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The urine patch diffusional area: An important N_2O source?

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ABSTRACT

Urine patches contribute greatly to greenhouse gas emissions within livestock grazed ecosystems. The effective area of a ruminant urine patch comprises the wetted area, the diffusional area and the pasture response area. This study specifically assesses the importance of considering the diffusional area for monitoring urine patch N_2O emissions. Spatial and temporal changes in N_2O emissions and potential drivers of emissions (soil pH, EC, redox potential, dissolved organic carbon and nitrogen, NO_3^- and NH_4^+) were measured in sheep urine amended Eutric Cambisol mesocosms, maintained at 50% or 70% water-filled pore space (WFPS). At 70% WFPS, over 10 weeks, the emission factor (EF) was greater when considering the wetted area plus a 9 cm diffusional area (EF = 2.75 ± 0.72% of applied N) than when considering the wetted area alone (EF = 1.44 ± 0.30% of applied N); differences were not statistically significant at 50% WFPS. Redox potential, total extractable N and WFPS contributed significantly to the observed variation in daily N_2O fluxes from the urine patch. We conclude that the urine patch diffusional area is an extremely important source of emissions from urine patches. This has implications when measuring EFs, as the lateral diffusion of solutes may be restricted by chamber walls resulting in an underestimate of N_2O emissions, particularly at higher soil moisture contents. Site-specific assessments of the urine patch diffusional area should be made, and accounted for, prior to monitoring emissions and calculating emission factors from urine patches applied within chambers.

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1. Introduction

Grazing returns of excreta to pasture soils are estimated to account for 40% of the total (direct and indirect) nitrous oxide (N_2O) emissions from animal production systems, globally (Oenema et al., 2005). Additions of labile carbon (C) and nitrogen (N) to soil in the form of urine (van Groenigen et al., 2005) fuel the major microbial N_2O producing processes of nitrification and denitrification, creating "hot spots" and "hot moments" for emissions within pastures (McClain et al., 2003; Groffman et al., 2009). The current default IPCC emission factors used in national inventories for excretal deposition to soils are 1% and 2% of deposited N for sheep and cattle, respectively (IPCC, 2006); yet, this Tier 1 approach lacks accuracy as it fails to account for variation in N_2O emissions due to environmental, edaphic or management related factors (Skiba and Smith, 2000; Skiba et al., 2012; Buckingham et al., 2014).

Variability in N_2O emissions from urine patches can arise due to differences in urine composition, the amount of N excreted and the volume and frequency of urine events (Dijkstra et al., 2013). Additionally, microbial N_2O production and consumption processes depend on several interacting environmental controls (Bouwman et al., 2013) such as N supply, soil temperature, soil moisture, oxidation–reduction potential (ORP), the availability of labile organic compounds, soil type, soil pH and climate (Skiba and Smith, 2000; Butterbach-bahl et al., 2013). Urine patches offer potential for emission reductions and improvements of nitrogen use efficiency (NUE) within the agricultural sector, yet a greater understanding of the spatial and temporal variability in N_2O emissions from urine patches (at several scales of magnitude) is required to improve emission estimates and provide information for emission reduction strategies, such as the use of nitrification inhibitors.

The urine patch "wetted area", where urine is directly voided, has been distinguished from the "effective area" which incorporates the diffusive edge of solutes and the plants able to access this pool of nutrients via root extension (Selbie et al., 2015). It is suggested that the effective area actually comprises the "wetted area", the "diffusional area" and the "pasture response area" in order to distinguish between these regions. The pasture response area can extend to twice the initial wetted area (Doak, 1952), however, the diffusive edge of urinary N has been shown not to

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exceed 20 cm beyond the initial wetted area in three soil types (granitic Brunisol, Neoluvisol and Calcosol; Decau et al., 2003). The diffusional area may vary with urinary volume, solute concentrations, soil texture (relating to tortuosity and cation exchange capacity), soil moisture content, topography and time. The pasture response area is likely to be dependent on the magnitude of the diffusional area, the vegetation type and the corresponding root architecture.

Dennis et al. (2011) maintain that for investigating soil nutrient cycling processes, the wetted area is more important than the pasture response area, however, the diffusive edge of solutes may also be important to consider. An overestimation of NO_3^- leaching losses from urine applied to lysimeters may occur if no room is allowed for the diffusional area (Selbie et al., 2015). Similarly, underestimations of N_2O emissions may occur if the urine patch diffusional area is not considered (e.g. applying urine uniformly to the entire area beneath a static chamber for gas flux measurements), due to chamber walls preventing the lateral movement of solutes into surrounding soil. Koops et al. (1997) have demonstrated that N_2O losses from the diffusive zone of an artificial urine patch, applied to a peat grassland, can reach the same order of magnitude as the area where urine was directly applied.

This experiment was predicated on the need to assess the importance of the urine patch diffusional area for different solutes, N_2O-regulating soil properties (e.g. dissolved organic C and ORP) and the accuracy of N_2O emission measurements. Eutric Cambisol mesocosms, amended with sheep urine, were established in order to 1) assess the spatial and temporal changes in soil properties in the wetted and diffusional area, 2) identify those soil properties which are key drivers of N_2O emissions under two moisture regimes, and 3) compare the N_2O emission factor from the wetted area with that of the wetted and diffusional areas combined. Two soil moisture regimes (50% and 70% WFPS) were included, as this was considered important with regards to both N_2O production processes, emissions and the spatial distribution of solutes within the urine patch.

2. Materials and methods

2.1. Soil sampling and analysis

Independent replicate ($n = 4$) samples of a Eutric Cambisol (0–10 cm) were collected from a sheep-grazed, fertilised grassland located at the Henfaes Agricultural Research Station, Abergwyngregyn, North Wales (53°14′N, 4°01′W). After collection the soil was sieved through a 10 mm mesh. Soil moisture content was determined by oven drying (105 °C, 24 h), and organic matter was determined by loss-on-ignition (450 °C, 16 h; Ball, 1964). Soil pH and electrical conductivity (EC) were measured using standard electrodes submerged in 1:2.5 (w/v) soil-to-distilled water suspensions. The oxidation–reduction potential (ORP) was measured directly in the soil using an ELIT 31C ORP combination electrode (EA Instruments Ltd., London, UK) connected to a mV reader.

Total soil C and N were determined on oven-dried, ground soil using a TruSpec® Analyzer (Leco Corp., St. Joseph, MI). Within 24 h of soil collection, 1:5 (w/v) soil-to-0.5 M K_2SO_4 extractions were performed; the total dissolved C and N (mineral and organic) in the resulting extracts were determined with a Multi N/C 2100S Analyzer (AnalytikJena, Jena, Germany). Nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (P) within the 0.5 M K_2SO_4 extracts were measured by the colorimetric methods of Miranda et al. (2001), Mulvaney (1996) and Murphy and Riley (1962), respectively. The cations (Na, K and Ca) within 1:5 (w/v) soil-to-1 M NH_4Cl soil extracts were measured using a Sherwood Model 410 Flame Photometer (Sherwood Scientific Ltd, Cambridge, UK). A summary of the soil characteristics is provided in Table 1.

2.2. Urine collection and analysis

Welsh mountain ewes ($n = 5$) were fed a diet of freshly cut grass (*Lolium perenne* L.; 80%) and white clover (*Trifolium repens* L.; 20%). Ewes were housed in individual pens on plastic slatted flooring designed for sheep (Rimco Ltd., Yorkshire, UK), with collection trays located beneath the flooring for urine collection. Urine samples were centrifuged (4000 *g*; 10 min) and immediately frozen (−20 °C) until required, to minimize losses of N. The urine collected from five replicate sheep was bulked, in order to provide sufficient urine of a homogenous composition for experimental use. The total dissolved C, total dissolved N, pH, EC, NO_3^-, NH_4^+, P and cations were determined directly within the urine samples, as described for the soil samples. The urea content of the urine was determined via the method of Orsonneau et al. (1992). A summary of the urine characteristics is provided in Table 2.

2.3. Soil mesocosm preparation, treatment application and sampling regime

Briefly, 5 kg of fresh soil ($n = 4$) was weighed into polypropylene trays (internal height: 11 cm, internal length: 35.5 cm, internal width: 26.5 cm), and repacked to a depth of 5 cm, resulting in a fresh bulk density of 1.10 g cm^{-3}. Bare pasture soil mesocosms were used, in order to gain a mechanistic understanding of processes that occur at the soil-urine interface, in the absence of competing factors (e.g. plant removal of nutrients, NO_3^- leaching). The soil mesocosms were wetted with distilled water using a fine mist sprayer to facilitate even coverage to achieve 50% and 70% water-filled pore space (WFPS), where the initial starting weights were recorded. The mesocosms were pre-incubated in a greenhouse maintained at 20 °C for 1 week before application of urine, to ensure any observed effects were not due to soil disturbance (e.g. sieving). Soil mesocosms were maintained under these conditions for the duration of the experiment, and rewetted weekly with distilled water to achieve initial starting weights using a fine mist sprayer. Urine (36 ml) was applied in a strip (24 cm × 3 cm) across the width of the mesocosms, resulting in an equivalent urine-to-soil surface area for an average sheep urine deposition (150 ml over 300 cm^2; Doak, 1952). This urine application resulted in an equivalent total N loading rate of ca. 200 kg N ha^{-1}, where other

Table 1
Properties of the Eutric Cambisol used to fill soil mesocosms. Values represent means ± SEM ($n = 4$) and results are reported on a dry weight basis.

Eutric Cambisol properties	
Texture	Sandy clay loam
Field wet bulk density (g cm^{-3})	1.57 ± 0.05
Moisture content (%)	21.9 ± 1.00
pH	6.91 ± 0.17
EC (μS cm^{-1})	65.4 ± 5.14
ORP (mV)	368 ± 10.3
Total C (%)	3.29 ± 0.22
Total N (%)	0.26 ± 0.15
C:N ratio	13.0 ± 0.99
Dissolved organic C (mg C kg^{-1})	102 ± 8.66
Total dissolved N (mg N kg^{-1})	13.8 ± 1.89
Extractable NO_3^- (mg N kg^{-1})	2.28 ± 0.32
Extractable NH_4^+ (mg N kg^{-1})	0.41 ± 0.24
Extractable P (mg P kg^{-1})	9.27 ± 0.91
Exchangeable Na (mg kg^{-1})	54.0 ± 5.98
Exchangeable K (mg kg^{-1})	181 ± 21.5
Exchangeable Ca (g kg^{-1})	1.09 ± 0.05

Table 2
Properties of sheep urine, applied to Eutric Cambisol mesocosms. Values represent means ± SEM ($n = 3$), where replicates are analytical replicates of urine combined from 5 individual sheep.

Urine properties	
pH	9.15 ± 0.01
EC (mS cm^{-1})	14.1 ± 0.20
Dissolved organic C (g C l^{-1})	6.03
Total N (g N l^{-1})	3.86
Urea (g N l^{-1})	2.71 ± 0.61
NH_4^+ (mg N l^{-1})	129 ± 5.30
NO_3^- (mg N l^{-1})	1.08 ± 0.02
P (mg P l^{-1})	11.6 ± 0.34
Na (mg l^{-1})	692 ± 1.59
K (g l^{-1})	4.00 ± 0.05
Ca (mg l^{-1})	48.4 ± 0.54

studies investigating sheep urine patches have used N application rates of ca. 300 kg N ha^{-1} (Haynes and Williams, 1993; Moir et al., 2013). Soil was sampled at increasing distances away from the centre of the urine patch, along a horizontal diffusional gradient. Briefly, 0–3 cm represents the centre of the urine patch, with further sampling conducted at 3–6, 6–9, 9–12, 15–18 and 27–30 cm away from the direct area of urine application, hereafter referred to as zones A, B, C, D, E and F, respectively (see Fig. 1). The final sampling distance (27–30 cm; Zone F) was considered to be the control, as we hypothesised that this zone would receive no effect from the urine application. Samples were taken from three parallel mesocosms for each replicate ($n = 4$), to provide enough

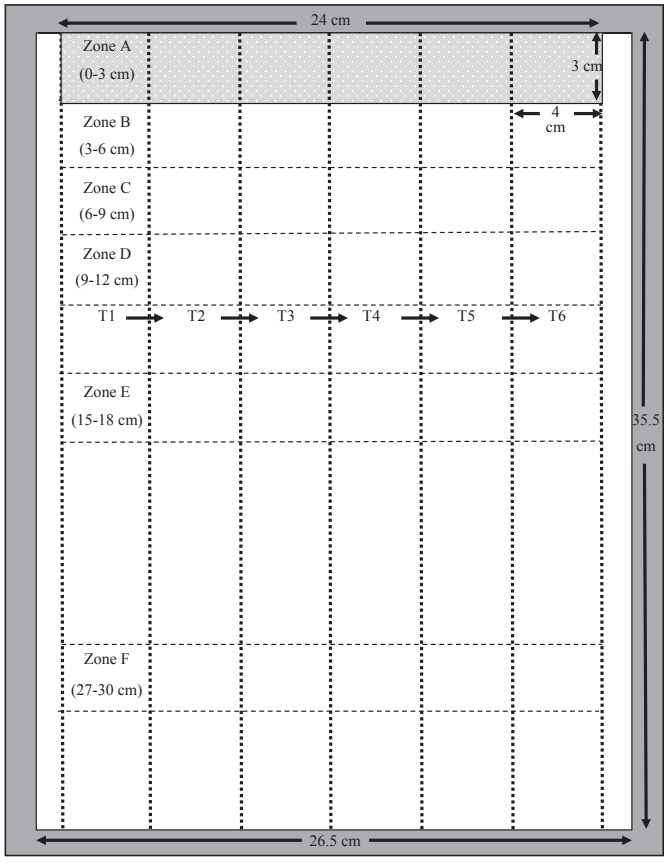

Fig. 1. Aerial schematic view of the Eutric Cambisol mesocosms, repacked to a depth of 5 cm. The shaded region represents the area of direct urine application, labelled rows display sampling regions and T1–T6 represent successive sampling time points, where further time points were sampled from parallel mesocosms.

soil sampling points for the duration of the experiment (10 weeks). Sampling was conducted 3 times a week for the first two weeks and once a week thereafter, until the end of the experiment.

2.4. Monitoring nitrous oxide emissions and changes in soil properties

Soil from each sampling zone (see Fig. 1; ca. 53 g) was removed, weighed and placed into gas-tight polypropylene containers fitted with a silicone Suba Seal® (VWR International, Lutterworth, UK). Gas samples (20 ml) were taken at 0 and 60 min following container lid closure and were stored in pre-evacuated 20 ml glass vials. The linearity of gas build up within the containers was checked by taking four gas samples (0, 20, 40 and 60 min) on each sampling day in zone A, as this was expected to have the highest emissions. Linearity of gas build up within containers was met ($R^2 > 0.95$) on 29 and 39% of occasions at 50% and 70% WFPS, respectively. Chadwick et al. (2014) state that non-linear fluxes can arise during occasions of no significant net flux. Our data supports this in that where the linear assumption was violated, the fluxes tended to be minimal and during periods of high emissions the data fitted well to the linear model. We, therefore, consider this acceptable as a poorly fitted linear model at periods of non-significant fluxes is unlikely to cause excessive bias in overall emission estimates.

Gas samples were analysed for N_2O with a Varian 450 GC (Agilent Technologies, Santa Clara, CA), fitted with a ^{63}Ni electron capture detector (ECD), where the column, injector and detector temperatures were 50, 100 and 330 °C, respectively. After gas sampling, the soil pH, EC and ORP were measured using the methods described previously. Total dissolved organic C, total dissolved N, NH_4^+ and NO_3^- were measured following extraction of the excavated soils with 0.5 M K_2SO_4, as described previously (Section 2.1).

2.5. Statistical analysis

All statistical analyses were performed in either SPSS Statistics 20.0 (IBM UK Ltd, Portsmouth, UK) or Minitab 17.1.0 (Minitab Inc., State College, PA). Spatial and temporal differences between soil pH, EC and ORP in Zones A to E were compared to the control (zone F), and differences between soil incubated at 50% and 70% WFPS were determined via one-way ANOVA, followed by Fisher's LSD post-hoc test. Normality and homogeneity of variance assumptions were tested on log-transformed data using Shapiro Wilk and Levene's test, respectively and significance was determined at the $p < 0.05$ level. Cumulative N_2O emissions were determined by integration using the trapezoidal rule, and differences between the cumulative emissions in each zone were compared via one-way ANOVA, as above. The N_2O emission factor (EF) for each treatment was calculated via the following equation:

$$EF = \text{treatment } N_2O\text{-N} - \text{control } N_2O\text{-N}/\text{Total N applied} \times 100\%. \quad (1)$$

Differences in emission factors between Zone A and the sum of Zones A–D were compared via one-way ANOVA, as above.

In order to determine the amount of variation in N_2O emissions explained by measured soil parameters, multiple linear regression was used. Data were ln transformed where the distribution was improved by the transformation, in order to approximate normality. During exploratory data analysis, best subset's regression was used; this procedure compares all models for a given set of predictor variables (pH, EC, ORP, Total N, DOC, NO_3^-, NH_4^+, and WFPS), and provides summary statistics (R^2, adjusted R^2, predicted

R^2, S and Mallows' Cp) for the best two candidate models with increasing numbers of fixed predictor variables. The number and type of predictor variables were chosen based on the criteria of having a high R^2, adjusted R^2 and predicted R^2, a low value of S (which represents the standard deviation of the error term) and Mallow's Cp values close to the number of terms in the model. The best candidate models were then inputted into the normal multiple regression regime in Minitab.

3. Results

3.1. Urine patch pH, EC and ORP

Spatial and temporal variation in soil pH, EC and ORP was observed in the Eutric Cambisol following sheep urine application (Fig. 2). In Fig. 2, data are only displayed for the zones A (Fig. 2a, e and i), B (Fig. 2b, f and j), C (Fig. 2c, g and k) and F (Fig. 2d, h and l), as data for zones D (Supplementary Information Fig. 1a, c and e) and E (Supplementary Information Fig. 1b, d and f) were similar to zone F. The initial soil pH was 6.87 ± 0.15 (50% WFPS) and 6.84 ± 0.16 (70% WFPS; Fig. 2d), which increased immediately to pH 8.58 ± 0.08 (50% WFPS) and 8.21 ± 0.14 (70% WFPS) following urine deposition to zone A (Fig. 2a). During the first 3 days of incubation the pH in zone A (50% WFPS) was more alkaline ($p < 0.01$) than that of the control (zone F; Fig. 2d). By day 7 the pH had returned to a similar ($p > 0.05$) value to zone F, however, after 10 days the pH was more acidic ($p < 0.01$) than soil previously unaffected by urine, and remained so for the duration of the experiment. Differences in pH, in comparison to the control, only extended to zone B (Fig. 2b); this zone was more alkaline ($p < 0.05$) in comparison to zone F (Fig. 2d) immediately after urine deposition and returned to the control value faster (after 2 days) than the immediate area of application (Fig. 2a). After 4 days, zone B was more acidic ($p < 0.05$) than that of the control for the duration of the experiment. The spatial and temporal changes in pH were generally very similar at 50% and 70% WFPS.

The Eutric Cambisol had an EC of 68.7 ± 6.1 and 74.8 ± 7.2 μS cm^{-1} (50% and 70% WFPS, respectively) without urine application (Fig. 2h), which increased to 367 ± 8.5 and 360 ± 30.8 μS cm^{-1} (50% and 70% WFPS, respectively) immediately following urine application (Fig. 2e). The EC of Zones A and B (Fig. 2e and f, respectively) of the 50% WFPS treatment was greater ($p < 0.001$) for all sample points in comparison to zone F (Fig. 2h). A greater EC was also observed in zones C (Fig. 2g) and D (Supplementary information Fig. 1c), however, these only became significant ($p < 0.05$) after 4 and 7 days, respectively, indicating a temporal delay in the lateral movement of

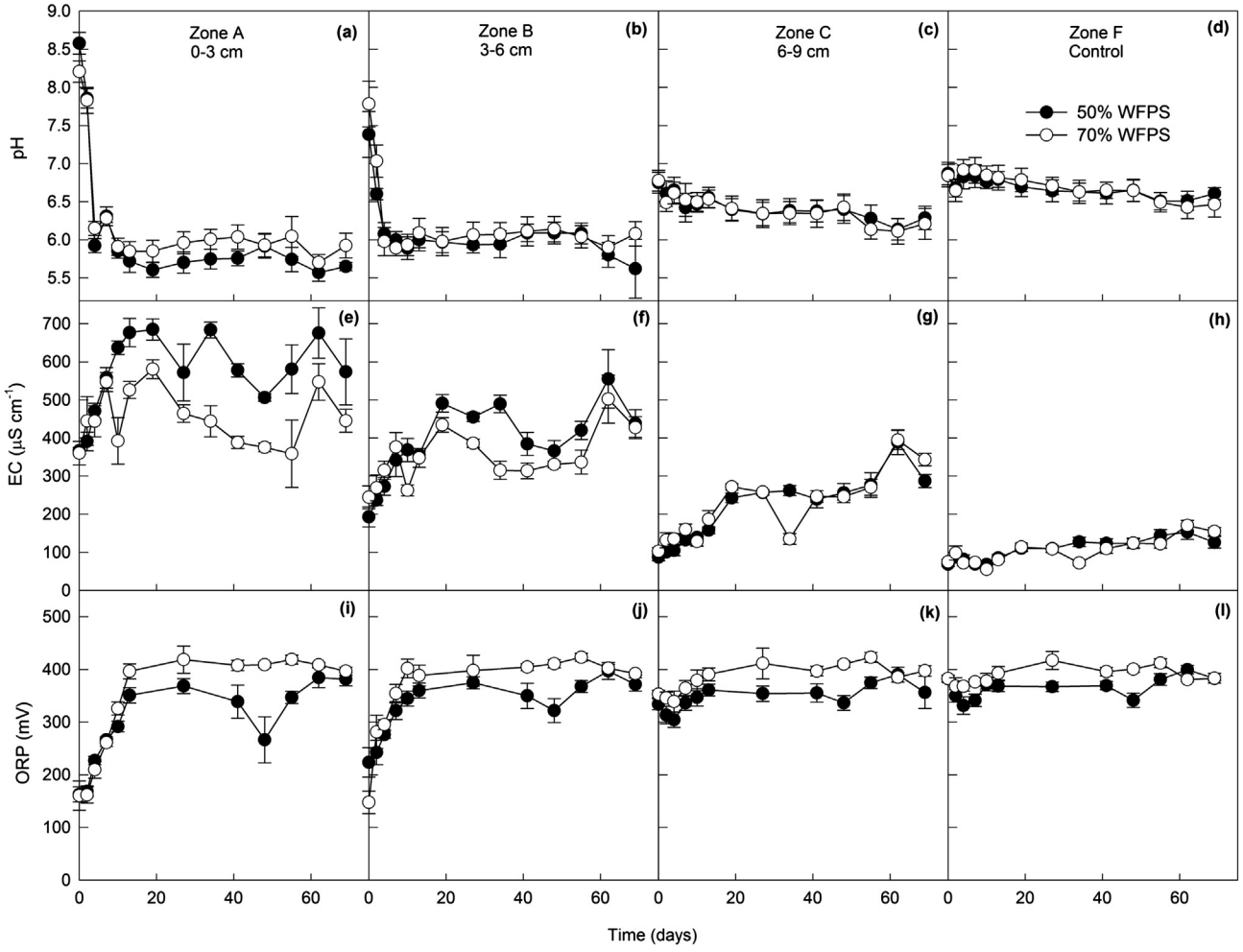

Fig. 2. Changes in soil pH (panels a, b, c and d), electrical conductivity (EC; panels e, f, g and h) and oxidation reduction potential (ORP; panels i, j, k and l) following sheep urine application to a Eutric Cambisol, maintained at either 50% or 70% water-filled pore space (WFPS), and sampled at increasing distances away from the direct area of application (Zone A, 0–3 cm: panels a, e and i; Zone B, 3–6 cm: panels b, f and j; Zone C, 6–9 cm: panels c, g and k; Zone F, control: panels d, h and l). Symbols represent means ± SEM ($n = 4$). Figure legend applies to all panels and text on the top row of panels applies to each respective column.

solutes. No difference ($p < 0.05$) in EC was observed in the 50% WFPS soil from zone E (Supplementary information Fig. 1d) in comparison to the control. At both 50% and 70% WFPS in zones A and B (Fig. 2e and f, respectively), the EC was immediately higher than the control (Fig. 2h), remaining so for the duration of the experiment; the EC in zone C (Fig. 2g) was immediately higher than the control (Fig. 2h) at 70% WFPS, however, at 50% WFPS it took 4 days for the EC in zone C to be greater than the control. After 10 days, the EC in zone D at 70% WFPS was greater ($p < 0.001$) than the control (Fig. 2h), and remained so for the duration of the experiment.

Following application of urine the ORP in zone A at 50% and 70% WFPS (163 ± 14 and 160 ± 27 mV, respectively; Fig. 2i) was lower ($p < 0.001$) than zone F (382 ± 6 and 383 ± 6 mV at 50% and 70% WFPS, respectively; Fig. 2l) and increased to control levels after 13 days. The ORP in zone B (Fig. 2j) at 50% and 70% WFPS (224 ± 28 and 148 ± 21 mV, respectively) was also lower ($p < 0.01$) than zone F (Fig. 2l), and increased to that of the control after one week following urine deposition. The ORP was lower ($p < 0.05$) at 50% WFPS in zone A as opposed to the 70% WFPS treatment (Fig. 2i), during days 13–48 after urine application.

3.2. Spatial and temporal dynamics of nitrogen and carbon in the urine patch

Nitrogen and carbon dynamics following sheep urine deposition to a Eutric Cambisol are shown in Fig. 3, which includes results for total extractable N (Fig. 3a, b, c and d), NH_4^+ (Fig. 3e, f, g and h), NO_3^- (Fig. 3i, j, k and l), total DOC (Fig. 3m, n, o and p) and N_2O emissions (Fig. 3q, r, s and t), as no major differences were observed in comparison to the control for Zones D (Supplementary Information, Fig. 2a, c, e, g and i) and E (Supplementary Information, Fig. 2b, d, f, h and j), only Zones A (Fig. 3a, e, i, m and q), B (Fig. 3b, f, j, n and r), C (Fig. 3c, g, k, o and s) and F (Fig. 3d, h, l, p and t) are displayed. Most of the applied urine-N was in the form of urea (Table 2), which quickly hydrolysed in the soil. This resulted in immediately high soil NH_4^+ concentrations in zone A (50% WFPS; Fig. 3e), which peaked at the first sample point at 240±44 mg NH_4^+-N kg^{-1} soil DW. In Zone A of the 70% WFPS soil, the NH_4^+ was high at the first sample point (93±25 mg NH_4^+-N kg^{-1} soil DW) but peaked 3 days following urine application at 140±45 mg NH_4^+-N kg^{-1} soil DW. The NH_4^+ did not diffuse far in the soil and only minor amounts were measured further than zone B.

As nitrification proceeded, the NH_4^+ concentration decreased and a concomitant increase in NO_3^- was observed. In zone A, the NO_3^- concentration peaked 19 days following urine application (Fig. 3i), where concentrations were higher in the soil incubated at 50% WFPS (268±9 mg NO_3^--N kg^{-1} soil DW) than the soil incubated at 70% WFPS (207±5 mg NO_3^--N kg^{-1} soil DW). Following the rapid increase in NO_3^- concentration, a decreasing trend was observed over 19–41 days following urine application. After 41 days, the NO_3^- concentration increased at similar rates to that of the control (zone F; Fig. 3l) in all zones, but the concentration remained higher than the control in zones A–D. The NO_3^- diffused further than the NH_4^+, and a temporal delay in the diffusion of NO_3^- into outer zones was observed.

The major peaks in N_2O emission occurred in zones A and B during the first 20 days following urine application (Fig. 3m and n, respectively), while NO_3^- concentrations were still increasing. Emissions peaked immediately following urine deposition, where 882 ± 190 and 1825 ± 774 μg N_2O-N m^{-2} h^{-1} were emitted from zone A at 50% and 70% WFPS, respectively. The greatest emissions observed in zone B were also on the day of urine application, and were lower than that of zone A at 431 ± 146 and 1048 ± 531 μg N_2O-N m^{-2} h^{-1} at 50% and 70% WFPS, respectively. Another peak in emissions was observed 13 days following urine deposition in zones A and B, and this was more pronounced in the soil incubated at 70% WFPS. After 20 days following urine application, no major N_2O emissions were measured, yet NO_3^- levels decreased beyond this point.

During the first day of urine application the concentration of total DOC in the control soil was 66.6 ± 8.6 and 79.1 ± 9.6 mg C kg^{-1} soil DW at 50% and 70% WFPS, respectively (Fig. 3t). Due to the presence of labile C within the sheep urine and the potential for urine to solubilise soil organic matter, the concentration of DOC in soil within zone A was 161 ± 12.0 and 169 ± 27.0 mg C kg^{-1} soil DW at 50% and 70% WFPS, respectively (Fig. 3q). This rapidly decreased over the course of one week following urine application to 54.0 ± 15.8 and 60.4 ± 10.4 mg C kg^{-1} soil DW in zone A at 50% and 70% WFPS, respectively. A similar trend was observed in zone B (Fig. 3r), but at lower initial concentrations (106 ± 1.9 and 131 ± 7.6 mg C kg^{-1} soil DW at 50% and 70% WFPS, respectively) indicating rapid movement and/or solubilisation of DOC into this zone.

3.3. Cumulative N_2O emissions and urine patch emission factors

The cumulative N_2O emissions within each zone are displayed in Fig. 4. Greater cumulative N_2O emissions ($p < 0.01$) were only observed within zone A at 50% WFPS, with respect to the control treatment. At 70% WFPS both zone A ($p < 0.01$) and zone B ($p < 0.05$) emitted greater amounts of N_2O in comparison to the control. Greater cumulative emissions were observed in the 70% WFPS treatment in comparison to the 50% WFPS in zones A, B and C ($p < 0.01$) but not D, E and F ($p > 0.05$). The emission factor when only considering zone A was greater ($p < 0.01$) in the soil maintained at 70% WFPS (1.44 ± 0.30% of applied N over 69 days) as opposed to the same soil maintained at 50% WFPS (0.44 ± 0.06% of applied N over 69 days). The N_2O emission factor at 70% WFPS was greater ($p < 0.05$) when summing zones A–D (2.75 ± 0.72% of N applied over 69 days) than when only considering zone A (1.44 ± 0.30% of applied N over 69 days); this was not the case in the 50% WFPS soil, where accounting for the diffusive area had no effect on the N_2O emission factor.

3.4. Multiple regression analysis

The data included in the multiple regression analysis were those from zones A and B as these regions emitted most N_2O; in addition, the control data (zone F) were also included. The results of the best subset's regression (see Table 3) revealed a potential model containing three predictor variables for individual 'daily' fluxes which fitted the selection criteria well. Increasing the number of variables beyond this did not substantially improve the predictive power of the model, therefore, in order to avoid over fitting, only three variables were used. The model contained total extractable soil N, ORP and WFPS as predictor variables (R^2: 0.56; adjusted R^2: 0.55, predicted R^2: 0.53; Mallows' Cp: 4.2; S: 0.72). The parameters were then entered separately into the least squares multiple regression model. The results of the regression are presented in Table 3 and the regression equations for 50% and 70% WFPS were:

50% WFPS: ln N_2O 'daily' flux $= 14.65 - 1.879 \ln \text{ORP} + 0.2364 \ln \text{Total N}$ (2)

70% WFPS: ln N_2O 'daily' flux $= 15.11 - 1.879 \ln \text{ORP} + 0.2364 \ln \text{Total N}$ (3)

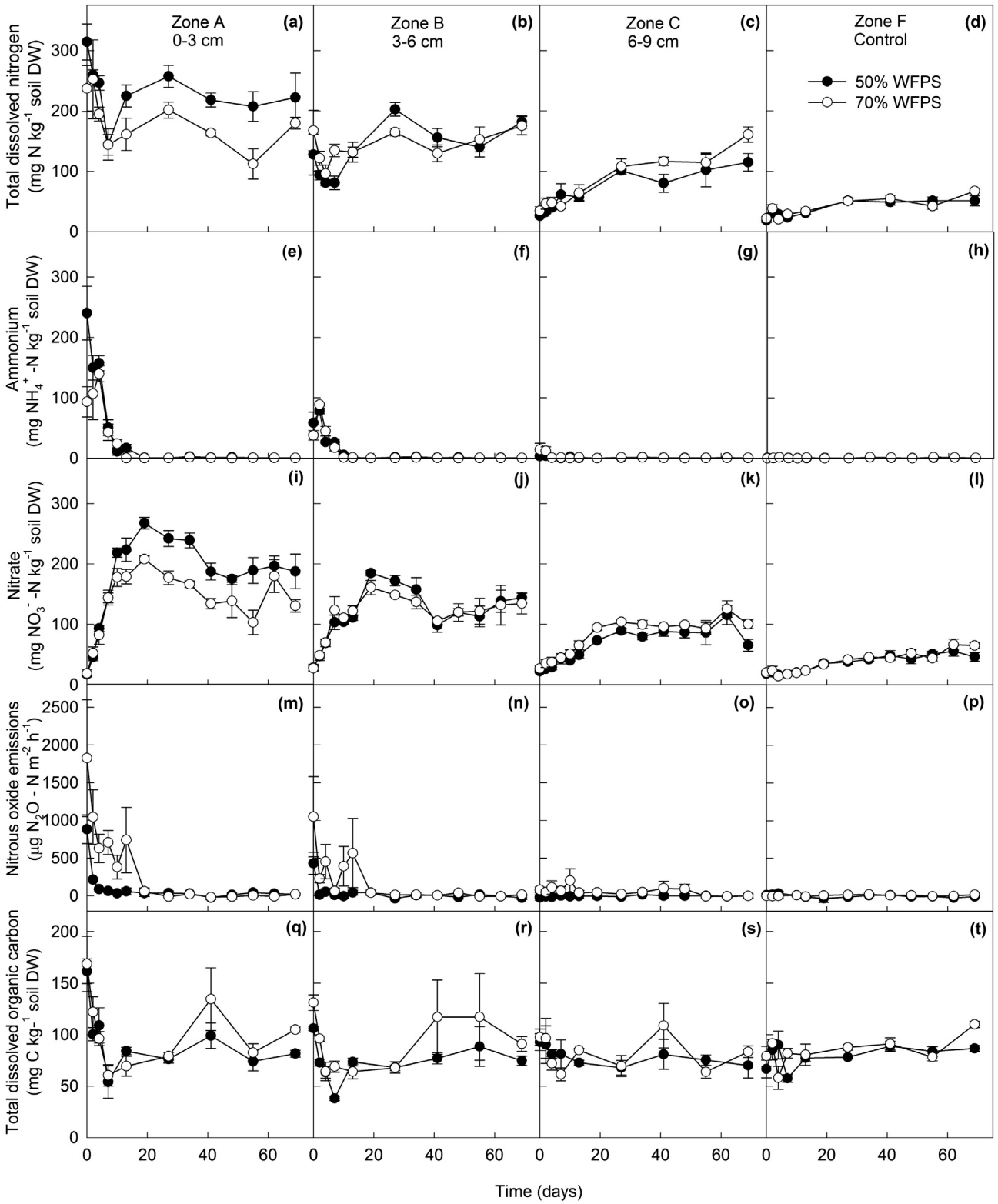

Fig. 3. Soil extractable total dissolved nitrogen (panels a, b, c, d), ammonium (panels e, f, g, h), nitrate (panels i, j, k and l), nitrous oxide emissions (panels m, n, o and p) and extractable dissolved organic carbon (panels q, r, s and t) following sheep urine application to a Eutric Cambisol, maintained at either 50% or 70% water-filled pore space (WFPS), and sampled at increasing distances away from the direct area of application. Symbols represent means ± SEM ($n = 4$). Figure legend applies to all panels and text on the top row of panels applies to each respective column.

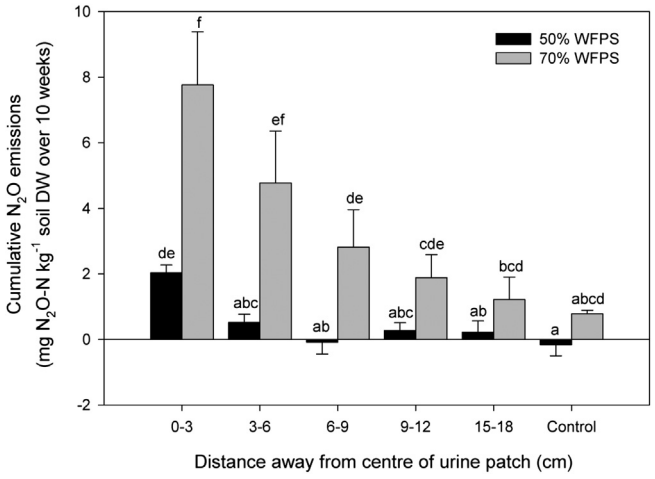

Fig. 4. Cumulative nitrous oxide emissions following sheep urine application to a Eutric Cambisol, maintained at either 50% or 70% water-filled pore space (WFPS) and sampled at increasing distances away from direct area of urine application. Bars represent means ± SEM ($n = 4$) and different letters indicate significant differences (Fisher's LSD, $p < 0.05$).

4. Discussion

4.1. Within-urine patch spatial and temporal variability

The first objective of this study was to determine changes in soil chemical properties of a sheep urine patch both spatially and temporally, and assess how this may influence N_2O production. Within the first few hours of sheep urine application the soil pH and EC, total extractable N and NH_4^+, N_2O emissions and total DOC had increased while the ORP had decreased in soil directly wetted by the urine. The same trend was observed in zone B, but to a lesser extent, indicating the spread of solutes by mass flow into the region of soil adjacent to the wetted area. The addition of urine to soil may have increased DOC within the soil, due to solubilisation of soil organic matter or lysis of microbial cells by the applied urine (Monaghan and Barraclough, 1993; Ambus et al., 2007; Lambie et al., 2012). The greatest spatial effects were observed for extractable total N and NO_3^-, and EC, whereas all other urine induced soil changes mainly occurred in zones A and B. This is likely to be due to rapid diffusion of NO_3^- and other ions present within ruminant urine through the soil.

In this study the soil pH increased by ca. 2–2.5 pH units, which can be attributed to the high carbonate content of the urine and to alkaline products generated during urea hydrolysis (van Groenigen et al., 2005; Carter, 2007). Soil pH returned to control levels after 7 days, following which it remained more acidic than the control due to the acidifying processes of ammonification, nitrification and urea hydrolysis (Bolan et al., 1991). The spatial changes in pH within a urine patch may be important for within-patch variability and source partitioning of N_2O, as the N_2O product ratios of nitrification, denitrification and dissimilatory NO_3^- reduction to NH_4^+ (DNRA) are all influenced by soil pH (Stevens et al., 1998; Šimek and Cooper, 2002; Mørkved et al., 2007). The pH optimum of nitrification is 6.5–8.0 (Šimek and Cooper, 2002), and these conditions are generally met when ruminant urine is deposited to agricultural soils. In our study the pH dropped below the optimum for nitrification, to values as low as pH 5.7. Denitrifying enzyme activity has been shown to be highest at, or near, the soils natural pH (Šimek et al., 2002), however, reductions in pH (from 6.82 ± 0.40 to 5.52 ± 0.48) over a 10 month period increased the N_2O/N_2 product ratio of denitrification (Čuhel et al., 2010). In either case, denitrification activity and N_2O release via denitrification are likely to increase once the initial high pH within the urine patch has subsided, whereas N_2O release from nitrification may occur immediately following urine deposition. This may explain the split peak observed in N_2O emissions in this (see Fig. 3m and n) and other studies involving urine deposition (e.g. Di and Cameron, 2012; Boon et al., 2014). Alternatively, the second peak may reflect emissions from a more recalcitrant N containing urine constituent. Advances in the use of stable isotopes, molecular techniques (Bateman and Baggs, 2005; Wrage et al., 2005; Baggs, 2008, 2011) and quantum cascade laser based absorption spectroscopy for the measurement of N_2O isotopomers (Waechter et al., 2008; Decock and Six, 2013) will facilitate our understanding of the source partitioning of N_2O following urine deposition to soils.

The differences observed between EC in the different zones revealed a faster lateral movement of solutes at 70% compared to 50% WFPS. This indicates that dilution, mixing and diffusion within soils of a high moisture content may lead to a faster movement of NO_3^- to anaerobic denitrifying microsites within the soil (Luo et al., 1999), where diffusion of soluble carbon may then become limiting (Myrold and Tiedje, 1985). For the majority of the incubation, the soils could be described as moderately oxidized, however, the urine influenced soil was poorly oxidised at the beginning of the study; this may be due to a localised increase in biological oxygen demand for degradation of the added urinary C (Azam et al., 2002; Baral et al., 2014). Interestingly, the ORP was lower ($p < 0.05$) at 50%

Table 3
Multiple regression analysis with ln N_2O as the dependent variable, ln total extractable soil nitrogen (TN) and ln oxidation reduction potential (ORP) as predictor variables and water-filled pore space (WFPS; 50% and 70%) as a categorical predictor variable.

Term	Unstandardized coefficients		Standardized coefficients	*T*	*p*	VIF[a]
	B	SEM	Beta			
(Constant)	5.05	0.09	–	57.0	0.00	–
TN	0.24	0.08	0.19	2.89	0.00*	1.31
ORP	−1.88	0.21	−0.61	−9.09	0.00*	1.32
WFPS (70% vs. 50%)	0.46	0.13	0.21	3.65	0.00*	1.00
Model summary	*F*	R^2	R^2 (adj)[b]	R^2 (pred)[c]		
	54.6*	0.56	0.55	0.53		

*$p < 0.05$.
[a] VIF = variance inflation factor, values close to 1 indicate predictors are not correlated.
[b] R^2 (adj) = R^2 adjusted for number of terms in the model.
[c] R^2 (pred) = Predicted R^2, a measure of how well the model predicts the dependent variable for new observations.

WFPS in zone A as opposed to the 70% WFPS treatment, during days 13–48 after urine application. Due to a reduction in the oxygen content of the soils, it may have been expected that the 70% WFPS treatment would have a lower ORP than the 50% WFPS treatment; however, pH and the abundance of oxidizing and reducing agents can also influence ORP. Here, it is postulated that a greater dilution of oxidizing and reducing agents may have occurred at 70% WFPS, which resulted in a lower ORP at this moisture content. The majority of N_2O emissions occurred when the ORP was between 160 and 350 mV, which is in line with results from studies of paddy and arable soils (Patrick and Jugsujinda, 1992; Yu et al., 2001).

The extractable soil NH_4^+ concentration was initially higher than the control in zone A, indicating rapid urea hydrolysis. The NH_4^+ concentration peaked at 50% WFPS on the day of urine application, however, at 70% WFPS the NH_4^+ concentration peaked 3 days after urine deposition. Increasing moisture increases urease activity up to field capacity, following which it decreases (Dharmakheerthi and Thenabadu, 1996). By using the soil water characteristics estimator of Saxton and Rawls (2006), the WFPS at field capacity was estimated to be 53% which may explain the slight delay in NH_4^+ generation at 70% WFPS as this was above field capacity. The time taken for completion of urea hydrolysis at both moisture contents is similar to that of other studies (e.g. Yadav et al., 1987). As the NH_4^+ was oxidised, the NO_3^- concentration in the urine influenced soil increased. The major emission period of N_2O took place during the first 20 days after urine application, whilst nitrification was still taking place. As the soils were not completely saturated it is suggested that both nitrification and denitrification contributed to the overall N_2O emissions, due to a combination of aerobic and anaerobic microsites within the soil. Less N_2O emissions at 50% WFPS are consistent with an inhibitory effect of a greater oxygen content upon denitrification. The magnitude of N_2O fluxes were similar to that measured by Allen et al. (1996), where dairy cow urine was applied to pasture blocks in a laboratory incubation.

Minimal N_2O fluxes were observed beyond 20 days of incubation, even though NO_3^- concentrations remained higher than the control, suggesting another factor may have been limiting N_2O production. As temperature and moisture were controlled in this study, it is suggested that labile C limitation prevented N_2O emissions from denitrification, and an NH_4^+ limitation prevented N_2O production via nitrification. Interestingly, NO_3^- concentrations continued to decrease following the major N_2O peak in the absence of plants; possible removal mechanisms are complete denitrification to N_2, immobilization and diffusion into surrounding soil. Studies investigating the effect of increasing DOC on denitrification rates commonly use glucose as a readily available C source e.g. Weier et al. (1993), however, further work is required to understand how differences in DOC molecular weight and concentration, may influence denitrification rates. Some studies have demonstrated more readily available C compounds stimulate denitrification more than complex molecules (Bremner and Shaw, 1958; deCatanzaro and Beauchamp, 1985) and therefore, determining the effect of DOC species specifically found within ruminant urine on N_2O emissions may explain some of the variability associated with emissions from urine patches related to urine composition.

4.2. Predicting N_2O emission by multiple regression analysis

The second objective of this study was to determine the amount of variation in N_2O emissions which could be predicted by the measured soil parameters. In the final multiple regression model changes in total extractable soil N, WFPS and ORP all contributed significantly ($p < 0.001$) to the variation in N_2O emissions, explaining 55.6% of the total variation. Provided all other variables are held constant, increasing the total N by 1% resulted in a 0.24% increase in N_2O emissions and decreasing ORP by 1% resulted in a 1.88% increase in N_2O emissions, under these experimental conditions. N_2O emissions were, on average, 58% higher in soil incubated at 70% in comparison to 50% WFPS, when holding ORP and total extractable N constant. Model parameters which contributed the most new information to the model followed the sequence ORP > WFPS > total extractable N. Low amounts of organic N were extracted from soils following urea hydrolysis and, therefore, the inclusion of total extractable N in the best subset's regression, as opposed to individual NO_3^- or NH_4^+ concentrations, supports the tenet that the majority of N_2O emissions were due to a combination of nitrification and denitrification.

4.3. The importance of urine patch edge effects

The third objective of this study was to determine how important considering urine patch edge effects are when calculating N_2O emission factors. The marked difference between the lateral distribution of NH_4^+ and NO_3^- within the urine patch highlights the importance of considering the urine patch diffusional area when monitoring N_2O emissions via chambers, or studies monitoring N loss via lysimeters. Under field conditions the mass of mineral N available for lateral diffusion may be influenced by plant uptake, the extent of vertical diffusion, leaching and preferential flow through soil macropores. The mesocosms used in this study used homogenised soil (i.e. preferential flow unlikely) and did not include effects of plant uptake, leaching, drainage and vertical diffusion beyond 5 cm. It would be expected that accounting for these processes would result in a lower mass of mineral N available for lateral diffusion than observed in this study. Nevertheless, the lateral diffusion of N in our study was 11 cm less than that observed by Decau et al. (2003), where cattle urine (3 L over 0.4 m^2) was applied to 1 m deep lysimeters, with a cross-sectional area of 2 m^2.

The NH_4^+ derived from the urine application remained central to the urine patch, with only small amounts diffusing up to 3 cm away from the initial wetted area. Conversely, the highly mobile NO_3^- diffused ca. three times as far from the centre of the urine patch and persisted in the soil for a longer period. These results suggest that N_2O production via nitrification would be limited by the lateral diffusion of NH_4^+, and are therefore only likely to occur in the initial wetted area and the area of soil influenced by mass flow of urine through soil immediately after deposition. On the other hand, denitrification of urinary nitrogen may occur both centrally and within a larger diffusional area of soil around the urine patch.

This suggests that in order for mitigation strategies such as synthetic or biological nitrification inhibitors to be effective at reducing N_2O emissions, it would be beneficial for the inhibitors to possess a similar charge and diffusion coefficient to NH_4^+. As roots can undergo death and decomposition in the direct urine deposition zone (Shand et al., 2002), it is likely that the biological (i.e. plant) delivery of nitrification inhibitors will be of most significance in the diffusive zone. Research regarding biological denitrification inhibition is still in its infancy (Bardon et al., 2014) and further research is required, yet, an effective denitrification inhibitor would ideally match the diffusive speed of NO_3^-.

In this study, emissions were ca. 1.5 and 2 (50% and 70% WFPS, respectively) times greater when considering the wetted and diffusional area (sum of zones A–D) in comparison to the wetted area only (zone A). Under field conditions this figure may be expected to be lower due to some removal of NO_3^- via plant uptake, draining, leaching and vertical diffusion however, it may be more representative of times where plant uptake is low or urine is deposited to areas of bare soil in the field. The walls of chambers for measuring gaseous emissions from soil are generally inserted to a depth of 5 cm. If a urine patch is applied uniformly throughout a

chamber, then the chamber walls may prevent lateral diffusion of NO_3^- and DOC into the surrounding soil, resulting in greater concentrations than would have been present otherwise or a deeper infiltration of urine. This could potentially overestimate denitrification, however, due to the limited diffusive speed of NH_4^+ perturbation to N_2O emissions from nitrification may be minimal. On the other hand, not considering the urine patch diffusional area may underestimate N_2O emissions, due to the smaller zone of soil influenced by the urine, resulting in fewer microbes exposed to the addition of N, DOC and moisture. Similarly, due to fewer microbes at soil depth, a greater vertical movement of urine may reduce direct emissions. It cannot be excluded that the opposing effects could cancel each other out. Further work is required to assess these potential processes, which could be investigated via the use of ^{15}N-labelled urine applied to larger and deeper pasture mesocosms with an intact sward, comparing source-partitioned emissions with and without a chamber wall to restrict diffusion.

4.4. Theoretical diffusion of NO_3^- and NH_4^+ through soil

A calculation of the theoretical diffusive speed of NO_3^- and NH_4^+ through soil may be useful for estimating the urine patch diffusional area in differing soils (and hence the additional area required within chambers to improve accuracy of emission measurements). To assess this we compared the theoretical linear distance of diffusive movement to the observed diffusive movement in the mesocosms. The effective diffusion coefficient (D_e) can be calculated using the equation

$$D_e = \frac{D_1 \times \theta f \times dC_1}{dC_s}, \quad (4)$$

where D_1 is the diffusion coefficient in pure water, θ is the soil volumetric moisture content, f is the impedance or pore tortuosity factor and dC_1/dC_s is the reciprocal of the buffer power (Nye and Tinker, 2000). To calculate D_e in the mesocosms we used D_1 values of 1.60 and 1.64 $cm^2\ d^{-1}$ for NO_3^- and NH_4^+, respectively (Lide, 2004), an f value of 0.3 (Jones et al., 2005) and the moisture contents of the mesocosms. Values for the buffer power in the same soil, were obtained from Jones et al. (2012). Further, the linear distance (L) of diffusive movement of NO_3^- and NH_4^+ through time can be calculated as

$$L = (2D_e t)^{1/2}, \quad (5)$$

where t is time. Using these parameters, the linear diffusive distance of NH_4^+ over 10 weeks was calculated as 1.50 and 1.72 cm at 50% and 70% WFPS, respectively. The calculated diffusive distance of NO_3^- was greater at 4.18 and 4.94 cm at 50% and 70% WFPS, respectively. In the soil mesocosms, increased NH_4^+ was observed up to 3 cm from the urine patch edge, whereas increased NO_3^- concentrations were observed up to 9 cm from the urine patch edge. Some disparity between observed and theoretical values may be due to the coarser scale of measurement (3 cm fractions) in the mesocosms and saturation of the exchange phase with urine derived K^+ (and other ions) which could lower the sorption of NH_4^+. The formation and subsequent diffusion of NO_3^- may have occurred after the NH_4^+ had diffused 1.50–1.72 cm, which may be the reason for the greater observed compared to theoretical diffusive distance of NO_3^-. Further validation of this method by comparison to measured urine patch diffusional areas in the field, across varying soil types, soil moisture contents, microtopography and urine patch N concentrations and volume need to be investigated prior to utilising this equation as a method for determining the chamber size required for an experimental urine patch.

5. Conclusions

The results of our study show that N_2O emissions can extend beyond the initial wetted area of a urine patch, and that this effect is greater under a high soil moisture content. For a typical sheep urine application to a Eutric Cambisol with an even surface, an additional 9 cm around the initial wetted area would have been required to capture the majority of N_2O emissions via a chamber based system. The additional area required around a urine patch may also vary alongside urine volume, patch area, the concentration of N applied, the soil type beneath the patch and the underlying microtopography. These conditions are likely to be highly site specific, therefore, preliminary assessments should be conducted in order to assess the magnitude of the urine patch diffusional area, and additional area inside chambers should be allowed for, prior to monitoring emissions.

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Appendix A. Supplementary material

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.10.011.

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