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Are nitrification inhibitors effective in reducing N₂O from farm-scale emission hotspots?

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Abstract Livestock congregation areas are nitrous oxide (N₂O) hot-spots and could be key areas to focus mitigation action. We tested whether combined cattle urine and fertiliser N₂O-N emission factors (EFs) would be higher from a farm gateway area compared to a standard pasture under sub-tropical conditions, and whether the nitrification inhibitor, dimethyl pyrazole phosphate (DMPP), would lower N₂O EFs from

the gateway area. Treatments ($n=3$) included: (i) fertiliser applied to a standard pasture (50 kg urea-N ha⁻¹), (ii) fertiliser (50 kg urea-N ha⁻¹) + urine (350 kg N ha⁻¹) applied to a standard pasture, (iii) fertiliser (50 kg urea-N ha⁻¹) + urine (350 kg N ha⁻¹) applied to the gateway area, and (iv) fertiliser (50 kg urea-N ha⁻¹) + urine (350 kg N ha⁻¹) + DMPP (1.5 kg ha⁻¹) applied to the gateway area. Emissions were monitored via an automated static chamber-based system and ¹⁵N-labelled urine treatments ($n=4$) used to assess N₂O + N₂ emissions, N₂O:N₂ and ¹⁵N recovery from the pasture. No significant differences ($p > 0.05$) were observed for EFs between the fertiliser + urine treatment for the standard pasture (1.10 ± 0.17%) or the gateway area (1.46 ± 0.40%). DMPP did not lower the N₂O-N EF from the gateway area (1.50 ± 0.22%), where wet and warm conditions may have accelerated DMPP degradation. In the ¹⁵N-labelled urine treatments, significantly ($p < 0.05$) greater N₂O + N₂ emissions occurred in the gateway compared to the standard pasture, but disaggregating EFs between the contrasting areas was not warranted.

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Introduction

The livestock sector accounts for 14.5% of global greenhouse gas (GHG) emissions (Gerber et al. 2013). Mitigation strategies for methane (CH₄) and nitrous oxide (N₂O), are required from this sector to sustainably produce food for a growing population (Grossi et al. 2019). The major sources of N₂O emissions from global pastures and rangelands between 1961 and 2014 include the application of mineral N fertilisers (7%; 0.17 Tg N₂O–N year⁻¹), manure (13%; 0.33 Tg N₂O–N year⁻¹) and livestock excreta (54%; 1.31 Tg N₂O–N year⁻¹) deposited within the pasture (Dangal et al. 2019). Emissions from excreta deposited on pastures represent a large source, due to the high concentrations of labile N and C present over relatively small areas, resulting in nutrient loads in excess of the requirements for grass growth (Selbie et al. 2015). The deposited N is, therefore, vulnerable to gaseous and leaching losses from the system (De Rosa et al. 2020). Emissions of N₂O result from the major soil processes of nitrification and denitrification of the deposited N (Di and Cameron 2012).

Livestock urinate and defecate in discrete patches within pastures resulting in emission hotspots within the pasture. Cattle can also congregate in contrasting areas of a pasture e.g. seeking shade or shelter, around water troughs under warm conditions or near gateways. Such areas may experience elevated excretal N and C loads, reduced plant cover and favour soil conditions for denitrification (Matthews et al. 2010; Luo et al. 2017) i.e. by causing reduced soil aeration by compaction, pugging and puddling of the soil. These areas represent small-scale emission hotspots, with increased chances of urine spread. Studies in New Zealand have quantified EF₃ values for urine deposited to hotspots (troughs, gateways, and raceways) within dairy farms (Adhikari et al. 2020; Saggarr et al. 2022), generally finding slightly higher N₂O emissions from these areas, but similar EF₃ values

due to the concomitant higher background emissions within the hotspots. This has led to the suggestion that the emissions may not need to be disaggregated within inventories to account for hotspot areas, however, the background emissions may still be anthropogenic in origin and need to be accounted for (Saggarr et al. 2022). Due to the relatively large emissions arising from proportionately small areas of a single farm, targeting emission hotspots for mitigation action is frequently mentioned (Cowan et al. 2017; Hénault et al. 2012; Mitchell et al. 2021; Turner et al. 2016) yet few studies have quantified the performance of mitigation practices within such areas.

Nitrification inhibitors such as DCD and DMPP are a technology used to delay the first and rate-limiting step of ammonia oxidation, by depressing *Nitrosomonas* bacteria activity in soil (Zerulla et al. 2001). This results in the added N remaining in the ammoniacal form for longer, providing added time for the pasture to take up the excess N before it is converted to the highly mobile NO₃⁻ form, which may leach from the system or be denitrified to N₂O and N₂. Under the conditions of poor pasture growth, the pasture will not be as effective in removing the ammoniacal N, which could lead to a reduction in the efficacy of inhibitors in reducing N₂O emissions. The soil compaction and increased inputs of C and N around livestock congregation areas, could also result in denitrification being the major N₂O production pathway, so nitrification inhibitors may have reduced efficacy in minimising N₂O emissions (Mitchell et al. 2021), but this has not been quantified. In a literature review, Adhikari et al. (2021) found DCD, DMPP and Nitrapyrin reduced N₂O emissions from urine patches by 44 ± 2%, 28 ± 38%, and 28 ± 5%, respectively; however, only two publications were available for DMPP highlighting the need for further field trials investigating the performance of DMPP applied with urine. Di and Cameron et al. (2011) found DCD applied at 10 kg ha⁻¹ and DMPP applied at 1 kg ha⁻¹ were equally effective at reducing N₂O emissions from cattle urine deposited on a New Zealand pasture, resulting in 62–66% reduction in N₂O emissions. However, under UK summer conditions, Marsden et al. (2017) found no significant effect of DMPP (1 kg ha⁻¹) in reducing N₂O emissions from sheep urine patches. Similarly, DCD has been found to have a high variability in its efficacy in reducing N₂O, producing an average of a 46% reduction in a study on

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cattle urine deposited to various sites across the UK (Chadwick et al. 2018). Friedl et al. (2017) conducted a study to investigate emissions of both N_2O and N_2 from urea fertiliser applied with DMPP in three subtropical pastures in Australia and found N_2 emissions were lowered by more than 70% but without a decrease in N_2O emissions. Whether a decrease in N_2 emissions would occur under a urine patch with DMPP, to our knowledge, has not been previously investigated under sub-tropical conditions, or where conditions may be conducive to denitrification e.g. within a cattle congregation area.

Data for N_2O emissions from the urine patch are also under-represented in the literature for tropical and sub-tropical conditions, as identified in a global meta-analysis of urine-derived N_2O emissions (López-Aizpún et al. 2019). Although a recent study by Mitchell et al. (2021) found that within an intensive dairy farm in sub-tropical Australia, a small proportion of the farm area of ca. 3% was responsible for ca. 28% of the total farm N_2O emissions. Emissions from urine deposited to hotspot areas under sub-tropical conditions could cause high N_2O emissions due to a combination of high temperatures and heavy rainfall events (Mitchell et al. 2021).

This study aimed to quantify both N_2O and N_2 emissions from cattle urine deposited to standard areas of pasture and an area with a history of high cattle occupation/disturbance (soil around a gateway). The performance of DMPP in reducing both N_2O and N_2 emissions was assessed for the gateway area. We hypothesised: (i) N_2O emission factors from urine deposited on the gateway soil would be greater than when deposited on an area of pasture, and (ii) DMPP would not be effective in reducing N_2O emissions associated with urine deposition near the gateway area. The work is anticipated to improve the accuracy of GHG emission estimates from sub-tropical grazed pasture ecosystems and provide information on the performance of nitrification inhibitors in an area with a history of high cattle occupation/disturbance. This information could contribute to more refined emission factor inventories and assist in the development of GHG offsetting methods in Australia.

Materials and methods

Study site

The study was conducted on an irrigated perennial dairy pasture in Casino, New South Wales, Australia. The soil at the site is a heavy clay Black Vertisol (FAO 1998) with shrink-swell characteristics. The pasture comprised Kikuyu (*Cenchrus clandestinus*) over-sown with Annual Ryegrass (*Lolium multiflorum x rigidum*). The climate at the site is humid sub-tropical and typically receives an average of 1272 mm of rainfall annually (based on 50 years of weather data). The study was conducted in autumn, where typical average daily autumn air temperatures are 19.4 °C. Paddocks were grazed on a 3-week cycle, with urea fertiliser applied at 30–50 kg N ha⁻¹, up to eight times per season.

Treatment details and plot layout

Two areas within the field were established, the first representing the middle or ‘standard’ area of the pasture and the second near to a gateway, representing an area where cattle congregate. The entire pasture was fertilised at 50 kg N ha⁻¹ applied as urea to reflect the interaction of fertiliser and urine, which is likely to occur under typical management (Di and Cameron 2011). The treatments ($n=3$) included (i) fertiliser applied to standard pasture conditions (50 kg N ha⁻¹), (ii) fertiliser (50 kg N ha⁻¹) + urine (350 kg N ha⁻¹) applied to standard pasture conditions, (iii) fertiliser (50 kg N ha⁻¹) + urine (350 kg N ha⁻¹) applied near a gateway, and (iv) fertiliser (50 kg N ha⁻¹) + urine (350 kg N ha⁻¹) + DMPP (1.5 kg ha⁻¹) applied near a gateway. A diagrammatic representation of the treatments can be seen in Fig. 1. Details of the plot layout can be seen in the Supplementary Information, Fig. S1 and photos of the field trial in Fig. S2.

Briefly, treatments were randomised within two separate blocks—one in the standard pasture and one near a gateway. Plots were laid out (1.5 m × 1.5 m) with an area where an automatic greenhouse gas chamber system was located and an adjacent soil sampling area, to which the treatments were replicated for ancillary soil and pasture measurements throughout the study. Treatments were applied to all plots on 15/04/19 (dd/mm/yy) and monitored for a period of

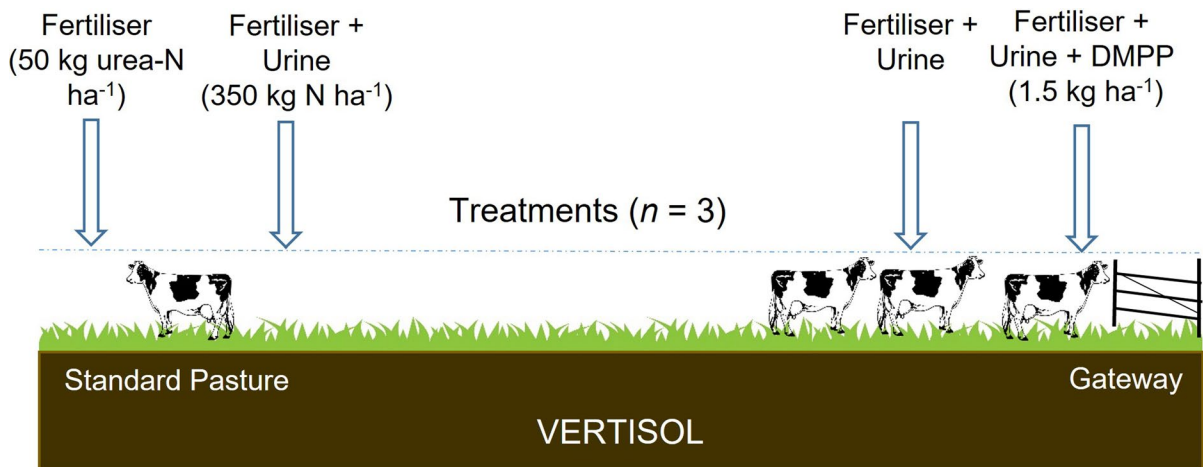


Fig. 1 Diagrammatic representation of treatments applied in the current study, where the application rates are the same as the first mention for the different treatments

59 days. Here, a watering can was used to apply 1.25 L of urine (6.88 g N L^{-1} ; ca. 350 kg N ha^{-1}) evenly within the chamber area and the surrounding area used for soil sampling. This resulted in a urine application rate of 5 L m^{-2} , which has been used to simulate cattle urine events in previous studies e.g. Cardenas et al. (2016). The DMPP treatment was applied in a 1 L solution immediately after urine application, at a rate of $1.5 \text{ kg DMPP ha}^{-1}$. This inhibitor application technique was used as it is more likely to occur in a commercial setting than being directly mixed with the urine (Adhikari et al. 2021). All non-DMPP treatments received an equivalent application of water, so the additional solution application did not become a confounding factor.

In addition to the automated GHG chambers, micro-plots ($n=4$) were established adjacent to the treatment plots to determine the urine-derived N_2O and N_2 fluxes via the ^{15}N gas flux method (Friedl et al. 2020; see below). Here, three treatments were established: (i) ^{15}N -urine applied under standard pasture conditions (350 kg N ha^{-1} equivalent loading rate), (ii) ^{15}N -urine applied to the gateway area (350 kg N ha^{-1} equivalent loading rate) and (iii) ^{15}N -urine+DMPP applied to the gateway area (350 kg N ha^{-1} equivalent loading rate and $1.5 \text{ kg DMPP ha}^{-1}$ equivalent loading rate). These areas were used for the application of ^{15}N -labelled urine for determination of N_2 emissions via the ^{15}N gas flux method (see below). The quantities of liquid

Table 1 Properties of the soil (0–10 cm, unless otherwise stated) from the standard pasture and gateway at the start of the experiment

Soil property	Standard pasture	Gateway
Bulk density (g cm^{-3}) [†]	$0.68 \pm 0.04 \text{ a}$	$0.96 \pm 0.05 \text{ b}$
Gravimetric moisture content (%)	40 ± 1	37 ± 1
Organic matter (%)	35.5 ± 0.9	33.6 ± 0.9
pH	6.65 ± 0.09	6.64 ± 0.09
EC ($\mu\text{S cm}^{-1}$)	120 ± 24	151 ± 36
Total C (%)	4.67 ± 0.13	4.08 ± 0.28
Total N (%)	$0.43 \pm 0.01 \text{ b}$	$0.35 \pm 0.03 \text{ a}$
C-to-N ratio	$10.9 \pm 0.1 \text{ a}$	$11.7 \pm 0.2 \text{ b}$
Dissolved organic C (mg C kg^{-1})	$103 \pm 5 \text{ a}$	$162 \pm 14 \text{ b}$
Total dissolved N (mg N kg^{-1})	35 ± 10	53 ± 20
Extractable NO_3^- (mg N kg^{-1})	10.9 ± 2.3	13.4 ± 5.2
Extractable NH_4^+ (mg N kg^{-1})	5.3 ± 1.6	20.3 ± 12.6

Values represent means ($n=6$) \pm SEM and where appropriate results are reported on a dry soil weight basis. Small letters indicate significant differences ($p < 0.05$) based on t-tests

[†]Conducted at 0–5 cm

added were scaled down to the same rates as those used within the ^{15}N micro-chambers. Irrigation was applied at two points within the monitoring period,

following farmers' practice, accounting for the estimated evapotranspiration based on previous years.

Soil and urine characteristics

To characterise the soil at each location a soil sample (0–10 cm) was taken from each plot 11 days prior to the treatment application ($n=6$ samples each; see Table 1). Cores for bulk density measurements were taken by driving 100 cm³ metal rings into the ground (0–5 cm) and drying the cores in an oven (105 °C; 24 h). Gravimetric soil moisture was determined by drying soils for the same time and at the same temperature as the cores used for determining the bulk density. Organic matter content was determined via the loss-on-ignition method of Ball (1964), by combusting soils in a muffle furnace (450 °C; 16 h). The soil pH and EC were determined using standard electrodes on a 1:2.5 soil-to-distilled water suspension, after shaking and allowing them to settle. The total soil C and N concentrations were determined on dried and ground soils on a LECO CNS-2000 analyser (Leco Corporation, St. Joseph, MI, USA). A soil extraction was conducted with 0.5 M K₂SO₄ (1:5 w/v, soil-to-solution), and the supernatant filtered through Whatman No.42 filter papers, prior to determination of NO₃⁻, NH₄⁺, dissolved organic C and total dissolved N. The NO₃⁻ and NH₄⁺ in the soil extracts were determined on a Gallery™ Discrete Analyser (ThermoFisher Scientific Australia Pty Ltd.). The dissolved organic C and total N were determined on a Shimadzu TOC-V (with TNM attachment) Total Organic Carbon Analyser (Shimadzu Instruments, NSW, Australia).

Urine samples were collected from cattle when they were in the milking parlour, after grazing the same vegetation as the field site, taking care to avoid faecal contamination. The samples were bulked to produce a homogenous urine sample to apply to the plots. The urine was maintained refrigerated for a period of <1 week prior to its application, to minimise urea hydrolysis (Charteris et al. 2021). Prior to application, the urine was sent to the Environment Analysis Laboratory in Southern Cross University for determination of total N, NH₄⁺ and NO₃⁻. The urine pH and EC were determined using standard electrodes. The urine contained 6.88 g N l⁻¹, the NH₄⁺ concentration was 244 mg N l⁻¹ indicating urea hydrolysis had not occurred at a substantial rate prior

to treatment application. The NO₃⁻ concentration was below the detection limit, and the pH and EC of the urine sample was 7.26 and 21.95 mS cm⁻¹, respectively. In addition, we created a ¹⁵N-enriched urine solution for application to the micro-plots for determination of N₂O and N₂ emissions. Here, cattle urine (6.8 g N l⁻¹) was mixed with a 98 atom % ¹⁵N-urea solution (6.8 g N l⁻¹) in a 4:6 ratio respectively, resulting in a 57.4 atom % ¹⁵N-mixture, with the same overall N concentration as the original urine sample.

Automated greenhouse gas flux measurements

GHG emissions (N₂O, CO₂ and CH₄) were analysed on a mobile automated GHG system, as described in detail in Scheer et al. (2014). Briefly, the system accommodates 12 automated chambers (50×50×15 cm) fixed on stainless steel bases (10 cm depth), connected to a gas chromatograph (SRI) for analysis of N₂O and CH₄ and a LICOR for analysis of CO₂. During uninterrupted measurement, the system produces eight measurements of GHG flux per day, with four headspace gas samples measured per chamber over a 1 h closure period. After every fourth gas sample a calibration standard is measured, with concentrations of 0.5, 4, and 800 ppm for N₂O, CH₄, and CO₂, respectively. Fluxes of N₂O were calculated from the linear increase in concentration of the headspace gases over the 1 h chamber closure period. Cumulative emissions over the monitoring period were calculated via trapezoidal integration. Unfortunately, data from a true control treatment in the original experimental design were compromised and could not be used. Therefore, to estimate emission factors, the control flux over the background measurement period (ca. 1 week prior to treatment application) was extrapolated over the length in time of the monitoring period (59 days) providing values of 3116 and 3899 μg N₂O–N m⁻² for the standard pasture and gateway areas, respectively. These values were subtracted from the cumulative treatment flux before dividing by the quantity of N applied in the treatment to approximate an emission factor. Thus, the reported emission factor values are subject to slightly higher uncertainty and are therefore referred to as *estimated* emission factors throughout the manuscript.

Micro-chamber measurements of N₂O and N₂

Here we used the closed static chamber technique to monitor N₂O and N₂ emissions from the micro-plots. The polyethylene chambers (22 cm × 22 cm × 32 cm), which had a 2-way Luer-lock tap to allow for headspace gas sampling, were attached to stainless steel bases. Gas samples were taken between 9 a.m. and 12 a.m. at 0, 1 h and 3 h after chamber closure. The 3 h closure time was chosen based on data from the increase of ¹⁵N–N₂ as shown in Friedl et al. (2016). Headspace gas samples (20 ml) were stored in 12 ml pre-evacuated exetainers (Labco, UK). Samples were taken on day 0, 2, 4, 8, 11 and 14 days after treatment application, targeting the most emission-intense period following urine application. Temperatures inside the chambers were monitored with HOBO onset UA-002-64 temperature loggers. Gas samples were analysed for N₂O via gas chromatography (Shimadzu 2015), with N₂O flux determined as above. The gas samples were analysed for the isotopologues of N₂ (¹⁵N¹⁴N, ¹⁵N¹⁵N) and N₂O ([¹⁴N¹⁵N¹⁶O + ¹⁵N¹⁴N¹⁶O] and ¹⁵N¹⁵N¹⁶O) using a Sercon 20–22 IRMS (Sercon, UK) with a Sercon Cryoprep trace gas concentration system. The ¹⁵N enrichment of the NO₃⁻ pool undergoing denitrification (*a_p*) and the fraction of N₂ emitted from this pool (*f_p*) were calculated using the equations given by Spott et al. (2006) and detailed in the Supplementary Information. The headspace concentrations of N₂ were multiplied by the respective *f_p* values giving N₂ produced via denitrification, allowing the calculation of the product ratio N₂O/(N₂O + N₂). Values for N₂O/(N₂O + N₂) were gap-filled using linear interpolation for the first 15 days after urine application. Daily N₂ + N₂O emissions were then calculated by dividing daily N₂O fluxes measured with the automated GHG system by the N₂O/(N₂O + N₂) product ratio, and daily N₂ emissions by subtracting the respective daily N₂O emissions. The precision of the IRMS for N₂ based on the standard deviation of atmospheric air samples (*n* = 10) at 95% confidence interval was 7.6×10^{-7} and 3.0×10^{-7} for ²⁹R and ³⁰R, respectively.

Ancillary soil and pasture measurements

Additional soil samples (0–10 cm) were taken from the urine patch sub-plots using an auger, bulking quadruplicate soil cores. Soil samples were processed

within 24 h of collection and stored within a fridge prior to analysis. The soils were analysed for gravimetric soil moisture, pH, EC, NO₃⁻, NH₄⁺, extractable dissolved organic C and total dissolved N as described for the soil characterisation. These measurements were taken on days –11, 0, 2, 4, 8, 11, 14, 22, 28, 35, 42 and 49 days after treatment application. Soil water content was also monitored via frequency domain reflectance probes (Sentek EnviroSCAN, South Australia), situated in the standard pasture and the gateway area (however, the probe located near the gateway failed, resulting in soil water data from the standard pasture only, apart from gravimetric soil moisture measurements taken during routine soil sampling). Soil water-filled pore space (WFPS) was calculated from soil moisture data using the ratio of volumetric water content to soil porosity. Pasture biomass cuts (> 5 cm grass height) were taken 15 and 42 days after treatment application, from the plots receiving treatments (adjacent to the GHG chambers) and from within the ¹⁵N-micro-plot chamber areas. Pasture biomass samples were dried in an oven (70 °C; 48 h), ground in a Rocklabs BenchTop Ring Mill and then analysed for total C and N as described for the soil characteristics. The ¹⁵N-labelled plant biomass samples were analysed for ¹⁵N enrichment using IRMS (as above). The contribution of urine-N to N uptake in the aboveground biomass (> 5 cm height) was calculated based on the recovery of ¹⁵N calculated based on mass ¹⁵N balance (Michener and Lajtha 2008).

Calculations and statistical analysis

We conducted t-tests to determine differences between the soil characteristics between the standard area of pasture and the soil from around the gateway. Cumulative N₂O emissions and estimated N₂O–N emission factors were compared via one-way ANOVA, after testing for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). The same procedure was conducted for the two pasture biomass cuts, pasture foliar N content and C-to-N ratio and the % recovery of the ¹⁵N tracer in the pasture biomass. The cumulative N₂O, N₂ and N₂O + N₂ arising from the ¹⁵N-labelled urine application were tested using location and inhibitor application as fixed factors. Data were log-transformed if normality or homogeneity of variance assumptions were violated.

Soil mineral N dynamics, total extractable dissolved organic C and total dissolved N were tested for significant differences via one-way ANOVA on the contrasting sampling dates. All statistical analyses were performed in R (R Core Team 2018).

Results

Soil properties

Results for the soil characteristics sampled from the standard pasture and gateway area can be seen in Table 1. Briefly, the soil sampled from near the gateway had a significantly greater bulk density ($p < 0.05$) and extractable dissolved organic C content ($p < 0.05$). Most of the other measured soil

parameters were similar ($p > 0.05$) between the two sampled areas except for the gateway area having a significantly higher C-to-N ratio ($p < 0.05$) and a significantly lower ($p < 0.05$) total N content. Extractable soil NH_4^+ was also higher in the gateway soil, although this was not significant ($p > 0.05$).

Weather and soil moisture data

Data for air temperature, daily rainfall and soil volumetric water content are displayed in Fig. 2. The mean air temperature across the study period was 17 °C, with minimum and maximum temperatures of -2 °C to 42 °C, respectively. Cumulative rainfall across the study period was 80 mm, with a peak in rainfall at a similar time to the treatment application. The volumetric water content displayed increasing

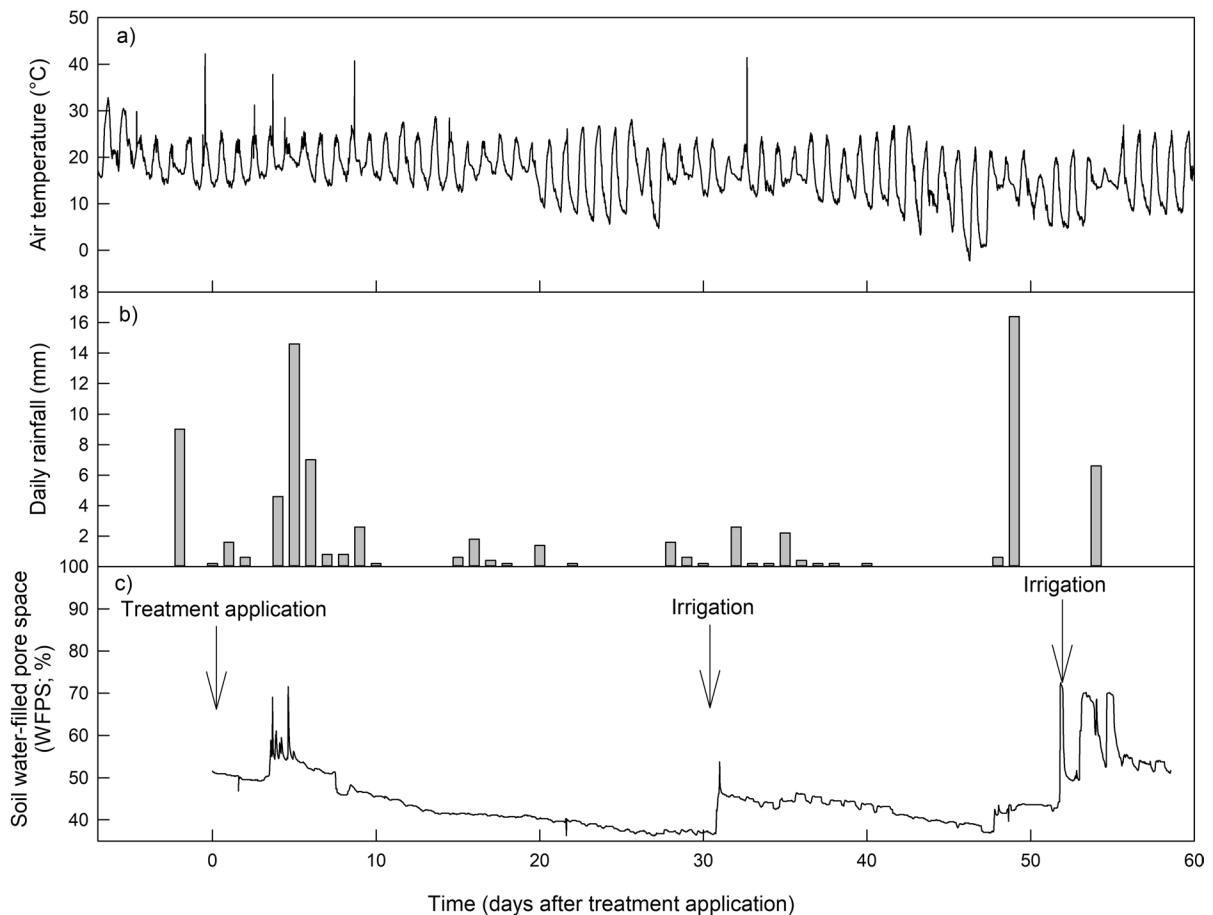
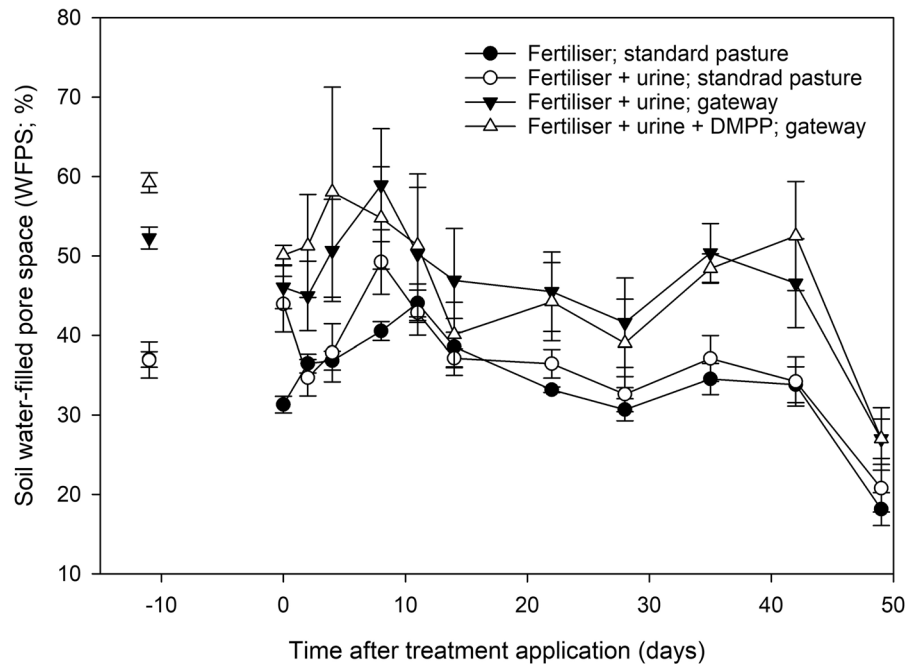


Fig. 2 Air temperature panel **a**, daily rainfall panel **b** and volumetric soil moisture content panel **c** across the study period, including timing of treatment application and irrigation events

Fig. 3 Soil water-filled pore space (%) derived from the soil sampling data. Symbols represent means ($n=3$) and error bars denote SEM



peaks correlating with the timing of rainfall and irrigation events, although this data was derived from only the standard pasture area (due to failure of the soil moisture probe located in the gateway). To visualise differences in soil moisture between the standard pasture and gateway areas, the gravimetric soil moisture data arising from sampling of the plots expressed as WFPS are displayed in Fig. 3. Briefly, we see slightly lower values of the soil WFPS in the standard pasture compared to the gateway area.

Soil N₂O emissions and estimated emission factors

Soil N₂O emissions following the fertiliser, urine and DMPP treatments can be seen in Fig. 4. There was an initial large peak of N₂O emissions in all treatments, rising to > 1000 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ in all treatments. This steadily declined to background levels, but further emission peaks coincided with the timing of irrigation, which was more pronounced in the soil near the gateway under the first irrigation application. Cumulative N₂O emissions and estimated N₂O-N emission factors over the 59-day monitoring period can be seen in Table 2. Although cumulative emissions were higher in the treatments applied near the gateway and lowest for the fertiliser applied alone to the standard pasture, no significant differences were

found between the groups ($p > 0.05$; ANOVA). The overall ANOVA for the N₂O-N emission factors was significant ($p = 0.009$), however, the EF from the fertiliser plus urine treatment was not significantly higher in the gateway compared to the standard pasture ($p > 0.05$; Tukey's HSD), nor was DMPP effective in reducing N₂O emission factors from the gateway location ($p > 0.05$; Tukey's HSD). Thus, the significance level of the overall ANOVA arose from the vastly differing N₂O-N EF values arising from the fertiliser only treatment, which had a much lower N application rate than those treatments receiving urine.

The N₂O/(N₂O + N₂) product ratio and N₂O + N₂ emissions

The cumulative N₂O, N₂ and N₂O + N₂ emissions can be seen in Table 3 and trends in the N₂O/(N₂O + N₂) product ratio can be seen in Fig. 5. The cumulative N₂ and N₂O + N₂ were both significantly greater ($p < 0.05$) in the gateway areas compared to the standard pasture. The difference in N₂O + N₂ was largely driven by higher N₂ emissions (rather than N₂O emissions) in the gateway area. No significant differences ($p > 0.05$) were found between the urine and nitrification inhibitor treatments applied to the gateway area.

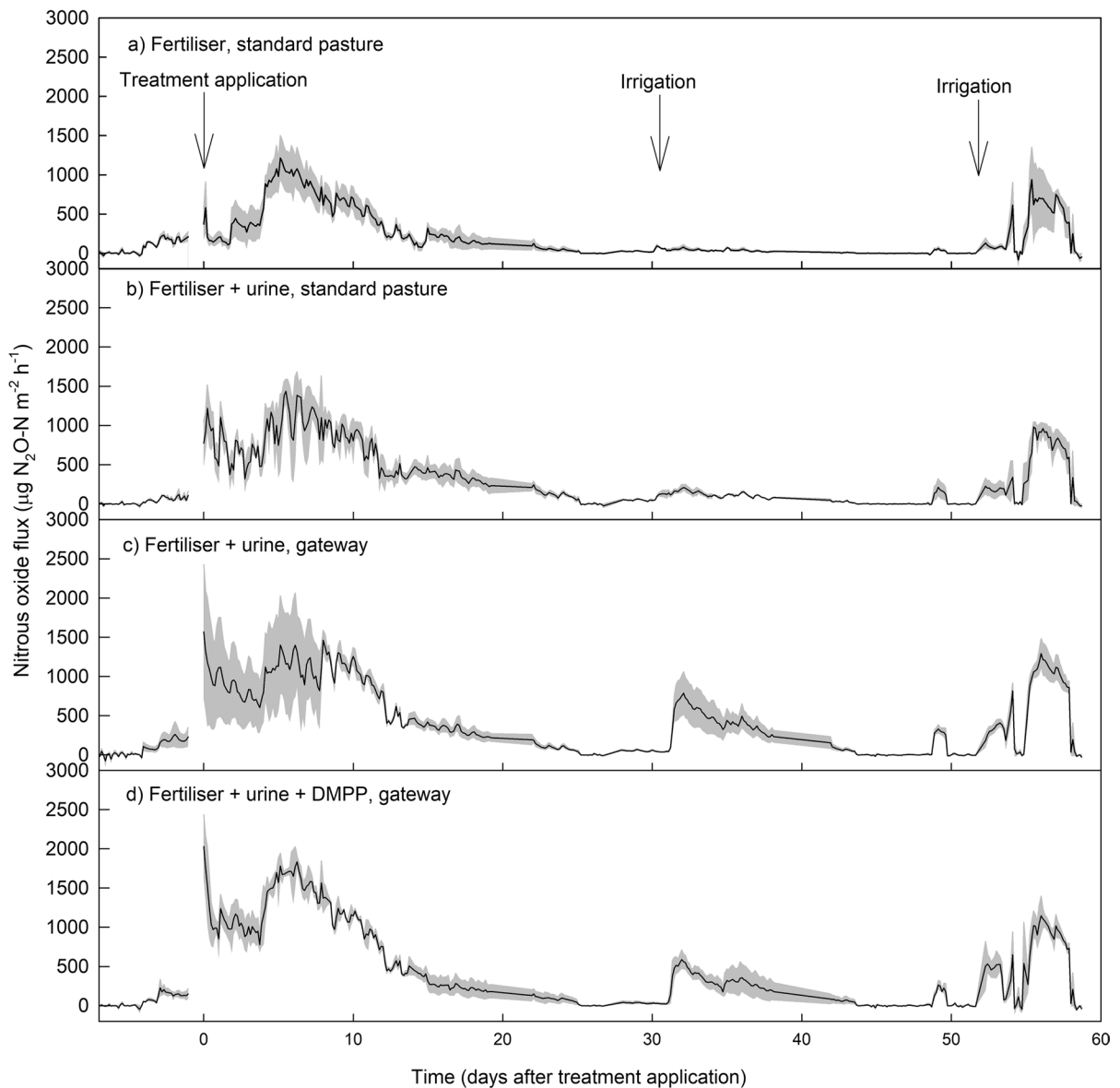


Fig. 4 Soil nitrous oxide emissions over the study period. Lines represent means ($n=3$) and shaded area represents the upper and lower bounds of the SEM, with treatment applica-

tion type and location of application shown on each panel. Treatment application and irrigation timing arrows apply to all panels

The $N_2O/(N_2O+N_2)$ ratio showed a declining trend in all treatments over the monitoring period (Fig. 5).

Soil extractable mineral nitrogen dynamics

Results for the soil extractable mineral nitrogen dynamics are displayed in Fig. 6. Additionally,

we provide the results of the ANOVA on contrasting sampling dates in Supplementary Information Tables S1 for NH_4^+ and S2 for NO_3^- . The soil NH_4^+ concentrations rapidly increased due to the treatment applications, declining to pre-treatment values by day 11. We only found a significant difference ($p < 0.05$) between treatments on the day of treatment application (Table S1), where the NH_4^+ concentration in the

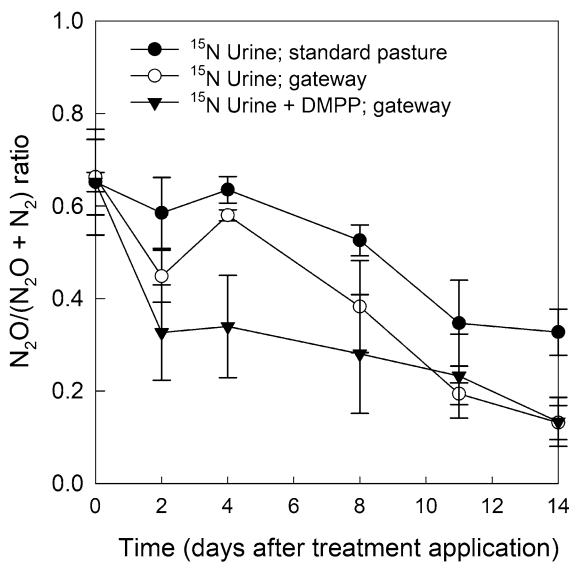
Table 2 Cumulative N₂O emissions and estimated N₂O-N emission factors over 59 days

Treatment	Cumulative N ₂ O emissions (mg N ₂ O-N m ⁻² over 59 days)	Estimated N ₂ O-N emission factor over 59 days (% of N applied)
Fertiliser in standard pasture	281 ± 70	5.56 ± 1.41 a
Fertiliser + urine in standard pasture	436 ± 68	1.10 ± 0.17 b
Fertiliser + urine in gateway	578 ± 158	1.46 ± 0.40 b
Fertiliser + urine + DMPP in gateway	594 ± 85	1.50 ± 0.22 b

Values represent means ± SEM (*n* = 3). Small letters indicate significant differences between groups (Tukey's HSD; significance level *p* < 0.05)

Table 3 Cumulative N₂O, N₂ and N₂O + N₂ over 14 days from the ¹⁵N-labelled microchambers. Values represent means ± SEM (*n* = 3)

Treatment	Cumulative N ₂ O emissions (mg N ₂ O-N m ⁻²)	Cumulative N ₂ emissions (mg N ₂ -N m ⁻²)	Cumulative N ₂ O + N ₂ emissions (mg N m ⁻²)
¹⁵ N-urine in standard pasture	255 ± 46	2868 ± 906	3123 ± 930
¹⁵ N-urine in gateway	274 ± 59	6092 ± 586	6366 ± 559
¹⁵ N-urine + DMPP in gateway	310 ± 25	5108 ± 1096	5418 ± 1099

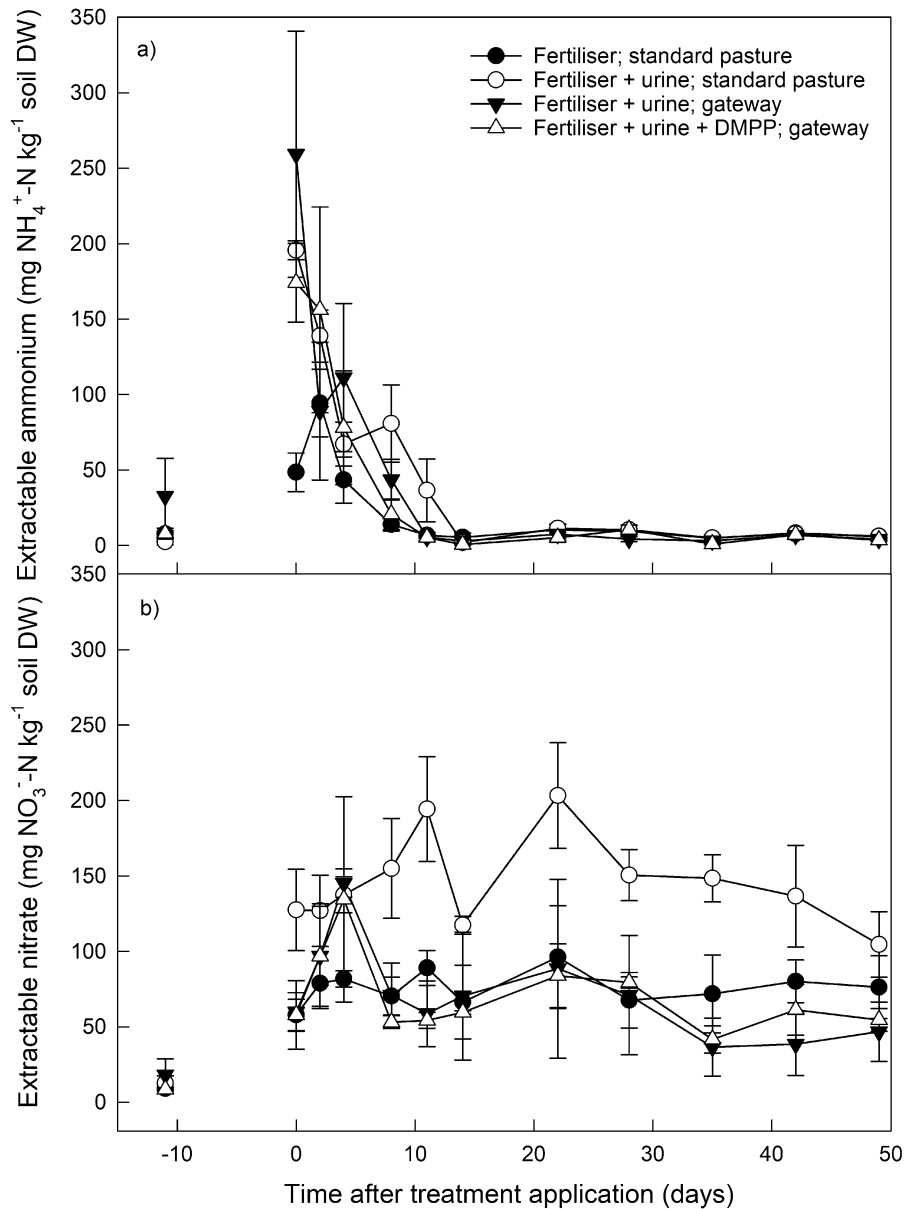
**Fig. 5** The N₂O/(N₂O + N₂) product ratio from the ¹⁵N-urine labelled micro-plots, over the first two weeks of the study period. Symbols represent means (*n* = 4), error bars denote SEM

fertiliser only treatment was significantly lower than the fertiliser + urine in the gateway treatment, reflecting the lower amounts of N applied. On all other

sampling dates, the values for NH₄⁺ were similar between treatments (*p* > 0.05).

The soil NO₃⁻ concentrations increased in all treatments in comparison to the pre-treatment sampling date, increasing as nitrification proceeded. The NO₃⁻ concentrations then remained stable during later sampling time points, not returning to the pre-treatment values. For the soil extractable NO₃⁻, we found significant differences (*p* < 0.05; Table S2) between treatments on days 8, 11 and 35. On day 8, the lowest NO₃⁻ concentration was found in the fertiliser + urine + DMPP applied to the gateway treatment (53.1 ± 4.2 mg NO₃-N kg⁻¹ soil DW), which was significantly lower (*p* < 0.05) than the fertiliser + urine applied to the standard pasture (154.9 ± 33.1 mg NO₃-N kg⁻¹ soil DW). The NO₃⁻ concentrations were significantly higher (*p* < 0.05) in the fertiliser + urine applied to standard pasture treatment (194.3 ± 34.7 mg NO₃-N kg⁻¹ soil DW) compared to all other treatments on day 11. On day 35 the NO₃⁻ concentrations were significantly lower (*p* < 0.05) in both treatments applied to the gateway compared to the fertiliser + urine applied to standard pasture. This indicates slight differences in nitrification rates between the two sampled locations.

Fig. 6 Dynamics of extractable soil ammonium **a** and nitrate **b** in the field trial. Symbols represent means ($n=3$) and error bars denote SEM. Legend applies to both panels and results are reported on a dry soil weight basis

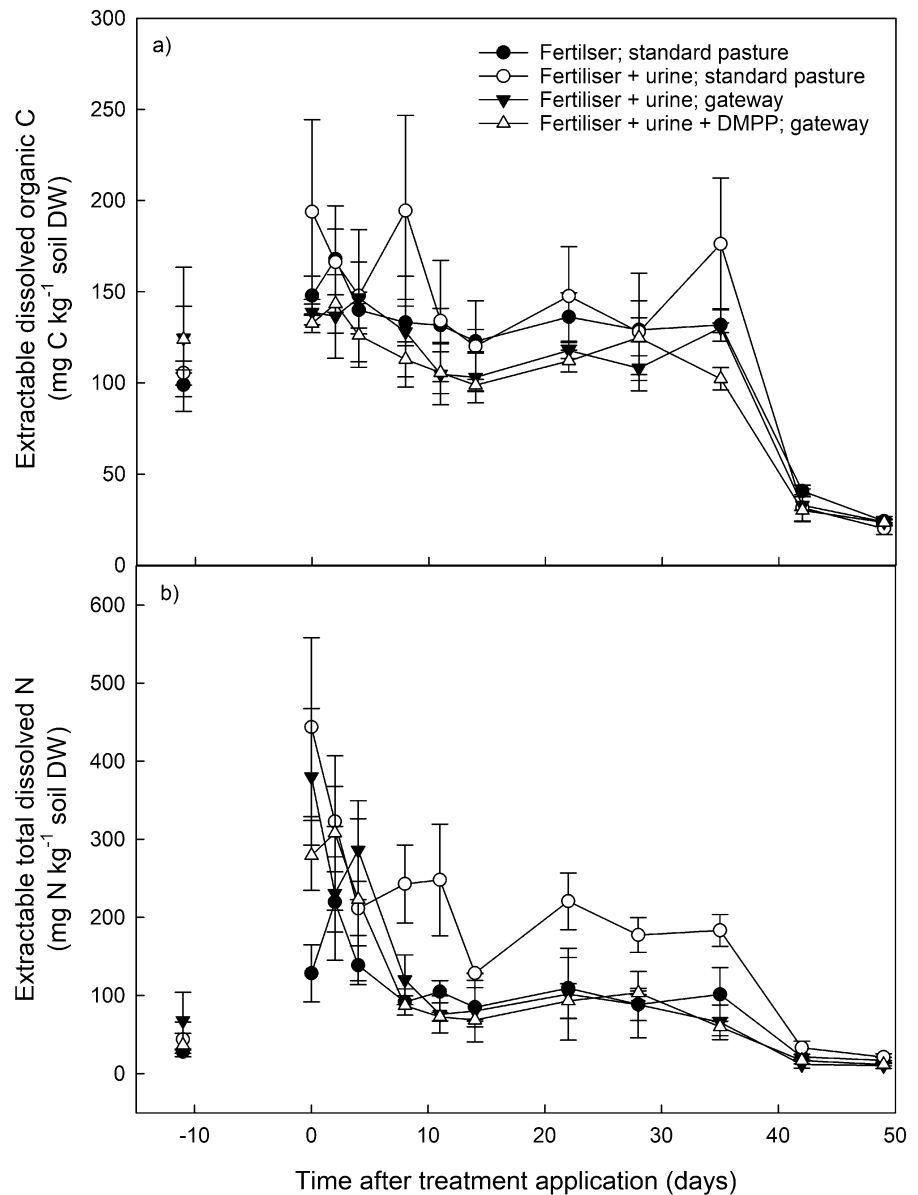


Soil extractable dissolved organic carbon and total dissolved nitrogen

Results for the soil extractable dissolved organic C and total dissolved N can be seen in Fig. 7 and the results of the ANOVA for the contrasting sampling

dates are displayed in Supplementary Information, Tables S3 and S4. Briefly, we did not find any differences in soil dissolved organic C contents between treatments on the different sampling days. As expected, the total dissolved N increased following all treatment applications and declined to background concentrations over the course of the study. Significant differences ($p < 0.05$) were found for the total extractable dissolved N on days 8, 11 and 35. Here, N concentrations were highest in the

Fig. 7 Dynamics of extractable dissolved organic carbon **a** and total dissolved nitrogen **b**. Symbols represent means ($n=3$) and error bars denote SEM. Legend applies to both panels and results are reported on a dry soil weight basis



fertiliser + urine applications to the standard pasture and were significantly greater in this treatment compared to the fertiliser + urine + DMPP treatment.

Soil pH and EC and pasture data

The soil pH and EC dynamics following the treatment applications are displayed in Supplementary Information, Fig. S3. Results for the biomass dry matter, N content and foliar C-to-N ratio for the two biomass cuts from the non-labelled plots can

be seen in Table 4 and the urine-N plant uptake data (as determined within the ¹⁵N-labelled micro-chambers) can be seen in Table 5. The results are described in Supplementary Information, as limited differences were observed in these measurements.

Table 4 Biomass weights and foliar N content and C-to-N ratio taken from the plots over the course of the study

Treatment	Biomass cut 1 (15 days after treatment application)			Biomass cut 2 (42 days after treatment application)		
	Biomass (g DM m ⁻²)	Foliar N content (%)	Foliar C-to-N ratio	Biomass (g DM m ⁻²)	Foliar N content (%)	Foliar C-to-N ratio
Fertiliser in standard pasture	75 ± 2	4.1 ± 0.3	10.8 ± 0.6	130 ± 6	3.5 ± 0.3	12.9 ± 1.4
Fertiliser + urine in standard pasture	109 ± 32	4.1 ± 0.2	10.8 ± 0.5	104 ± 15	3.6 ± 0.1	12.3 ± 0.4
Fertiliser + urine in gateway	87 ± 24	4.6 ± 0.1	9.5 ± 0.4	136 ± 22	3.5 ± 0.3	12.1 ± 1.0
Fertiliser + urine + DMPP in gateway	160 ± 21	4.0 ± 0.4	11.0 ± 1.7	131 ± 25	3.6 ± 0.3	11.8 ± 1.6

Values represent means ± SEM ($n = 3$)

Table 5 Uptake of urine-N in the above ground biomass

Treatment	Uptake of urine-N into the above ground biomass (%)		
	1st biomass cut (15 days after treatment application)	2nd biomass cut (42 days after treatment application)	Sum of 1st and 2nd biomass cuts
Urine in standard pasture	5.47 ± 1.75	7.02 ± 2.01	12.5 ± 3.53
Urine in gateway	10.25 ± 1.98	5.82 ± 1.38	16.1 ± 3.03
Urine + DMPP in gateway	4.80 ± 0.89	7.08 ± 0.40	11.9 ± 1.04

Data represent means ± SEM ($n = 4$), no significant differences were found between treatments (one-way ANOVA; $p > 0.05$)

Discussion

Soil compaction near gateway did not increase N₂O emissions

We observed a higher bulk density and extractable dissolved organic C concentrations in the soil near the gateway, which is likely due to the greater impact of livestock in these areas due to treading and dung deposition. Greater mineral N concentrations may have been expected in the gateway area due to the potential for more urination events, although significant differences may have been obscured by the patchy nature of excretal events adding to the high variability observed. Gravel had been added to the gateway area to prevent excessive pugging and puddling at the entrance, thus we positioned the chambers where the gravel was not present. Thus, vegetative cover beyond the gravel area was no different (confirmed with no significant differences in the pasture biomass between treatments) to the standard pasture, which is an important factor to consider when interpreting N losses from such areas.

Despite observing some differences in the soil properties between the gateway and the standard pasture, the measured cumulative N₂O emissions and estimated emission factors from these areas were not significantly different. We expected that the soil conditions in the gateway would have been conducive to higher N₂O emissions, as a higher bulk density would have reduced the air-filled pore spaces and the additional labile C could have also provided additional electron donors contributing to denitrification. Although cumulative N₂O and N₂O–N emissions factors were not significantly different, the N₂O:N₂ ratios demonstrated that N₂ dominated the N₂O+N₂ losses and supports the tenet of greater rates of denitrification in the gateway compared to the standard pasture. Harrison-Kirk et al. (2015) demonstrated in a laboratory study using ¹⁵N-labelled artificial urine that compaction altered N₂O emissions and N₂O:N₂ ratios, with N₂O and N₂ emissions being more sustained under elevated compaction. At the same field site to our study, De Rosa et al. (2020) found emissions of N₂O from nearby gateways were up to 11 times higher than the N₂O average from the total field

area. Nevertheless, the sampling of this study was targeted towards greater spatial coverage (grid sampling with 90 sampling points) which possibly was able to detect differences with greater resolution across these potentially highly variable areas. It is possible that a greater number of chambers located in these areas would have been required to observe any significant differences, but this would have resulted in a lower temporal resolution compared to the data within this study.

Our emission factors for N_2O were not significantly higher in the gateway area compared to the standard area of pasture. This was similar to the findings of Adhikari et al. (2020), where N_2O emission factors were not significantly different from urine patches applied to gateways, water troughs and raceways compared to typical pasture areas. Our results are in contrast, however, with the findings of Luo et al. (2017) who found emission factors that were five times higher in gateway and water trough areas compared to the rest of the paddock. Our results do not support the notion that areas with urine patches should be disaggregated from pasture areas for GHG inventories, despite the edaphic conditions being potentially favourable for greater N_2O emissions.

Adequate vegetative cover around the gateway may have obscured observing differences in N_2O emissions

We did not find differences in our treatments for the pasture biomass, N contents, foliar C-to-N ratio or the quantity of the ^{15}N -urine tracer taken up by the plants. We may have expected the biomass would have been lower in the gateway area compared to the standard pasture area, due to greater trampling in this area. The fact that we found similar values across the two pasture areas suggests the impact by livestock in terms of the pasture cover was minimal in this study. We suggest that areas which have reduced pasture cover due to livestock may enhance N_2O emissions due to reduced plant N uptake, similar to the findings of Chirinda et al. (2019). The adequate pasture cover in the gateway observed in this study may, therefore, have resulted in the absence of any differences in the cumulative N_2O emissions. Management practices that ensure good pasture cover could, therefore, potentially alleviate N losses from gateways, which could be achieved by moving the animals quickly off

pastures that have been recently grazed to avoid congregation or having more than one entry/exit point within the field.

We found no effect of DMPP on increasing pasture biomass, similar to two other studies investigating DMPP efficacy in Australian pastures (Dougherty et al. 2016; Nauer et al. 2018). In a subtropical pasture, however, DMPP applied with a urease inhibitor to urea fertiliser increased pasture biomass and N uptake by 20–30% (Lam et al. 2018). The meta-analyses conducted by Abalos et al. (2014) summarised available data on urease and nitrification inhibitors on crop productivity (including forages), but the authors highlight the lack of available data for DMPP within pastures, with only one study available at the time of publication. Similarly, the later review by Adhikari et al. (2021) found limited information for the effect of DMPP applied to urine patches on pasture biomass, citing only Marsden et al. (2017) where no effect was observed. Our data contribute to this knowledge gap, and we also suggest further field studies with greater spatial resolution should be conducted, to examine areas that have lower vegetative cover, as the vegetation is an important sink for the urine-N returns.

DMPP was ineffective in reducing nitrification and N_2O emissions from the gateway

In this study with the rates of DMPP applied (1.5 kg ha^{-1}), we saw no effect in terms of a reduction in the rates of nitrification (based on soil extractable NH_4^+ and NO_3^- concentrations) or the overall N_2O emissions and estimated emission factors. While some studies have demonstrated DMPP can be effective in reducing urine patch N_2O emissions by > 60% (Di and Cameron 2012) others have found no effect, such as when DMPP was applied to sheep urine under temperate summer conditions (Marsden et al. 2017). Dougherty et al. (2016) saw no effect of DMPP and a urease inhibitor (NBPT) on pasture yield, N_2O emission or nitrate leaching from urea fertiliser in a hot-dry climate. Similarly, on-farm studies in North-Eastern Victoria demonstrated no effect of DMPP applied with urea fertiliser on N_2O emissions or crop/pasture yields in broad-acre cropping farms and dairy pasture farms (Nauer et al. 2018). Under tropical conditions in Brazil, DCD was ineffective in mitigating cumulative N_2O emissions from bovine urine patches on an Oxisol (Mazzeto et al. 2015).

Under the temperatures to which soils were exposed in the current study, it is possible that DMPP was rapidly degraded. It has been shown, for instance, that DMPP efficacy is lower at higher soil temperatures and higher soil water contents, where denitrification is expected to be the main source of N_2O (Irigoyen et al. 2003; Menéndez et al. 2012). McGeough et al. (2016) report a similar phenomenon on the strong impact of increasing temperature on the efficacy of DCD in inhibiting nitrification. Unequal mixing of the DMPP with the urine-substrate may have also occurred leading to spatial dislocation of the DMPP with the urine substrate. When investigating the performance of DCD in reducing N_2O emissions from subtropical pastures, Simon et al. (2018) found the inhibitor was more effective when dissolved directly in the urine compared to when spray-applied to the urine patch as was done in this study. Adhikari et al. (2021) also state that having a delay between the application of the nitrification inhibitor and the urine may result in the urine traveling down the soil profile before the nitrification inhibitor, thus reducing the contact between them, and reducing its efficacy. Additionally, higher rates of DMPP may be required to sufficiently inhibit nitrification, as the rates of N deposited within urine are typically much greater than that applied in fertilisers. We suggest further work should be conducted to investigate rates of degradation at contrasting soil moistures and temperatures, over the range of temperatures and moisture conditions which can occur in these sub-tropical conditions. In addition, it would be useful to investigate nitrification inhibition efficacy under a range of DMPP application rates, application methodologies and DMPP to N ratios to be able to make specific recommendations to farm managers for targeting urine patches and potential emission hot-spot areas.

Urine patch N_2O emission factors are underrepresented from sub-tropical and irrigated pastures

The estimated N_2O -N emission factor for the combined urine and fertiliser in the standard pasture ($1.10 \pm 0.17\%$ of the N applied) is higher than the Australian annual urine EF of 0.4% (Singh et al. 2021), although we did not measure for a full year after urine application to soil. Additionally, the N was applied as a combination of both urea fertiliser and

urine, however, the overall N loading rate of 400 kg N ha^{-1} is still well within the ranges of N application rates that could occur under a cattle urine patch alone (and most of this N would be in the urea form). In the review paper by Selbie et al. (2015) typical cattle urine N loading rates found in the literature varied between 200 and $2000 \text{ kg N ha}^{-1}$. N application rates as high as $3920 \text{ kg N ha}^{-1}$ have been applied to a subtropical Brazilian pasture (Sordi et al. 2014), although this high loading rate was simulating a urination event with 1.5 times the average urine volume found in their study. Nevertheless, our study provides an indication of the potential interaction of urine and fertiliser-N in terms of N_2O emission factors, which has not been well-studied (Maire et al. 2020). This could be one factor contributing to the higher cattle urine N_2O emission factor found in this study compared to the Australian default N_2O emission factor, but alternatively it could have been due to the sub-tropical conditions—warm temperatures accelerate microbial N transformations and heavy rainfall events result in increased soil water-filled pore space, known to stimulate anaerobic denitrification losses. Irrigation was also applied as and when the farmer would have irrigated their field normally, which could have also maintained the WFPS at appropriate levels for continued N_2O production. The review by López-Aizpún et al. (2020) highlights the limited information that is available for urine-N dynamics in sub-tropical and tropical systems. Simon et al. (2020) found urine N_2O -N emission factors of 1.2% on average in sub-tropical Brazil, similar to that determined in this study for fertiliser + urine in the standard pasture conditions of $1.10 \pm 0.17\%$. Therefore, the data in this study contribute to information on N_2O emissions from grazed grasslands in sub-tropical regions. Caution should be exercised in the use of these estimated emission factors due to the absence of a true control treatments for background emissions. This could have resulted in a slight under or over estimation of the true EF if the collected background data was not representative of the entire monitoring period.

Conclusions

In conclusion we reject our first hypothesis that cumulative N_2O emissions would be higher from a gateway compared to a standard pasture area but confirm our

second hypothesis that DMPP would not be effective in reducing emissions from the gateway area. Our data does not support the notion of disaggregating urine patch N_2O -N emission factors based upon differing areas within the same pasture, although further measurements with increased spatial resolution would be useful to confirm this. Investigation of emissions from non-vegetated areas would be particularly important, as the presence of vegetation limits the amount of N available for production of N_2O . The cumulative $N_2O + N_2$ losses were significantly higher from the gateway compared to the standard pasture. We found no effect of DMPP in reducing N_2O emissions from a gateway, which may have been due to the application rate not being adequate and the interaction of high soil moisture and temperature contributing to rapid degradation of the inhibitor in the soil. Further work is required to determine whether higher rates of DMPP application ($> 1.5 \text{ kg ha}^{-1}$) would be effective in emission reductions in sub-tropical conditions and the reasons behind this ineffectiveness including the impact of inhibitor degradation dynamics, the optimum inhibitor delivery method and the spatial location of DMPP application in relation to urine-N deposition.

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Declarations

Competing interests The authors have no competing interests to declare.

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