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Nitrification represents the bottle-neck of sheep urine patch N_2O emissions from extensively grazed organic soils

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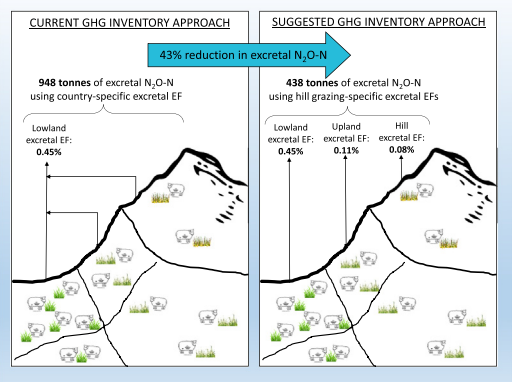
HIGHLIGHTS

- Mountains, moorlands and heath occupy 18% of the UK land area.
- These areas are understudied in terms of N_2O emissions from livestock urine.
- N_2O-N EFs for sheep urine were determined across two seasonal grazing periods.
- Low rates of nitrification of urine-N resulted in extremely low N_2O-N EFs (<0.01%).
- National inventory sheep excretal N_2O-N estimates reduced by 43% using new EFs.

GRAPHICAL ABSTRACT



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ABSTRACT

Extensively grazed grasslands are understudied in terms of their contribution to greenhouse gas (GHG) emissions from livestock production. Mountains, moorlands and heath occupy 18% of the UK land area, however, in situ studies providing high frequency N_2O emissions from sheep urine deposited to such areas are lacking. Organic soils typical of these regions may provide substrates for denitrification-related N_2O emissions, however, acidic and anoxic conditions may inhibit nitrification (and associated emissions from nitrification and denitrification). We hypothesised urine N_2O-N emission factors (EFs) would be lower than the UK country-specific and IPCC default value for urine, which is based on lowland measurements. Using automated GHG sampling chambers, N_2O emissions were determined from real sheep urine (930 kg N ha^{-1}) and artificial urine (920 kg N ha^{-1}) applied in summer, and from an artificial urine treatment (1120 kg N ha^{-1}) and a combined NO_3^- and glucose treatment (106 kg N ha^{-1}; 213 kg C ha^{-1}) in autumn. The latter treatment provided an assessment of the soils capacity for denitrification under non-substrate limiting conditions. The artificial urine-N_2O EF was 0.01 ± 0.00% of the N applied in summer and 0.00 ± 0.00% of the N applied in autumn. The N_2O EF for real sheep urine applied in summer was 0.01 ± 0.02%. A higher flux was observed in only one replicate of the real urine treatment, relating to one chamber where an increase in soil solution NO_3^- was observed. No lag phase in N_2O emission was evident following application of the NO_3^- and glucose treatment, which emitted

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0.69 ± 0.15% of the N applied. This indicates nitrification rates are the bottle-neck for N_2O emissions in upland organic soils. We calculated the potential impact of using hill-grazing specific urine N_2O EFs on the UK inventory of N_2O emissions from sheep excreta, and found a reduction of ca. 43% in comparison to the use of a country-specific excretal EF.

1. Introduction

Mountains, moorlands and heath comprise 18% of the total UK land area (Van der Wal et al., 2011) and extensive livestock grazing in these ecosystems allows the maintenance of an open habitat of grass and heath (Worrall and Clay, 2012; Leiber-Sauheitl et al., 2015). The impact of livestock urine on greenhouse gas (GHG) emissions from extensively grazed agroecosystems is understudied, especially those from organic soils (e.g. Histosols). Organic soils are renowned for either being large sources or sinks of GHGs e.g. under water-saturated conditions they are a source of CH_4 and a sink for CO_2, due to the retarded degradation of plant residues (Martikainen et al., 1995; Berglund and Berglund, 2011). Organic soils drained for agriculture, forestry or peat extraction produce large amounts of the powerful GHG nitrous oxide (N_2O; Regina et al., 1999; Andert et al., 2011; Taft et al., 2017). Drained peat soils emit high amounts of N_2O due to enhanced mineralisation and nitrification of stored and/or added N. Pristine peat soils, however, have negligible N_2O emissions (Regina et al., 2004), due to the highly competitive demand for available N between plants and microorganisms (Repo et al., 2009). Atmospheric N deposition is also the only major input of N to these systems i.e. inputs of N as fertilisers do not occur (Batey, 1982; Chapman et al., 2001).

The main explanatory factors for high or low N_2O emissions from peat soils do not hold under the conditions of a livestock urine patch, which forms a potential hotspot of N_2O emissions (Selbie et al., 2014; Krol et al., 2017; Chadwick et al., 2018). Here, the substrates (labile N and C) required to produce N_2O are directly added to the soil within urine, without prior need for mineralisation of native organic matter to produce these substrates. Whether negligible N_2O emissions occur under these circumstances is unclear - on one hand, soil conditions can be considered optimal for denitrification-related N_2O losses e.g. potentially high levels of soil water-filled pore space (WFPS) and dissolved organic C (Weier et al., 1993). Conversely, the highly acidic (Ineson, 1987) and waterlogged conditions may inhibit the aerobic process of nitrification, preventing formation of the substrate (NO_3^-) for denitrification (Marushchak et al., 2011) and emissions associated with the process of nitrification.

Recent studies have demonstrated low N_2O emissions from urine patches deposited to extensively grazed upland mineral soils in the UK (e.g. Orthic Podzol; Marsden et al., 2018) and from silt loam soils typical of hill grazing in New Zealand (Hoogendoorn et al., 2008; Van der Weerden et al., 2011; Luo et al., 2013). However, urine-derived N_2O emissions can differ markedly between mineral and organic soils, as demonstrated by Clough et al. (1996), who found N_2O-N losses were higher in mineral compared to organic soils. Leiber-Sauheitl et al. (2015) investigated GHG emissions and the priming effect of sheep excreta from microcosms of a nutrient-poor peat grassland and reported N_2O emission factors (EFs) close to zero, and no priming effect on peat-derived C and N. Allen et al. (1996) applied cattle urine to extensively grazed peat soil in an incubation study and did not find any significant emission of N_2O, and limited formation of NO_3^-, in contrast to the other mineral soil types investigated. Skiba et al. (2013) measured GHG emissions in situ from an extensively managed acid moorland in Scotland, however chambers were moved around to account for grazing, rather than measuring from a urine patch directly. They found the GHG budget was dominated by CO_2 fluxes, with the contribution from N_2O and CH_4 being minimal (only impacting net ecosystem exchange flux buy 3%). Other studies of urine patches deposited to peat soil include lowland intensively grazed peat soils, which have generally been drained and have high N_2O emission potentials (Koops et al., 1997; Boon et al., 2014). Emissions of N_2O from urine deposited to lowland peat in the Netherlands, for example, was found to be as high as 2.2% of the urine-N applied (Koops et al., 1997). In summary, studies conducted to date have only: monitored emissions from upland mineral soils; from laboratory incubations of organic soils; from peat soils but not directly from a urine patch; or from intensively grazed lowland peat soils.

Current estimates (based on 2017 data) of N_2O emissions from livestock excreta deposited to pasture, range and paddock comprise ca. 10% of the direct N_2O emissions from UK agriculture (UNFCCC, 2019), however, these estimates are based on data generated from the lowlands. The aim of this study was to quantify N_2O EFs for sheep urine deposited to organic soils, typical of extensive grazing systems at high altitudes, across two contrasting periods of the grazing season (summer and autumn). We focused on the urine fraction of excreta as, in comparison to faeces, it is more susceptible to N_2O losses due to the highly labile nature of the substrates added. We hypothesised that EFs would be lower than that used to underpin the UK country-specific $EF3_{PRP}$ value (0.69% for urine-N_2O and 0.45% for excretal N_2O; Chadwick et al., 2018), due to acidic and water-logged soil conditions inhibiting nitrification of the urine-N. We assessed the capacity for denitrification in these organic soils, to assess if either nitrification or denitrification were limiting N_2O emissions. The potential impact of using hill-grazing specific urine N_2O EFs on the national agricultural GHG inventory is discussed.

2. Materials and methods

2.1. Study site

The study took place on an area of common grazing land on the Carneddau mountain range (556 m a.s.l.), within the Snowdonia National Park (53°22′N, 3°95′W), Wales, UK. The collective graziers have rights to stock 15,000 sheep (Welsh Mountain ewes; *Ovis aries*) across 2836 ha (equivalent to 5.29 sheep ha^{-1} or 0.42 LU ha^{-1}). However, management of the flock(s) determines the stocking levels at given times of the year e.g. stocking levels in April, during lambing, can be as low as 0.71 ewes ha^{-1} (0.06 LU ha^{-1}) ranging to a maximum of 3.53 ewes ha^{-1} (0.28 LU ha^{-1}) towards the end of the grazing season. All sheep are removed from this common land from the end of October until the beginning of April. The vegetation at the field site is comprised of NVC classification H12 (*Calluna vulgaris – Vaccinium myrtillus* heath; Elckington et al., 2001), overlaying Dystric Histosol and Humic Gleysol soil types (Avery, 1990).

The experimental site was excluded of stock from 15th May 2017, to prevent confounding effects of recent excretal events on the results of the study. Two experimental areas were established to measure GHG emissions from urine patches applied in either summer (17/07/18) or autumn (12/10/18). A rain gauge (HOBO® RG3 Data Logging rain gauge with a Pendant Event data logger, Tempcon Instrumentation Ltd., Sussex, UK) was installed at the study site and soil (5 cm) and air temperatures were also monitored using a HOBO® U23–004 ProV2 temperature/external temperature data logger.

2.2. Soil characteristics

To characterise the soil at each site, soil was sampled from control plots in both seasonal studies ($n = 4$; 0–10 cm). Some soil characteristics differed between the seasonal application experiments (Table 1), despite their proximity in location (<10 m apart). Briefly, bulk density cores (0–5 cm; 100 cm^3) were taken, dried in an oven (105 °C; 24 h) and subsequently ground and sieved (<2 mm) to record stone weight and volume. The gravimetric soil moisture was determined by drying soils in a crucible (105 °C; 24 h). Soil organic matter content was determined via the loss-on-ignition in a muffle furnace (450 °C; 16 h; Ball, 1964). Soil pH and electrical conductivity (EC) were determined on 1:2.5 *w/v* soil-to-distilled water suspensions using standard electrodes. The soil (oven dried and ground) C and N content were determined on a TruSpec® CN Analyzer (Leco Corp., St. Joseph, MI). N mineralisation rates were determined via the method of Waring and Bremner (1964), where 1 M KCl extractable (1:5 *w/v*, soil-to-solution) NH_4^+ concentrations were determined before and after anaerobic incubation of the soil in the dark (1 week; 40 °C). The NH_4^+ concentrations in the extracts were analysed colorimetrically, via the method of Mulvaney (1996). Extractions with 0.5 M K_2SO_4 (1:5 *w/v*, soil-to-solution) were also performed, to determine dissolved organic C, total dissolved N and mineral N (NH_4^+ and NO_3^-) concentrations. Dissolved organic C and total dissolved N were determined on a Multi N/C 2100S analyzer (AnalytikJena, Jena, Germany). Microbial biomass C and N were determined via the chloroform fumigation procedure of Voroney et al. (2008), using K_{EC} and K_{EN} values of 0.35 and 0.5, respectively. Extractable NH_4^+ was determined as described above, and NO_3^- was determined via the method of Miranda et al. (2001). An additional extract (0.5 M acetic acid; 1:5, *w/v*, soil-to-0.5 M acetic acid) was conducted to determine available P and exchangeable cations. P was measured in the extracts via the method of Murphy and Riley (1962) and cations were measured using a Sherwood Model 410 flame photometer (Sherwood Scientific Ltd., Cambridge, UK).

2.3. Treatment details

Treatments ($n = 4$) applied in summer (17/07/18) included: i) control (no urine application), ii) artificial sheep urine (920 kg N ha^{-1}), and iii) real sheep urine (930 kg N ha^{-1}). The artificial sheep urine was made up according to Lucas and Jones (2006), but modified by increasing the proportion of urea to provide 6 g N l^{-1}, providing a N concentration value approximately in the middle of the range reported for sheep and cattle urine (2–12 g N l^{-1}) in Selbie et al. (2015). Welsh Mountain ewe ($n = 6$) urine was collected by allowing sheep to graze vegetation present in a grazing pen situated at the field site (see Supplementary Information, Fig. S1). Sheep urine was collected utilising urine collection pens with slatted flooring and trays situated beneath (see Supplementary Information, Fig. S2), described in Marsden et al. (2017), approved by Bangor University's College of Natural Sciences Ethics Committee (Ethics approval code CNS2016DC01). Individual urination volumes were recorded and frozen (−20 °C), but prior to application the sheep urine was defrosted and bulked ($n = 24$ urine events), to provide a homogeneous urine sample to apply across the plots (see Supplementary Information, Fig. S3). This method of collection has been shown to not cause excessive volatilisation of NH_3 from the urine samples (data not shown). Treatments applied in autumn (12/10/18) were: i) control, ii) artificial urine (prepared as described above; 1120 kg N ha^{-1}), and iii) NO_3^- and glucose (106 kg N ha^{-1}; 213 kg C ha^{-1}). The purpose of the artificial urine was to provide a reference treatment to allow comparison between seasons. The combined NO_3^- and glucose treatment was applied to determine the capacity for denitrification-related N_2O emissions without substrate limitation (i.e. it was not meant to replicate a urine patch) under the prevalent weather conditions (the mean water-filled pore space was 60% and assumed not to limit denitrification). A C-to-N ratio of 2:1 was chosen for the glucose/NO_3^- treatment to optimise denitrification efficiency, as shown in Her and Huang (1995).

We used the mean individual urine event volume (195 ± 54 ml) of Brilliant Blue dye (2 g dye l^{-1}; $n = 5$) to simulate a urine patch (see Supplementary Information, Fig. S4) and determine the area of soil to apply the urine to (both artificial and real urine). The wetted area was determined by tracing the spatial extent of the dye, using a sheet of acetate, resulting in patch sizes of 100 ± 4 cm^2 and an application rate of 20 l urine m^{-2}. The urine patch treatments in both seasons were all applied in triplicate within the GHG chambers, where 12% of the chamber basal area received urine treatment. Additional urine patches were applied around the GHG chambers ($n = 7$ for the artificial urine patches in both seasons), and marked out with stakes to allow for soil sampling. Due to limited quantities of real sheep urine, only two additional urine patches were applied around chambers for soil sampling, for three out of four of the real urine plots, resulting in $n = 3$ for the real urine soil sampling data. For the NO_3^- and glucose treatment, 1 l of solution (1.7 g N l^{-1}; 3.4 g C l^{-1}) was applied across a 40 × 40 cm square inside the chamber to create the targeted N and C application rate, with a replicate square outside each chamber for soil sampling. A different application method for the NO_3^- and glucose treatment was used, as these treatments were not meant to be directly compared to the urine treatments. Schematics of all experimental plot layouts can be seen in Supplementary Information (Fig. S5).

Table 1
Characteristics of the Dystric Histosol (0–10 cm) used in the summer (sampled on 18/07/17) and Humic Gleysol in autumn (sampled on 17/10/17) field studies. Results are expressed on a dry soil weight basis, as means ($n = 4$) ± SEM with letters denoting significant differences (two-sample t-test).

Soil properties	Summer	Autumn
Bulk density (g cm^{-3})	0.33 ± 0.05	0.40 ± 0.04
Gravimetric moisture content (%)	222 ± 37 b	88 ± 6 a
Organic matter (%)	47.2 ± 8.0 b	14.7 ± 1.8 a
pH	4.44 ± 0.06	4.36 ± 0.04
Electrical conductivity (μS cm^{-1})	36 ± 2 a	59 ± 3 b
Total C (%)	24.9 ± 4.6 b	7.7 ± 0.5 a
Total N (%)	1.39 ± 0.24 a	2.05 ± 0.04 b
C:N ratio	17.8 ± 1.1	15.7 ± 1.0
N mineralisation rate (mg N kg^{-1} d^{-1})	63.2 ± 6.6 b	33.7 ± 5.6 a
Dissolved organic C (mg C kg^{-1})	915 ± 58 b	394 ± 25 a
Total dissolved N (mg N kg^{-1})	128 ± 6 b	55 ± 7 a
Microbial biomass C (g C kg^{-1})	7.19 ± 0.64 b	4.45 ± 0.18 a
Microbial biomass N (mg N kg^{-1})	861 ± 80 b	352 ± 32 a
Extractable NO_3^- (mg N kg^{-1})	7.48 ± 4.31	2.30 ± 0.13
Extractable NH_4^+ (mg N kg^{-1})	14.9 ± 2.5 b	5.8 ± 0.3 a
Extractable P (mg P kg^{-1})	5.93 ± 2.21	1.88 ± 0.19
Exchangeable Na (mg kg^{-1})	80 ± 14 b	25 ± 7 a
Exchangeable K (mg kg^{-1})	137 ± 14	140 ± 19
Exchangeable Ca (mg kg^{-1})	32 ± 10	16 ± 5

2.4. Greenhouse gas flux monitoring

Fluxes of N_2O, CO_2 and CH_4 were monitored from the chambers (50 cm × 50 cm) using an automated GHG measurement system (Queensland University of Technology, Institute for Future Environments, Brisbane, Australia), connected to a diesel generator and battery system to provide power at the remote field site. A detailed description of the measurement system can be found in Marsden et al. (2018). Briefly, the system can provide eight gas flux measurements per 24 h period, per chamber, during uninterrupted measurement. For treatments applied in summer, automated measurements were conducted for 80 days following treatment application. For treatments applied in autumn, automated measurements were conducted for 45 days after treatment application. The shorter measurement period in autumn was due to adverse weather conditions (snow and ice) at the field-site.

After the automated measurement period, further gas samples were taken manually from the same chambers (used for automated

sampling) in both seasonal experiments. Briefly, these gas samples were taken using the static chamber technique where four gas samples (20 ml) were taken (over a 45 min chamber closure period) and injected into evacuated 20 ml glass vials. Manual gas samples were taken approximately once per month for an additional three months following the summer application and once per month for an additional two months following the autumn application. The manual gas samples were analysed on a Perkin Elmer 580 Gas Chromatograph (GC), served with a Turbo Matrix 110 auto sampler (Perkin Elmer Inc., Beverly, CT, USA). Gas samples passed through two Elite-Q mega bore columns via a split injector, with one connected to an electron capture detector (ECD) for N_2O determination, and the other to a flame ionisation detector (FID) for CO_2 and CH_4 determination.

2.5. Soil sampling and analysis following treatment application

To monitor chemical changes in the soil solution directly pertaining to the GHG fluxes, Rhizon® soil solution samplers (2.5 mm diameter, 5 cm porous part, 12 cm length tubing; Rhizon Research Products, Wageningen, Netherlands) were inserted at a 45° angle in relation to the soil surface, within the urine patch and control treatments inside the chambers. Successful sample collection was normally achieved in a minimum of three out of the four replicate treatments, resulting in a minimum of $n = 3$. Soil solution (ca. 1 ml) was collected from the chambers periodically (−3, 0, 2, 4, 7, 9, 14, 21, 28, 37, 42, 56, 70, 85, 112, 119, 144 and 177 days after treatment application in the summer and 0, 2, 5, 7, 9, 15, 22, 29, 41, 55, 84 and 117 days after treatment application in the autumn) using evacuated vials to collect the sample. The soil solution was analysed for NO_3^-, NH_4^+ and dissolved organic C and N as described in Section 2.2.

In case soil solution could not be collected (e.g. under dry conditions), soil cores were also taken from the control area ($n = 4$) around the chamber using an auger (1.3 cm diameter), or from within replicated urine patch treatments applied around the chamber, where resulting holes were back-filled with non-urine influenced soil. The summer plots were sampled 0, 2, 4, 7, 9, 14, 21, 28, 42, 56 and 85 days after treatment application. The autumn plots were sampled 0, 2, 5, 7, 9, 15, 22, 28, 40, 54, 83 and 117 days after treatment application. Soils were taken back to the laboratory and processed within 24 h of sample collection. The soil % WFPS was estimated by calculating the ratio of volumetric water content to soil porosity, where soil porosity was calculated assuming particle densities of 2.65 g cm^{-3} for the mineral fraction and 1.4 g cm^{-3} for the organic fraction (Rowell, 1994). Soils were homogenised and large roots were removed by hand, where necessary. The soil pH and EC were determined and extractions were performed with 0.5 M K_2SO_4, with resulting extracts analysed for NO_3^-, NH_4^+, and total extractable dissolved organic C and N as described in Section 2.2.

2.6. Statistical analyses

In order to determine the similarity between the two experimental areas (plots receiving treatments in summer and plots receiving treatments in autumn), the soil characteristics were compared via two-sample *t*-tests, after testing the data conformed to normality (Shapiro-Wilk test) and homogeneity of variance (F-test). If data failed the assumptions, then Welch's two-sample *t*-tests were conducted. Tests were conducted using the 'stats' package in R (R Core Team, 2018). Due to differences in soil characteristics, urinary N-content and length of study time between the summer and autumn studies, further results were only statistically compared within each season of application.

Cumulative GHG emissions (N_2O, CO_2 and CH_4) were calculated via trapezoidal integration using the 'pracma' package (Borchers, 2018) in R. For the summer experiment, cumulative N_2O emissions were $\log_{10}$-transformed to meet homogeneity of variance (Levene's test: 'car' package in R; Fox and Weisberg, 2011) and normality assumptions (Shapiro-Wilk test). A one-way ANOVA was then conducted, to test whether there were differences in cumulative N_2O emissions between the control, artificial urine and real urine treatments. EFs for N_2O were calculated by first correcting for the area under the chamber not influenced by urine, and then expressing as a percentage of the urine-N applied emitted as N_2O. A two-sample t-test was used to compare the summer-applied artificial and real urine N_2O EFs. Cumulative N_2O emissions from the autumn-applied artificial urine and the NO_3^- and glucose treatment were compared to the control via *t*-tests as above.

The soil solution NH_4^+ and NO_3^-, dissolved organic C and N in the summer applied treatments were compared via one-way ANOVA across each sampling date, followed by Tukey's HSD post-hoc test. If the test assumptions were violated after $\log_{10}$ transformation then a non-parametric equivalent was conducted (Kruskal-Wallis rank sum test). For the study in autumn, the soil solution NH_4^+, NO_3^-, dissolved organic C and N in either the artificial urine or the NO_3^- and glucose treatment were compared to the control via t-tests (as described above, due to large differences in N contents applied). Bonferroni adjusted *p* values were used to determine statistical significance of all tests, to compensate for type I errors associated with multiple comparisons. As the soil solution data was collected from within the GHG chambers, we believe these data were more useful in understanding the observed N_2O fluxes. Therefore, soil extraction data (NO_3^-, NH_4^+, dissolved organic C and N), pH, EC and % soil WFPS are provided as supplementary material, with statistical analysis conducted only on the soil solution data.

3. Results

3.1. Rainfall, air and soil temperature

The air temperature, soil temperature and hourly rainfall across both seasonal application dates can be seen in Fig. 1. The air temperature displayed a general declining trend moving from the summer to winter months (Fig. 1a). The soil temperature (Fig. 1b) also displayed a declining trend moving from the summer to autumn months, with the expected smaller diurnal amplitude compared to air temperature. See Supplementary Information for further details on soil and air temperature during the experimental monitoring periods. The hourly rainfall can be seen in Fig. 1c, where a large rainfall event occurred in the middle of December 2017, causing localised flooding in the area. Over the summer automated monitoring period the cumulative rainfall was 444 mm, and the cumulative rainfall over the entire monitoring period for the summer-applied treatments was 1512 mm. In the autumn automated experimental period there was 261 mm of rainfall and 1025 mm rainfall over the entire experimental period.

3.2. Urine patch greenhouse gas fluxes

Fluxes of N_2O from the control and urine treatments (artificial and real) in both seasons can be seen in Fig. 2. The cumulative N_2O emissions and calculated EFs can be seen in Table 2. An analysis of variance showed no significant differences of the cumulative N_2O emissions between the treatments applied in summer ($p > 0.05$), despite the peak in emissions observed in one chamber of the real urine treatment. Although a clear emission peak was observed, it was still fairly small in magnitude (<100 μg N_2O-N m^{-2} h^{-1}), where urine patch N_2O fluxes can often be >1000 μg N_2O-N m^{-2} h^{-1}. No significant difference ($p > 0.05$) was found between the artificial and real urine treatments in the summer. In autumn, the cumulative N_2O emissions were not significantly different between the control and artificial urine treatments ($p > 0.05$). Fluxes of N_2O following the application of NO_3^- and glucose can be seen in Fig. 3. The cumulative N_2O emissions from this treatment were significantly greater ($p < 0.05$) than the control cumulative emissions over the same period. The CO_2 and CH_4 fluxes can be found in Supplementary Information, Fig. S5 and S6, respectively.

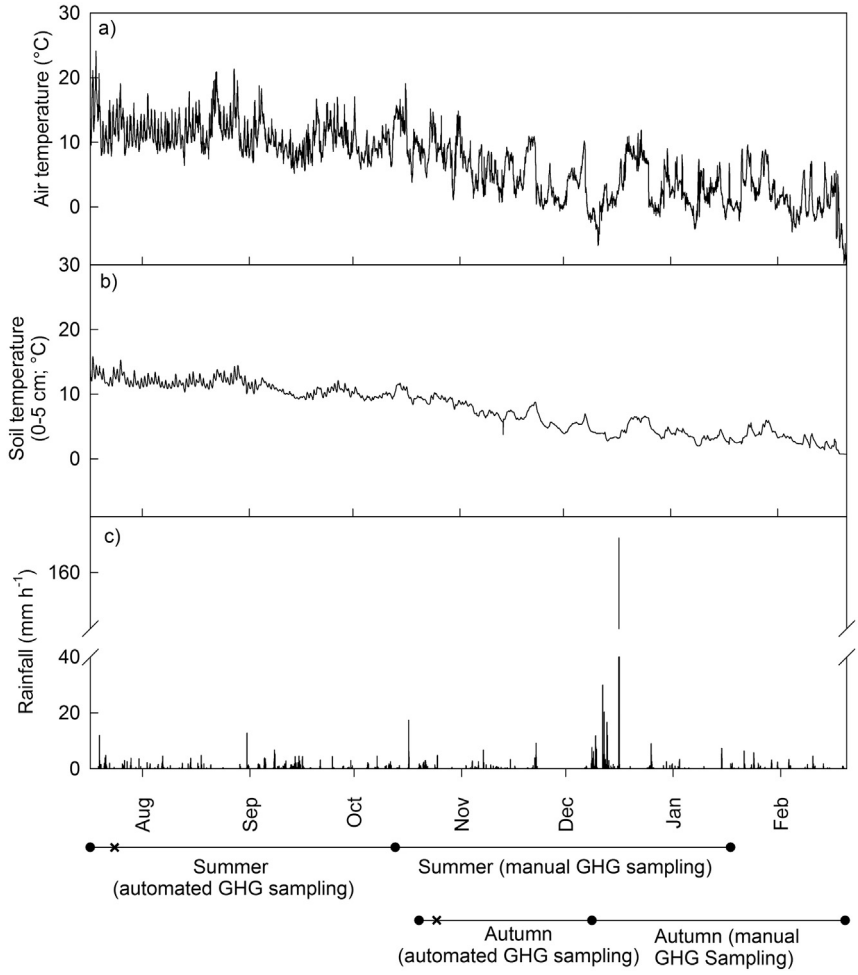

Fig. 1. Weather data over the two seasonal study periods, displaying a) soil temperature (°C; 0–5 cm), b) air temperature (°C) and c) rainfall (mm h^{-1}). Lines at the bottom of the figure represent the experimental monitoring periods for summer (treatments applied on 17/07/18) and autumn (treatments applied on 12/10/18). The circle symbols on this line displays the duration of automated and manual sampling and the cross symbols represent the point of treatment application.

3.3. Soil solution ammonium and nitrate

3.3.1. Summer experiment

The soil water mineral N dynamics within the chambers (measured via Rhizon® soil solution samplers) can be seen in Fig. 4. A summary of results of the analysis of variance for the soil solution NH_4^+ across the sampling days in summer can be seen in Supplementary Information, Table S1. Here, the soil solution NH_4^+ increased following application of either urine type, where the real urine resulted in a significantly higher soil solution NH_4^+ concentration on the day of urine application ($p < 0.05$), whereas the soil solution NH_4^+ concentration in the artificial urine patches did not become significantly greater than the control until two days after treatment application ($p < 0.01$). The soil solution NH_4^+ concentration peaked four days after application in both the artificial and real urine treatments (at 22.5 ± 4.8 and 52.0 ± 14.6 mg NH_4^+-N l^{-1}, respectively). Following this the concentrations declined to background levels, remaining significantly higher than the control in the artificial urine treatment for up to three weeks ($p < 0.05$), and for up to four weeks in the real urine treatment ($p < 0.05$). Generally, across the different sampling dates, the soil solution NH_4^+ concentrations were not significantly different between the artificial and real urine, and differences were only significant with respect to the control treatment. No further differences in soil solution NH_4^+ concentration were observed beyond four weeks after treatment application, except on day 119, however, these concentrations were very low (<0.4 mg NH_4^+-N l^{-1} soil solution).

The soil solution NO_3^- concentrations from the summer-applied treatments can be seen in Fig. 4c, and a summary of the results of the analysis of variance conducted across the sampling days in Supplementary Information, Table S2. There were no significant differences in the soil solution NO_3^- concentration between treatment means on any of the sampling dates ($p > 0.05$). A build-up of soil solution NO_3^- was only detected in one replicate chamber in the real urine treatment, corresponding to the same chamber that emitted N_2O. In all other replicates of the real urine treatment a build-up of NO_3^- in the soil solution did not occur.

3.3.2. Autumn experiment

The soil solution NH_4^+ concentrations in the autumn applied treatments are shown in Fig. 4b. A summary of the *t*-tests conducted for the soil solution NH_4^+ concentrations in either the artificial urine or the NO_3^- and glucose treatment (both in comparison to the control) can be seen in Supplementary Information, Table S3. Following artificial urine application in autumn, the soil solution NH_4^+ increased with respect to the control, peaking on day 15 at 54.3 ± 15.2 mg NH_4^+-N l^{-1}. The soil solution NH_4^+ was significantly greater than the control on days 0, 5, 22, 55 and 117. The soil solution NH_4^+ was significantly higher than the control at the end of the study in the artificial urine treatment, however, values had decreased to 6.19 ± 0.62 mg NH_4^+-N l^{-1} and were displaying an overall declining trend. As expected, there were no significant difference in the soil solution NH_4^+ in the NO_3^- and glucose

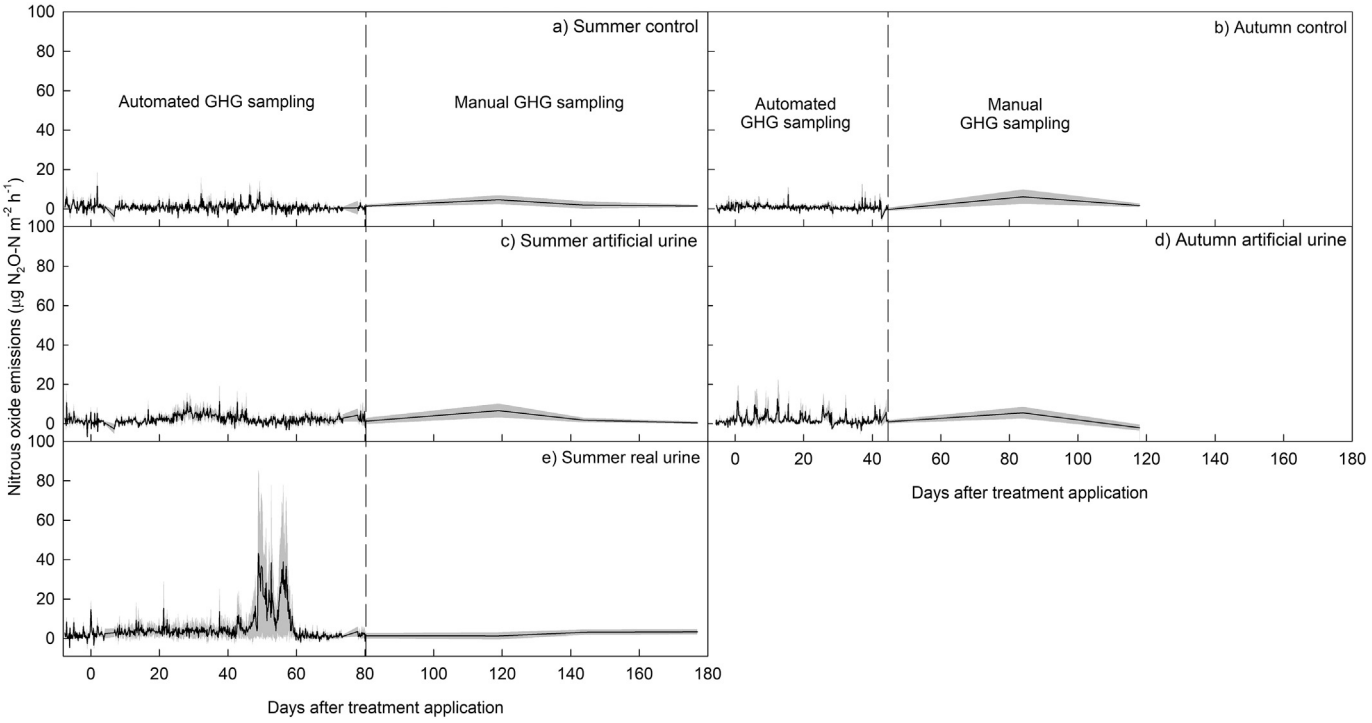

Fig. 2. Nitrous oxide (μg N_2O-N m^{-2} h^{-1}) emissions from a) control in summer, b) control in autumn, c) artificial sheep urine in summer, d) artificial sheep urine in autumn, and e) and real sheep urine patch treatments, applied to an upland Histosol. Amendments were made on day 0, black lines represent the treatment means ($n = 4$) and the shaded area represents the upper and lower bounds of the SEM.

treatment, apart form on one date (day 22), but soil solution NH_4^+ concentrations were low (0.86 ± 0.11 mg NH_4^+-N l^{-1}) at this time.

The soil solution NO_3^- concentration across the autumn experimental period is displayed in Fig. 4d, with a summary of the results of the t-tests in Supplementary Information, Table S4. There were no significant differences detected on any day after treatment application for soil solution NO_3^- in the artificial urine treatment compared to the control. As expected, the NO_3^- and glucose treatment caused a significant increase in soil solution NO_3^- with respect to the control, on days 2, 5, 7, 9 and 15. Following this, no further significant differences were detected in soil solution NO_3^- in comparison to the control treatment.

3.4. Soil solution dissolved organic C and N

3.4.1. Summer experiment

The soil solution dissolved organic C and N, sampled from within the GHG chambers can be seen in Fig. 5. A summary of the statistical analysis for the soil solution dissolved organic C in the summer applied treatments can be seen in Supplementary Information, Table S5, where no significant differences were observed between treatment means on any sampling day. The real sheep urine had numerically higher values than the control, and followed a declining trend, yet values were highly variable across the replicates. A summary of the analysis of variance for the soil solution N in summer can be seen in Supplementary Information, Table S6. Overall, significant differences in soil solution dissolved N were observed on days 2, 4, 7, 9, 14, 21, 28 and 85. The real urine peaked in soil solution dissolved N on the day of treatment application, at 77.6 ± 37.4 mg N l^{-1}, and was significantly higher (Tukey's HSD) than the control (but not the artificial urine treatment) on day 2 ($p < 0.01$), 4 ($p < 0.05$), 7 ($p < 0.01$), 9, 14, 21 and 28 (all $p > 0.05$). The soil solution N in the real urine treatment was also significantly greater than the control on days 85 and 119 (both $p < 0.01$), although the magnitude of soil solution N was smaller than at the beginning of the study (< 8 mg N l^{-1} soil solution). The artificial urine treatment soil solution N also peaked on the day of urine application at 134.5 ± 81.6 mg N l^{-1}. In this treatment, the soil solution N content was significantly greater than the control on days 2, 7 and 9 (all $p < 0.05$). The artificial urine soil solution N was also significantly higher than the control on day 85 ($p < 0.05$), but the amount of soil solution N was low (1.6 ± 0.1 mg N l^{-1}) at this point in time.

3.4.2. Autumn experiment

A summary of the *t*-tests conducted for the soil solution dissolved organic C in the autumn applied treatments, can be seen in Supplementary Information, Table S7. The soil solution dissolved organic C was only significantly greater than the control on the day of artificial urine application ($p < 0.01$). Although numerically the mean soil solution dissolved organic C in the artificial urine treatment was higher than control values for most of the measurement period, the variability between replicates was very high. No significant differences in soil solution dissolved organic C were detected between the NO_3^- and glucose treatment and the control, at any time point following treatment application. A summary of the t-tests conducted for the soil solution dissolved N in the autumn applied treatments can be seen in Supplementary Information, Table S8. In the artificial urine treatment the soil solution N was

Table 2
Cumulative N_2O emissions and emission factors for the artificial and real sheep urine applied in summer and for artificial urine and nitrate and glucose applied in autumn.

Treatment	Summer (177 days)			Autumn (118 days)		
	Control	Artificial urine	Real urine	Control	Artificial urine	Nitrate and glucose
Cumulative N_2O (mg N_2O-N m^{-2})	0.31 ± 0.08	0.48 ± 0.11	0.62 ± 0.47	0.28 ± 0.11	0.28 ± 0.13	11.7 ± 2.6
Emission factor (% of N applied)	NA	0.01 ± 0.00	0.01 ± 0.02	NA	0.00 ± 0.00	0.69 ± 0.15

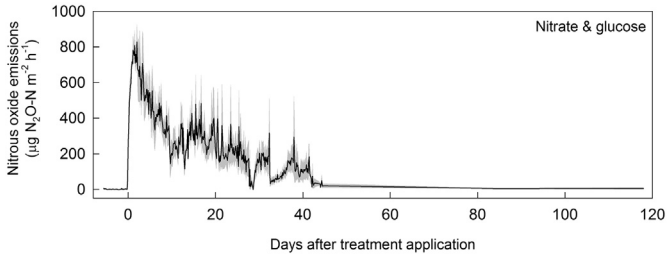

Fig. 3. Nitrous oxide (μg N_2O-N m^{-2} h^{-1}) emissions from a NO_3^- and glucose treatment applied to an upland Histosol in autumn (12/10/18). Amendments were made on day 0, black line represents the treatment mean ($n = 4$) and shaded area represents the upper and lower bounds of the SEM.

significantly greater than the control on nearly all sampling days (Fig. 5d, Supplementary Information Table S8). The soil solution dissolved N was highest in the artificial urine treatment on day 0 at 92.3 ± 29.0 mg N l^{-1}, following which the concentrations declined through time. By the end of the study (day 117), the soil solution N in the artificial urine treatment was not significantly different compared to that of the control ($p > 0.05$). For the NO_3^- and glucose treatment, the soil solution N was significantly higher than the control on days 7 and 9 (both $p < 0.01$), day 15 ($p < 0.05$) and 22 ($p < 0.01$).

3.5. Soil extractable ammonium, nitrate, dissolved organic C and N

The soil extractable NH_4^+ and NO_3^- as sampled from the experimental plots across both seasonal studies can be seen in Supplementary Information, Fig. S8. The soil extractable NH_4^+ and NO_3^- followed similar general trends to those observed in the soil solution across both seasons, however, the increase in soil solution NO_3^- which was detected in the single replicate of the real urine treatment in summer was not found in the corresponding soil extractions (sampled from urine patches outside the chambers).

Soil extractable dissolved organic C and N, sampled from the experimental plots can be seen in Supplementary Information, Fig. S9. The mean extractable dissolved organic C ranged between 380 and 884 mg C kg^{-1} soil DW across all treatments applied in summer. The total extractable N in the artificial and real urine treatments followed similar temporal trends, generally declining through time reaching similar values to that of the control towards the end of the soil sampling period (day 85). The mean soil extractable organic C ranged between 285 and 747 mg C kg^{-1} soil DW across all treatments applied in autumn. The soil extractable N content displayed a larger response in the artificial urine treatment compared to the NO_3^- and glucose treatment, as would be expected from the difference in N application rates between these treatments e.g. the peak extractable N content occurred on day 9 at 270 ± 109 mg N kg^{-1} soil DW in the artificial urine treatment, and the peak extractable N in the NO_3^- and glucose treatment occurred on day 15, at 121 ± 27 mg N kg^{-1} soil DW.

3.6. Soil water-filled pore space

The soil % WFPS, as sampled from the experimental plots during both seasonal studies can be seen in Supplementary Information, Fig. S10. In the summer experimental plots the mean WFPS ranged from 41 ± 5 to 75 ± 20% in the control, from 44 ± 5 to 88 ± 17% in the artificial urine plots and from 41 ± 4 to 78 ± 24% in the real urine plots. The lowest % soil WFPS values were recorded in the same individual plot where a build-up of NO_3^- was detected in the soil solution, e.g. a value as low as 20% WFPS was recorded two days after treatment application, and during the period where NO_3^- peaked in the soil solution (days 21 to 28), soil WFPS was in the range of 33 to 35%. In the autumn study, the mean % soil WFPS ranged between 42 ± 2 and 81 ± 24% in the control plots, between 37 ± 3 and 81 ± 14% in the artificial urine plots and between 42 ± 8 and 82 ± 18% in the NO_3^- and glucose treated plots.

3.7. Soil pH and EC

The soil pH and EC across both seasonal studies can be seen in Supplementary Information, Fig. S10. In the summer study, mean soil pH in the control plots ranged between 4.2 ± 0.0 and 4.7 ± 0.1. The soil pH reached higher values in the urine treatments over this period e.g. artificial urine treatment pH ranged between 4.3 ± 0.2 and 5.4 ± 0.4, and the real urine treatment pH ranged between 4.5 ± 0.2 and 5.1 ± 0.2. During the summer experimental period the soil EC peaked on the day of treatment application in the artificial urine (128 ± 30 μS cm^{-1}) and real urine (159 ± 34 μS cm^{-1}) treatments, compared to the control (36 ± 2 μS cm^{-1}). The soil EC in the urine treatments gradually declined over time, and by the end of the soil sampling period (day 85) the soil EC was similar to the control (58 ± 6 μS cm^{-1}) in the artificial urine (57 ± 8 μS cm^{-1}) and real urine (76 ± 10 μS cm^{-1}) treatments.

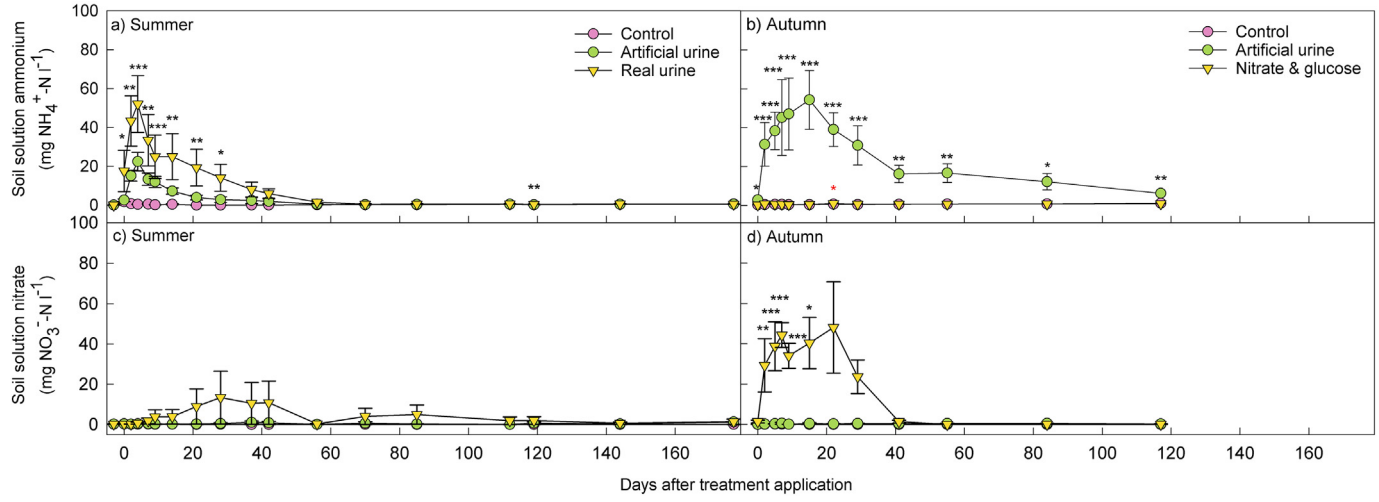

Fig. 4. Soil solution ammonium (panels a and b; mg NH_4^+-N l^{-1}) and nitrate (panels c and d; mg NO_3^--N l^{-1}), measured from Rhizon soil water samplers within the GHG monitoring chambers. Amendments were made on day 0, symbols represent means ($n = 3$ or 4), error bars represent SEM and legends are specific to each column of panels. For panels a and c, asterisks represent significance levels of the analysis of variance. For panels b and d, black asterisks represent significance levels of *t*-tests for artificial urine compared to the control and red asterisks represent significance levels of t-tests for the NO_3^- and glucose compared to the control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

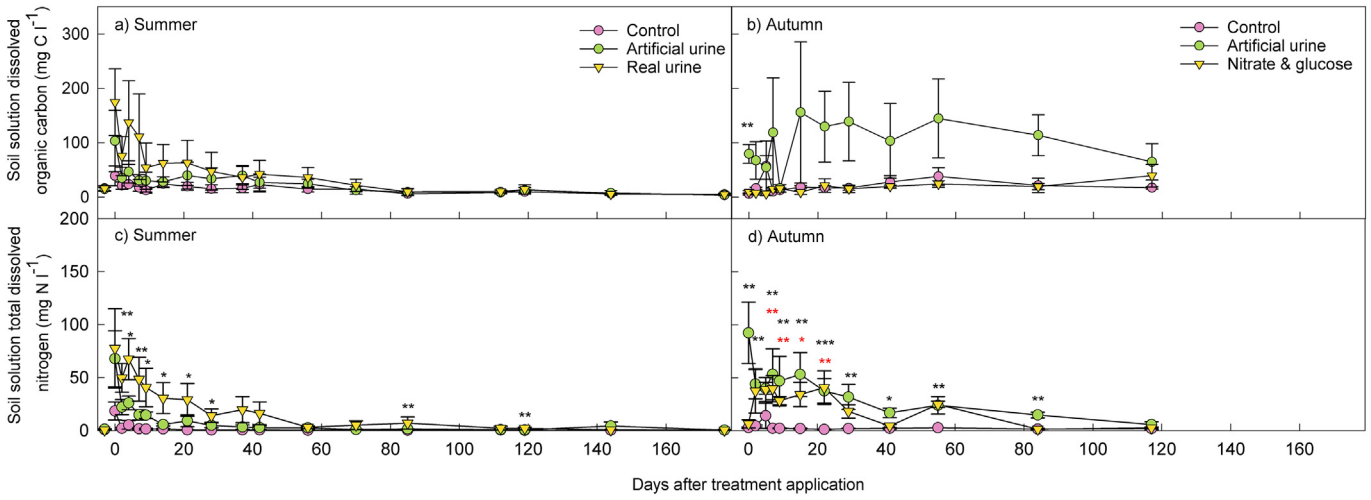

Fig. 5. Dissolved organic carbon (panels a and b; mg C l^{-1}) and total dissolved nitrogen (panels c and d; mg N l^{-1}) in soil solution, measured from Rhizon soil water samplers located within the GHG monitoring chambers. Amendments were made on day 0, symbols represent means ($n = 3$ or 4), error bars represent SEM and legends are specific to each column of panels. For panels a and c, asterisks represent significance levels of the analysis of variance. For panels b and d, black asterisks represent significance levels of t-tests for artificial urine compared to the control and red asterisks represent significance levels of t-tests for the NO_3^- and glucose compared to the control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In the autumn applied treatments, mean soil pH was fairly consistent temporally. Mean soil pH ranged between 4.2 ± 0.0 and 4.6 ± 0.0 in the control treatment, between 4.4 ± 0.1 and 4.9 ± 0.1 in the artificial urine treatment and between 4.1 ± 0.0 and 4.8 ± 0.1 in the NO_3^- and glucose treatment. The peak in EC values were observed two days after treatment application in the autumn study, where the soil EC was 64 ± 9 μS cm^{-1} in the control, 143 ± 33 μS cm^{-1} in the artificial urine treatment and 127 ± 17 μS cm^{-1} in the NO_3^- and glucose treatment. By the end of the study (day 117) the soil EC values were similar to the control (42 ± 8 μS cm^{-1}) in the artificial urine (48 ± 8 μS cm^{-1}) and the NO_3^- and glucose treatments (48 ± 7 μS cm^{-1}).

4. Discussion

4.1. Urine patch N_2O emission factors in organic soils

To our knowledge, this study represents the first to provide in situ, high frequency measurements of N_2O fluxes from sheep urine deposited to upland peat soils globally. In the summer study, real sheep urine was collected from the site, providing urine representative in chemical composition for the study area. Although fluxes were not monitored for a full year, which is recommended to provide IPCC compliant N_2O-N EFs, we believe we have captured the main N_2O emission window caused by the urine application, as concentrations of both NH_4^+ and NO_3^- were similar to control values by the end of the summer study. While some studies have shown urine N_2O emissions continuing beyond four months (e.g. Cardenas et al., 2016; Luo et al., 2013; Nichols et al., 2016), several other studies have shown the emission period to be over within four months (Marsden et al., 2018; de Klein et al., 2011; Van der Weerden et al., 2011). By the end of the autumn study, although NH_4^+ was still significantly higher than the control, it had been displaying a consistent declining trend and there had been no evidence of nitrification of this NH_4^+-N in this treatment, even when NH_4^+ concentrations were at their highest. The urine patch N_2O-N EFs across both seasons in this study were negligible, similar to the findings of Marsden et al. (2018) on an extensively grazed upland mineral soil. The N_2O-N EFs were also much lower than that used to underpin the UK's country-specific $EF3_{PRP}$ (pasture, range and paddock) for N_2O from urine deposited by grazing livestock (0.69% for urine-N, Chadwick et al., 2018).

We hypothesised that urine patch N_2O EFs from an organic upland soil would be low, due to low rates of nitrification. This hypothesis is supported by our data in a number of ways: i) the N_2O-N EFs arising from the urine patch treatments (both real and artificial) were negligible, across both seasons of study, ii) levels of soil solution NO_3^- were not significantly greater than the control at any time point following the application of the different urine types, demonstrating a general lack of nitrification, iii) a sustained peak in N_2O emissions above base-line levels was observed in one of the replicate real urine patch treatment, which corresponded to the only chamber where a build-up of NO_3^- in the soil solution was detectable, suggesting nitrification was limiting N_2O emissions in all other chambers, iv) the lowest values of soil % WFPS were recorded in the same plot where nitrification occurred, and during the period of active nitrification soil WFPS was below 40%, and v) the NO_3^- and glucose treatment produced a clear and sustained N_2O flux, without a lag phase, ruling out the possibility of N_2O emissions being low due to a lack of denitrifying microbial communities at the site.

4.2. Possible mechanisms of low nitrification rates in upland organic soils

The results of this study raise questions of the mechanisms behind the low levels of nitrification and resulting low N_2O emissions from the urine patches in upland organic soils. Possible explanations for a lack of nitrification include a small or functionally inactive population of nitrifiers, high soil acidity, limited O_2 concentrations (Allen et al., 1996), or some combination of the above. The detection of nitrification in the soil solution in one chamber suggests that the potential for nitrification exists in these upland peat soils. Nitrification rates, however, have been found to be lowest in moorlands and bogs in comparison to grasslands and woodlands, and are highest in arable and improved grasslands (Yao et al., 2013). We suggest plant and microbial uptake were likely to be the main cause of the decline in soil solution NH_4^+ concentrations in the urine treatments, with the decline occurring faster in the summer compared to the autumn treatments. The potential for NH_3 volatilisation was low due to acidic soil conditions, and leaching losses unlikely due to the limited build-up of NO_3^- in the soil solution. Complete denitrification to N_2 was also unlikely to occur due to production of N_2O reductase being sensitive to low soil pH (<6.1; Liu et al., 2010; Liu et al., 2014).

Soil acidity can influence the community composition of organisms capable of nitrification. At low soil pH, the protonation of NH_3 to NH_4^+ occurs, and typically ammonia oxidizing archaea (AOA) dominate in environments with low NH_3 concentrations (Stopnišek et al., 2010; Zheng et al., 2017). Indeed, AOA have contrasting NH_3 acquisition systems and

possess energy-dependent NH_4^+ transporters, compared to ammonia oxidizing bacteria (AOB) which have NH_3 transporters (Offre et al., 2014). In addition, low soil pH has a greater negative impact on the abundance of AOB in comparison to AOA (Yao et al., 2013). Extensively grazed acidic soils are likely to harbour greater numbers of AOA adapted to low NH_3 concentrations, as they do not receive fertiliser applications and inputs of excreta are minimal and 'patchy' due to low stocking densities. The addition of urine to intensively managed grassland soils has been found to stimulate AOB, rather than AOA growth (Di et al., 2009; Podolyan et al., 2014), yet the response of AOA and AOB to urine events in extensively grazed systems are less well understood. We suggest that the high concentrations of urea within urine, which rapidly hydrolyses to produce high concentrations of NH_4^+ in the soil, do not favour AOA growth, and the acidic conditions hinder AOB growth, resulting in limited nitrification from either prokaryotic domain.

Soil hydrology can influence N_2O sources and sinks (Rubol et al., 2012), e.g. the higher the soil moisture, the lower the O_2 content, which would hinder the aerobic process of nitrification. In the individual chamber where nitrification was detected, the N_2O-N EF was still only 0.06% of the N applied, therefore, we suggest that the magnitude of nitrification may have been limited by additional factors. It is clear from our data that understanding the causes of spatial variability in nitrification rates are key to understanding the magnitude of N_2O emissions from these upland organic soils. Enhanced understanding of the soils hydrology and the interactive effect of soil pH, aeration status and other soil characteristics on nitrification of urine-N would be useful to investigate the upper limits of urine-N_2O-N EFs from extensively grazed peat soils.

4.3. Denitrification potential of upland organic soils

The combined NO_3^- and glucose treatment provided an indication of the soils capacity for denitrification. We expected a high potential for N_2O fluxes from this soil type when adding this treatment, and 0.69% of the N applied was emitted as N_2O. No lag phase was observed, with N_2O emissions proceeding immediately following treatment application. We, therefore, conclude denitrifying communities are present and active at this site. This further indicates that nitrification is the bottle-neck of N_2O emissions from urine patches (from both nitrification and denitrification) in upland organic soils. In de Sosa et al. (2018), the addition of glucose stimulated denitrification in an extensively grazed riparian area, to a greater extent than the addition of urea. We suggest the addition of a labile C source may have been important for the high N_2O emissions observed in the NO_3^- and glucose treatment, as in these organic soils labile C could be bound up in more recalcitrant forms. It would be useful to further study the effects of NO_3^- and glucose applied alone in addition to in combination, to determine the importance of labile C on N_2O fluxes from these soils.

4.4. Potential impact of variation in soil and urine characteristics on urine N_2O fluxes

Given the limited spatial extent of the current study, it is important to consider whether these data are typical for such environments. Despite the close proximity of the two seasonal studies, the soils differed markedly in their characteristics. This highlights the spatial complexity of these upland habitats in terms of the underlying soil, the hydrology and the overlaying vegetation, which are often mosaics of upland heath and montane grassland communities. Despite the differences in some of the soils characteristics between the two seasonal studies, the urine patch N_2O EFs were negligible across both the experimental sites. The artificial and real urine also behaved in a similar fashion in the summer study. We believe the general lack of nitrification may have obscured any further differences related to soil characteristics, season or urine chemical composition. In this study, treatments were not applied to sheep camping areas, where a disproportionate amount of N_2O emissions are possible due to an alteration of microbial dynamics, soil biochemical properties (Haynes and Williams, 1999) and nitrification potential (Letica et al., 2006). The measurement of urine patch N_2O EFs from these areas would also be useful to fully account for N_2O production from hill grazing systems.

The urine patch simulation resulted in a high urine volume-to-soil surface area application rates, at 20 l urine m^{-2}. This value is slightly higher than the 17 l m^{-2} reported for a mineral soil in the uplands (Marsden et al., 2018). The wetted area of a sheep urine patch applied to these organic soils in the uplands is small, potentially due to the sponge-like action of live bryophytes on the soil surface and senescent bryophytes in the soil. This has the potential to cause N loading rates much higher than those generally reported in the literature, depending on the N concentration of the voided urine. However, if the results of this study are representative, then the concentration of N applied may not be important for N_2O emissions if nitrification does not occur at an appreciable rate.

4.5. Implications for the greenhouse gas inventory

Utilising the urine-N_2O EFs from organic soils in this study and those from an upland mineral soil reported in Marsden et al. (2018), we aimed to quantify the effect of including hill-grazing specific sheep urine N_2O EFs on the national inventory of GHG emissions from livestock production systems in a heterogeneous landscape. Currently, all excretal-N from grazing livestock is considered to have an EF based on country-specific data, collected from cattle excreta deposited to lowland fertile grasslands, on mineral soils; this recent improvement to the UK agriculture greenhouse gas inventory is in place of the IPCC default of 1% for livestock excreta. Excretal N is partitioned into faeces and urine via an empirical function of feed N content (Brown et al., 2018). The UK country specific ruminant N_2O-N EFs are 0.19% for faeces and 0.63% for urine, resulting in an overall excretal EF of 0.45% (Brown et al., 2018). As we did not measure faecal EFs in the current study, we used the country-specific faecal EF in our calculations, representative of the lowlands. The mean sheep urine EF across spring and autumn was 0.05% in the semi-improved uplands (Marsden et al., 2018) and 0.01% on the unimproved moorland (representative of hill grazing, reported in the current paper). This resulted in excretal EFs of 0.45% for the lowlands, 0.11% for the uplands and 0.08% on the hill land. N excretion rates were adjusted based on maintenance energy requirements, using crude protein contents of 200, 150 and 100 g kg^{-1} for lowland, upland and hill grazing, respectively. Using these excretal EFs and N excretion rates we calculated an annual reduction in the N_2O-N emission from the UK sheep flock at grazing, from 948 t of N_2O-N to 538 t of N_2O-N, i.e. a reduction of 43%. Clearly, this revised inventory total for grazing sheep should be viewed with caution, as the upland and hill urine-N_2O data only come from one regional area and faecal N_2O EFs are assumed to be the same in lowland, upland and hill areas. Nevertheless, it provides an indication that with further research it may be worthwhile disaggregating the inventory by lowland, upland and hill areas, as recommended by Kelliher et al. (2014) for New Zealand grazing sheep and cattle.

While we suggest that excretal EFs could be separated along altitudinal gradients (lowland, semi-improved upland and unimproved moorland) and their inherent differences in management intensity, our data indicate that sheep excretal EFs could also be disaggregated by areas with low soil pH and high levels of soil anaerobicity. These two contrasting ways of defining lower urine N_2O emissions may overlap to some extent, although it may not include lowland areas which could also possess these features. Further regional data would be required to assess the most effective method of disaggregating such emissions. In addition to the potential impact on the GHG inventory, the low N_2O EF values for sheep urine in upland regions also have the potential implication of reducing the carbon footprint of upland-reared livestock products

(although other GHG sources e.g. enteric CH_4 and net CO_2 emissions would need to be taken into account).

5. Conclusions

Urine patch N_2O-N EFs from an upland organic soil in this study were minimal. Nitrification of urine-N was found to limit N_2O emissions from urine patches in organic upland soils. The potential for denitrification of urine-N exists if nitrification occurs, therefore, understanding spatial variability in nitrification rates are key to understanding the potential magnitude of N_2O emissions from urine patches in extensively grazed organic soils. Assuming our data are typical for extensively grazed systems, utilising hill-grazing specific urine patch N_2O-N emission factors would reduce the annual estimate of N_2O derived from UK sheep excreta deposited during grazing by ca. 43%.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.133786.

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