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Nitrous Oxide Fluxes and Soil Oxygen Dynamics of Soil Treated with Cow Urine

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Ruminant urine deposition onto pasture creates hot-spots where emissions of nitrous oxide (N_2O) are produced by aerobic and anaerobic microbial pathways. However, limited measurements of in situ soil oxygen (O_2)–N_2O relationships hinder the prediction of N_2O emissions from urine-affected soil. This study tested whether soil O_2 concentration or relative diffusivity of O_2 (D_P/D_O) could explain N_2O emissions from urine patches. Using a randomized plot design, N_2O emissions were measured daily from a perennial ryegrass (*Lolium perenne* L.) pasture for 56 d following bovine (*Bos taurus*) urine deposition to an imperfectly drained silty loam soil. Soil O_2, volumetric water content, pH, conductivity, and extractable N and C were measured in urine-amended and non-amended soil. Values of water-filled pore space (WFPS) and D_P/D_O were modeled. When data from treatments were pooled together, daily mean D_P/D_O explained 73% of the total variance in mean daily N_2O flux, compared with 65, <60, and <20% for WFPS, O_2 and other measured variables, respectively. Soil pH, O_2, volumetric water content, WFPS and D_P/D_O all explained more of the variance in the urine-amended compared with the non-amended soil. Daily N_2O fluxes increased substantially at D_P/D_O values around 0.006, which was consistent with past laboratory studies. These results demonstrate for the first time an O_2 diffusion threshold for elevated N_2O fluxes in the field, expressed as $D_P/D_O \approx 0.006$. Further studies should examine the consistency of this threshold under varying N and C substrates and a range of soil pH.

Abbreviations: CWC, cold water carbon; DOE, day of experiment; DI, deionized water; HWC, hot water carbon; GLM, general linear model; N_2OR, nitrous oxide reductase; SWLR, structure-dependent water-induced linerar reduction model; WFPS, water-filled pore space.

Core Ideas

- **Heavy irrigation, surface flooding, and urine decrease soil oxygen.**
- **When soil oxygen decreases, N_2O fluxes rapidly increase when nitrogen is available.**
- **Nitrous oxide fluxes are explained well using relative soil gas diffusivity.**

Nitrous oxide is a potent greenhouse gas (GHG) that contributes to climate change, and it is projected to be the dominant ozone-depleting substance emitted in the 21st century (Ravishankara et al., 2009). Increases in atmospheric N_2O concentrations are linked to N-based fertilizer inputs and excretal returns from grazing ruminant livestock to agricultural soils. High inputs of N from these sources can cause soil N concentrations to be greater than plant requirements. This excess soil N is available for microbial processes such as nitrification, denitrification, and nitrifier-denitrification, the latter two processes dominate the production of N_2O (Wrage et al., 2001; Davidson, 2009; Kool et al., 2010; Zhu et al., 2013).

Nitrous oxide is produced from denitrification and nitrifier-denitrification when soil O_2 is low (Goreau et al., 1980; Firestone and Davidson, 1989; Venterea, 2007; Zhu et al., 2013). Soil O_2 distribution in situ is variable (Butterbach-Bahl et al., 2013) as even soils considered aerobic can have anaerobic microsites where N_2O production may occur (Robertson et al., 1989; Laughlin and Stevens, 2002; Müller et al., 2004). Soil O_2 concentrations and the distribution of soil O_2 are influenced by chemical reactions, microbial activity, and hydrological events. For

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example, urine deposition onto soil from grazing ruminant animals increases soil water content, initiates urea hydrolysis, and increases microbial respiration rates (Uchida et al., 2008). The combination of these factors can intensify O_2 depletion under a urine patch (Norton and Stark, 2011) and in turn, increase soil-to-atmosphere N_2O emissions (Owens et al., 2016). Increasing soil moisture content alone can reduce soil O_2 concentrations because water impedes the diffusion of O_2 into and through soil thereby restricting soil O_2 distribution in soil (Farquharson and Baldock, 2008). The relative volumes and distribution of water and O_2 in soil are regulated by soil properties, including structure (Farquharson and Baldock, 2008), texture (Schjønning et al., 1999), and soil pore-size distribution (Horn and Smucker, 2005). The extent to which chemical, hydrological, and soil physical properties influence soil O_2, and in turn, influence surface N_2O emissions, is difficult to quantify. Previous work has added labeled ^{18}O- and ^{15}N-labeled compounds to evaluate scenarios of O_2 exchange in various microbial pathways (Kool et al., 2009) but few studies have simultaneously measured both N_2O emissions and soil O_2 concentrations in the field (Simojoki and Jaakkola, 2000; Owens et al., 2016).

Diffusion of O_2 in and through soil can be modeled or inferred using soil physical and hydrological data. Relative soil gas diffusivity of O_2 (D_P/D_O) describes the rate of gas diffusion within soil (D_P) relative to free air (D_O). It can be calculated as a function of relative air-filled porosity—which is derived from soil bulk density, soil particle density, and volumetric water content—and total porosity (Schjønning et al., 1999; Moldrup et al., 2001). Relative soil gas diffusivity is a good predictor of O_2 diffusion through a soil because it accounts for the interaction between soil bulk density, the resulting pore-size distribution, and the ensuing soil moisture content (Moldrup et al., 2013). Relative soil gas diffusivity has been shown to explain the rapid increase in rates of N_2O fluxes under controlled laboratory conditions when NO_3^- and C are available, with peak N_2O fluxes occurring at a D_P/D_O value of 0.006 when N substrate was not limiting (Balaine et al., 2013, 2016). However, D_P/D_O may also be a valuable tool to explain N_2O fluxes in situ, but more data are needed.

Since soil D_P/D_O has outperformed WFPS as a predictor of N_2O fluxes in controlled laboratory studies using repacked soil cores (Balaine et al., 2013, 2016), this field study aimed to build on this work by relating the same concepts in the field under variable hydrological conditions. The objective of this field study was to assess how well different measures of soil moisture, soil O_2, and D_P/D_O, as well as a range of other soil chemical variables, explained N_2O emissions from a poorly drained pasture soil, with and without ruminant urine addition.

MATERIALS AND METHODS

Study Site

The experiment was conducted at Lincoln University (−42° 38′ 81.4″ S. long., 172° 27′ 63.3² E. lat., elevation 9 m above sea level) in July–August 2014 (southern-hemisphere winter). The soil was a stone-free, imperfectly drained Wakanui Mottled Immature Pallic Silty Loam Soil in the New Zealand Soil Classification (Hewitt, 2010), or an Endoaquept in the USDA Classification (Soil Survey Division Staff, 1999). The experimental plot was situated on an established, long-term unfertilized pasture sown with perennial ryegrass. Previously, the pasture has been grazed by sheep. Currently, the area is an established research station and has not been grazed for 10 yr. During the growing season, between October and April, the pasture is mown about once a month.

Experimental Design

The experiment used two treatments replicated four times. The urine treatment applied bovine urine once at the beginning of the experiment, subsequently referred to as "day of experiment" zero (DOE 0). The urine was collected from cows grazing perennial ryegrass and white clover (*Trifolium repens L.*) pasture. A subsample of the urine was immediately analyzed after urine collection on a CN elemental analyzer (Elementar Vario-Max CN Elemental Analayzer, Elementar GmbH, Hanau, Germany) to determine total N content. The total N content of the urine was increased from 4.9 to 7.5 g N L^{-1} using urea [$CO(NH_2)_2$], the dominate N source in ruminant urine, and then the urine was stored in a sealed container with no headspace. Two liters of urine (equaling 10.2 mm of water) was applied within each chamber area for the urine treatment at a rate of 750 kg N ha^{-1}, a typical cattle urine deposition event (Haynes and Williams, 1993). The urine treatment was applied to the soil within 24 h of urine total N determination. Nothing was applied to the soil in the no urine treatment. This was done to mimic actual field conditions of an area that is not affected by urine deposition.

A 4- by 6-m experimental area was subdivided into plots for gas sampling, manual sampling of soil, and for installation of automated instrumentation (Supplemental Fig. S1). Within each of the plots, circular stainless steel gas-flux chamber bases (0.196 m^2) were installed for gas sampling, to delineate areas soil sampling, and to distinguish areas where the automated instrumentation was installed (Supplemental Fig. S1). Each chamber base was inserted into the soil DOE -21 to a depth of 100 mm.

The experimental plot was covered with a tunnel house (Torto, Hamilton, New Zealand) between DOE -2 and 20. The original goal was to exclude precipitation so that soil water content could be controlled using irrigation. Between DOE 2 and 20, there was a total of 48 mm of precipitation. This resulted in surface flooding of the experimental area between DOE 19 and 22. Because of this, the tunnel house was removed on DOE 20. Periodically, the soil was manually irrigated in an attempt to decrease soil O_2 concentrations. On DOE 6, 11, and 13, 6 mm of irrigation was applied. On DOE 29, 14 mm of irrigation was applied, and 20 mm of irrigation was applied on DOE 49, 50, and 51 (Fig. 1a). In addition to irrigation, 16 mm of rain fell between DOE 21 and 56 directly onto the experimental plot.

Nitrous Oxide Fluxes

Soil-to-atmosphere N_2O fluxes were measured daily from DOE -1 to DOE 56, except between DOE 49 and 51, using non-steady state vented and insulated chambers (headspace volume = 19.63 L). To attain fluxes representative of the daily average, sampling occurred between 1000 and 1200 h local time (van der Weerden et al., 2013). Four N_2O samples were taken at 0, 15, 30, and 45 min following placement of the chamber lids onto the chamber base. Gas (9 mL) was collected and transferred to 6 mL pre-evacuated (−1 atm) glass Exetainers (Labco Ltd., Lampeter, UK) using a syringe fitted with a three-way stop cock. Gas samples were analyzed with an automated gas chromatograph equipped with an electron capture detector (SRI 8610c GC, SRI Instruments, Torrance, CA), as previously described (Clough et al., 1996). The detection limit of the gas chromatograph was 0.01 $\mu L\ L^{-1}$ and the furnace temperature was 310°C. Nitrous oxide concentrations were converted to mass per volume concentration using the ideal gas law and air temperature at the time of sampling. Flux calculations used the change in N_2O concentration over time, along with the chamber volume and area. Initially, both quadratic regression (Wagner et al., 1997) and linear regression were used to determine the change in N_2O concentration. The quadratic regression fluxes were evaluated using the LINEST function in Microsoft Excel (Version 2013). Flux calculations used quadratic regression unless the second derivative of the quadratic regression was ≥ 0 (Venterea et al., 2009; Venterea, 2013). All measured fluxes were above the detection limit (Parkin et al., 2012). Of the 408 flux calculations, 34% were calculated using quadratic regression and 66% using linear regression. A correction factor was applied to account for chamber-induced errors (Venterea, 2010). This required knowing the soil bulk density within each gas chamber base. Soil bulk density was determined from an average of three intact soil cores (height = 75 mm, i.d. = 75 mm) which were removed at the end of the experiment from the area within each gas-flux measurement area.

Cumulative N_2O emissions from each chamber were determined by summing the daily N_2O flux estimates. Nitrous oxide fluxes from days without a flux measurement were derived using linear interpolation. The emission factor was determined by subtracting the cumulative N_2O emissions in the no urine treatment from the cumulative N_2O emissions in the urine treatment, then dividing the sum by the rate of urine N applied, and expressing this as a percentage of N applied (de Klein et al., 2003).

Soil and Environmental Variables

Precipitation (mm) data were acquired from a Lincoln weather station 2 km northwest of the experiment site (Broadfield, Lincoln, −43° 37′ 57.2″ S. long., 172° 28′ 22.4″ E. lat.). Environmental instrumentation was installed in the center of the experimental plot within urine and no urine treatments (Supplemental Fig. S1). Soil temperature sensors (107 temperature sensor, Campbell Scientific, Logan, UT) and volumetric water content (θ_v) sensors (CS 616 Reflectometer, Campbell

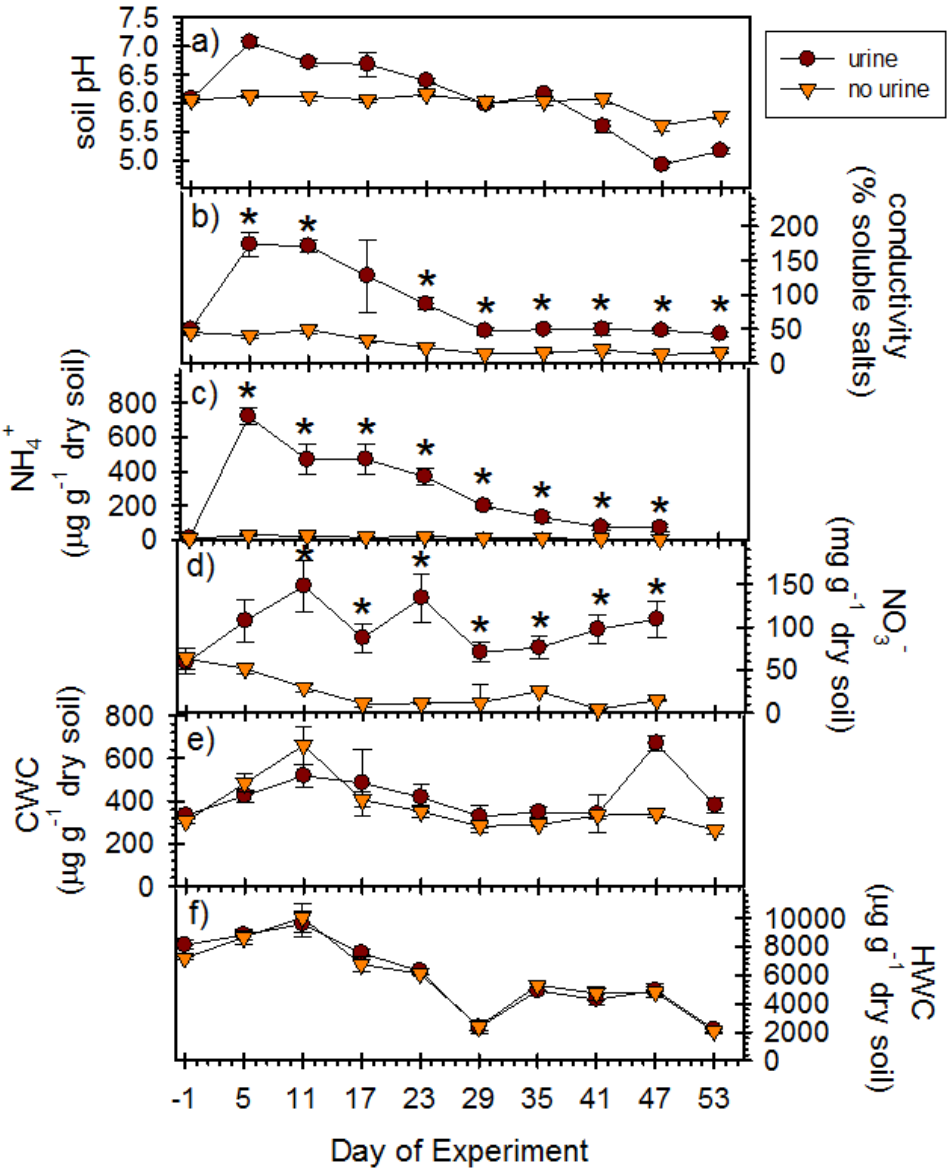

Fig. 1. Means and standard error of the means (±SEM, n = 4) of the soil chemical data over time, (a) soil pH, (b) conductivity, (c) ammonium (NH_4^+–N), (d) nitrate (NO_3^-–N), (e) cold water carbon (CWC), and (f) hot water carbon (HWC), where the asterisks (*) indicate a significant difference between the treatments at $P < 0.05$.

Scientific, Logan, UT) were installed horizontally into the soil at the 50-mm depth. Soil O_2 sensors (SO-110, Apogee Instruments, Logan, UT) were installed vertically at 10-, 50-, and 100-mm soil depths in both treatments. Two soil O_2 sensors were installed in each treatment and soil depth, one with the diffusive head attached and one without the diffusive head. The purpose of the different diffusive head configurations was to measure different sized areas within the soil. The motivation was that these different measurement areas may show different soil O_2 concentrations and dynamics in response to changes to soil moisture or urine deposition; the smaller measurement area captured from the sensors without the diffusive head may reveal a relatively finer resolution measurement of soil O_2 compared with the sensors with the diffusive head. Data from the O_2 sensor without the diffusive head at the 50-mm depth in the no urine treatment are not reported because the sensor malfunctioned. The instrumentation was powered and controlled by two data loggers and a multiplexer (CR3000, CR1000, AM416, Campbell Scientific, Logan, UT). Samples were taken every 15 min from DOE -1 (000 h, 2 July 2014) until DOE 56 (1200 h, 27 Aug. 2014). Manual tensiometer readings (2900F1 Quick Draw Tensiometer, Soilmoisture Equipment Corp. Santa Barbara, CA) were also taken once daily, from within each gas chamber base at two depths (≈20 and ≈70 mm), to measure soil matric potential (ψ) from DOE 19 to DOE 56, during and after surface flooding. Soil WFPS was calculated using measured θ_v at the 50-mm soil depth (Linn and Doran, 1984). Soil bulk density was measured as described above. The capillary rise equation (Supplementary Table S1), which can use a given ψ value to determine an equivalent pore radius that remains full of water at that ψ value (Scott, 2000; Hillel, 1998), and is used to determine the size of soil pore which was water-filled at the minimum measured ψ. Calculations used in this paper are available in Supplemental Table S1. Pasture was harvested to ≈50 mm height using hand held shears on DOE −1, 26, and 56 to simulate grazing.

The soil sampling areas within the manual sampling chambers and the instrumentation chambers for each treatment were treated the same as the soil in the gas sampling chambers. The soil was sampled from these areas to a depth of 70 mm using an auger. Soil was sampled every 6 d between DOE −1 and 56 for soil chemical analysis. Soil pH was determined by mixing 10 g of air-dried soil with 25 mL deionized water (DI) and the solution was measured (SevenEasy, Mettler Toledo, Port Melbourne, Australia) after 12 h of settling (Blakemore et al., 1987). Conductivity was determined by combining 10 g of dry weight equivalent of soil with 50 mL of DI, mixing for 30 min and measuring (SevenEasy, Mettler Toledo, Port Melbourne, Australia) following 5 min of centrifuging at 1500 rpm (Blakemore et al., 1987). Nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were determined by extracting 4 g dry weight equivalent of soil with 40 mL of 2 M KCl. Samples were mixed for 1 h, centrifuged for 20 min at 2000 rpm, and gravity filtered through Whatman 42 filters (Blakemore et al., 1987). Extracts were frozen until flow injection analysis (FIAstar 5000 Analyzer, FOSS Analytical, Hilerød, Denmark).

Extractable cold water carbon (CWC), indicative of water soluble C, was extracted by combining 3 g dry weight equivalent of soil and 30 mL of DI followed by 30 min of mixing, centrifuging for 20 min (3500 rpm), and filtering through Avantec 5C filters (Ghani et al., 2003). Then the soil was extracted again to obtain hot water-extractable carbon (HWC), which is related to microbial biomass. After adding DI as before, the soil-DI mixture was placed in a hot water bath at 80°C for 16 h before mixing, centrifuging, and filtering as noted above (Ghani et al., 2003). The CWC and HWC samples were frozen after extraction until analysis with a Total Organic Carbon Analyzer (TOC 5000A, Shimadzu, Sydney, Australia).

The structure-dependent water-induced linear reduction (SWLR) model (Moldrup et al., 2013) was used to calculate D_P/D_O values using the previously measured soil bulk density from within the chambers, and the daily air-filled porosity (Supplemental Table S1).

Data Analyses

Unless otherwise stated, data analyses were performed using Minitab (Minitab Inc. 2010, version 17) with parametric statistics. Data were transformed (Supplemental Table S2) if needed. Analysis of urine treatment effects on overall means included only data collected after urine application (from DOE 1 onward). If data were transformed, conclusions were drawn from the analysis on the transformed scale. Figures present untransformed data unless otherwise noted.

A linear mixed model run in SPSS (IBM Corp., 2011) using a significance criteria of 0.05 was used to test for treatment effects on mean daily N_2O emissions. This model was used to compensate for repeated measures and heterogeneity of variance between treatments. A heterogeneous first-order autoregressive covariance structure was used for the repeated measures. The effect of urine, DOE, and urine × DOE were treated as fixed effects, and DOE as a repeated measure. Tukey's multiple comparisons was used as a post hoc test (Steel et al., 1997).

A general linear model (GLM) was used to test for treatment effects on overall means for all soil and environmental data (except ψ). For NH_4^+, NO_3^-, HWC, CWC, soil pH, and conductivity, the urine, DOE, and urine × DOE were treated as fixed effects. For overall mean soil temperature at the 50-mm soil depth, θ_v at the 50-mm soil depth, WFPS, soil O_2 at the 10- and 100-mm soil depths from sensors with and without the diffusive heads, and modeled D_P/D_O, only urine and DOE were treated as fixed effects. Tukey's multiple comparison test at $P < 0.05$ was used for post hoc tests (Steel et al., 1997). A two-sample student *t* test was used to test for differences in soil bulk density and total porosity between the treatments.

Least squares linear regression was used to evaluate the relationships between daily N_2O fluxes, and the daily mean measured and calculated factors noted above.

RESULTS

Soil and Environmental Variables

The soil bulk density of the urine treatment was higher than from the no urine treatment ($P = 0.086$). Soil bulk density averaged 1.01 (±0.054 SEM; $n = 4$) and 1.24 Mg m^{-3} (±0.098 SEM; $n = 4$) in the no urine and urine treatments, respectively. Total porosity was lower from urine treatment compared with the no urine treatment ($P = 0.086$), averaging 62 (±2.1 SEM; $n = 4$) and 53% (±3.7 SEM; $n = 4$) in the no urine and urine treatments, respectively.

Air temperature averaged 7.2°C over the course of the experiment, with minimum and maximum values of −2.4 and 20.6°C, respectively. Soil temperatures followed a diel cycle with overall mean soil temperatures ranging from 7.2 to 7.6°C, and there was no difference between the two treatments.

There was no difference in overall mean pH between the treatments. Soil pH increased after urine deposition but was lower in the urine treatment compared with the no urine treatment at the end of the experiment (Fig. 1a). Overall mean soil conductivity, NH_4^+ concentrations, and NO_3^- concentrations were 211 ($P < 0.001$), 95 ($P = 0.016$), and 80% ($P < 0.001$) greater in the urine treatment compared with the no urine treatment (Fig. 1b–1d). Overall mean CWC (Fig. 1e) and HWC (Fig. 1f) concentrations were not affected by urine. All extractable soil environmental factors varied with DOE (Fig. 1).

Rapid increases in θ_v occurred following urine application, heavy irrigation, and precipitation (Fig. 2a,b). Surface flooding resulted in high θ_v at the 50-mm soil depth in both treatments between DOE 19 and 22 (Fig. 2b). Overall daily mean θ_v was 6% higher in the urine treatment compared with the no urine treatment ($P < 0.001$). The highest N_2O fluxes were observed between 0.70 and 0.80 m^3 m^{-3} WFPS (Fig. 2c). The overall daily mean WFPS was 17% higher in the urine treatment compared with the no urine treatment ($P < 0.001$), consistent with the relatively higher bulk density in the urine treatment. Matric potential ranged from ≈0 kPa during surface flooding, to a minimum of −11 kPa on DOE 45 (Fig. 2c). There was some spatial variability in ψ. At each depth, the surface flooding differed by a maximum of 6 kPa between gas chambers on each day (Supplemental Fig. S2).

At 10-mm soil depth, the overall mean soil O_2 concentration was higher in the urine treatment compared with the no urine treatment by 4.5 ($P < 0.001$) and 6.1% ($P < 0.001$) with and without the diffusive head present, respectively. Soil O_2 concentrations decreased following urine deposition for a period of ≈24 h at both the 50- and 100-mm soil depths regardless of the presence or absence of the diffusive head (Fig. 2e–2f). Minimum soil O_2 concentrations at the 50- and 100-mm soil depths occurred following surface flooding, and prior to drainage on DOE 23, regardless of O_2 sensor diffusive

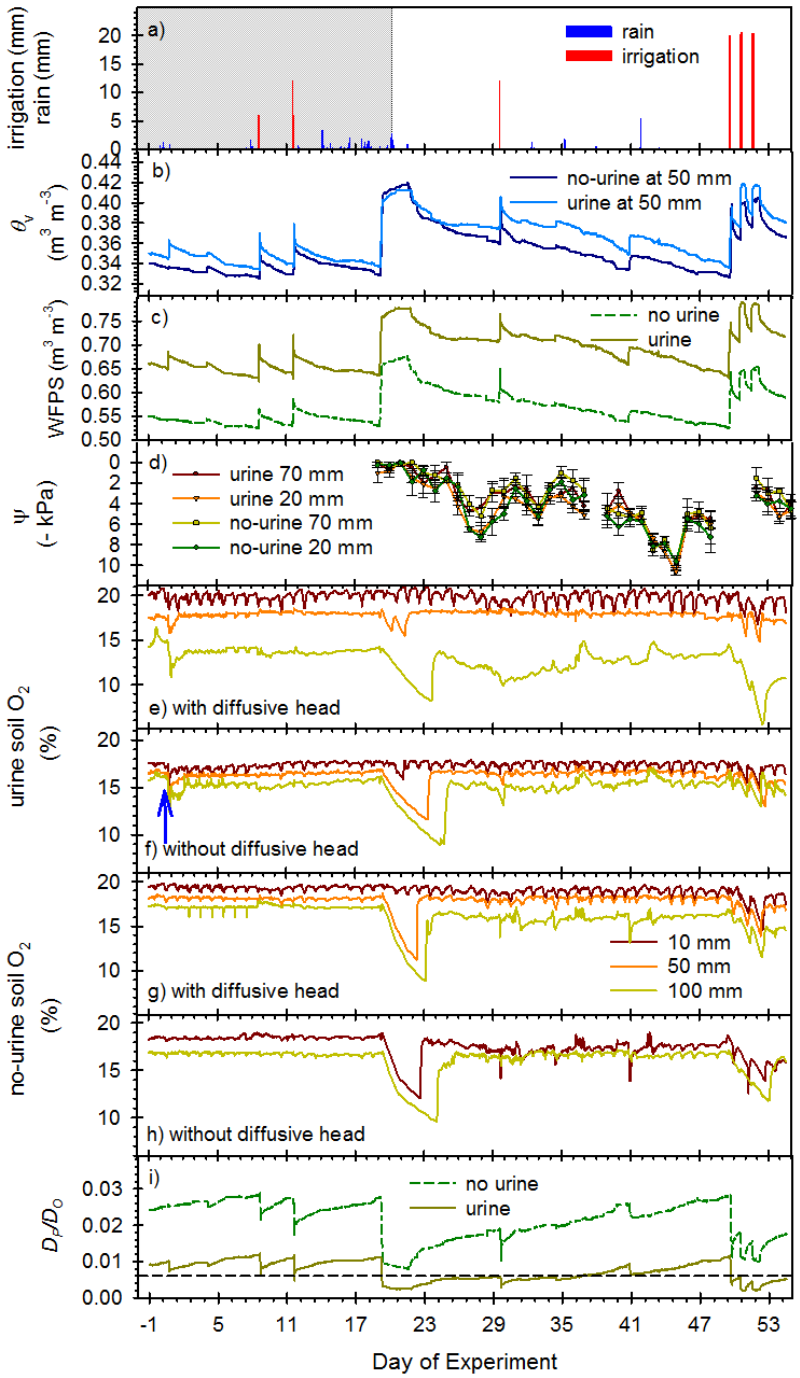

Fig. 2. (a) Rain (blue) and irrigation (red) with the gray shaded area representing when the experimental plot was covered with the tunnel house, (b) the volumetric water content (m^3 m^{-3}) over time from the urine and no urine treatment at the 50-mm soil depth, (c) water-filled pore space over time from urine and no urine, and (d) daily tensiometer readings from the gas collars ($n = 4$, ±SEM) from the urine and no urine treatment, at the 20- and 70-mm soil depths. Soil oxygen (O_2) from the urine treatment at the 10-, 50-, and 100-mm soil depths from the sensors with the diffusive head (e) and without the diffusive head (f), and soil O_2 from the no urine treatment at the 10-, 50-, and 100-mm soil depths from the sensors with the diffusive head (g) and without the diffusive head (h). Relative soil gas diffusivity (i) modeled using the SWLR model (Moldrup et al., 2013) where the dashed line marks 0.006. The arrow indicates the time of urine application.

head configuration (Fig. 2e–2h). At the 100-mm depth, the overall mean soil O_2 concentration was lower in the urine compared with the no urine treatment by 27% ($P < 0.001$) when the diffusive head was present, and 17% ($P < 0.001$) when the diffusive head was absent, respectively.

In situ modeled D_p/D_O decreased when θ_v increased (Fig. 2i). Due to the higher average bulk density and lower average porosity in the urine treatment compared with the no urine treatment, the overall mean D_p/D_O was 149% lower in the urine treatment ($P < 0.001$).

Nitrous Oxide Fluxes

Daily average N_2O fluxes were 16 times greater from the urine treatment compared with the no urine treatment ($P < 0.001$), and there was a significant urine × DOE interaction effect ($P < 0.001$, Fig. 3). Differences by DOE were associated with increased N_2O fluxes following urine deposition, surface flooding (DOE 19–22), and heavy irrigation on DOE 52 (Fig. 2 and Fig. 3). Fluxes of N_2O increased as D_p/D_O declined toward ≈ 0.006 and negative N_2O fluxes were also observed on DOE 53 and 54, also at a D_p/D_O value of ≈ 0.006 (Fig. 3, Fig. 4 c). Cumulative N_2O emissions were also greater from the urine treated soil ($P = 0.016$), with an emission factor of 2.1%.

Individually, soil temperature, NO_3^-, NH_4^+, HWC, CWC, soil pH, and conductivity explained ≤17% of the variability in N_2O fluxes when all data were considered, ≤15% of the variability in N_2O fluxes when only the urine data were considered, and ≤9% of the variability in N_2O fluxes when only the no urine data were considered (Table 1).

When all data were pooled, soil O_2 explained ≤59% of the variability in N_2O fluxes (Table 1). All O_2 data, except that measured at 50 mm with the diffusive head, were significantly related with N_2O fluxes. However, negative relationships were observed between O_2 and N_2O fluxes at the 50- and 100-mm depth, and a weaker but positive relationship was observed between O_2 and N_2O fluxes at 10 mm. In only the urine treatment, daily average soil O_2 at the 100-mm soil depth explained ≤69% of the variability of N_2O both with and without the diffusive head (Table 1). Soil O_2 was not significantly related to N_2O fluxes in the no urine treatment fluxes (Table 1).

Volumetric water content, WFPS, and D_p/D_O were significantly related to N_2O fluxes within individual treatments, and when both treatments were pooled (Table 1). The strongest relationship in the urine treatment was between WFPS and N_2O fluxes, but when both treatments were considered together, the strongest relationship was between D_p/D_O and N_2O fluxes (Table 1).

There was an increase in N_2O fluxes associated with the increase in WFPS. However, the high N_2O fluxes occurred over a WFPS range of ≈0.70 to ≈0.80 m^3 m^{-3} (Fig. 4). The increase in N_2O fluxes associated with a decrease in D_p/D_O, showed that the high N_2O fluxes occurred over a relative narrow D_p/D_O range of 0.004 to 0.006 (Fig. 4).

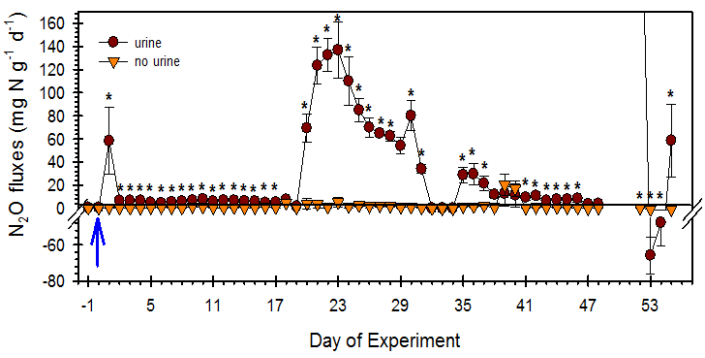

Fig. 3. Daily mean N_2O fluxes (±SEM, $n = 4$) from the urine and no urine treatment over time where the asterisks (*) represents a difference between the treatments at $P < 0.05$. On DOE 52, mean fluxes for the urine treatment go up to 330.3 mg N m^{-2} d^{-1} (±SEM 123.2). The arrow indicates the time of urine application.

DISCUSSION

Nitrous oxide fluxes from the urine treatment in the current study, as well as the emission factor, were similar to previously reported values from poorly drained pasture soils (Luo et al., 2008, Kelliher et al., 2014). Urine application onto a pasture soil induces hydrolysis reactions, which are followed by biological nitrification and denitrification (Baral et al., 2014). These processes can increase inorganic N concentrations and N_2O emissions (Orwin et al., 2010; Taghizadeh-Toosi et al., 2011; Owens et al., 2016). The weak relationships observed between all environmental variables and daily N_2O fluxes in the no urine treatment were due to limited inorganic N substrate availability for N_2O production in that treatment. Higher N_2O emissions from the urine treatment were due to greater substrate availability (Fig. 1). However, soil nutrient concentrations were not correlated with N_2O fluxes (Table 1). Instead, variables pertaining to soil aeration, including soil O_2 measurements, WFPS, and D_P/D_O explained the variability in N_2O fluxes when N was not limiting.

Oxygen concentrations are a proximal controller of the microbial processes responsible for N_2O production (Knowles, 1982; Firestone and Davidson, 1989; Wrage et al., 2001). While soil O_2 often related with N_2O fluxes in the current study, especially in the urine treatment (Table 1), neither of the diffusive head configurations used with the soil O_2 sensor consistently explained N_2O fluxes in all treatments. The results suggest that both O_2 sensor configurations captured changes to bulk soil O_2 concentrations. For there to be a consistent relationship between N_2O fluxes and soil O_2 when substrates are not limited, there must be a measure of soil O_2 that correlates with the physical scale of the microbial processes producing N_2O in the soil. If N_2O is produced in anaerobic microsites, a measure of soil O_2 at the microscale level is needed, and the sensors need to be measuring at the same depth of N_2O production. We suspect that both diffusive head configurations measured an area that was too large, and lacked the resolution to observe O_2 dynamics at the soil macropore–micropore scale (<0.2 μm to approximately >600 μm), which were significant to N_2O production.

Nitrous oxide fluxes increased by 1 to 3 orders of magnitude when soil O_2 decreased following heavy irrigation or surface flooding (Fig. 3). Decreases in bulk soil O_2 can increase nitrifier-denitrification and/or denitrification rates (Goreau et al., 1980; Venterea, 2007; Zhu et al., 2013). Nitrification drove the decline in NH_4^+ from DOE 5 onward in the urine treatment (Fig. 1c), implying that both nitrification and nitrifier-denitrification were potential sources of urine-induced N_2O fluxes during this study. During surface flooding, Ψ read 0 kPa. The soil then drained to a minimum Ψ of −11 kPa, which meant that 26.8 μm or smaller diameter soil pores remained water-filled based on the capillary rise equation, suggesting macropores and some mesopores would have drained but not the micropores (Luxmoore, 1981). These water-filled micropores may have led to the development of anaerobic microsites in the soil following drainage suggesting denitrification was a potential source of N_2O fluxes (Müller et al., 2004) during this time. Further evidence of denitrification after drainage is the disparity between the decline in NH_4^+ concentrations and the increase in NO_3^- concentrations, which indicates NO_3^- was removed from the soil. While this may be partially due to NO_3^- leaching, which was not measured during this study, high N_2O fluxes coupled with high WFPS and low D_P/D_O suggest that some soil NO_3^- was denitrified and emitted as N_2O.

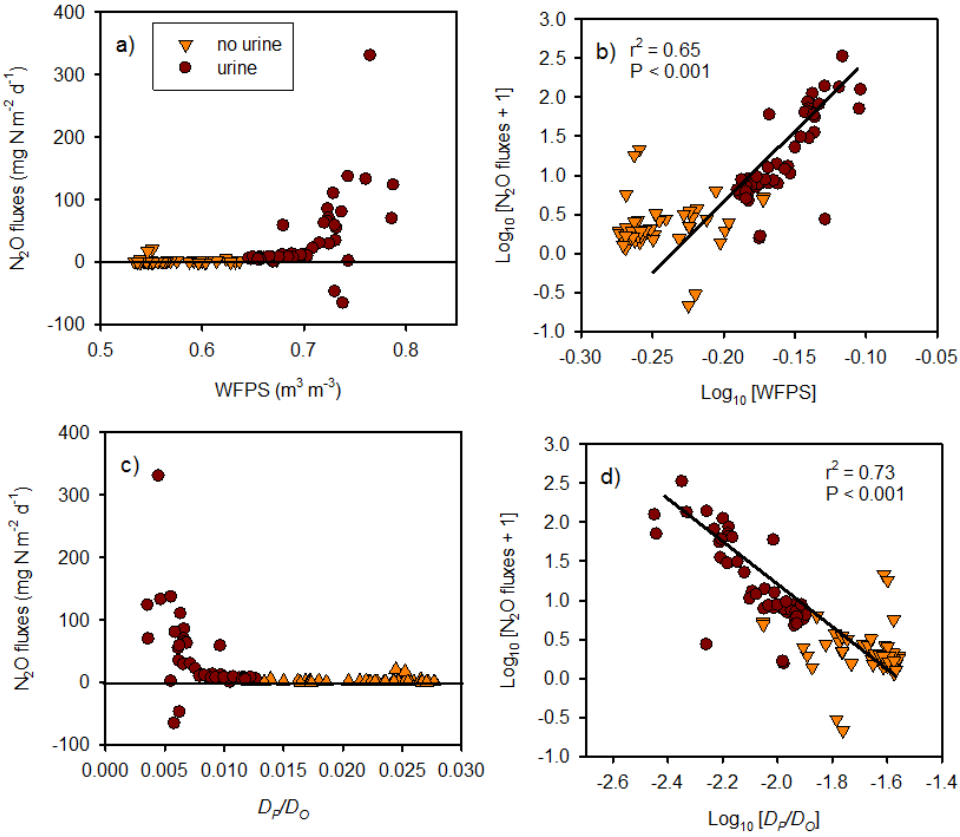

Fig. 4. The daily average N_2O fluxes and (a) water-filled pore space (WFPS) and (c) relative soil gas diffusivity (D_P/D_O), and (b, d) the same data represented with both variables log-transformed, and a linear regression through both the urine and no urine treatment data.

The relationship between WFPS and N_2O fluxes was strongest when only data from the urine treatment were considered, with less variability explained when N_2O fluxes from both treatments were pooled. Conversely, D_P/D_O explained more variability in N_2O fluxes when data from both treatments were considered (Table 1, Fig. 4). This is because WFPS fails to account for the interactive effects of soil bulk density and ψ. The difference in bulk density influenced the strength of the relationship between N_2O fluxes, and WFPS and D_P/D_O. The differences in bulk density and total porosity between the treatments occurred despite the randomization of the treatments. Bulk density was determined at the end of the experiment so this could not be accounted for during the experimental design. The differences in bulk density between the treatments highlight the issue with relying solely on the use of WFPS to explain N_2O fluxes. An integrative measure of the soil

Table 1. P-values and regression analyses relating daily average N_2O fluxes and daily average environmental variables. The direction of the relationship between the two variables is represented as positive by (+) and negative by (-).

Variable	Units	Depth	All data	Urine	No urine
		mm		r^2	
Ammonium	mg N g^{-1} dry soil	0–70	+0.17***	+0.03	+0.03
Nitrate	mg N g^{-1} dry soil	0–70	+0.12**	+0.04	–0.02
Hot water carbon	mg g^{-1} dry soil	0–70	+0.04	+0.07	+0.00
Cold water carbon	mg g^{-1} dry soil	0–70	+0.00	–0.00	+0.00
pH	—	0–70	+0.14**	+0.15*	+0.09
Conductivity	% soluble salts	0–70	–0.04	–0.03	+0.00
Temperature	°C	50	–0.02	+0.00	+0.02
O_2 with diffusive head	%	10	+0.28***	+0.03	+0.00
		50	+0.04	–0.09	–0.03
		100	–0.59***	–0.69***	–0.08
O_2 without diffusive head	%	10	+0.16***	–0.03	–0.06
		50	–0.41***	–0.27***	N/A
		100	–0.56***	–0.43***	–0.04
Volumetric water content	m^3 m^{-3}	50	–0.35***	–0.50***	–0.11*
Water-filled pore space	m^3 m^{-3}	50	+0.65***	+0.82***	+0.11*
Relative soil gas diffusivity	—	50	–0.73***	–0.65***	–0.11*

*Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

physical characteristics that directly affect soil O_2 supply, including air-filled porosity and pore-size distribution is encompassed by D_P/D_O (Moldrup et al., 2013).

Nitrous oxide emissions are episodic, and high N_2O fluxes can occur over a wide range of WFPS values, between 0.60 to 0.90 $m^3\ m^{-3}$ (Dobbie et al., 1999; Davidson et al., 2000; Müller and Sherlock, 2004). This makes it difficult to predict when high fluxes will occur. In the current study, high N_2O fluxes occurred at WFPS values ranging from ≈ 0.70 to ≈ 0.80 $m^3\ m^{-3}$. This variation occurs because WFPS is not quantifying the fraction of the total soil volume that is either water- or air-filled, and so it is not a direct measure of N_2O production/consumption regulation mechanisms (Farquharson and Baldock, 2008, Balaine et al., 2016). The SWLR from Moldrup et al. (2013) to model D_P/D_O includes provisions for variability in soil moisture content, soil texture, and soil compaction (Fig. 4).

Controlled laboratory studies noted N_2O fluxes increased substantially as D_P/D_O lowered to a value of 0.006 (Balaine et al., 2013, 2016). In the current study, N_2O fluxes also increased as D_P/D_O declined to this value from the urine treatment, as there was available substrate. There were also negative N_2O fluxes observed on DOE 53 and 54 (Fig. 3) which occurred below the D_P/D_O value of 0.006 (Fig. 2i) due to the reduction of N_2O to dinitrogen (N_2; Chapuis-Lardy et al., 2007; Balaine et al., 2016). The enzyme responsible for the reduction of N_2O to N_2, nitrous oxide reductase (N_2OR), is highly sensitive to the presence of O_2 (Knowles, 1982; Firestone and Davidson, 1989; Wrage et al., 2001) and takes 33 to 48 h to synthesize after the onset of anaerobic conditions (Smith and Tiedje, 1979; Dendooven and Anderson, 1994). The surface flooding and wet soil conditions between DOE 20 and 35 likely primed the N_2OR pathway. After heavy irrigation between DOE 49 and 51, whereby D_P/D_O was reduced to <0.006, net N_2O consumption occurred on DOE 53 and 54 resulting in negative fluxes (Fig. 3). The role of antecedent moisture conditions on N_2O fluxes, and prior wet conditions priming N_2OR followed by reduction in N_2O fluxes on rewetting, has been noted in previous studies (Smith and Patrick, 1983; Groffman and Tiedje, 1988; Dendooven et al., 1996; Bergstermann et al., 2011; Guo et al., 2014; Uchida et al., 2014; Owens et al., 2016). The concept of a D_P/D_O threshold where maximum N_2O fluxes occur, and N_2O is reduced N_2, may provide opportunities to modify soil management to minimize N_2O fluxes. If the soil were kept aerated with high D_P/D_O, then N_2O production could be limited. Alternatively, lowering D_P/D_O could encourage reduction of N_2O to N_2. Strategies could involve, for example, careful timing irrigation or ensuring soil management reduced soil compaction.

A limitation of D_P/D_O is that it did not capture chemically induced reductions in O_2 from urea hydrolysis after urine deposition (Fig. 2), and where increases in N_2O flux rates occurred. Following urea hydrolysis, the carbonate ions produced are further hydrolyzed. The ensuing re-equilibration of the inorganic-C pools results in carbon dioxide production occurring, and lowering O_2 concentrations, despite D_P/D_O > 0.006. Similar observations were noted in the only other study to investigate this (Owens et al., 2016), where a reduction of soil O_2 and a peak in N_2O emissions were observed about 2 d after urine deposition, without D_p/D_o dropping below 0.006. Relative soil gas diffusivity is a physical parameter that assumes negligible biological or chemical consumption of soil O_2 (Rolston and Moldrup, 2002). Future N_2O studies are needed to explore the potential interactions between D_P/D_O and different permutations of environmental conditions such as substrate supply and pH, and respiration rates, which will influence soil O_2 supply and may modify the D_P/D_O threshold of 0.006 for maximum N_2O production or reduction of N_2O to N_2 (Petersen et al., 2013).

CONCLUSION

In summary, soil O_2 concentrations in a poorly drained pasture decreased with hydrological events such as flooding, or chemical hydrolysis events following urine deposition. Decreases in soil O_2 induced rapid increases in N_2O fluxes. It was found that hydrological variables such as WFPS work well to explain N_2O emissions so long as other soil physical properties do not vary. Relative soil gas diffusivity explained N_2O fluxes better when all treatments were considered because it compensated for how soil properties and soil moisture interacted to influence soil O_2 diffusion. These results demonstrate for the first time an O_2 diffusion threshold for elevated N_2O fluxes in the field, occurring at a value of D_p/D_o ≈ 0.006. Further studies should examine the consistency of this threshold under varying microbial substrate and soil pH conditions.

SUPPLEMENTARY MATERIAL

The supplementary data includes a map of the experimental plot (Supplemental Fig. S1), a graphical representation of the spatial distribution of matric potential within the gas collars after surface flooding (Supplemental Fig. S2), a reference to the equations used during this study (Supplemental Table S1), and a reference to the transformations for statistics (Supplemental Table S2).

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