Nitrous Oxide Fluxes, Soil Oxygen, and Denitrification Potential of Urine- and Non-Urine-Treated Soil under Different Irrigation Frequencies

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Abstract
Despite increased use of irrigation to improve forage quality and quantity for grazing cattle (Bos taurus, Linnaeus), there is a lack of data that assess how irrigation practices influence nitrous oxide (N₂O) emissions from urine-affected soils. Irrigation effects on soil oxygen (O₂) availability, a primary controller of N₂O fluxes, is poorly understood. It was hypothesized that increased irrigation frequency would result in lower N₂O emissions by increasing soil moisture and decreasing soil O₂ concentrations. This would favor more N₂O reduction to dinitrogen (N₂). We examined effects of high (3-d) versus low (6-d) irrigation frequency with and without bovine urine addition to pasture. Nitrous oxide fluxes were measured daily for 35 d. Soil O₂, temperature, and water content were continuously measured at multiple depths. Inorganic nitrogen, organic carbon, and soil pH were measured at 6-d intervals. Measurements of denitrification enzyme activity with and without acetylene inhibition were used to infer the N₂O/(N₂O + N₂) ratio. The N₂O/(N₂O + N₂) ratio was lower under high- compared with low-frequency irrigation, suggesting greater potential for N₂O reduction to N₂ with more frequent irrigation. Although N₂O fluxes were increased by urine addition, they were not affected by irrigation frequency. Soil O₂ decreased temporarily after urine deposition, but O₂ dynamics did not explain N₂O dynamics. Relative soil gas diffusivity (D_o/D_w) was a better predictor of N₂O fluxes than O₂ concentration. On a free-draining soil, increasing irrigation frequency while providing the same total water volume did not enhance N₂O emissions under ruminant urine patches in a grazed pasture.

Core Ideas
• Irrigation effects on N₂O emissions from ruminant urine patches are rarely studied.
• Irrigation frequency influenced soil oxygen and N₂O reductase enzyme.
• N₂O emission was unaffected by irrigation frequency on a free-draining soil.
• Soil gas diffusivity (D_o/D_w) was a strong predictor of cumulative N₂O emissions.

Nitrous oxide (N₂O) is a potent greenhouse gas (GHG) and is the dominant ozone-depleting substance currently emitted (Ravishankara et al., 2009). Agricultural soils are the primary source of anthropogenic N₂O (IPCC, 2007) due to nitrogen (N) inputs from fertilizer application and animal excreta (Davidson, 2009), especially ruminant urine (Oenema et al., 2005). Upward of 300 million ha of the world's agricultural soils receive irrigation (FAO, 2010), which helps provide food security but may also alter soil N cycling, thereby affecting N₂O emissions (Trost et al., 2013).

Irrigation improves forage quality and quantity in grazed pastures (McBride 1994), where annual spatial coverage of urine patches can reach ~20% of a paddock (Moir et al., 2011). Few studies have examined how irrigation affects N₂O emissions from urine patches (Di and Cameron, 2002). Irrigation studies on cropped systems have reported conflicting results; irrigation either increases or has no effect on N₂O emissions (Horváth et al., 2010; Maharjan et al., 2014; Scheer et al., 2013; Simojoki and Jaakkola, 2000).

Irrigation may decrease soil oxygen (O₂) concentrations by increasing soil moisture (Trost et al., 2013). Soil O₂ is a proximal controller of biological pathways producing N₂O (Firestone and Davidson, 1989). Anaerobic conditions promote N₂O reductase enzyme (N₂O:OR) activity, which reduces N₂O to dinitrogen (N₂) during denitrification (Knolle and Cameron, 2002). The degree of anaerobiosis determines the relative ratio of N₂O to N₂ emitted (Knowles, 1982; Wrage et al., 2001; Zhu et al., 2013). In situ soil O₂ concentrations in pastures have never been intensively measured, with only sporadic measurements available (Eccles et al., 1990; Simojoki and Jaakkola, 2000). It is unknown how soil O₂ in pastures changes under different irrigation regimes, and such data may help elucidate controls over N₂O fluxes and potential N₂O:OR activity.

Measures of soil moisture content, such as water-filled pore space (WFPS), are generally used as a proxy for soil O₂ – N₂O flux variation (Dobbie et al., 1999; Ruser et al., 2006). However, the WFPS calculation (Linn and Doran, 1984) fails

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Abbreviations: CWC, cold water–extractable carbon; DEA, denitrification enzyme activity; DM, dry matter; DOE, day of experiment; HWC, hot water carbon; N₂O:OR, N₂O reductase enzyme; WFPS, water-filled pore space.
to account for pore connectivity and tortuosity (Farquharson and Baldock, 2008), which are key factors determining soil gas transport. Relative soil gas diffusivity, $D_r/D_p$, which is the ratio of the soil–gas diffusion coefficient to the free-air gas diffusion coefficient (Moldrup et al., 2013), incorporates these factors. It describes the ease of movement of gases through the soil profile and the exchange of gases between the soil and the atmosphere by accounting for the total porosity and air-filled porosity (Moldrup et al., 2013). Relative soil gas diffusivity has been shown to explain the variability in $N_2O$ emissions in a controlled lab study using repacked cores (Balaine et al., 2013) and from intact soil cores from different cropping systems (Petersen et al., 2013).

This study aimed to quantify the effect of two irrigation frequencies on urine-affected pasture soil with respect to (i) the timing and magnitude of $N_2O$ emissions, (ii) soil $O_2$ concentrations through direct measurements and estimates of soil $D_r/D_p$, and (iii) the potential $N_2O/(N_2O + N_2)$ ratio, which is indicative of potential $N_2O$ OR. It was hypothesized that more frequent irrigation would keep soil moisture higher, reducing soil $O_2$ concentrations and thereby promoting $N_2O$ OR, leading to a lower $N_2O/(N_2O + N_2)$ ratio and to lower total $N_2O$ emissions.

## Materials and Methods

### Study Site

The experiment was conducted during the summer on an intensively managed dairy farm in Canterbury, New Zealand ($43^\circ 35^\prime 30.6^\prime\prime$ S, $171^\circ 55^\prime 36.6^\prime\prime$ E). The soil was a free-draining Lismore stony silt loam, known as a Pallic Farm Brown Soil in the New Zealand Soil Classification (Hewitt, 2010) or as a Xercepts Udepts Typic Dystrudepts in the USDA classification (Soil Survey Division Staff, 1999), with a 150-mm-deep A (Ap) horizon consisting of fractions of 0.29, 0.12, and 0.58 of clay, sand, and silt, respectively (S. Carrick, T. Webb, J. Scott, and J. Payne, unpublished data, 2013). The pasture consisted of perennial ryegrass (Lolium perenne L.) and white clover (Trifolium repens L.). A 6 × 6 m experimental area on the grazed paddock was fenced to exclude animals for 90 d before the start of the experiment and was shielded from irrigation and precipitation using a tunnel house covered with a transparent plastic cover (Torto). The paddock is normally mob-grazed every 3 to 4 wk throughout the growing season and is irrigated every 3 d when rainfall is insufficient.

### Experimental Design

The experiment was a split-plot randomized block design with irrigation frequency as the main plot and urine addition or non-urine as the subplots. Each treatment combination was replicated four times (Supplemental Fig. S1). At the sampling locations, circular gas flux collars for gas sampling, supplementary collar bases for soil sampling, and instrumentation bases for marking the placement of automated sensors (area, 0.19635 m²) were inserted into the soil to a depth of 100 mm. Irrigation frequency was either every 3 d (with 12 mm applied over a 10-min irrigation event, equivalent to $72$ mm h⁻¹) or every 6 d (with 24 mm applied over a 10-min irrigation event, equivalent to $144$ mm h⁻¹) and was applied over a ~0.2 m² area within each collar base. The 3-d treatment followed the current on-farm practice. The 6-d treatment reduced the frequency but increased the intensity. Irrigation was applied using an eight-branch manifold equipped with nozzles (Fulljet FL-5VG, Tecjet Technologies) positioned 200 mm above the ground and controlled by an automated timer.

The day before urine treatment application is referred to herein as day of experiment (DOE) –1 (20 Feb. 2014). Urine was collected from the Lincoln University Dairy Farm on DOE –1 from cows fed ryegrass/white clover pastures, and 2 L of urine was applied to the soil within each urine-treated chamber base on DOE 0. The urine was applied once at a rate of 750 kg N ha⁻¹, which is typical of cattle urine (Haynes and Williams, 1993). The N content of the urine was determined by analyzing a subsample on a CN elemental analyzer (Vario-Max, Elementar GmbH). The non-urine subplots received neither urine nor water on this day to mimic actual field differences between soil affected and unaffected by urine patches.

### $N_2O$ Fluxes

Soil-to-atmosphere $N_2O$ fluxes were measured using vented insulated non–steady-state chambers (headspace volume, 19.625 L) following standardized protocols (Parkin et al., 2012). Fluxes were measured daily between 10:00 AM and 12:00 PM (van der Weerden et al., 2013) and were expressed as daily fluxes from DOE –1 and 29 and also on DOE 32 and 35. To seal chambers during sampling, annular moats on the bases were filled with water. Gas samples were taken at 0, 15, 30, and 45 min from each chamber using a 20-mL glass syringe fitted with a three-way stopcock and immediately transferred to 6-mL pre-evacuated (–1 atm) glass Exetainers (Labco Ltd.). Gas samples were analyzed on an automated gas chromatograph system equipped with an electron capture detector (SRI 8610c GC, SRI Instruments) as described in Clough et al. (1996). Flux calculations used the ideal gas law, air temperature, chamber volume and area, and the change in $N_2O$ concentration over time, which was assessed using both quadratic regression (Wagner et al., 1997) and linear regression. The quadratic regression flux was selected unless the second derivative of the regression model was ≥20 (Venterea, 2013; Venterea et al., 2009) according to the LINEST function in Microsoft Excel (version 2013). A correction factor was applied to account for chamber-induced artifacts using soil bulk density (Venterea, 2010). Fluxes below the detection limit (Parkin et al., 2012) were assigned a value of zero. Of the 528 fluxes, 75% were calculated using the quadratic regression method, and 21% were calculated using the linear regression method. The remaining 4% were below the detection limit.

Cumulative $N_2O$ emissions ($\text{kg N ha}^{-1}$) were determined by summing the daily fluxes. Emission factors (%) for $N_2O$ lost as a proportion of urine-N were also determined (de Klein et al., 2003).

### Ancillary Soil and Pasture Measurements

Sensors for soil $O_2$ (SO-110, Apogee Instruments), temperature (Probe 107, Campbell Scientific), and volumetric water content ($\theta$1) (CS 616 Reflectometer, Campbell Scientific) were installed in the center of the experimental plots inside the instrumentation collar bases (Supplemental Fig. S1). Soil $O_2$ and temperature sensors were installed at depths of 10, 50, and 100 mm, and the $\theta$1 sensors were installed at depths of 50 and 100 mm. A
Potential denitrification enzyme activity (DEA) was determined using the acetylene (C,H₂) block technique (Drury et al., 2008; Groffman et al., 2006). Briefly, 25 mL of a solution containing 50 µg g⁻¹ of NO₃⁻–N (as KNO₃) and 300 µg g⁻¹ of C (as HWC extracted from the same soil used for the denitrification potential measurement) was mixed with 20 g dry weight equivalent of soil and placed in a 250-mL Mason jar with a gas-tight lid fitted with a rubber septum. The jar headspace was made anaerobic by flushing the jar with N₂ (instrument grade, <0.0001% O₂) for 10 min and then incubating with acetylene (+C,H₂, instrumentation grade C,H₂ >98%, <2% air) or without acetylene (–C,H₂) at 20°C for 48 h. The headspace of the jars was sampled using a closed-loop circulating system attached to the photo-acoustic analyzer (multi-gas monitor type 1302, Bruel and Kjaer) to measure N₂O. The jars and the closed-loop system were flushed with N₂ gas; exhaust was directed into a container of water to keep pressure equilibrated within the closed loop, and the jar, and to minimize O₂ leakage back into the system. During sampling for N₂O, the inlet for the N₂ and the outlet to the water were closed. The change in N₂O concentration was measured every 2 min for 8 min. Each jar was measured every 4 h for the first 24 h and every 8 h thereafter. Total N₂O evolved over each 48-h incubation period represented either DEA-N₂O + N₂ (from the +C,H₂ samples) or DEA-N₂O (from the –C,H₂ samples), which were then expressed as the N₂O/(N₂O + N₂) ratio; herein this ratio is referred to as DEA-N₂O/(DEA-N₂O + N₂).

Data Analyses

All analyses were performed in Minitab (Minitab Inc., 2010) unless otherwise specified. Data were transformed (Supplemental Table S1) to meet assumptions of parametric statistics when required (Steel et al., 1997). Statistical analyses for treatment effects did not include data prior to urine application (DOE – 1 and 0), but these data are presented for reference. When data were transformed, conclusions were drawn from the analysis on the transformed scale; however, the mean and error values presented in tables and figures are from untransformed data.

Treatment effects on mean daily N₂O emissions were evaluated using a linear mixed model in SPSS (IBM Corp., 2011). Irrigation frequency, urine, and DOE were treated as fixed effects, with DOE as a repeated measure using a heterogeneous first-order autoregressive covariance structure. P-values of ≤0.10 are considered significant. For NH₄⁺–N, NO₃⁻–N, NO₂⁻–N, HWC, CWC, soil pH, and θ, a general linear model was used to evaluate treatment effects. Volumetric water content data could not be transformed to normal because the distribution was bimodal, so these data were not analyzed statistically for treatment effects. Irrigation frequency, urine, DOE, and interactions were treated as fixed factors. Main effects were tested using Tukey’s multiple comparison test (Steel et al., 1997).

A general linear model was used to test for treatment effects with irrigation frequency and urine as factors and with interactions assessed between urine × irrigation frequency for cumulative N₂O emissions acquired individually from each chamber; DM yield; pasture N content; daily averaged soil temperature at 50 mm; daily average soil O₂ at 10, 50, and 100 mm; and the ratio of DEA-N₂O/(DEA-N₂O + N₂).

Least squares linear regression was used to evaluate relationships with daily N₂O fluxes, cumulative N₂O fluxes, or
DEA-N\(_2\)O/(DEA-N\(_2\)O + N\(_2\)) as the response variables and with NH\(_4\)\(^+\)–N; NO\(_3\)–N; NO\(_2\)–N; HWC; CWC; soil pH; \(\theta_2\); daily average soil temperature at 50 mm; daily average soil \(O_2\) at 10, 50, and 100 mm; daily average WFPS; and daily average \(D_P/D_O\) as the explanatory variables.

**Results**

**Soil Physical Properties**

Spikes in \(\theta_2\) were observed after irrigation events and after the urine deposition event (Fig. 1c,d). Overall mean \(\theta_2\) (Fig. 1a,b) was 7% higher under the 3-d irrigation treatment than under the 6-d irrigation treatment (\(P < 0.001\)) and 17% higher in the urine-treated compared with the non–urine-treated soil (\(P < 0.001\)). Total irrigation exceeded total evapotranspiration in the non-urine and urine treatments by 41.0 and 52.4 mm, respectively.

Overall mean soil temperatures at 50 mm from the urine, non-urine, 3-d, and 6-d irrigation treatments were 15.4 ± 0.22, 15.7 ± 0.19, 16.1 ± 0.22, and 15.0°C ± 0.18, respectively. Overall mean soil temperatures were higher under the 3-d irrigation treatment than under the 6-d irrigation treatment (\(P < 0.05\)). The addition of urine did not influence soil temperature (Supplemental Fig. S2).

Soil \(O_2\) showed diel variation (Supplemental Fig. S3). After the urine application, soil \(O_2\) decreased to a minimum of 13% at 100 mm soil depth and recovered to pretreatment concentrations within 24 h. Between DOE 1 and 35 (the data used for statistical analysis), daily mean soil \(O_2\) concentrations varied between 17 and 20% (Fig. 1e–h). Overall mean soil \(O_2\) concentrations in the 3-d irrigation treatment were 1.09 and 0.79% lower at 50 (\(P < 0.001\)) and 100 mm (\(P < 0.001\)) soil depths, respectively, when compared with the 6-d irrigation treatment. The overall average soil \(O_2\) concentration at 10 mm was 0.32% lower in the urine treatment compared with the non-urine treatment (\(P < 0.01\)). Lower soil \(O_2\) was found with both urine and 3-d irrigation treatment at 50 and 100 cm (\(P < 0.05\)).

Relative soil gas diffusivity, \(D_P/D_O\), ranged from 0.026 to 0.101, averaging 0.050, 0.029, 0.089, and 0.031 in the 3-d non-urine, 3-d urine, 6-d non-urine, and 6-d urine treatments, respectively. The WFPS ranged from 0.24 to 0.45 m\(^3\) m\(^{-3}\), averaging 0.26, 0.41, 0.29, and 0.34 m\(^3\) m\(^{-3}\) from the 3-d non-urine, 3-d urine, 6-d non-urine, and 6-d urine treatments, respectively. Urine increased overall mean WFPS (\(P < 0.001\)) and decreased \(D_P/D_O\) (\(P < 0.001\)). Under the 6-d irrigation treatment, WFPS was lower (\(P < 0.001\)) and \(D_P/D_O\) was higher (\(P < 0.001\)) compared with the 3-d irrigation treatment. There was an interaction between urine and irrigation treatments, with \(D_P/D_O\) being lower under the 3-d irrigation treatment with urine application (\(P < 0.001\)).

**Soil Chemical Properties**

Urine application increased overall mean concentrations of NO\(_3\)–N (Fig. 2d) and NH\(_4\)\(^+\)–N (Fig. 2b) and increased soil pH (\(P < 0.05\)) (Fig. 2h), with NH\(_4\)\(^+\)–N peaking shortly after urine deposition (Fig. 2a) and NO\(_3\)–N increasing with time since urine deposition (Fig. 2b). The addition of urine did not affect the HWC values (Fig. 2k), but the 6-d irrigation frequency resulted in 20% higher HWC (\(P < 0.05\)) (Fig. 2l). Urine and irrigation treatments interacted to produce greater soil NO\(_3\)–N and NH\(_4\)\(^+\)–N concentrations under urine in the 6-d irrigation treatment (\(P < 0.10\)). Concentrations of NO\(_3\)–N (Fig. 2e) and CWC (Fig. 2i) differed with DOE but were not influenced by urine or irrigation treatments (Fig. 2f,j).

**Pasture Yield**

Irrigation frequency did not influence DM yield. Urine application increased total DM yield by 35% (\(P < 0.05\)) over the whole experimental period from 2634.7 kg ha\(^{-1}\) (SEM, 227.0)
to 3754.0 kg ha⁻¹ (SEM, 146.2). Dry matter yields were 19% higher from the urine treatment compared with the non-urine treatment at the first harvest \((P < 0.10)\) and were 47% higher from the second cut \((P < 0.05)\).

**N₂O Fluxes**

The daily \(\text{N}_2\text{O}\) fluxes from the urine treatment varied with DOE \((P < 0.001)\) (Fig. 3a). Overall mean daily \(\text{N}_2\text{O}\) fluxes from the urine treatment were 440% higher compared with the non-urine treatment \((P < 0.001)\) (Fig. 3b). Non-urine \(\text{N}_2\text{O}\) fluxes were low \((≤1.2 \text{ mg N m}^{-2} \text{ d}^{-1})\), with an overall average of 0.47 mg N m⁻² d⁻¹. Daily \(\text{N}_2\text{O}\) fluxes did not differ with irrigation treatment.

The cumulative \(\text{N}_2\text{O}\) emissions (data not shown) reflected the trends observed in the daily \(\text{N}_2\text{O}\) fluxes and were higher under urine by a factor of 4.9 \((P < 0.001)\) compared with the non-urine treatment. Irrigation frequency did not influence cumulative \(\text{N}_2\text{O}\) emissions. When expressed as an emission factor, cumulative \(\text{N}_2\text{O}\) emissions from the 3-d and 6-d irrigation treatments equaled 0.09%.

Nitrous oxide fluxes were highest between 0.4 and 0.6 m³ m⁻³ WFPS (Fig. 4a) and were highest from \(D_j/D_o\) values between ~0.06 and ~0.02 (Fig. 4b). Pooling all \(\text{N}_2\text{O}\) flux data, irrespective of treatment, and performing linear regression analysis of log-transformed WFPS or \(D_j/D_o\) versus log-transformed daily \(\text{N}_2\text{O}\) fluxes showed that \(D_j/D_o\) best explained the variation in the daily \(\text{N}_2\text{O}\) fluxes (Fig. 4c,d). Overall mean WFPS and \(D_j/D_o\) explained 16% (not significant) and 87% \((P < 0.05)\) of the variability in cumulative \(\text{N}_2\text{O}\) emissions from urine-treated soils, respectively (Fig. 4c,f).

Concentrations of \(\text{NO}_3^−\text{--N}\) and \(\text{NH}_4^+\text{--N}\) and soil pH explained 18 \((P < 0.05)\), 28 \((P < 0.001)\), and 32% \((P < 0.001)\) of the variability in daily \(\text{N}_2\text{O}\) fluxes, respectively, under the
3-d irrigation frequency. However, there were no relationships observed between daily N₂O fluxes and environmental variables under the 6-d irrigation treatment. When all of the data were pooled, irrespective of treatment, NO₃⁻–N, NH₄⁺–N, NO₂⁻–N, and pH explained 10 (P < 0.05), 18 (P < 0.001), 12 (P < 0.05), and 13% (P < 0.05) of the variability in daily N₂O fluxes, respectively.

**Fig. 3.** (a) Mean daily N₂O fluxes from each treatment (± SEM; n = 4). The arrow represents the timing of urine deposition. *Differences between the urine and non-urine treatments (P < 0.05) on each day. (b) A box plot comparison of daily N₂O emission from each treatment as analyzed statistically (± SEM; n = 256). In the box plots, the gray line represents the median, and the red line represents the mean. The box represents the 25th and 75th percentiles, and the open circles represent outliers.

**Fig. 4.** The daily average nitrous oxide (N₂O) fluxes and (a) water-filled pore space (WFPS) and (b) relative soil diffusivity (Dp/Do). (c and d) Linear regression between average log₁₀ [1 + N₂O] and (c) log₁₀ [WFPS] or (d) log₁₀ [Dp/Do] from data from all treatments. (e and f) Linear regression between cumulative N₂O fluxes from the urine treatment and (e) overall mean WFPS from the urine treatment or (f) overall mean Dp/Do from the urine treatment.

**Ratios of DEA-N₂O/(DEA-N₂O + N₂) from Denitrification Enzyme Assays**

The overall mean ratio of DEA-N₂O/(DEA-N₂O + N₂) was greater from the 6-d (0.83) compared with the 3-d (0.65) irrigation treatment (P < 0.05) and was lower from the non-urine (0.67) compared with the urine (0.81) treatments (P <
0.05) (Fig. 2n). There was an interaction between the treatments, with a lower ratio observed from the 3-d and non-urine treatments \((P < 0.05)\). These treatment differences were also reflected in the temporal trends. By DOE 17 and 23, the ratios of \(\text{DEA-N}_2\text{O}/(\text{DEA-N}_2\text{O} + \text{N}_2)\) were 0.98 and 0.95, respectively, under the 6-d irrigation treatment and 0.81 and 0.60, respectively, under the 3-d irrigation treatment (Fig. 2m). The ratio of \(\text{DEA-N}_2\text{O}/(\text{DEA-N}_2\text{O} + \text{N}_2)\) was positively related to CWC \((R^2 = 0.23; P < 0.10)\) and negatively related to \(\text{NO}_3^{-}-\text{N}\) \((R^2 = 0.28; P < 0.05)\).

**Discussion**

Other studies have reported similar \(\text{N}_2\text{O}\) emissions from free-draining soil both for the peak urine-induced (Di and Cameron, 2002) and the average non-urine emissions (Horváth et al., 2010). Cumulative \(\text{N}_2\text{O}\) emissions (Di and Cameron, 2002) and emission factors (de Klein et al., 2014) are within the range of those reported by others from free-draining soil that received cow urine of similar concentrations. Urine application results in a series of hydrolysis reactions, followed by biological nitrification and denitrification (Baral et al., 2014), which subsequently change the soil pH and inorganic N concentrations (Orwin et al., 2010; Taghizadeh-Toosi et al., 2011). Although these factors are known regulators of \(\text{N}_2\text{O}\) fluxes (Firestone and Davidson, 1989), individually they were not robust predictors of \(\text{N}_2\text{O}\) fluxes in this study. Rather, they contributed to the variability in \(\text{N}_2\text{O}\) fluxes observed between urine treatments. The lack of any irrigation frequency effects on \(\text{N}_2\text{O}\) emissions can be explained by considering how \(\text{N}_2\text{O}\) regulators varied, specifically soil \(\text{O}_2\) concentration and \(D_j/D_o\). As originally hypothesized, more frequent irrigation produced higher soil moisture and lower soil \(\text{O}_2\), and the \(\text{DEA-N}_2\text{O}/(\text{DEA-N}_2\text{O} + \text{N}_2)\) ratio was lower, inferring greater potential for \(\text{N}_2\text{O}\) production and thus a greater reduction of \(\text{N}_2\text{O}\) to \(\text{N}_2\). However, this did not result in lower \(\text{N}_2\text{O}\) emissions.

The higher overall mean soil \(\theta\) under the urine treatment could have resulted from the additional water embodied in the applied urine, equal to 10.8 mm irrigation or 7.5% more total water. Despite equal volumes of water being applied in total, the soil was drier under the 6-d irrigation treatment most of the time. Higher irrigation intensity can increase preferential flow through macropores as a consequence of an increasing hydrostatic head (Gjettermann et al., 1997). The relatively drier soil conditions under the 6-d irrigation treatment suggest this occurred.

Although \(\text{N}_2\text{O}\) fluxes were not affected by irrigation, daily average \(\text{N}_2\text{O}\) fluxes did increase with increasing WFPS and declining \(D_j/D_o\) (Fig. 4a,b). Soil \(D_j/D_o\) is a measure of the relative rate at which \(\text{O}_2\) diffuses through soil and takes into account pore water blockage effects. Oxygen diffuses about 10\(^6\) times slower in water than in free air, and thus soil moisture content exerts a major influence on soil \(D_j/D_o\) (Farquharson and Baldock, 2008; Moldrup et al., 2001, 2013). Soil WFPS is often used to explain \(\text{N}_2\text{O}\) flux magnitude (Dobbie et al., 1999; Smith et al., 1998; Velthof and Onema, 1995), but the relationship does not account for the interaction between bulk density and matric potential (Balaine et al., 2013). Soil \(D_j/D_o\) does account for these variations, and this explains the strong relationship observed between \(\text{N}_2\text{O}\) fluxes and \(D_j/D_o\) (Fig. 4d,f). In this study, log-transformed daily average \(\text{N}_2\text{O}\) fluxes related well to both log-transformed WFPS and log-transformed \(D_j/D_o\) under the controlled range of soil moisture. However, the inclusion of physical differences in the soil using \(D_j/D_o\) provides a repeatable threshold for \(\text{N}_2\text{O}\) production and consumption (Balaine et al., 2013; Harrison-Kirk et al., 2015).

Soil anaerobiosis has been reported to begin at \(D_j/D_o<0.02\) (Stepniewski, 1981), suggesting the soils were well aerated during the current experiment (Fig. 4b,f). This is supported by the fact that soil \(\text{O}_2\) concentrations did not fall below 17% except immediately after the urine application. Higher soil water content under the 3-d irrigation treatment impeded soil \(\text{O}_2\) replenishment via diffusion from the atmosphere to the soil. This, combined with the low variability in daily mean soil \(\text{O}_2\) concentrations, explains the lower soil \(\text{O}_2\) observed at 50 and 100 mm in the 3-d irrigation treatment.

The diel variation in soil \(\text{O}_2\), which lagged soil temperature, was most likely driven by heterotrophic soil respiration (Lloyd and Taylor, 1994). Despite the soil being well aerated \((D_j/D_o > 0.02\) and soil \(\text{O}_2 > 17\%\) ), daily \(\text{N}_2\text{O}\) fluxes from the urine treatments after DOE 17 imply \(\text{N}_2\text{O}\) emissions occurred via denitrification because \(\text{NO}_3^{-}-\text{N}\) was the only available substrate. Denitrification or nitrifier–denitrification in anaerobic microsites must have contributed to \(\text{N}_2\text{O}\) emissions under otherwise aerated soil conditions (Morley et al., 2008; Müller et al., 2004). Thus, measured \(\text{O}_2\) concentrations during this study did not reflect soil \(\text{O}_2\) concentrations at microsites, and a method to measure soil \(\text{O}_2\) in situ at the microscale is still required.

Urine addition decreased soil \(\text{O}_2\) for ~24 h. This is consistent with urea hydrolysis reactions that occur after urine deposition, which take between 24 and 48 h (Sherlock and Goh, 1983). The hydrolysis reactions create \(\text{OH}^-\) ions, increase pH, and generate \(\text{NH}_4^+\) and bicarbonate ions, with the latter hydrolyzing to generate \(\text{CO}_2\) (Avnimelech and Laher, 1977). Fluxes of \(\text{CO}_2\) have been previously observed immediately after urine deposition (Uchida et al., 2008). Rapid anoxia from \(\text{CO}_2\) production may trigger denitrification (Sherlock and Goh, 1983), accounting for high \(\text{N}_2\text{O}\) fluxes after urine deposition.

Nitrous oxide production and \(\text{N}_2\text{O}\) OR activity via heterotrophic denitrification and nitrifier–denitrification pathways occur under anaerobic or anoxic conditions, respectively (Wrage et al., 2001; Zhu et al., 2013). The strong relationship between net \(\text{N}_2\text{O}\) emissions and average \(D_j/D_o\) suggests \(D_j/D_o\) (Fig. 4f) could provide insight into the potential for \(\text{N}_2\text{O}\) uptake. The \(\text{N}_2\text{O}/(\text{DEA-N}_2\text{O} + \text{N}_2)\) ratios were positively related to C, which is a driver of denitrification (Barnard et al., 2005) and negatively related to \(\text{NO}_3^{-}-\text{N}\), which is preferentially used over \(\text{N}_2\text{O}\) as a terminal electron acceptor during denitrification (Barnard et al., 2005). The denitrification enzyme assays were run under nonlimiting conditions and therefore do not directly reflect in situ conditions. These assay results demonstrate a proof-of-concept; even when bulk soil \(\text{O}_2\) is not anaerobic, the contribution of anaerobic microsites can have a significant impact on the ratio of \(\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)\) emitted. Future research using \textsuperscript{15}N isotopes for partitioning \(\text{N}_2\text{O}/\text{N}_2\) ratios, along with direct measurements of \(\text{N}_2\text{O}\) OR, are required and should be linked to \(D_j/D_o\) to refine its use for predicting \(\text{O}_2^{-}\text{N}_2\text{O}\) relationships in grazed pasture soils.
From the perspectives of farm and water management, this study shows that, on a free-draining soil, increasing the irrigation frequency while providing the same total volume of water does not enhance N\textsubscript{2}O emissions or alter DM production rates within ruminant urine patches. There may be the potential for higher N\textsubscript{2}O losses as irrigation intensity increases, but this needs to be confirmed with further study.

Conclusions
Daily and cumulative N\textsubscript{2}O emissions, and DM yields, were not influenced by irrigation frequency. A lower ratio of DEA-N\textsubscript{2}O/(DEA-N\textsubscript{2}O + N\textsubscript{2}O) indicated greater potential for N\textsubscript{2}O activity and therefore greater potential for N\textsubscript{2}O to be reduced to N\textsubscript{2} in the more frequently irrigated treatment, but this was not reflected in the field N\textsubscript{2}O emissions. Estimates of D\textsubscript{2}O/D\textsubscript{H} are a good indicator of cumulative N\textsubscript{2}O emissions in urine-treated soils and explain well the variability in daily N\textsubscript{2}O emissions. Future work linking D\textsubscript{2}O/D\textsubscript{H} and soil O\textsubscript{2} is needed in other soil types and under different climatic and moisture conditions to improve our understanding of the effects of irrigation frequency on N\textsubscript{2}O emissions and DEA-N\textsubscript{2}O/(DEA-N\textsubscript{2}O + N\textsubscript{2}O) ratios.

Supplementary Material
The supplementary data include more information on data transformations for statistics, a map of the experimental plot, soil temperature time series, and an example of the diel cycling of soil O\textsubscript{2} and soil temperature.

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