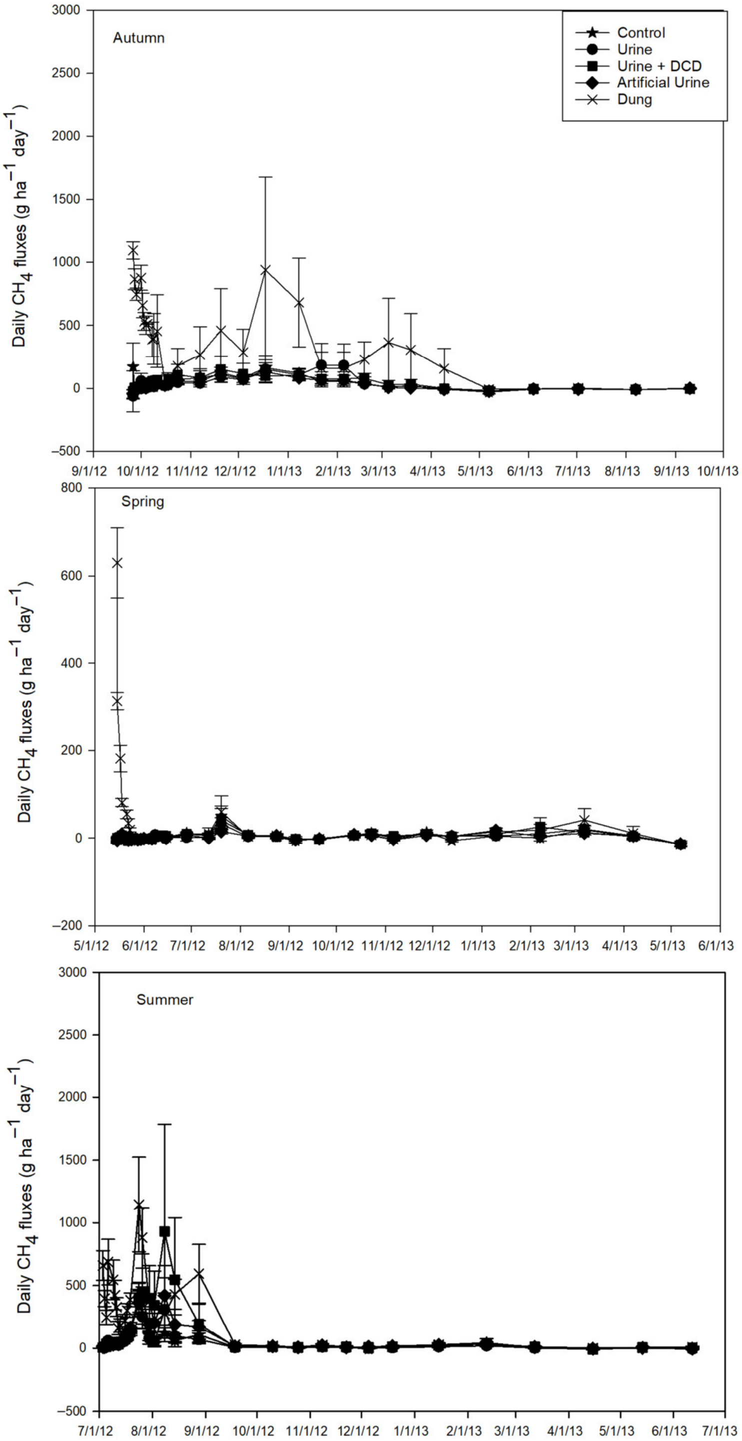

Figure 3. Soil CH_4 fluxes as influenced by the different treatments in autumn, spring, and summer ($N = 3$). Note, different y-axis scales.

2.3.2. Soil CO_2 Fluxes

At the commencement of the autumn experiment, the D treatment displayed large CO_2 fluxes (205.8 ± 92.8 kg ha^{-1} day^{-1}) immediately after treatment application. The large CO_2 fluxes observed in the D treatment immediately after treatment application were followed closely by the U treatment with 180.8 ± 22.2 kg ha^{-1} day^{-1}, while the remainder of the treatments had fluxes < 165 kg ha^{-1} day^{-1}. After this peak, fluxes in all treatments remained at ~100 kg ha^{-1} day^{-1} until a second peak was captured at the beginning of May 2013. During this peak, the AU treatment emerged with the CO_2 flux of 255.5 ± 68.7 kg ha^{-1} day^{-1} but was overtaken by the D treatment on the succeeding sampling event with a flux of 275.3 ± 49.2 kg ha^{-1} day^{-1}.

The soil CO_2 fluxes peaked at a high of 266.1 ± 21.7 in the U treatment within the first two weeks after amendment application in the summer experiment. Immediately after this peak, CO_2 fluxes took a downward spiral, with the D treatment often predominantly having more significant fluxes until the end of the investigation. Immediately after the amendment application in the summer experiment, the D treatment recorded a CO_2 flux of up to 168.6 ± 38.2 kg ha^{-1} day^{-1}, when the other treatments had fluxes of <115 kg ha^{-1} day^{-1}. The same treatment emerged with significant CO_2 of 490.3 ± 133.6 kg ha^{-1} day^{-1} within three weeks after amendment application, followed by the U treatment with 466.7 ± 35.4 kg ha^{-1} day^{-1} (Figure 4). After these peaks, CO_2 fluxes remained at ~200 kg ha^{-1} day^{-1} in all the treatments until the end of the experiment.

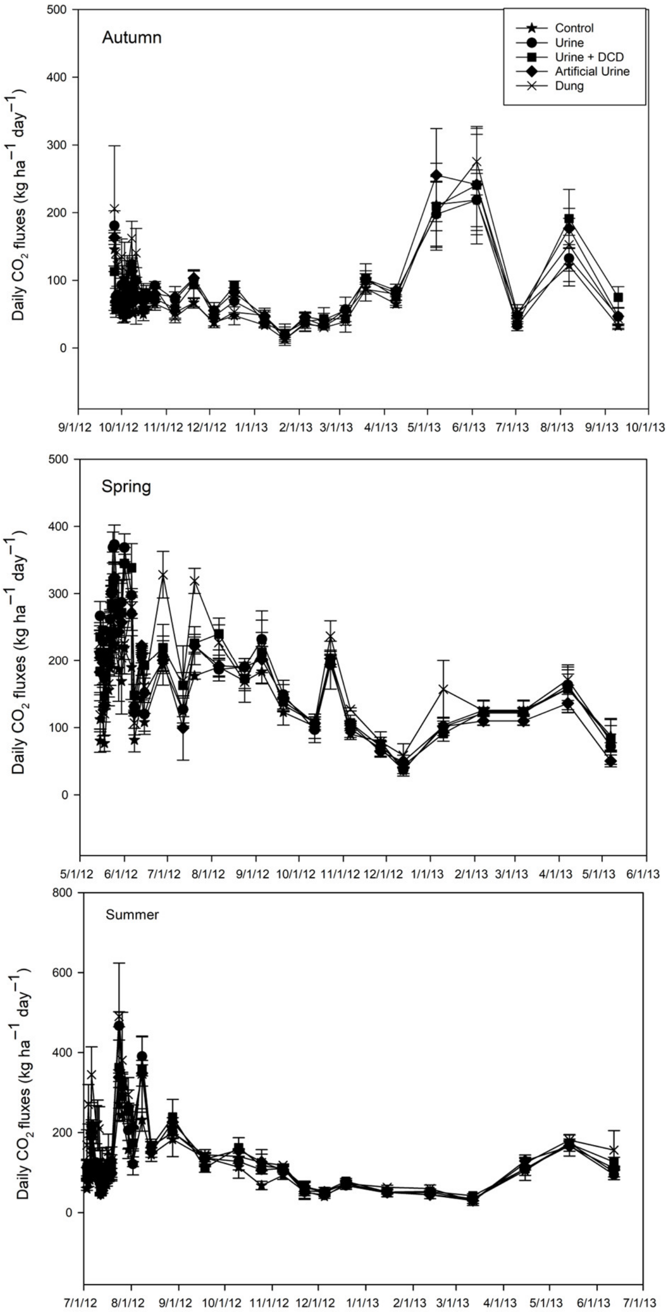

Figure 4. Soil CO_2 dynamics as influenced by the different treatments in the autumn, spring, and summer ($N = 3$). Note, different y-axis scales.

2.4. Cumulative CH_4 and CO_2 Emissions

2.4.1. CH_4 Emissions

The largest cumulative soil CH_4 fluxes were reported in the D treatment (up 76.8 ± 51.1 kg ha^{-1} $year^{-1}$) in the autumn experiment, which was not significantly larger than in other treatments, except for the AU treatment. Again, in the spring experiment, the D (up to 5.32 ± 0.9 kg ha^{-1} $year^{-1}$) maintained the largest CH_4 cumulative fluxes, significantly larger than all other treatments except for the U + DCD treatment. Similarly, to the autumn and spring experiments, in the summer experiment, the D treatment had significantly larger cumulative CH_4 fluxes, which were, however, not significantly different from the other treatments, except for the U treatment (Figure 5).

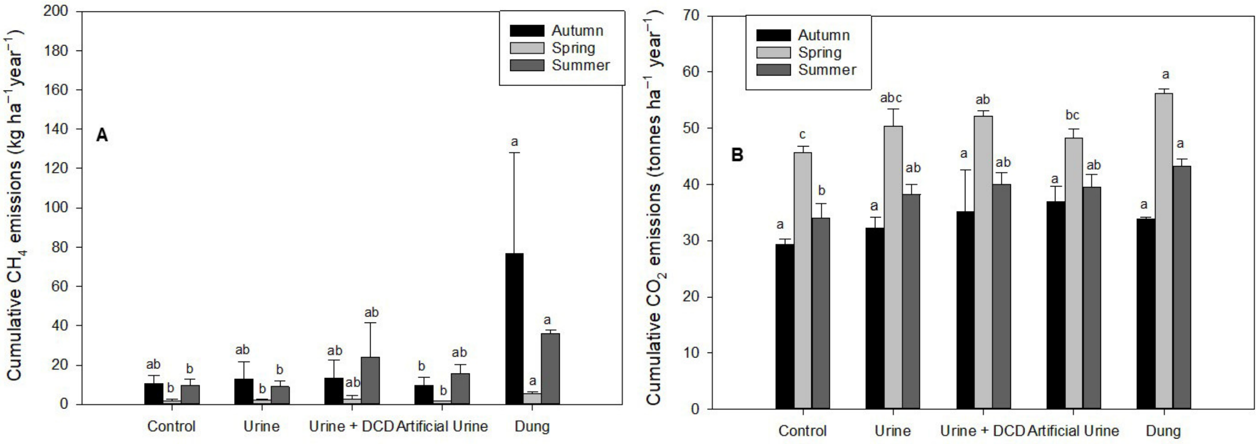

Figure 5. Cumulative (**A**) CH_4 and (**B**) CO_2 emissions as influenced by the different treatments in autumn, spring, and summer. Vertical lines are standard errors of the mean (N = 3). Treatments with the same letter are not significantly different to each other.

The results from the correlations between parameters showed that in the spring experiment, there were significant relationships between soil CH_4 and NO_3^- ($p = 0.049$; $r = -0.997$) in the C treatment and yield ($p = 0.036$; $r = 0.998$) in the D treatment in the spring experiment.

2.4.2. CO_2 Emissions

In the autumn experiment, cumulative soil CO_2 emissions showed no significant difference within treatments, despite the largest CO_2 observed in the AU treatment (36.9 ± 2.9-ton ha^{-1} $year^{-1}$). On the other hand, in the spring experiment, the D treatment (56.1 ± 0.92-ton ha^{-1} $year^{-1}$) showed significantly greater cumulative soil CO_2, which was, however, not significantly higher than the U treatment (50.5 ± 2.9 tons ha^{-1} $year^{-1}$) and the U + DCD treatment (52.2 ± 0.97-ton ha^{-1} $year^{-1}$), but to the remainder of the treatments. The D treatment (43.8 ± 1.3-ton ha^{-1} $year^{-1}$) again emerged with the largest cumulative soil CO_2, which was, however, only significantly different from the C treatment (Figure 5). We found a significant relationship between cumulative soil CO_2 and N-offtake ($p = 0.041$; $r = 0.998$) in AU, and herbage yield ($p = 0.044$; $r = 0.998$) in D treatment during the autumn experiment.

2.5. Net Global Warming Potential (GWP)

We estimated the net GWP from the treatments using the total CO_2 and CH_4 fluxes in this analysis and the cumulative N_2O fluxes from [24] corrected by their GWP (1, 27, and 273 for CO_2, CH_4, and N_2O, respectively). The results showed that net global warming potential was significantly greater in the D treatment compared to the remainder of the treatments (Table 1). Notably, the U, U + DCD, and AU treatments were not significantly different from each other, but they were compared to the C treatment.

Table 1. Cumulative CH_4, CO_2, and net GWP emissions (CO_2e) for the different treatments.

Treatment	**CH_4**	**CO_2**	**Net GWP**
	(kg CO_2e ha^{-1} yr^{-1})	**(kg CO_2e ha^{-1} yr^{-1})**	**(kg CO_2-eq. ha^{-1} yr^{-1})**
Control	278.8 ± 60.5 $^{c,¥}$	363.4 ± 10.3 ^{c}	110,513.8 ± 5072.2 ^{c}
Urine	287.1 ± 131.5 ^{c}	403.1 ± 20.6 ^{b}	127,039.8 ± 7359.2 ^{b}
Urine + DCD	569.8 ± 295.0 ^{b}	424.5 ± 16.1 ^{b}	131,864.9 ± 12,266.2 ^{b}
Artificial Urine	377.8 ± 100.1 ^{b}	415.5 ± 20.1 ^{b}	130,616.0 ± 7537.5 ^{b}
Dung	1390.8 ± 491.1 ^{a}	444.2 ± 1.1 ^{a}	139,463.4 ± 4345.2 ^{a}

[¥] All values are mean ± standard error. Values within a column for each treatment followed by the same letter are not significantly different at the $\alpha = 0.05$ probability level.

3. Discussion

3.1. Soil and Environmental Controls of Gas Flux Dynamics

3.1.1. Soil CH_4 Fluxes

The larger soil CH_4 fluxes immediately after application in the D treatment, compared to other treatments in the current experiment (Figure 3), are similar to values reported in other studies, particularly [25]. This previous study reported CH_4 fluxes up to 161 µg m^{-2} h^{-1} on the first day after dung application. The increase in CH_4 fluxes immediately after dung application could presumably be dissolved CH_4 in the dung [26]. Ref. [27] reported that the decline after the peak CH_4 in the dung could be due to drying and aerobic conditions, as the dung was continually exposed to the air. The decline in CH_4 fluxes could be linked to methanogenesis inhibition, as the process is known to be extremely sensitive to oxygen concentrations, and hence oxygen diffusion into the dung may have inhibited it [27]. The high soil CH_4 fluxes immediately after dung application in the autumn experiment could have been produced and released from the dung as it was deposited, similar to other authors, particularly [28,29]. The previous authors observed that short-term CH_4 pulses immediately after dung deposition were partly due to CH_4 production in fresh dung, as fresh dung is a vital host for the methanogenic population in an anaerobic environment supplied with labile C and the release of CH_4 of enteric origin embedded in the dung. It could also have been partly due to the high soil moisture stimulating the production of soil CH_4, similar to [30]. The previous authors reported that wetter soils in wet seasons had greater CH_4 fluxes because of low gas diffusivity.

Interestingly, at the beginning of the autumn and spring experiments, there was a large soil CH_4 flux in all treatments coinciding with higher soil NH_4^+ and NO_3^- (please see [24] and Figure 3), in line with findings by other authors, particularly [31] with fluxes of up to 84.2 and 108 µg CH_4 m^{-2} h^{-1}, respectively. The previous authors observed that high soil mineral N inhibits the capability of CH_4 to be oxidized into CO_2, thus reducing the soil's ability to be a CH_4 sink. Further, the low soil CH_4, coinciding with low NH_4^+ and NO_3^- until the end of the experiment in all treatments, further reiterates the findings of the previous authors that NH_4^+ plays an inhibitory role in the oxidation of CH_4. The role of mineral N in inhibiting CH_4 oxidation is attributed to the fact that CH_4 monooxygenase of methanotrophs can oxidize various substrates besides soil CH_4 [32,33]. The inhibition is also considered a general salt effect [34] with a competition between ammonia (NH_3) and CH_4 for monooxygenase enzymes [35].

3.1.2. Soil CO_2 Fluxes

The large CO_2 fluxes observed immediately after treatment application in all treatments in the three different experiments were similar to findings by other authors, particularly [36] who observed high cumulative soil CO_2 immediately after urine and manure/dung deposition. These studies credited the high fluxes immediately after urine or dung deposition to urea hydrolysis to CO_2 or labile C compounds introduced by the dung, stimulating microbial respiration. Also, larger soil CO_2 fluxes in all treatments and seasons of the current study coincided with higher soil temperatures (Figures 1 and 4), similar to findings by [37]. These studies showed increasing soil CO_2 fluxes with higher soil temperature. They credited this to high soil temperatures having a beneficial effect on soil respiration and bacterial respiration in urine and dung. The higher soil moisture at the beginning of all the experiments could also have stimulated soil CO_2 production, similar to other authors, particularly [5,38]. The previous authors reported a rapid CO_2 pulse from dung and urine patches coinciding with high water-filled pore space. Also, ref. [39] reported a peak of CO_2 fluxes immediately after a rainfall event.

3.2. Seasonal Effects on Gas Emissions

3.2.1. Soil Cumulative CH_4 Fluxes

In all three experiments, the D treatment in autumn and summer application also resulted in larger CH_4 cumulative fluxes compared to the remainder of the treatments in all the other seasons, similar to [32]. The previous authors reported predominantly large cumulative CH_4 fluxes between 13 and 30 mg m^{-2} in dung patches, which were significantly more than the values (<5 mg m^{-2}) obtained in urine patches of the same studies. The large CH_4 flux in the D treatment occurred despite the fact that this treatment sometimes had lower soil mineral N (autumn and spring), known to inhibit soil CH_4 oxidation into CO_2 [32,40] compared to the remainder of the treatments, except for the C treatment. This could signify that in the current study, other drivers of soil CH_4 significantly influenced the uptake inhibition by mineral N, but we did not test this in the present study. Reference [41] reported that since non-inundated soils are known CH_4 sinks, deposited dung creates localized CH_4 production hotspots due to high C content soil moisture, and the methanogen population in the material. This then explains the larger CH_4 emissions in the dung of the current treatment compared to the other treatments used in all three experiments.

3.2.2. Soil Cumulative CO_2 Fluxes

"Priming effect" is the stimulation of accelerated organic matter (OM) mineralization upon the addition of fresh C substrates (i.e., labile C) [42,43], which produces CO_2. Thus, in our study herein, the large cumulative CO_2 from the dung in the spring and summer experiments could have been a result of the "priming effect". This is because the dung may have introduced a labile C source, which then stimulated C grassland OM mineralization, thus producing soil CO_2. The "priming effect" is known to be high in instances where the soil has high NH_4^+ coupled with high C content [44]. However, some treatments with high soil NH_4^+ (i.e., U + DCD) had lower soil CO_2 compared to the D treatment. These results highlight the interactive role of soil NH_4^+ and soil C in promoting OM mineralization via the "priming effect" since the other treatments did not introduce fresh C, except for the dung.

3.3. Net Global Warming Potential

The significantly large net GWP from the D treatment implies that dung deposited from grazing cattle may pose an atmospheric GHG risk. This was a result of the high CH_4 being released from the dung upon deposition as well as the release of the CH_4 of enteric origin embedded in the dung [30,31]. The high net GWP in the dung could also be a result of high soil CO_2 immediately after deposition as a result of labile C compounds introduced by the dung stimulating microbial respiration [41].

4. Materials and Methods

4.1. Experimental Site

The three experiments (i) spring, (ii)summer, and (iii) autumn applications of different treatments were conducted on a permanent grassland site in Rothamsted Research North Wyke farm, Devon, UK (50°45′ N, 3°50′ W), between March 2012 and September 2013. For three years leading up to the commencement of the current experiment, the site was used for silage production with no livestock grazing. The climate record of North Wyke between 1982 and 2012 shows that the site has a 30-year mean annual precipitation of 1055 mm and a mean annual air temperature of 9.6 °C. The site has poorly drained silty clay loam soils of the Halstow series [45].

4.2. Initial Soil Analysis

At the commencement of the current experiment, soil analysis was carried out to determine pH (1:2.5 soil:water), phosphorus P (Olsen P) [25], and available P, available potassium (K), and available magnesium (Mg) were measured using ICP-AES (model 9800, Shimadzu, Tokyo, Japan) after ammonium lactate and acetic acid extraction. Total N was analyzed by a thermal conductivity detector (Elementar Rapid MAX N Exceed, Elementar, Berlin, Germany) after sample combustion, and total organic carbon © by modified Walkley and Black [46] and loss on ignition (LOI) methods, where the oven dry sample was placed in a furnace at 450 °C for 11 h [47]. Particle size distribution (PSD) (sand, silt, and clay) was analyzed using a laser diffraction particle sizer and bulk density by using the core-cutter method [48]. Key soil characteristics are summarized in Table 2.

Table 2. Soil characteristics at the commencement of the experiment at the Beacon Field, North Wyke.

Soil Variable	Mean ± Standard Error
Soil texture	Silty clay loam
Sand (0.063–2.0 mm)	13.6 ± 5.6
Silt (0.002–0.063)	43.2 ± 3.0
Clay (<0.002 mm)	43.2 ± 6.4
pH	5.73
Available P (mg kg^{-1} dry soil)	28.3
Available K (mg kg^{-1} dry soil)	197.3
Available Mg (mg kg^{-1} dry soil)	102.7
Organic C (%)	5.37
Total N (%w/w)	0.52
Bulk Density (g cm^{-3})	0.62 ± 0.01

4.3. Treatments Applied, Experimental Design, and Plot-Layout

4.3.1. Treatments Applied

Treatments were applied on 15th May, 3rd July, and 26th September 2012, for the spring, summer, and autumn experiments, respectively. The aim of these timings was to reflect deposition at different stages of the grazing season (early, mid, and late [24]. And, also for each application timing, new plots were established. The applied treatments were as follows: (i) control (nothing applied), (ii) natural urine (NU), (iii) natural urine + DCD (NU +DCD), (iv) artificial urine (AU), and (v) dung (D) (Table 3). The natural urine and dung were collected from 6-year-old Holstein dairy cows at Reading University, UK, which were mostly fed on grass and maize silage. The urine and dung were collected from randomly selected cows in the herd of 24 cows, which coincidentally happened to urinate and defecate during the collection of experimental materials. Fresh urine and dung were collected during each of the experiments and characterized for N-loading before application to the field (Table 3). The collected urine was stored in sealed vials at <4 °C and remained unfrozen until application. The urine and dung were stored for up to 2 days and were removed from the cold room the night before application to allow them to attain ambient temperature before application [24].

Table 3. Description of the three experiments in the current study.

	Spring	Summer	Autumn
Start date	15 May 2012	3 July 2012	26 September 2012
End date	9 May 2013	11 June 2013	10 September 2013
Natural urine N loading (kg N ha^{-1})	405	429	435
Artificial urine N loading (kg N ha^{-1})	440	481	423
Dung N loading (kg N ha^{-1})	911	625	771
NU + DCD loading (kg N ha^{-1})	395	436	454
Grass harvest dates	1st: 19 June 2012 2nd: 28 August 2012	1st: 9 August 2012 2nd: 25 May 2013	25 May 2013

NB: For the NU + DCD, the N in the DCD was considered in the total N applied.

The artificial urine was prepared using a recipe suggested by [49] and detailed in [24]. The artificial and natural urine, as well as the dung were analyzed for pH (ratio urine/dung to water 1:6), dry matter (DM) (drying at 105 °C ± 5 for at least 12 h), total N and C (by sample combustion followed by separation by a GC column and analyzed using TCD detector), and readily available N, i.e., ammonium (NH_4^+) and nitrate (NO_3^-)] by the formation of a diazo compound between nitrite and sulphanilamide, which is then coupled with N-1-Napthyle-thyleneddiamine dihydrochloride to give a red azo dye (color measured at 540 nm) [50]. Total organic carbon (modified Walkley-Black) was analyzed by acidification followed by carbon oxidation, and further analysis was carried out using a Leco Combustion analyzer (TruSpec MicroCHN/CHNS/O, Lakeview, Michigan, USA).

Subsamples of the bulked artificial and natural urine were prepared and applied as detailed in [24]. In the field, urine and dung were applied at rates of 5 L m^{-2} and 20 kg m^{-2}, which are rates adapted from ranges of real-life deposition during grazing [51,52]. DCD (99% purity) (Sigma–Aldrich, Merck Life Science, Gillingham, UK) was mixed with urine before application and was applied at a commercially recommended rate of 10 kg ha^{-1}; equivalent to 6.5 kg N ha^{-1} [16] (Table 2).

4.3.2. Experimental Design

Each experiment (spring, summer, and autumn) was set up as a randomized block design, with three replicates per treatment. Plot dimensions were 6 m × 3 m, with areas for measurements of GHG emissions, soil mineral N and moisture, grass DM production, and plant N uptake, for example [53]. On each plot, there were five urine patches and five dung patches, each measuring 60 cm × 60 cm. Each urine patch received 1.8 L of urine (a typical volume for a cattle urination event [54], which was applied using a watering can that was fitted with a perforated spray head. The application area was bordered by a frame to prevent the urine from running off the area during application. The frame was removed once the urine had soaked into the soil. Each dung patch received 4 kg of fresh dung, spread to an even thickness across the 60 × 60 cm^2 area. On each plot, an additional 2 m × 2 m area was treated with dung or urine. This area was used for soil mineral N and grass DM production and N uptake measurements. For the NU + DCD treatment, the inhibitor was mixed with the urine prior to application to give a DCD application rate of

10 kg ha^{-1}. DCD was mixed with the urine to maximize the uniformity of distribution of the very small amount of product over the treatment area and would simulate the effect of adding DCD to feed [55].

4.4. Measurements and Sampling Strategies

4.4.1. Soil CH_4 and CO_2 Fluxes

Soil CH_4 and CO_2 fluxes were measured using the static chamber technique [56,57]. In order to account for spatial variability, five of the polyvinyl chloride (PVC) chambers (40 cm width × 40 cm length × 25 cm height) were installed in each of the replicate plots using a steel base to a soil depth of 5 cm. Gas sampling was performed periodically for a whole year, between 10:00 and 13:00, using 60 mL syringes and pre-evacuated 22 mL vials fitted with a rubber septa. Sampling was performed more frequently immediately after treatment application, i.e., five times per week during the first two weeks, which was then reduced to twice per week up to week 24 and then to monthly, totaling 30 occasions for the year. During each gas sampling occasion, ten ambient air samples were taken as a surrogate for the chamber air sample (T_0), and a gas sample was taken from each chamber at 40 min (T_{40}) after closure [56]. Both CH_4 and CO_2 were analyzed using a Perkin Elmer Clarus 500 gas chromatograph (GC Model 107, Perkin Elmer, Buckinghamshire, UK) fitted with a flame ionization detector (FID). Gas separation was achieved employing a Perkin Elmer Elite-PLOT megabore capillary column, 30 cm long and 0.53 mm i.d., maintained at 35 °C, and N_2 was used as a carrier gas. Cumulative gas emissions for each treatment and block were computed from the daily means and estimated using the trapezoidal integration method [58]. When measurements were stopped only for a few days short of a full year from the day of application, the last measured value was assumed to apply also for the last day of the year [24]. The global warming potential (GWP) of CH_4 and N_2O is, respectively, 27 and 273 times that of CO_2 in a 100-year horizon [13]. Therefore, GWP was estimated by multiplying annual CO_2, CH_4, and N_2O fluxes by 1, 27, and 273, respectively, and summing the results to obtain the net GWP [38]. N_2O data used in the calculations were sourced from [24].

4.4.2. Soil and Meteorological Measurements

During each gas measurement event, a bulk soil sample (up to 10 cm) was collected from each block to determine soil moisture content using the gravimetric method. During fourteen predetermined events, soil samples were colometrically analyzed for NH_4^+ and NO_3^- from 2 M KCl soil extracts using a Skalar SAN^{PLUS} Analyzer (Skalar Analytical B.V., Breda, The Netherlands). Daily rainfall, soil (up to a 5 cm depth), and air (min. and max.) temperature were monitored for a whole year after treatment application. In order to adjust gas concentrations to standard temperature for flux calculations, soil temperature was measured at every gas sampling occasion using a digital thermometer (Thermo Fisher Scientific, Birmingham, UK).

4.4.3. Statistical Analysis

Genstat (version 21, VSN International, Hemel Hempstead, UK) was used for statistical analysis. Prior to statistical tests, the data were checked for normal distribution using the Shapiro–Wilk test [59,60], and the homogeneity of variance was satisfied using Levene's test [61]. Analysis of variance (ANOVA) at $p < 0.05$ was performed according to the General Linear Model (GLM) procedure in the Statistical Analysis System (Version 9.4, Cary, NC, USA) when the Shapiro–Wilk test was significant ($p > 0.05$), and the data were confirmed to be normal. This was carried out to assess treatment differences in cumulative gas emissions of each gas, as well as differences in the measured soil characteristics between treatments. All our data were normally distributed according to the Shapiro–Wilk test. All tests were

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performed at the 5% probability level, and all graphs were made using SigmaPlot (Systat Software Inc., San Jose, CA, USA).

5. Conclusions

Daily soil CH_4 and CO_2 fluxes were controlled by soil moisture content, temperature, mineral N availability, and the addition of fresh C. The cumulative fluxes reported herein showed that dung produces high soil CH_4 and CO_2 under the temperate conditions of the current study. The results further highlight that dung deposition in grasslands may result in the emissions of environmentally harmful gases, including CH_4 and CO_2, and may predominantly result in high net GWP. Hence, exploring mitigation measures is necessary to reduce the intensities of these gases as a result of dung deposition in grazing systems.

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