

NITROUS OXIDE EMISSIONS FROM COW URINE PATCHES WITH DIFFERENT NITROGEN LOADINGS AT TWO SOIL MOISTURES IN AN INTENSIVELY MANAGED AUSTRALIAN GRASSLAND

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Abstract

In intensively grazed pastures, urine patches deposited during livestock grazing are the hotspots for nitrous oxide (N₂O) emissions and nitrate leaching. The impacts of spatial and temporal variability in urine N concentration and volume on N₂O emissions required to accurately estimate country-specific N₂O emission factors (EFs) have not been thoroughly evaluated under variable warmer and drier temperate environments (e.g., Menangle, NSW, Australia). Here we quantify and compare N₂O emissions and EFs from a naturally expanding effective area (NEEA), with that from a uniformly wetted area (UWA) of urine application in large versus small chambers, respectively.

The results show that over 146 days (early winter to late spring), there was the least cumulative N₂O emissions with low urine-N loading (141–282 kg N ha⁻¹) under NEEA, relative to the urine-N loading of 709 kg N ha⁻¹ under UWA. In NEEA, there was no difference in N₂O emissions with different urine volume treatments applied at the below field capacity (BFC) soil moisture condition. In contrast, there was a significant difference in N₂O emissions at the field capacity (FC), for example, 0.52±0.06 kg N₂O-N ha⁻¹ (1.5 L urine) *versus* 0.36±0.05 kg N₂O-N ha⁻¹ (1.0 L urine). In UWA, cumulative N₂O emissions were 2.3 times higher at FC (1.96 kg N₂O-N ha⁻¹) than BFC (0.87 kg N₂O-N ha⁻¹). The EF values in NEEA did not vary significantly with urine-N loading and soil moisture conditions and ranged between 0.07±0.01% to 0.10±0.02% in the BFC, and 0.09±0.02% to 0.16±0.03% in the FC. The EF values in UWA were 0.09±0.02% and 0.26±0.05% in the BFC and FC, respectively. The N₂O EF was higher in UWA than NEEA only at the FC soil moisture condition. The results suggest that the cattle urine-derived EFs for N₂O emissions (over winter to spring) in the drier temperate environment are lower than the country-specific EFs of 0.4 and 1.0% currently used in the Australian and New Zealand inventories, respectively.

Introduction

Livestock production systems with nitrogen (N) deposited through animal excreta are a major contributor (30–50%) to total nitrous oxide (N₂O) emissions in intensively grazed pasture soils (Oenema et al., 2005). The Australian Greenhouse Gas Inventory attributes more than 50% of direct and indirect N₂O emissions through the return of cattle excreta to pasture systems, and the soil emissions category accounts for approximately 57% of total N₂O emissions reported in the Australian Inventory (Department of Environment 2015). In intensively managed grassland systems, urine patches deposited from dairy cows during grazing contribute N inputs of >500–1000 kg N ha⁻¹ (Selbie et al., 2015). Since urine inputs from grazing animals are a major source of labile C and N, this creates “hotspots” to fuel the major microbial processes and expedite N loss from nitrification/denitrification, ammonia volatilisation, and nitrate leaching, thus creating a threat to the environment (Saggar et al., 2015; Luo et al., 2017).

The processes of N losses from real urine are likely to vary through uniformly distributed *versus* naturally expanded urine patches onto soils with different urine volumes, urine-N concentrations, soil moistures, and climate conditions (Selbie et al., 2015; Marsdsen et al., 2016; Forrestal et al., 2017; López-Aizpún et al., 2020). Globally, urine patches cover effective surface areas of ~0.24 to 0.68 m² while each urine patch contains ~2 times more N than pastures require for maintenance and growth (Selbie et al. 2015). Understanding these factors and processes of urine-derived N losses from dairy pastures can assist in accurate estimation of emission factors (EFs) (Chadwick et al., 2018; López-Aizpún et al., 2020). Cattle urine patches usually contain high amounts of soluble N, and urea is the dominant form of N in cattle urine, causing highly variable soil N₂O emissions (Chadwick et al., 2018). Initially, 80% of urine-N is rapidly hydrolysed to ammonia or ammonium, leading to a further N loss through nitrification, nitrification-denitrification, and denitrification (Selbie et al., 2015).

The non-random spatial distribution of urine patches and their variable shapes and sizes would result in a non-uniform distribution of urine-N (Selbie et al. 2015), relative to UWA, which are more uniform than urine patches in NEEA. For this reason, the N₂O emissions estimated from conventional UWA approaches may be inaccurate. Moreover, the ability to accurately estimate N₂O EFs from the NEEA urine patches with variable urine-N concentration/volume remains more challenging in intensively managed pasture-based systems. The reported urine derived N₂O EF values in inventories are often default values rather than country specific or land-use specific EFs (López-Aizpún et al., 2020). In the current Intergovernmental Panel on Climate Change (IPCC, 2019), the EF value for excretion of urine patches during cattle grazing is 0.4% (0.2–0.6%), which is lower than the previous IPCC estimates of 2% EF (IPCC, 2006). The urine derived N₂O EFs, particularly under variable dry-warm temperate environments, may be considerably lower than that estimated in country specific inventories, including those estimated in the current IPCC 2019 (López-Aizpún et al., 2020). Multiple studies have examined N₂O emissions and EFs from the uniformly wetted urine patches in static small chambers (Saggar et al., 2004; Luo et al., 2019; Forrestal et al., 2017). There is however limited information on how the naturally deposited urine with different volumes and N contents during livestock grazing affects N₂O emissions and EFs (Marsdsen et al., 2016; Forrestal et al., 2017; Hoogendoorn et al., 2018).

In the current study, the main objective was to quantify and compare the effect of real urine patches from dairy cows on N₂O EFs under two deposition methods using different urine volumes, and across two different soil moisture conditions [i.e., at field capacity (FC) and below field capacity (BFC)], in a moderately well-drained soil under a dry-warm temperate environment of Australia. The different urine deposition methods were (i) naturally expanding effective area (NEEA) where urine was applied to a central point with varying volumes (~ 2 to 4 L m⁻²) in a large chamber (Forrestal et al., 2017), and (ii) uniformly wetted area (UWA) where urine was applied at the whole measurement point in a small chamber as per a standard method (10 L m⁻²) in the soil (Selbie et al., 2015; Luo et al., 2019).

Methods

A field trial was established on a moderately well-drained soil ~6 months before the commencement (mid-June 2019) of this research in New South Wales, Australia (34°07'30.1"S 150°42'17.5"E). The soil at the site was classified as Eutrophic Red Chromosol (Isbell, 2002). This site had been under permanent pasture for dairy production from the last 21 years (1996-2017), and over this period, the site received regular irrigation and fertiliser N inputs. The pasture at the site was a mix of ryegrass (*Lolium perenne* and *L. multiflorum* L.) and kikuyu (*Pennisetum clandestinum*) system (Dougherty et al., 2016). Before the commencement of the field trial, key soil chemical and physical properties from 0–10 cm depth at the experimental field site were analysed using methods described in Rayment and Higginson (1992), i.e., Total carbon (C) 2.4%, Total N 0.2%. Total P 0.05%, pH_w 5.6, clay 18%, sand 58%, and bulk density 1.09 t m⁻³. Soil texture is sandy loam (0-10 cm, 10–20 cm) and sandy clay loam (20-30 cm).

Since 2018, the selected experimental area was fenced-off from livestock to avoid fresh dung and urine inputs. Two levels of soil moisture, i.e., at 100% field capacity (FC) and at 60% field capacity (BFC), were established and maintained through irrigation before initiating the urine patches and calibrated for the water-filled pore space (WFPS) in the range of 66–72% at FC and 48–54% at BFC. After the application of urine for the continuous track of WFPS, volumetric moisture content (VMC) (Delta-T HH2 moisture meter) was regularly measured throughout after gas sampling within each chamber as an indirect representative of WFPS. The VMC was then calculated by multiplying the GMC by the soil bulk density (t m⁻³). Soil temperature was also measured by using a handheld digital thermometer (Dig-stem-1 Digital Stem) at the times of gas sampling while inserted into the soil up to 10cm depth. The year 2019 was remarkably dry for the whole of Australia (278 mm), with a total annual rainfall of 304 mm in the Camden, NSW (BOM, 2019).

Urine was collected from dairy cows and stored in a laboratory at 4°C for up to four days before applying it in the field on 19 June 2020. Immediately after the collection, the total N content of the urine was analysed using the Flow Injection Analysis system (Lachat Quickchem 8500) from a NATA (National Association of Testing Authorities, Australia) facility accredited to ISO17025. The N content of the collected urine was 7.085 g L⁻¹.

Experimental design and urine application rates

The field trial was established in a completely randomised design for all urine patch treatments established in small and large chambers under both soil moistures (FC and BFC). Both the soil moisture sites were established on the same block of land separated with a buffer of 3 m. For the urine application, two types of urine deposition methods were performed. In NEEA, urine was applied at the height of 1.3 m to a central point of the base chamber in the soil with varying volumes (1, 1.5 and 2 L) in a large chamber and allowed to spread naturally. In UWA, urine was applied slowly and evenly at the base of a small chamber as per a standard method (10 L m⁻²) (Luo et al., 2019). The four NEEA-based urine patches were established in the large chamber with four different volumes [i.e., no urine (control), 1.0 L (NEEA-1), 1.5 L (NEEA-2), and 2.0 L (NEEA-3), equivalent to 0, 2, 3 and 4 L m⁻², respectively; **Table 1**], with five replicates for each treatment. The four treatments of NEEA were randomly assigned and hence constituting 20 plots of each under both soil moisture conditions. The size of each NEEA treatment plot was 2.5 × 2.5 m. In the NEEA blocks, two additional urine patch treatments in the small chamber [i.e., no urine (control) and 0.452 L urine (UWA) per chamber, equivalent to 0 and 10 L m⁻², respectively; **Table 1**] were also allocated randomly (n = 5) at a distance of 1-m from the edge of the large chamber under both soil moisture conditions.

Table 2: Urine-N application and loading rates in small and large chambers. The N content of the collected urine was 7.085 g L⁻¹.

Urine patches treatment	Chamber		Urine volume (L)	Urine volume (L m ⁻²)	Urine content (g N m ⁻²)	Urine loading kg N ha ⁻¹
	Type	Area (m ²)				
Control (NEEA)	Large	0.5027 (∅ 0.80m)	0	0	0	0
NEEA-1	Large	0.5027 (∅ 0.80m)	1.0	2	14.1	141
NEEA-2	Large	0.5027 (∅ 0.80m)	1.5	3	21.1	211
NEEA-3	Large	0.5027 (∅ 0.80m)	2.0	4	28.2	282
Control (UWA)	Small	0.0452 (∅ 0.24m)	0	0	0	0
UWA-1	Small	0.0452 (∅ 0.24m)	0.452	10	70.9	709

(where, ∅ - diameter symbol; UWA = uniformly wetted area; NEEA = naturally expanding effective area)

Nitrous oxide emissions

A static chamber technique was used to measure N₂O emissions from the UWA and NEEA treatments requiring different sized chambers. Both closed static chambers were made up of PVC consisting of two parts, i.e., base-part and lid. In brief, urine was applied in (i) the small chamber covered an area of 0.0452 m² and (ii) the large chamber covered an area of 0.5024 m². All the chambers were covered with Mylar sheeting to minimise temperature fluctuations inside the chambers during a closed time. Following the urine applications, N₂O flux measurements were conducted 2-hr post-urine application (day 1), then on days 2, 3, 6, 8 and 10, followed by bi-weekly (twice weekly) measurements for one month. After that, the N₂O emission measurements were carried out once per week or one time per two weeks for another 3.5 months depending upon the rainfall events that occurred. Gas samples were collected

between 8:30 am to 11:30 am by using a gas-tight 50 mL syringe. Gas samples were taken immediately after closure of the chamber and subsequently at 30 and 60 minutes.

During the chamber closer period, ~25 ml gas samples were taken by the syringe at t_0 , t_{30} , and t_{60} through a 3-way stopcock connected to a nylon tube pinned to the middle of the top part of the closed chamber. The gas samples were then transferred into 12-mL pre-evacuated exetainer tubes (Labco Ltd., UK) and transported to a laboratory for analysis. The concentrations of N_2O in the gas samples were analysed by an Agilent 7890A gas chromatograph (GC), equipped with a μ -electron capture detector and fitted with a Gerstel multi-purpose auto-sampler, programmed to inject 2,500 μ L from the sample vial into the injection port. The relative standard deviation of gas samples analysed in GC was <2% based on 7-replicate injections for N_2O .

The hourly N_2O fluxes were calculated for each chamber using linear regression and the ideal gas law, according to Eqs. 1 and 2. All N_2O flux rates were estimated ($g N_2O-N ha^{-1} d^{-1}$):

$$F_{N_2O} = b \times \frac{V_{ch}}{A_{ch}} \times \frac{MW_{N_2O-N}}{MV_{corr}} \times \frac{60}{10^5} \times 24 \quad (1)$$

where, A_{ch} is a basal area of the measuring chamber (m^2); b is an increase in concentration ($ppb min^{-1}$); MW_{N_2O-N} is the molecular weight of N_2O-N ($28 g mol^{-1}$); MV_{corr} is temperature-corrected molecular volume [$m^3 mol^{-1}$]; V_{ch} is the volume of the measuring chamber (m^3).

$$MV_{corr} = 0.02241 \times \left(\frac{273.15+T}{273.15} \right) \quad (2)$$

where, MV_{corr} is as defined; T is air temperature ($^{\circ}C$) in the chamber during the measurements; $0.02241 m^3$ is the molar volume of an ideal gas at 1-atm, 273.15 K. The cumulative emission ($gN_2O-N ha^{-1}$) was calculated by integrating the hourly fluxes measured at different times over winter to late spring (146 days).

The EFs over the 146 days were calculated using the Eq. 3 (Luo et al., 2019):

$$EF (\%) = \frac{N_{Treatment} - N_{Control}}{N_{load}} \times 100 \quad (3)$$

where, EF% is emission factor (N_2O-N emitted as a percentage of urine-N applied); $N_{Treatment}$ is the cumulative N_2O-N emissions from the urine treated soil ($kg N ha^{-1}$), $N_{Control}$ is the cumulative N_2O-N emissions from the non-urine treated soil ($kg N ha^{-1}$), and N_{load} is the amount of urine N applied ($kg N ha^{-1}$).

Statistical analysis

Repeated measures ANOVA was performed to test the main effects of urine treatment (between-subjects factor) and sampling time (within-subjects factor), and their interactive effects on N_2O emission rates. The one-way analysis of variance (ANOVA) was used to test the effect of urine-N loadings (across the different chamber sizes) on total N_2O EFs under each soil moisture (BFC or FC). The one-way ANOVA was used to test the effect of urine-N loading

treatments (in the large chamber) on cumulative N₂O emissions under each soil moisture. Data were log-transformed where needed to remove the variance heterogeneity. When the F-statistic was significant, the means were compared using the least significant difference (at 5% level, LSD0.05) unless stated otherwise. All statistical analyses were performed using IBM SPSS statistics 22.

Results

There were only a few rainfall events over the 21-week trial period (18 June to 11 November 2019), with the 114 mm total rainfall (Fig. 1a) being lower than the 30-year average (~240 mm) (BOM 2019). During the trial period, the minimum and maximum air temperatures ranged from -2.9 °C and 13.9 °C in end-August 2019 (low values) to 18.1 °C and 38.0 °C in end-October 2019 (high values) (Fig. 1a). Soil temperature in topsoil (0–0.1 m soil) under both soil moisture conditions (BFC and FC) ranged from 7.4 °C in early-July to 20.4 °C in early-November 2019 (Fig. 1b). After the maintenance of two levels of soil moistures, i.e., BFC (WFPS = 52%; VWC = 30%) and FC (WFPS = 69%; VWC = 40%) at the field site in both chambers, these soil moisture levels were continuing at the same standards after urine application in winter for about five weeks (Fig. 1b). Then, soil moisture levels decreased rapidly to 32–36% WFPS from 5 to 10 weeks (Fig. 1b). Although there were a few rainfall events during weeks 15–20 weeks (ranging from 2–19 mm), soil moistures decreased rapidly to 8–9% WFPS. Soil temperatures over 11 to 21 weeks increased from 11 °C to 20 °C (Fig. 1b).

The N₂O fluxes were greatest 2-h after urine application from both UWA and NEEA (Fig. 2). The N₂O fluxes then decreased sharply over three days in the UWA urine patches, and up to 3–10 days across different urine volumes in NEEA, under both soil moistures (Fig. 2a–d). The N₂O emissions were higher ($P < 0.05$) from all the urine treatments (UWA and NEEA) than the control treatment. In the UWA, only one N₂O emissions peak was obtained on day 20 in the BFC and two apparent N₂O emissions peaks on days 20 and 41 in the FC (Fig. 2c,d), but there were multiple peaks of N₂O fluxes from the NEEA. Because urine-N loading (per hectare basis) was greater under UWA than NEEA (i.e., 709 versus 141–282 kg N ha⁻¹) (**Table 1**), cumulative N₂O emissions were ~2 times higher from UWA (0.87 kg N₂O-N ha⁻¹) than all other NEEA treatments (0.39–0.47 kg N₂O-N ha⁻¹) at BFC, and were ~4–5 times higher from UWA (1.96 kg N₂O-N ha⁻¹) than all other NEEA treatments (0.36–0.52 kg N₂O-N ha⁻¹) at FC (**Table 2**). In UWA, the cumulative N₂O emissions was 2.3 times higher in the FC than BFC. In NEEA, cumulative N₂O emissions through different urine loadings were mostly similar under both soil moistures (FC and BFC). In NEEA, the N₂O EF values under different urine-N volumes at each soil moisture were not significantly different ($P > 0.05$) and ranged from 0.07±0.01% to 0.10±0.02% at the BFC, and from 0.09±0.02% to 0.16±0.03% at the FC (**Table 2**). In UWA, the N₂O EFs were 0.26±0.05% at the FC and 0.09±0.02% at the BFC (Table 3). The N₂O EF in UWA (0.26%) was significantly ($p < 0.05$ –0.1) higher compared to N₂O EFs from different NEEA treatments (0.09–0.16%) at the FC only. However, the EFs were similar in UWA (0.09%) and NEEA (0.07–0.10%) at the BFC (**Table 2**).

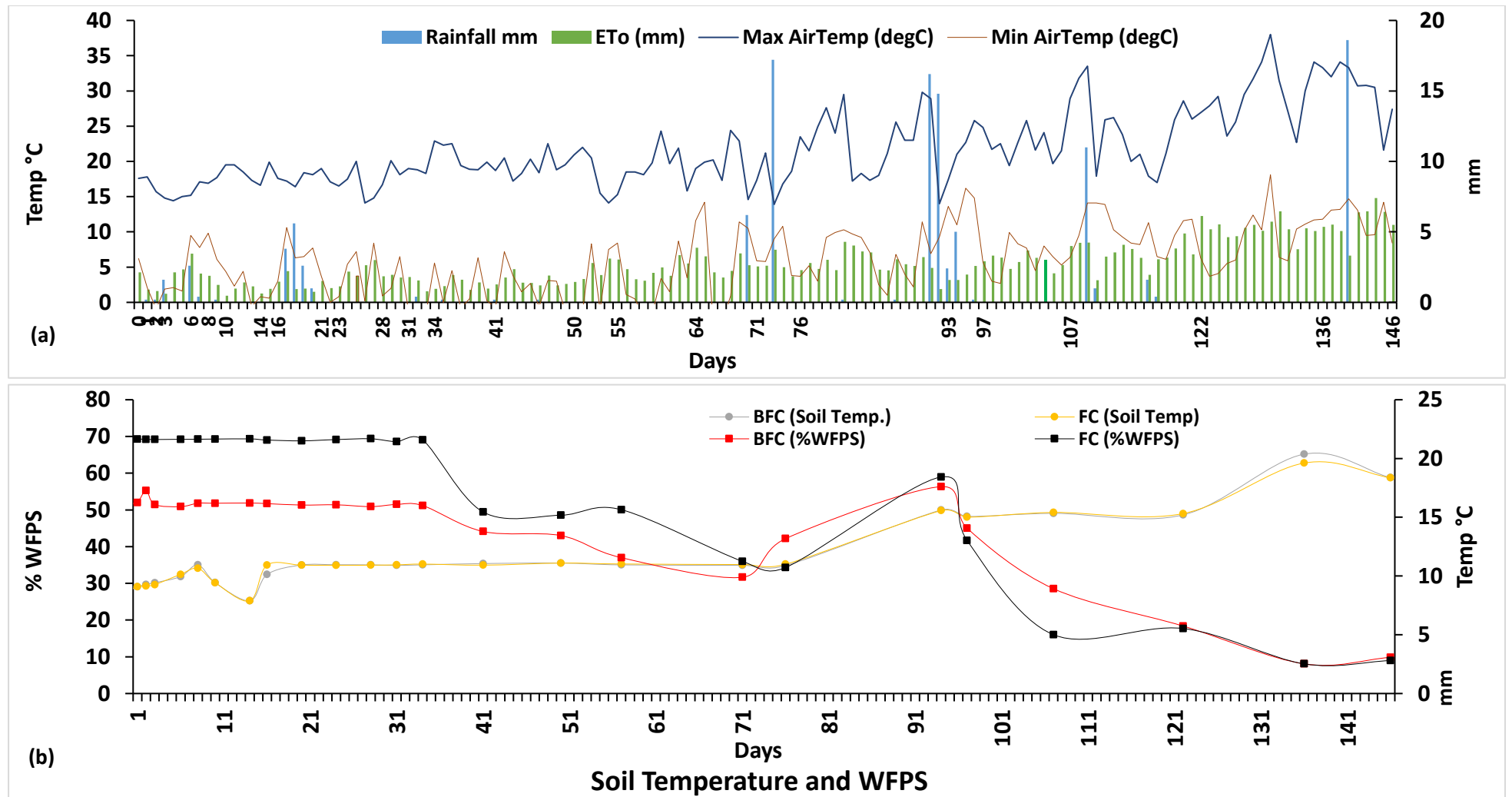


Fig. 1 (a) Weather data representing rainfall, evapotranspiration, maximum and minimum air temperature from the weather station installed within 500 m of the site; and (b) regularly monitored mean soil temperature and water filled pore space (WFPS) data across all chambers (0–0.1 m) at the experimental site from 18 June 2019 to 11 Nov 2019 (146 days). (*BFC* = *Below field capacity*; *FC* = *Field capacity*).

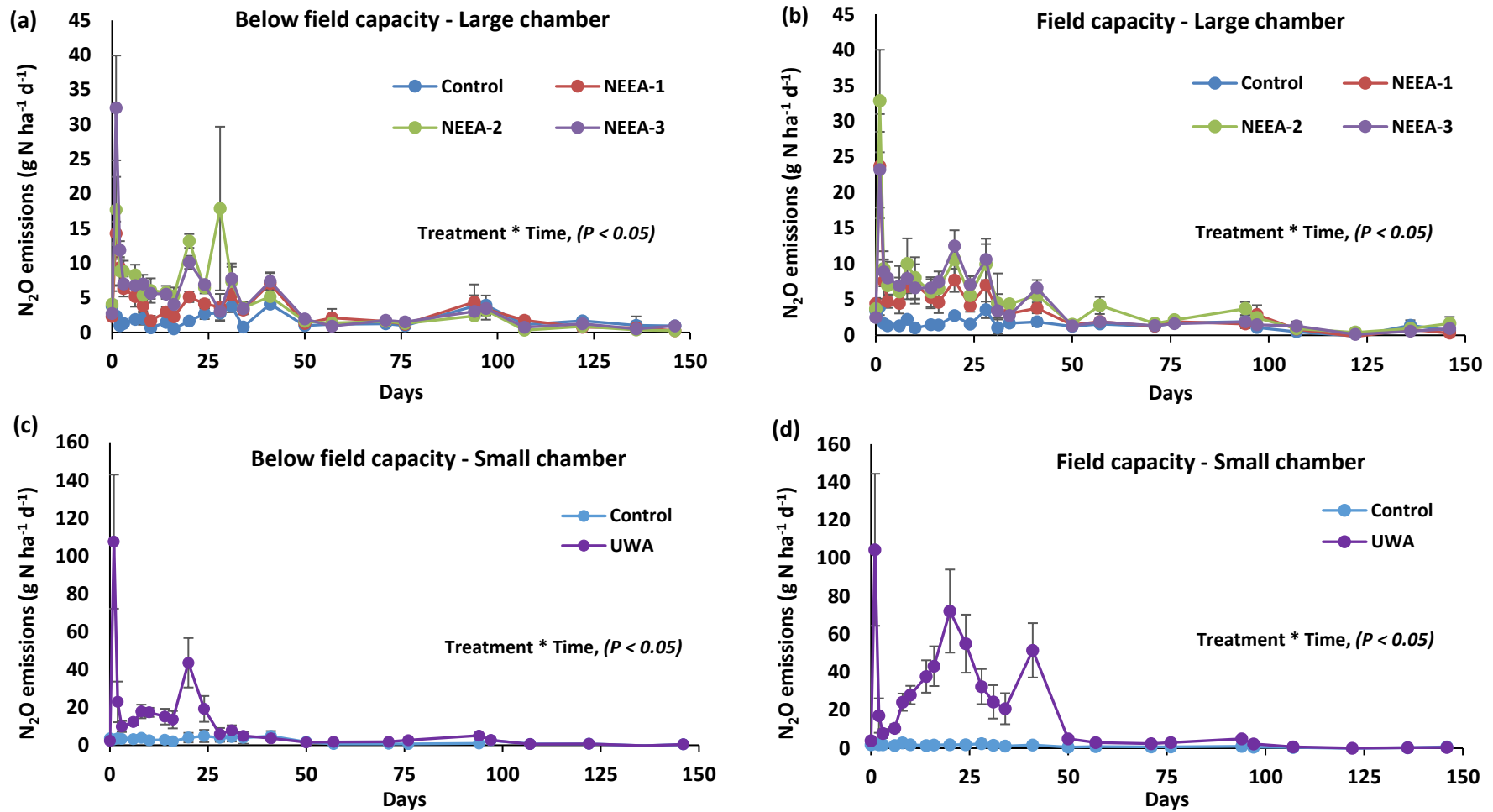


Fig. 2 Nitrous oxide (N_2O) emission rates from urine patches under different urine-N loadings: (a, b) large chambers representing naturally expanding effective area (NEEA-1, 2, 3) receiving 2L, 3L and 4L m^{-2} urine volumes, respectively; and (c, d) small chambers representing uniformly wetted area (UWA) receiving 10 L m^{-2} urine volume, plus the control treatment (zero urine-N) from both NEEA and UWA urine patches under different soil moistures (below field capacity, and at field capacity). The emission data at the experimental site were collected from 18 June 2019 to 11 Nov 2019 (146 days), where each treatment plot displays the mean values ($n=5$) with vertical bars representing standard error of the mean).

Table 3: Cumulative N₂O emissions and emission factor (EF%) from different urine-N loadings in small chamber (UWA) and large chamber (NEEA) under different soil moisture conditions (BFC = “Below field capacity”; FC = “Field capacity”).

Treatments	BFC	FC	BFC	FC
	N ₂ O (kg N ha ⁻¹)		EF%	
Control (non-urine)	0.263 (±0.023) ^a	0.194 (±0.029) ^a	NA	NA
NEEA-1	0.389 (±0.040) ^b	0.365 (±0.052) ^b	0.09 (±0.03) ^A	0.12 (±0.04) ^A
NEEA-2	0.475 (±0.049) ^b	0.522 (±0.064) ^c	0.10 (±0.02) ^A	0.16 (±0.03) ^{AB}
NEEA-3	0.465 (±0.023) ^b	0.456 (±0.043) ^{bc}	0.07 (±0.01) ^A	0.09 (±0.02) ^A
Control (non-urine)	0.251 (±0.099)	0.130 (±0.018)	NA	NA
UWA	0.867 (±0.115)	1.959 (±0.387)	0.09 (±0.02) ^A	0.26 (±0.05) ^B

All the values are the mean values (n=5); values followed by ‘±’ are standard errors. The superscript lowercase letters on cumulative N₂O emissions indicate least significant differences (at 5% level, LSD_{0.05}) with different urine-N loadings in NEEA at each soil moisture condition. The superscript uppercase letters on EF% indicate least significant differences (LSD_{0.05}) across treatments (i.e., two chamber sizes and different urine-N loadings) at each soil moisture condition. Control= non-urine. NEEA = naturally expanding effective area, NEEA-1 = 2.0 L m⁻², NEEA-2 = 3.0 L m⁻², NEEA-3 = 4.0 L m⁻². UWA = uniformly wetted area, UWA = 10 L m⁻² (see details in Table 1). NA = not applicable.

Discussion

This study provides information on how the urine patch configuration in the NEEA (compared to UWA) affects total N₂O emissions and EFs during grazing in a dairy pasture system in the dry–warm temperate region in Australia. The EF values in the NEEA from cow urine patches in the pasture soil are not significantly different under contrasting urine-N loadings and soil moistures. These results confirm the hypothesis because the EFs in NEEA were not controlled by variable urine volumes and soil moisture conditions over early-winter to late-spring period (in 2019) with a relatively low rainfall (114 mm). Although a few rainfall events occurred in the late winter to spring, they were not sufficiently large to trigger emissions through nitrification and denitrification (Dougherty et al., 2016; Hoogendoorn et al., 2018).

Here the study demonstrated maximum peaks of N₂O emission fluxes on the day of urine application in the winter across all urine patches and soil moisture treatments. Nevertheless, multiple peaks of daily N₂O emissions also occurred up to two months, mainly from the NEEA versus UWA treatments under both soil moistures (FC > BFC) (Fig. 2a-d). Recent studies have also observed multiple peak N₂O emissions, initially and over time, from different urine application methods (Forrestal et al., 2017) or urine input treatments (Luo et al., 2019). These results of high N₂O flux rates in the present study may be attributed to the maintenance of two separate %WFPS contents (i.e., FC and BFC) until mid-winter that may have enhanced N mineralisation and N loss (e.g., through emissions) from the urine patches (Luo et al., 2019). Moreover, the urine deposition may increase soil dissolved organic carbon and soil pH, thus enhancing the initial N₂O fluxes from nitrification at BFC or denitrification at FC (Luo et al.,

2019). The high urine-N loading in UWA may have induced only two to three high peaks of N₂O fluxes during winter (relative to multiple but lower peaks of N₂O fluxes with low urine N loadings in NEEA), which may likely be through denitrification with limited oxygen availability in the soil (Forrestal et al., 2017).

Since the calculated EFs were linked to the ratio of total net N₂O-N emissions and the amount of urine-N applied, the EFs ranged from 0.07 to 0.16% in NEEA, which were not significantly influenced by different urine-N loadings [as observed in natural patches from dairy cattle (Forrestal et al., 2017)] and variable soil moisture conditions (Fig. 1b). Nonetheless, in UWA, where only one type of urine-loading was applied as per the standard method (Selbie et al., 2015) but that was not mimicking natural urine patches (as used in NEEA), the N₂O EF was strongly influenced by soil moisture, e.g., high EF value (0.26%) at FC versus low EF value (0.09%) at BFC (Fig. 3). These EF values from both NEEA and UWA urine patches with different soil moistures in the dry-warm temperate region were lower than the default annual EFs of 0.4% currently used in the Australian and National Greenhouse Gas inventories (Department of Environment 2015; IPCC 2019). Moreover, the EF value from cattle urine on flatland and low slopes in dairy pastures was 0.98%, that is currently updated in the New Zealand inventory (van der Weerden et al., 2020). However, the EF values in our study were within (e.g., 0.02 to 0.19%) or lower than the range (e.g., >0.20–0.47%) for dairy cattle urine with variable volumes, reported in earlier studies from Australia with similar or different rainfalls, soil water contents, and soil temperature conditions (Galbally et al., 2005; Ward et al., 2018). As per the hypothesis, the moderately well-drained soil used in the present study may lead to lower total N₂O emissions from all urine volumes and soil moistures, which returned to background levels within two months post-urine application, also in agreement with the study from New Zealand (Van Der Weerden et al., 2013). However, some studies have found higher N₂O emissions and EFs from urine-N deposition in poorly- versus well-drained soils, possibly linked to higher nitrification or denitrification, depending on soil WFPS and rainfall conditions (Luo et al., 2019). Since the global IPCC default methodology pretends a constant EF value for the entire year (Paustian et al., 2006), N₂O measurements after cow urine deposited in several months of the whole year are needed to obtain annual EFs from different soils and moistures/rainfalls in dairy grazed pastures.

The results revealed that the N₂O EFs did not differ from urine applied with different volumes to a central point, allowing for natural expansion in a large chamber under both BFC and FC soil moistures. However, the N₂O EF from the urine applied uniformly to the entire measurement area (in a standard small chamber) was higher in the FC than BFC soil moistures, and higher in UWA versus NEEA at the FC only. In summary, the N₂O EFs from cattle urine in the warmer and drier temperate environment were much lower (by 35% to 82%) than the EFs of 0.4 currently used in the Australian inventory, and in the recent IPCC 2019 guidelines for National Greenhouse Gas Inventories. Future studies are needed to accurately estimate N₂O EFs and NUE through natural urine deposition by grazing dairy cattle over different climatic seasons, urine-N composition (based on different diets to dairy cows), soil types (poor- vs. well-drained soil with different hydrological regimes), and with variations of low or high soil moisture conditions in dry and warm temperature environments.

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