Published July 7, 2016

Journal of Environmental Quality TECHNICAL REPORTS
ATMOSPHERIC POLLUTANTS AND TRACE GASES

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

Jen Owens,* Tim J. Clough, Johannes Laubach, John E. Hunt, Rodney T. Venterea, and Rebecca L. Phillips

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 (N_2O) emissions from urine-affected soils. Irrigation effects on soil oxygen (O_2) availability, a primary controller of N_2O fluxes, is poorly understood. It was hypothesized that increased irrigation frequency would result in lower N_2O emissions by increasing soil moisture and decreasing soil O_2 concentrations. This would favor more N_2O reduction to dinitrogen (N_2). 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 O_2, 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 $N_2O/(N_2O + N_2)$ ratio. The $N_2O/(N_2O + N_2)$ ratio was lower under high- compared with low-frequency irrigation, suggesting greater potential for N_2O reduction to N_2 with more frequent irrigation. Although N_2O fluxes were increased by urine addition, they were not affected by irrigation frequency. Soil O_2 decreased temporarily after urine deposition, but O_2 dynamics did not explain N_2O dynamics. Relative soil gas diffusivity (D_P/D_O) was a better predictor of N_2O fluxes than O_2 concentration. On a free-draining soil, increasing irrigation frequency while providing the same total water volume did not enhance N_2O emissions under ruminant urine patches in a grazed pasture.

Core Ideas

- Irrigation effects on N_2O emissions from ruminant urine patches are rarely studied.
- Irrigation frequency influenced soil oxygen and N_2O reductase enzyme.
- N_2O emission was unaffected by irrigation frequency on a free-draining soil
- Soil gas diffusivity (D_P/D_O) was a strong predictor of cumulative N_2O emissions.

J. Environ. Qual. 45:1169–1177 (2016)
doi:10.2134/jeq2015.10.0516

Supplemental material is available online for this article.
Received 14 Oct. 2015.
Accepted 10 Feb. 2016.
*Corresponding author (jen.owens@lincolnuni.ac.nz).

Nitrous oxide (N_2O) 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 N_2O (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 N_2O 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 N_2O 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 N_2O emissions (Horváth et al., 2010; Maharjan et al., 2014; Scheer et al., 2013; Simojoki and Jaakkola, 2000).

Irrigation may decrease soil oxygen (O_2) concentrations by increasing soil moisture (Trost et al., 2013). Soil O_2 is a proximal controller of biological pathways producing N_2O (Firestone and Davidson, 1989). Anaerobic conditions promote N_2O reductase enzyme (N_2OR) activity, which reduces N_2O to dinitrogen (N_2) during denitrification (Knowles, 1982). The degree of anaerobiosis determines the relative ratio of N_2O to N_2 emitted (Knowles, 1982; Wrage et al., 2001; Zhu et al., 2013). In situ soil O_2 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 O_2 in pastures changes under different irrigation regimes, and such data may help elucidate controls over N_2O fluxes and potential N_2OR activity.

Measures of soil moisture content, such as water-filled pore space (WFPS), are generally used as a proxy for soil O_2–N_2O flux variation (Dobbie et al., 1999; Ruser et al., 2006). However, the WFPS calculation (Linn and Doran, 1984) fails

J. Owens and T.J. Clough, Dep. of Soil and Physical Sciences, Lincoln Univ., P.O. Box 85084, Lincoln 7647, New Zealand; J. Laubach, J.E. Hunt, and R.L. Phillips, Landcare Research, PO Box 69040, Lincoln 7640, New Zealand; R.T. Venterea, USDA–ARS, Soil and Water Management Research Unit, and Dep. of Soil and Water, and Climate, Univ. of Minnesota, St. Paul, MN 55108. Assigned to Associate Editor April Leytem.

Abbreviations: CWC, cold water–extractable carbon; DEA, denitrification enzyme activity; DM, dry matter; DOE, day of experiment; HWC, hot water carbon; N_2OR, N_2O 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_P/D_O, 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_P/D_O, and (iii) the potential $N_2O/(N_2O + N_2)$ ratio, which is indicative of potential N_2OR. It was hypothesized that more frequent irrigation would keep soil moisture higher, reducing soil O_2 concentrations and thereby promoting N_2OR, 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°35′30.6″ S, 171°55′36.6″ E). The soil was a free-draining Lismore stony silt loam, known as a Pallic Firm Brown Soil in the New Zealand Soil Classification (Hewitt, 2010) or as a Xerepts 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 slit, 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 m^2) 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 m^2 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, Teejet 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 ≥0 (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 (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 (θ_v) (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 θ_v sensors were installed at depths of 50 and 100 mm. A

three-point linear calibration (0.5, 30, and 99% O_2 concentration) was used to calibrate the soil O_2 sensors. A change of 1% O_2 is equivalent to a 0.6-mV change in the sensor reading, and at an O_2 concentration of 20.95% (ambient), the measurements are repeatable at <0.1 mV (~0.2% O_2) (Apogee Instruments Inc., 2015). Each O_2 sensor was equipped with a diffusive head, which integrated an area of ~385 mm^2 around the sensor when placed in soil. Air temperature (Probe 107, Campbell Scientific) at 1.5 m above the soil surface and barometric pressure (SB-100, Apogee Instruments) at the soil surface were also measured. Two data loggers and a multiplexer powered and controlled the instrumentation (CR3000, CR1000, AM416, Campbell Scientific), with samples taken every 15 min from DOE −1 onward. Daily evapotranspiration (ET) was estimated from the Penman–Monteith equation (Allen et al., 1998) using wind speed ($m\ s^{-1}$), net radiation ($MJ\ m^{-2}\ d^{-1}$), and relative humidity (%) measured at a nearby meteorological station.

Bulk density was determined from within the chamber bases at the end of the experiment using the sand replacement method (Maynard and Curran, 2008). Soil WFPS was calculated using the θ_v at soil depth of 50 mm (Linn and Doran, 1984). Soil D_p/D_O was calculated using the structure-dependent, water-induced linear reduction model (Moldrup et al., 2013), which uses air-filled pore space (Farquharson and Baldock, 2008), total porosity, and a media complexity factor of 2.1 (Moldrup et al., 2013).

The pasture was harvested to ~50 mm height on DOE 16 and 35. Dry matter (DM) yield ($kg\ ha^{-1}$) was determined after oven-drying for 48 h at 50°C.

Soil samples were collected on DOE −1, 5, 11, 17, 23, and 29 using a 70-mm-long auger from the supplementary bases allotted for soil collection for a total of four samples, which were not composited, from each treatment combination at each sampling time. Soils were extracted or analyzed within 24 h of collection and stored at 4°C until extraction or analysis. Gravimetric soil moisture (θ_g) was determined by oven-drying soil subsamples at 105°C for 24 h. Soil pH was determined with a pH probe (SevenEasy, Mettler Toledo) after mixing 10 g air-dried soil with 25 mL deionized water (Blakemore et al., 1987) after 12 h of settling. Nitrate (NO_3^-–N) and ammonium (NH_4^+–N) concentrations were determined by extracting 4 g dry weight equivalent soil with 40 mL 2 mol L^{-1} KCl. Samples were shaken for 1 h followed by 20 min of centrifuging at 2000 rpm before gravity filtering through Whatman no. 42 filters (Blakemore et al., 1987). Nitrite (NO_2^-–N) was extracted from 10 g dry weight equivalent soil using 40 mL 2 mol L^{-1} KCl adjusted to pH 8.0 (Stevens and Laughlin, 1995). Extracts were shaken for 10 min and centrifuged for 5 min at 1500 rpm followed by gravity filtering through Whatman 42 filters (Stevens and Laughlin, 1995). The NO_2^-–N extracts were analyzed within 24 h of extraction, and NO_3^-–N and NH_4^+–N extracts were frozen until flow injection analysis (FIAstar 5000 Analyzer, FOSS Analytical).

Cold water–extractable carbon (CWC) was measured using 3 g of soil and 30 mL of deionized H_2O shaken for 30 min and centrifuged at 3500 rpm followed by filtering through Avantec 5C filters (Ghani et al., 2003). After filtration, soil was extracted a second time for hot water carbon (HWC), as described by Ghani et al. (2003). The CWC and HWC extracts were frozen until analysis on a total organic carbon analyzer (TOC 5000A, Shimadzu).

Potential denitrification enzyme activity (DEA) was determined using the acetylene (C_2H_2) block technique (Drury et al., 2008; Groffman et al., 2006). Briefly, 25 mL of a solution containing 50 $\mu g\ g^{-1}$ of NO_3^-–N (as KNO_3) and 300 $\mu g\ g^{-1}$ 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 N_2 (instrument grade, <0.0001% O_2) for 10 min and then incubating with acetylene ($+C_2H_2$, instrumentation grade C_2H_2 >98%, <2% air) or without acetylene ($-C_2H_2$) 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, Brüel and Kjaer) to measure N_2O. The jars and the closed-loop system were flushed with N_2 gas; exhaust was directed into a container of water to keep pressure equilibrated within the closed loop, and the jar, and to minimize O_2 leakage back into the system. During sampling for N_2O, the inlet for the N_2 and the outlet to the water were closed. The change in N_2O 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 N_2O evolved over each 48-h incubation period represented either DEA-N_2O + N_2 (from the $+C_2H_2$ samples) or DEA-N_2O (from the $-C_2H_2$ samples), which were then expressed as the $N_2O/(N_2O + N_2)$ ratio; herein this ratio is referred to as DEA-N_2O/(DEA-N_2O + N_2).

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 N_2O 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 NH_4^+–N, NO_3^-–N, NO_2^-–N, HWC, CWC, soil pH, and θ_g, 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 interaction effects assessed between urine × irrigation frequency for cumulative N_2O emissions acquired individually from each chamber; DM yield; pasture N content; daily averaged soil temperature at 50 mm; daily average soil O_2 at 10, 50, and 100 mm; and the ratio of DEA-N_2O/(DEA-N_2O + N_2).

Least squares linear regression was used to evaluate relationships with daily N_2O fluxes, cumulative N_2O fluxes, or

DEA-N_2O/(DEA-N_2O + N_2) as the response variables and with NH_4^+–N; NO_3^-–N; NO_2^-–N; HWC; CWC; soil pH; θ_g; 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 θ_v were observed after irrigation events and after the urine deposition event (Fig. 1c,d). Overall mean θ_g (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 ± 022, 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)

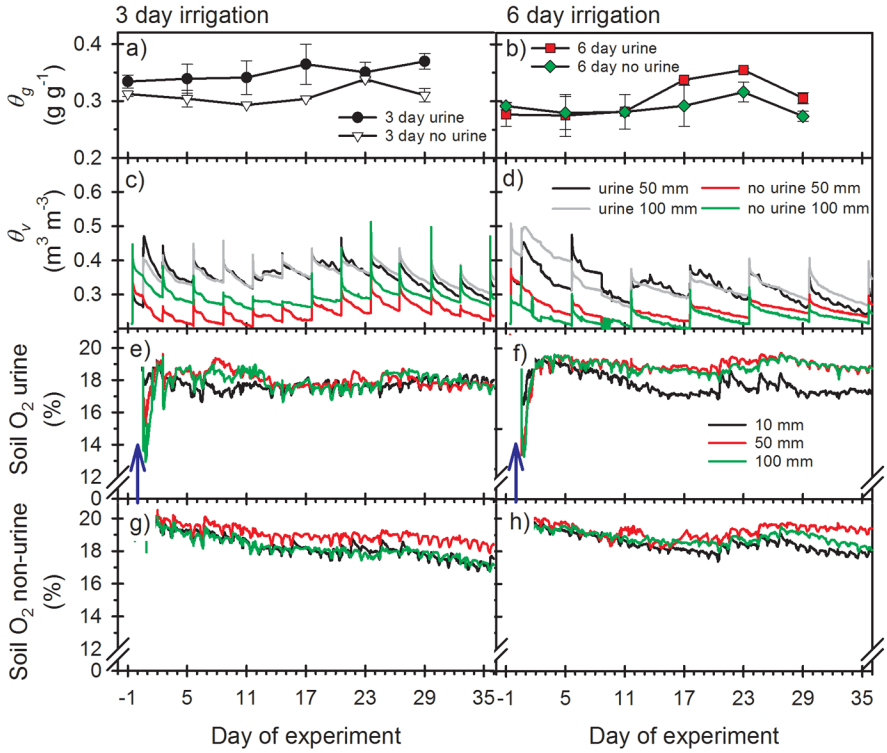

Fig. 1. Average gravimetric soil moisture (θ_g), volumetric water content (θ_v), soil oxygen (O_2) from the urine and non-urine treatments from the 3-d (a, c, e, and g) and 6-d irrigation treatment (b, d, f, and h). The arrow represents the timing of urine deposition.

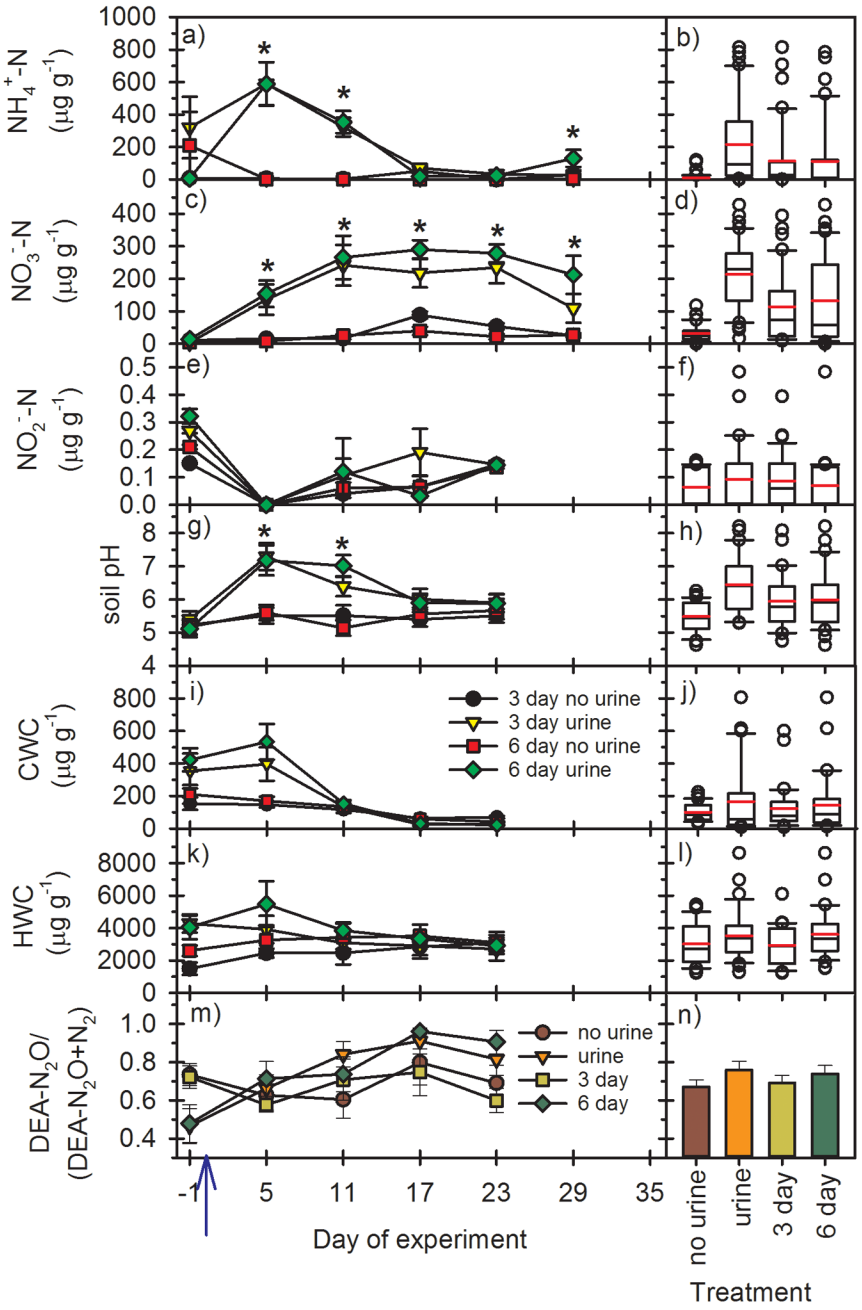

Fig. 2. Changes to means over time (± SEM; *n* = 4) for each treatment combination and box plots for each irrigation frequency and urine treatment (± SEM; *n* = 32) for ammonium (NH_4^+–N) (a, b), nitrate (NO_3^-–N) (c, d), nitrite (NO_2^-–N) (e, f), soil pH (g, h), cold-water carbon (CWC) (i, j), hot-water carbon (HWC) (k, l), and ratio of $N_2O/(N_2O + N_2)$ from denitrification enzyme assays (DEA) (± SEM; *n* = 6) and overall mean (± SEM; *n* = 6) of the ratios of $N_2O/(N_2O + N_2)$ from each treatment (m, n). The blue arrow represents the timing of urine deposition. *Differences at $P < 0.05$ between urine and non-urine treatments. The box plots represent the data by treatment as analyzed statistically. In the box plots, the median is represented by the gray line, and the mean is represented by the red line. The box represents the 25th and 75th percentiles, the whiskers represent the smallest and largest values that are not considered outliers, and the circles represent outliers.

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 ($P < 0.05$).

N_2O Fluxes

The daily N_2O fluxes from the urine treatment varied with DOE ($P < 0.001$) (Fig. 3a). Overall mean daily N_2O fluxes from the urine treatment were 440% higher compared with the non-urine treatment ($P < 0.001$) (Fig. 3b). Non-urine N_2O fluxes were low (≤1.2 mg N m^{-2} d^{-1}), with an overall average of 0.47 mg N m^{-2} d^{-1}. Daily N_2O fluxes did not differ with irrigation treatment.

The cumulative N_2O emissions (data not shown) reflected the trends observed in the daily N_2O 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 N_2O emissions. When expressed as an emission factor, cumulative N_2O emissions from the 3-d and 6-d irrigation treatments equaled 0.09%.

Nitrous oxide fluxes were highest between 0.4 and 0.6 m^3 m^{-3} WFPS (Fig. 4a) and were highest from D_P/D_O values between ~0.06 and ~0.02 (Fig. 4b). Pooling all N_2O flux data, irrespective of treatment, and performing linear regression analysis of log-transformed WFPS or D_P/D_O versus log-transformed daily N_2O fluxes showed that D_P/D_O best explained the variation in the daily N_2O fluxes (Fig. 4c,d). Overall mean WFPS and D_P/D_O explained 16% (not significant) and 87% ($P < 0.05$) of the variability in cumulative N_2O emissions from urine-treated soils, respectively (Fig. 4e,f).

Concentrations of NO_3^-–N and NH_4^+–N and soil pH explained 18 ($P < 0.05$), 28 ($P < 0.001$), and 32% ($P < 0.001$) of the variability in daily N_2O fluxes, respectively, under the

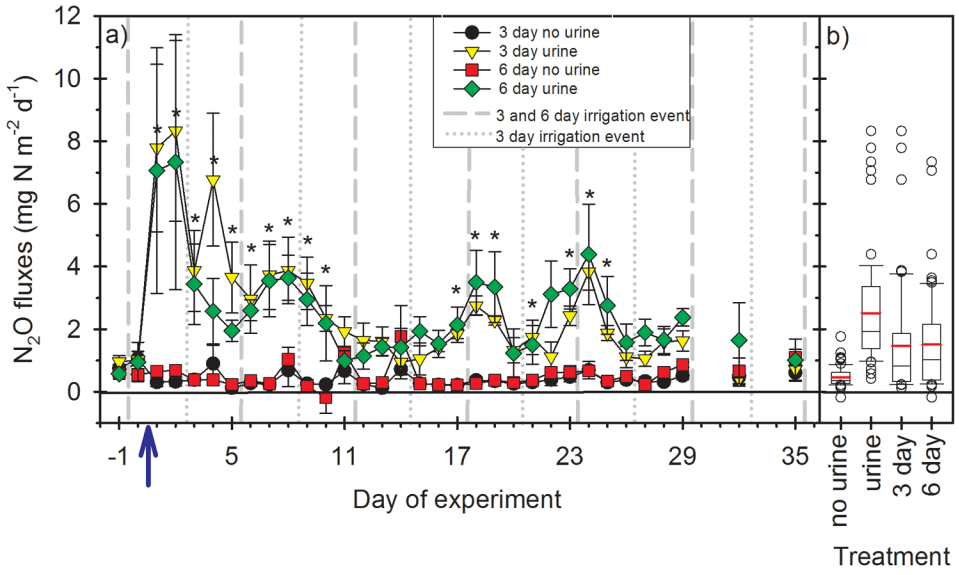

Fig. 3. (a) Mean daily N_2O fluxes from each treatment (± SEM; $n = 4$). The arrow represents the timing of urine deposition. *Differences between the urine and non-urine treatments ($P < 0.05$) on each day. (b) A box plot comparison of daily N_2O emission from each treatment as analyzed statistically (± 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.

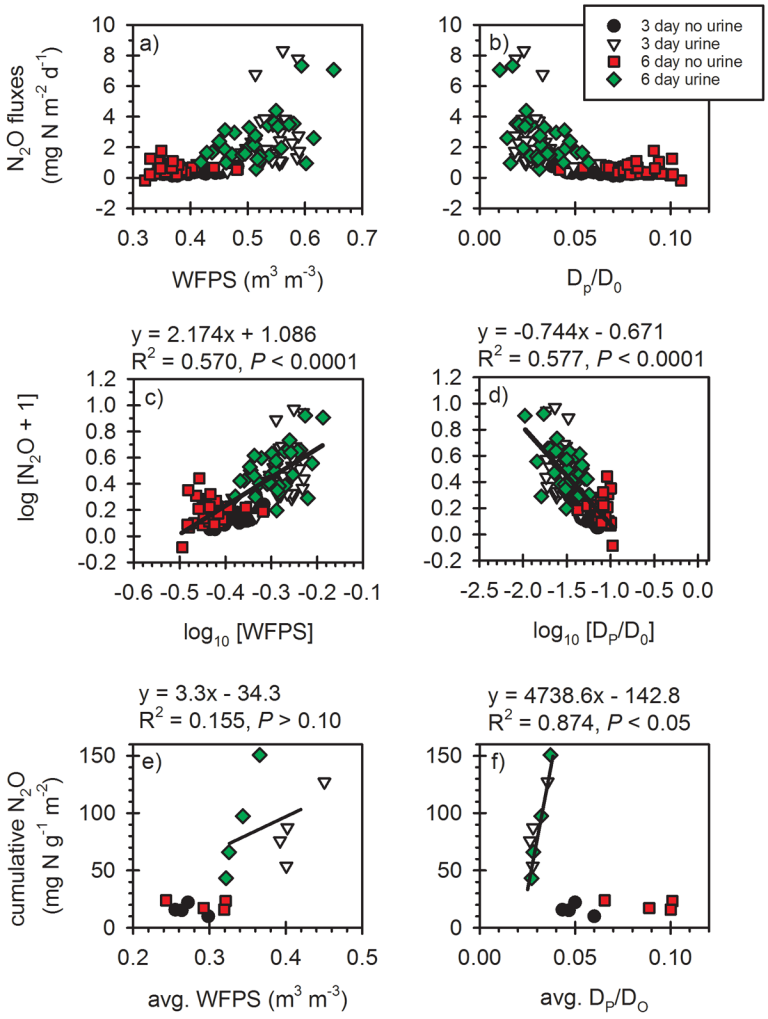

Fig. 4. The daily average nitrous oxide (N_2O) fluxes and (a) water-filled pore space (WFPS) and (b) relative soil diffusivity (D_P/D_O). (c and d) Linear regression between average $\log_{10}$ [1 + N_2O] and (c) $\log_{10}$ [WFPS] or (d) $\log_{10}$ [D_P/D_O] from data from all treatments. (e and f) Linear regression between cumulative N_2O fluxes from the urine treatment and (e) overall mean WFPS from the urine treatment or (f) overall mean D_P/D_O from the urine treatment.

3-d irrigation frequency. However, there were no relationships observed between daily N_2O fluxes and environmental variables under the 6-d irrigation treatment. When all of the data were pooled, irrespective of treatment, NO_3^-–N, NH_4^+–N, NO_2^-–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 N_2O fluxes, respectively.

Ratios of DEA-N_2O/(DEA-N_2O + N_2) from Denitrification Enzyme Assays

The overall mean ratio of DEA-N_2O/(DEA-N_2O + N_2) 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 treatment ($P < 0.05$). These treatment differences were also reflected in the temporal trends. By DOE 17 and 23, the ratios of DEA-N_2O/(DEA-N_2O + 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 DEA-N_2O/(DEA-N_2O + N_2) was positively related to CWC ($R^2 = 0.23$; $P < 0.10$) and negatively related to NO_3^-–N ($R^2 = 0.28$; $P < 0.05$).

Discussion

Other studies have reported similar N_2O 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 N_2O 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 N_2O fluxes (Firestone and Davidson, 1989), individually they were not robust predictors of N_2O fluxes in this study. Rather, they contributed to the variability in N_2O fluxes observed between urine treatments. The lack of any irrigation frequency effects on N_2O emissions can be explained by considering how N_2O regulators varied, specifically soil O_2 concentration and D_P/D_O. As originally hypothesized, more frequent irrigation produced higher soil moisture and lower soil O_2, and the DEA-N_2O/(DEA-N_2O + N_2) ratio was lower, inferring greater potential for N_2OR activity and thus a greater reduction of N_2O to N_2. However, this did not result in lower N_2O emissions.

The higher overall mean soil θ_g 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 N_2O fluxes were not affected by irrigation, daily average N_2O fluxes did increase with increasing WFPS and declining D_P/D_O (Fig. 4a,b). Soil D_P/D_O is a measure of the relative rate at which O_2 diffuses through soil and takes into account pore water blockage effects. Oxygen diffuses about 10^4 times slower in water than in free air, and thus soil moisture content exerts a major influence on soil D_P/D_O (Farquharson and Baldock, 2008; Moldrup et al., 2001, 2013). Soil WFPS is often used to explain N_2O flux magnitude (Dobbie et al., 1999; Smith et al., 1998; Velthof and Oenema, 1995), but the relationship does not account for the interaction between bulk density and matric potential (Balaine et al., 2013). Soil D_P/D_O does account for these variations, and this explains the strong relationship observed between N_2O fluxes and D_P/D_O (Fig. 4d,f). In this study, log-transformed daily average N_2O fluxes related well to both log-transformed WFPS and log-transformed D_P/D_O under the controlled range of soil moisture. However, the inclusion of physical differences in the soil using D_P/D_O provides a repeatable threshold for N_2O production and consumption (Balaine et al., 2013; Harrison-Kirk et al., 2015).

Soil anaerobiosis has been reported to begin at D_P/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 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 O_2 replenishment via diffusion from the atmosphere to the soil. This, combined with the low variability in daily mean soil O_2 concentrations, explains the lower soil O_2 observed at 50 and 100 mm in the 3-d irrigation treatment.

The diel variation in soil 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_P/D_O value >0.02 and soil O_2 >17%), daily N_2O fluxes from the urine treatments after DOE 17 imply N_2O emissions occurred via denitrification because NO_3^-–N was the only available substrate. Denitrification or nitrifier–denitrification in anaerobic microsites must have contributed to N_2O emissions under otherwise aerated soil conditions (Morley et al., 2008; Müller et al., 2004). Thus, measured O_2 concentrations during this study did not reflect soil O_2 concentrations at microsites, and a method to measure soil O_2 in situ at the microscale is still required.

Urine addition decreased soil 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 OH^- ions, increase pH, and generate NH_4^+ and bicarbonate ions, with the latter hydrolyzing to generate CO_2 (Avnimelech and Laher, 1977). Fluxes of CO_2 have been previously observed immediately after urine deposition (Uchida et al., 2008). Rapid anoxia from CO_2 production may trigger denitrification (Sherlock and Goh, 1983), accounting for high N_2O fluxes after urine deposition.

Nitrous oxide production and N_2OR 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 N_2O emissions and average D_P/D_O suggests D_P/D_O (Fig. 4f) could provide insight into the potential for N_2O uptake. The DEA-N_2O/(DEA-N_2O + N_2) ratios were positively related to C, which is a driver of denitrification (Barnard et al., 2005) and negatively related to NO_3^-–N, which is preferentially used over N_2O 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 O_2 is not anaerobic, the contribution of anaerobic microsites can have a significant impact on the ratio of N_2O/(N_2O + N_2) emitted. Future research using ^{15}N isotopes for partitioning N_2O/N_2 ratios, along with direct measurements of N_2OR, are required and should be linked to D_P/D_O to refine its use for predicting O_2–N_2O 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_2O emissions or alter DM production rates within ruminant urine patches. There may be the potential for higher N_2 losses as irrigation intensity increases, but this needs to be confirmed with further study.

Conclusions

Daily and cumulative N_2O emissions, and DM yields, were not influenced by irrigation frequency. A lower ratio of DEA-N_2O/(DEA-N_2O + N_2) indicated greater potential for N_2OR activity and therefore greater potential for N_2O to be reduced to N_2 in the more frequently irrigated treatment, but this was not reflected in the field N_2O emissions. Estimates of D_P/D_O are a good indicator of cumulative N_2O emissions in urine-treated soils and explain well the variability in daily N_2O emissions. Future work linking D_P/D_O and soil O_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_2O emissions and $N_2O/(N_2O + N_2)$ 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_2 and soil temperature.

Acknowledgments

This research was undertaken with CRI Core Funding to Landcare Research from New Zealand's Ministry of Business, Innovation and Employment. The authors thank Neil Smith and Greg Curlin for help in the field, the Centre for Environment and Soil Research at Lincoln University for the loaned equipment, and Synlait Farms (now renamed Purata) for allowing access to one of their dairy farms.

References

Allen, R., L. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration: Guidelines for computing crop water requirements. FAO Irrigation and Drainage Paper 56. FAO, Rome, Italy.

Apogee Instruments Inc. 2015. Apogee Instruments owner's manual oxygen sensor models SO-110, SO-120, SO-210, and SO-220. Apogee Instruments, Logan, UT.

Avnimelech, Y., and M. Laher. 1977. Ammonia volatilization from soils: Equilibrium considerations. Soil Sci. Soc. Am. J. 41:1080–1084. doi:10.2136/sssaj1977.03615995004100060013x

Balaine, N., T. Clough, M. Beare, S. Thomas, E. Meenken, and J. Ross. 2013. Changes in relative gas diffusivity explain soil nitrous oxide flux dynamics. Soil Sci. Soc. Am. J. 77:1496–1505. doi:10.2136/sssaj2013.04.0141

Baral, K., A. Thomsen, J. Olesen, and S. Petersen. 2014. Controls of nitrous oxide emission after simulated cattle urine deposition. Agric. Ecosyst. Environ. 188:103–110. doi:10.1016/j.agee.2014.02.029

Barnard, R., P. Leadley, and B. Hungate. 2005. Global change, nitrification, and denitrification: A review. Global Biogeochem. Cycles 19:GB1007. doi:10.1029/2004GB002282

Blakemore, L.C., P.L. Searle, and B.K. Daly. 1987. Methods for chemical analysis of soils. NZ Soil Bureau Scientific Report. NZ Soil Bureau, Wellington, NZ.

Clough, T.J., R.R. Sherlock, K.C. Cameron, and S.F. Ledgard. 1996. Fate of urine nitrogen on mineral and peat soils in New Zealand. Plant Soil 178:141–152. doi:10.1007/BF00011172

Davidson, E. 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. Nat. Geosci. 2:659–662. doi:10.1038/ngeo608

de Klein, C., L. Barton, R. Sherlock, Z. Li, and R. Littlejohn. 2003. Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. Aust. J. Soil Res. 41:381–399. doi:10.1071/SR02128

de Klein, C., J. Luo, B. Woodward, T. Styles, B. Wise, S. Lindsey, and N. Cox. 2014. The effect of nitrogen concentration in synthetic cattle urine on nitrous oxide emissions. Agric. Ecosyst. Environ. 188:85–92. doi:10.1016/j.agee.2014.02.020

Di, H.J., and K.C. Cameron. 2002. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. Soil Use Manage. 18:395–403. doi:10.1111/j.1475-2743.2002.tb00258.x

Dobbie, K.E., I.P. McTaggart, and K.A. Smith. 1999. Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. J. Geophys. Res. 104:26891–26899. doi:10.1029/1999JD900378

Drury, C.F., D.D. Myrold, E.G. Beauchamp, and W.D. Reynolds. 2008. Denitrification techniques for soil. In: M.R. Carter and E.G. Gregorich, editors, Soil sampling and methods of analysis. CRC Press, Boca Raton, FL. p. 471–493.

Eccles, W.J., C. Matthew, and A.C.P. Chu. 1990. Response of Matua prairie grass and Ellett perennial ryegrass to excess soil moisture in sand, silt and clay soils. Proc. N. Z. Grassl. Assoc. 51:127–130.

FAO. 2010. Statistical yearbook. FAO, Rome, Italy.

Farquharson, R., and J. Baldock. 2008. Concepts in modelling N_2O emissions from land use. Plant Soil 309:147–167. doi:10.1007/s11104-007-9485-0

Firestone, M., and E. Davidson. 1989. Microbial basis of NO and N_2O production and consumption in soil. In: M.O. Andreae and D.S. Schimel, editors, Exchange of trace gases between ecosystems and the atmosphere. John Wiley & Sons, New York. p. 471–493.

Ghani, A., M. Dexter, and K.W. Perrott. 2003. Hot-water extractable carbon in soils: A sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol. Biochem. 35:1231–1243. doi:10.1016/S0038-0717(03)00186-X

Gjettermann, B., K.L. Nielsen, C.T. Petersen, H.E. Jensen, and S. Hansen. 1997. Preferential flow in sandy loam soils as affected by irrigation intensity. Soil Technol. 11:139–152. doi:10.1016/S0933-3630(97)00001-9

Groffman, P., M. Altabet, J.K. Böhlke, K. Butterbach-Bahl, M. David, M. Firestone, A. Giblin, T. Kana, L.P. Nielsen, and M. Voytek. 2006. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecol. Appl. 16:2091–2122. doi:10.1890/1051-0761(2006)016[2091:MFMDDA]2.0.CO;2

Harrison-Kirk, T., S. Thomas, T. Clough, M. Beare, T. van der Weerden, and E. Meenken. 2015. Compaction influences N_2O and N_2 emissions from ^{15}N-labeled synthetic urine in wet soils during successive saturation/drainage cycles. Soil Biol. Biochem. 88:178–188. doi:10.1016/j.soilbio.2015.05.022

Haynes, R.J., and P.H. Williams. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Adv. Agron. 49:119–199. doi:10.1016/S0065-2113(08)60794-4

Hewitt, A.E. 2010. New Zealand soil classification. Landcare Research Science Series No. 1. 3rd ed. Manaaki-Whenua Press, Lincoln, New Zealand.

Horváth, L., B. Grosz, A. Machon, Z. Tuba, Z. Nagy, S.Z. Czóbel, J. Balogh, E. Péli, S.Z. Fóti, and T. Weidinger. 2010. Estimation of nitrous oxide emission from Hungarian semi-arid sandy and loess grasslands: Effect of soil parameters, grazing, irrigation and use of fertilizer. Agric. Ecosyst. Environ. 139:255–263. doi:10.1016/j.agee.2010.08.011

IBM Corp. 2011. IBM SPSS Statistics, version 20.0. IBM Corp., Armonk, NY.

Intergovernmental Panel on Climate Change (IPCC). 2007. Climate change: Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.

Knowles, R. 1982. Denitrification. Microbiol. Rev. 46:43–70.

Linn, D.M., and J.W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48:1267–1272. doi:10.2136/sssaj1984.03615995004800060013x

Lloyd, J., and J.A. Taylor. 1994. On the temperature dependence of soil respiration. Funct. Ecol. 8:315–323. doi:10.2307/2389824

Maharjan, B., R. Venterea, and C. Rosen. 2014. Fertilizer and irrigation management effects on nitrous oxide emissions and nitrate leaching. Agron. J. 106:703–714. doi:10.2134/agronj2013.0179

Maynard, D.G., and M.P. Curran. 2008. Bulk density measurement in forest soils. In: M.R. Carter and E.G. Gregorich, editors, Soil sampling and methods of analysis. Taylor & Francis Group, New York. p. 863–869.

McBride, S.D. 1994. Pasture yield responses to irrigation in Canterbury. Proc. N. Z. Grassl. Assoc. 56:165–168.

Minitab Inc. 2010. Minitab Statistical Software, version 17. Minitab Inc., State College, PA.

Moir, J.L., K.C. Cameron, H.J. Di, and U. Fertsak. 2011. The spatial coverage of dairy cattle urine patches in an intensively grazed pasture system. J. Agric. Sci. 149:473–485. doi:10.1017/S0021859610010120

Moldrup, P., T.K.K. Chamindu Deepagoda, S. Hamamoto, T. Komatsu, K. Kawamoto, D.E. Rolston, and L. Wollesen de Jonge. 2013. Structure-dependent water-induced linear reduction model for predicting gas diffusivity and tortuosity in repacked and intact soil. Vadose Zone J. doi:10.2136/vzj2013.01.0026

Moldrup, P., T. Olesen, T. Komatsu, P. Schonning, and D.E. Rolston. 2001. Tortuosity, diffusivity, and permeability in the soil liquid and gaseous phases. Soil Sci. Soc. Am. J. 65:613–623. doi:10.2136/sssaj2001.653613x

Morley, N., E. Baggs, P. Dörsch, and L. Bakken. 2008. Production of NO, N_2O and N_2 by extracted soil bacteria, regulation by NO_2^- and O_2 concentrations. FEMS Microbiol. Ecol. 65:102–112. doi:10.1111/j.1574-6941.2008.00495.x

Müller, C., R.J. Stevens, R.J. Laughlin, and H.J. Jäger. 2004. Microbial processes and the site of N_2O production in a temperate grassland soil. Soil Biol. Biochem. 36:453–461. doi:10.1016/j.soilbio.2003.08.027

Oenema, O., N. Wrage, G. Velthof, J. van Groenigen, J. Dolfing, and P. Kuikman. 2005. Trends in global nitrous oxide emissions from animal production systems. Nutr. Cycl. Agroecosyst. 72:51–65. doi:10.1007/s10705-004-7354-2

Orwin, K.H., J.E. Bertram, T.J. Clough, L.M. Condron, R.R. Sherlock, M. O'Callaghan, J. Ray, and D.B. Baird. 2010. Impact of bovine urine deposition on soil microbial activity, biomass, and community structure. Appl. Soil Ecol. 44:89–100. doi:10.1016/j.apsoil.2009.10.004

Parkin, T.B., R.T. Venterea, and S.K. Hargreaves. 2012. Calculating the detection limits of chamber-based soil greenhouse gas flux measurements. J. Environ. Qual. 41:705–715. doi:10.2134/jeq2011.0394

Petersen, S., P. Ambus, L. Elsgaard, P. Schjønning, and J. Olesen. 2013. Long-term effects of cropping system on N_2O emission potential. Soil Biol. Biochem. 57:706–712. doi:10.1016/j.soilbio.2012.08.032

Ravishankara, A.R., J. Daniel, and R. Portmann. 2009. Nitrous oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st Century. Science 326:123–125. doi:10.1126/science.1176985

Ruser, R., H. Flessa, R. Russow, G. Schmidt, F. Buegger, and J.C. Munch. 2006. Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: Effect of compaction, soil moisture and rewetting. Soil Biol. Biochem. 38:263–274. doi:10.1016/j.soilbio.2005.05.005

Scheer, C., P. Grace, D. Rowlings, and J. Payero. 2013. Soil N_2O and CO_2 emissions from cotton in Australia under varying irrigation management. Nutr. Cycl. Agroecosyst. 95:43–56. doi:10.1007/s10705-012-9547-4

Sherlock, R.R., and K.M. Goh. 1983. Initial emission of nitrous oxide from sheep urine applied to pasture. Soil Biol. Biochem. 15:615–617. doi:10.1016/0038-0717(83)90061-5

Simojoki, A., and A. Jaakkola. 2000. Effect of nitrogen fertilization, cropping and irrigation on soil air composition and nitrous oxide emission in a loamy clay. Eur. J. Soil Sci. 51:413–424. doi:10.1046/j.1365-2389.2000.00308.x

Smith, K.A., P.E. Thomson, H. Clayton, I.P. McTaggart, and F. Conen. 1998. Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. Atmos. Environ. 32:3301–3309. doi:10.1016/S1352-2310(97)00492-5

Soil Survey Division Staff. 1999. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. USDA, Washington, DC.

Steel, G.C., J.H. Torrie, and D.A. Dikey. 1997. Principles and procedures of statistics: A biometrical approach. McGraw-Hill, New York.

Stepniewski, W. 1981. Oxygen diffusion and the strength as related to soil compaction: II. Oxygen diffusion coefficient. Pol. J. Soil Sci. 14:3–13.

Stevens, R.J., and R.J. Laughlin. 1995. Nitrite transformations during soil extraction with potassium chloride. Soil Sci. Soc. Am. J. 59:933–938. doi:10.2136/sssaj1995.03615995005900030044x

Taghizadeh-Toosi, A., T. Clough, L. Condron, R. Sherlock, C. Anderson, and R. Craigie. 2011. Biochar incorporation into pasture soil suppresses in situ nitrous oxide emissions from ruminant urine patches. J. Environ. Qual. 40:468–476. doi:10.2134/jeq2010.0419

Trost, B., A. Prochnow, K. Drastig, A. Meyer-Aurich, F. Ellmer, and M. Baumecker. 2013. Irrigation, soil organic carbon and N_2O emissions: A review. Agron. Sustain. Dev. 33:733–749. doi:10.1007/s13593-013-0134-0

Uchida, Y., T. Clough, F. Kelliher, and R. Sherlock. 2008. Effects of aggregate size, soil compaction, and bovine urine on N_2O emissions from a pasture soil. Soil Biol. Biochem. 40:924–931. doi:10.1016/j.soilbio.2007.11.007

van der Weerden, T.J., T.J. Clough, and T.M. Styles. 2013. Using near-continuous measurements of N_2O emission from urine-affected soil to guide manual gas sampling regimes. N. Z. J. Agric. Res. 56:60–76. doi:10.1080/00288233.2012.747548

Velthof, G.L., and O. Oenema. 1995. Nitrous oxide fluxes from grassland in The Netherlands: II. Effects of soil type, nitrogen fertilizer application and grazing. Eur. J. Soil Sci. 46:541–549. doi:10.1111/j.1365-2389.1995.tb01350.x

Venterea, R. 2010. Simplified method for quantifying theoretical underestimation of chamber-based trace gas fluxes. J. Environ. Qual. 39:126–135. doi:10.2134/jeq2009.0231

Venterea, R. 2013. Theoretical comparison of advanced methods for calculating nitrous oxide fluxes using non-steady state chambers. Soil Sci. Soc. Am. J. 77:709–720. doi:10.2136/sssaj2013.01.0010

Venterea, R., K. Spokas, and J. Baker. 2009. Accuracy and precision analysis of chamber-based nitrous oxide gas flux estimates. Soil Sci. Soc. Am. J. 73:1087–1093. doi:10.2136/sssaj2008.0307

Wagner, S.W., D.C. Reicosky, and R.S. Alessi. 1997. Regression models for calculating gas fluxes measured with a closed chamber. Agron. J. 89:279–284. doi:10.2134/agronj1997.00021962008900020021x

Wrage, N., G.L. Velthof, M.L. van Beusichem, and O. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol. Biochem. 33:1723–1732. doi:10.1016/S0038-0717(01)00096-7

Zhu, X., M. Burger, T. Doane, and W. Horwath. 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. Proc. Natl. Acad. Sci. USA 110:6328–6333. doi:10.1073/pnas.1219993110