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Effect of cattle urine addition on the surface emissions and subsurface concentrations of greenhouse gases in a UK peat grassland

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ABSTRACT

Grazing systems represent a substantial percentage of the global anthropogenic flux of nitrous oxide (N_2O) as a result of nitrogen addition to the soil. The pool of available carbon that is added to the soil from livestock excreta also provides substrate for the production of carbon dioxide (CO_2) and methane (CH_4) by soil microorganisms. A study into the production and emission of CO_2, CH_4 and N_2O from cattle urine amended pasture was carried out on the Somerset Levels and Moors, UK over a three-month period. Urine-amended plots (50 g N m^{-2}) were compared to control plots to which only water (12 mg N m^{-2}) was applied. CO_2 emission peaked at 5200 mg CO_2 m^{-2} d^{-1} directly after application. CH_4 flux decreased to −2000 μg CH_4 m^{-2} d^{-1} two days after application; however, net CH_4 flux was positive from urine treated plots and negative from control plots. N_2O emission peaked at 88 mg N_2O m^{-2} d^{-1} 12 days after application. Subsurface CH_4 and N_2O concentrations were higher in the urine treated plots than the controls. There was no effect of treatment on subsurface CO_2 concentrations. Subsurface N_2O peaked at 500 ppm 12 days after and 1200 ppm 56 days after application. Subsurface NO_3^- concentration peaked at approximately 300 mg N kg dry $soil^{-1}$ 12 days after application. Results indicate that denitrification is the key driver for N_2O release in peatlands and that this production is strongly related to rainfall events and water-table movement. N_2O production at depth continued long after emissions were detected at the surface. Further understanding of the interaction between subsurface gas concentrations, surface emissions and soil hydrological conditions is required to successfully predict greenhouse gas production and emission.

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1. Introduction

Nitrous oxide (N_2O) is an important greenhouse gas (GHG) with 298 times the Global Warming Potential (GWP) of CO_2 (Forster et al., 2007). N_2O is produced as a result of microbial processes operating in the soil profile, whereby it is a by-product of the reduction of nitrate (NO_3^-) to nitrogen gas (N_2) (denitrification), the ammonification of nitrate and the oxidation of ammonium (NH_4^+) to NO_3^- (nitrification) (Firestone et al., 1980; Baggs, 2011). Agricultural systems, comprising both livestock and arable production, return substantial amounts of mineral N to the soil and therefore contribute significantly to global emissions of N_2O (IPCC, 2001). Grazing systems are thought to represent 16% of the global anthropogenic flux of N_2O (IPCC, 2001) as livestock add nitrogen (N) to the soil in the form of excreta.

Cattle urine has been shown to stimulate N_2O production to a larger extent than dung due to the dual effect of a large pool of readily available N and C and increased soil water content (e.g. Allen et al., 1996; van Groenigen et al., 2005a). Cattle urine supplies greater amounts of N to the patch than the pasture N demand, thereby facilitating losses through leaching and gaseous emissions (Di and Cameron, 2002). Cattle urine N content varies between 1 and 20 g L^{-1} due to differences in water intake and diet (Oenema et al., 1997; Leterme et al., 2003) and is on average 6 g N L^{-1} (Leterme et al., 2003; Bristow et al., 1992). Urine patch radius is generally around 0.32–0.35 m but ranges between 0.1 and 0.6 m for dairy cattle (Moir et al., 2011). The surface area of urine patches is generally between 0.34 and 0.40 m^2 (Moir et al., 2011; Oenema et al., 1997) giving rise to an N deposition of 20–80 g N m^{-2} (200–800 kg N ha^{-1}) on each urination event (Oenema et al., 1997;

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Whitehead, 1986). For beef cattle urine the typical N loading is 700 kg N ha^{-1} (Haynes and Williams, 1993).

On contact with the soil, urea-N is rapidly hydrolysed to ammonia (NH_3), catalysed by the enzyme urease which is ubiquitous in soils as a result of microbial activity. This process is dependent. The hydrolysis process also reduces the available carbon from the urea, as CO_2 is a by-product of the reaction. Hydrolysis can account for over 50% of the added urine-C depending on soil moisture (Lambie et al., 2013). The remaining C provides a substrate for respiration (and therefore emission of CO_2) or for CH_4 production in anoxic soils (Yamulki et al., 1999; Liebig et al., 2008). Studies have also shown that addition of cattle urine can increase the solubility of soil C, leading to increased soil C decomposition and therefore potentially increased CO_2 emission (Clough et al., 2003a) and leaching (Lambie et al., 2012). In addition to potential for increased N_2O and CO_2 production in urine patched, NH_4^+ is known to inhibit oxidation of CH_4 and therefore promote increased CH_4 emission (Mosier et al., 1991; Dobbie and Smith, 1996).

Studies indicate that even short-term grazing can cause a significant increase in N_2O emissions, particularly when combined with compaction and seasonal water-table rise (van Groenigen et al., 2005b; van Beek et al., 2011). There is a wide body of research into the effect of cattle excreta on soils, with focuses on soil moisture, N content, urine volume and interactions with dung and fertilisers (e.g. Allen et al., 1996; Velthof et al., 1996; van Groenigen et al., 2005a,b; Maljanen et al., 2007) but few focus exclusively on peat soils (Koops et al., 1997; van Beek et al., 2011) and few include observations of all three greenhouse gases under urine patches (Liebig et al., 2008; Lin et al., 2009). Peat soils by definition have higher organic matter content than mineral soils. This leads to physical differences between peat and mineral soils; in particular higher porosity and gas diffusion coefficient (Boon et al., 2013). Additionally, due to the tendency of peat soils shrink and swell with changing soil moisture, they exhibit strong variations soil hydraulic properties such as moisture retention (Kechavarzi et al., 2010) compared to mineral soils. Peat soils also generally have higher mineralisation rates than mineral soils leading to higher available N, which combined with higher moisture retention leads to increased N_2O emission through denitrification (Koops et al., 1997). Peat soils have been shown to have increased N_2O emissions with respect to mineral soils as a result of a combination of these factors, particularly when amended with fertilisers or livestock excreta (Velthof and Oenema, 1995). Due to the increased availability of soil organic carbon, peat soils are substantial sources of CH_4 when in an anaerobic state and CO_2 when in an aerobic state (Moore and Dalva, 1993).

Subsurface concentrations of greenhouse gases, when combined with measurements of soil nitrogen and carbon, can be used to identify the key processes contributing to the accumulation of gases that may be subsequently emitted to the surface (Li and Kelliher, 2005; Li and Kelliher, 2007). These measurements can be used to determine zones of production and storage of greenhouse gases in the soil, particularly when combined with soil physical measurements such as bulk density, air-filled porosity and the gas diffusion coefficient, all important predictors of greenhouse gas emissions (Ball, 2013; Balaine et al., 2013). Measurements of subsurface greenhouse gases are currently limited from peat soils (e.g. Clark et al., 2001; Elberling et al., 2011), particularly when these soils are subjected to agricultural amendments, and especially where measurements have been made of soil physical parameters.

Many lowland peatland environments in the UK are under seasonal grazing management, often as a contribution to conservation management schemes on tenanted farmland or nature reserves. Sheep production is regularly practiced on 85% of UK upland peat; but cattle and ponies are being introduced to manage fen vegetation in lowland peatland and little study of the potential effect on GHG budgets for these environments has been conducted (Worrall et al., 2011). In this study, we aim to simulate small urination events on an area of UK peat grassland that is intensively grazed by beef steers for short period of time during autumn seasonal water-table rise. The main objective of this experiment was to quantify the difference between subsurface concentrations and surface fluxes of CO_2, CH_4 and N_2O in plots treated with cattle urine and control plots treated with water. Secondary objectives were to examine the relative importance of water-table depth (WTD), water soluble (available) carbon (WSOC) and soil NO_3^- and NH_4^+ concentrations on CO_2, CH_4 and N_2O production and emission and thereby draw conclusions on the dominant greenhouse gas producing processes during short term cattle grazing on peat soils. We also consider the importance of measured soil physical parameters (porosity, bulk density and gas diffusion coefficient) for transport of greenhouse gases from the surface layers of soil to the atmosphere. We hypothesise that addition of cattle urine to the soil will produce significant differences in GHGs relative to the control plots and that water-table depth is the key control on these processes throughout the autumn rewetting period.

Table 1
Characterisation of field soil (dry weight basis) between 0 and 30 cm depth (averaged data ± SE) (data collected May 2009–June 2010).

	Soil depth		
	0–10 cm	10–20 cm	20–30 cm
Texture	Clay loam	Loamy clay	Peat
Total C (%)	23.84 ± 0.74	16.08 ± 0.75	26.35 ± 2.08
Total N (%)	2.05 ± 0.05	1.54 ± 0.04	2.02 ± 0.12
C/N ratio	11.63 ± 0.15	10.41 ± 0.21	12.84 ± 0.48
SOM content (%)	48.57 ± 2.09	63.07 ± 1.45	43.13 ± 3.98
pH	5.05 ± 0.14	5.46 ± 0.15	5.57 ± 0.15
Bulk density (g soil cm^{-3})	0.44 ± 0.01	0.40 ± 0.01	0.14 ± 0.002

2. Materials and methods

2.1. Site description

The experimental site was located at West Sedgemoor, Somerset in SW England, UK (51°0.1.11′N, 2°55.16′W); a 1035 ha peatland site that forms part of the Somerset Levels and Moors Environmentally Sensitive Area (ESA). The site is managed by the Royal Society for the Protection of Birds (RSPB) for wetland bird conservation with the majority of land grazed in rotation with hay cutting a minimum of one year in three. The land is grazed by mixed breed beef steers belonging to a single tenanted farm holding. Approximately 30 animals graze 4.2 ha of land in rotation for two weeks in early autumn. It is known that little or no organic or inorganic fertiliser has been applied to the site for over 20 years.

The climate of the region is characterised by warm winters and cool summers with an average rainfall of 1005 mm annually and an average annual temperature of 10 °C. According to Findlay et al. (1984) and Heathwaite and Ross (1987), the soil profile of West Sedgemoor comprises three clear horizons within the surface 0–30 cm. The uppermost horizon between 0 and approximately 10 cm is loamy clay, resulting from the decay of surface vegetation and is significant despite cutting/grazing activity. Beneath this is a deposit of silty clay arising from periodic inundation by the nearby River Parrett. Below the clay, at between 25 and 30 cm depth in most cases, is black fibrous sedge peat (fibric histosol) of up to 8 m depth. Characterisation of the soil is given in Table 1. These data were collected as part of a separate field trial conducted between May 2009 and June 2010.

The selected field has its water-table controlled by two different drainage ditch management practices. The north and west ditches

are managed by the Parrett Internal Drainage Board (IDB) and the south and east ditches are managed by the RSPB for wetland conservation. The water-level is maintained in the IDB ditches via a large inlet flow into the River Parrett, which lies approximately 2.5 km to the east of the study field. The RSPB ditches are separated from the IDB ditches via a blockade at the north end of the south ditch and a removable pipe at the end of the east ditch that connects the two systems during times of high water level in both ditches but isolates them when the IDB ditches are drained. The IDB-managed ditches have a lower water level than the RSPB ditches between December and March for flood prevention and drainage of agricultural land and are higher between April and June. During the period of this research it was considered unlikely that there would be significant difference in water-table across the field. Kechavarzi et al. (2007) showed that without installation of subsurface irrigation channels, the ditch water level did not have a significant impact on the water-table towards the centre of the field.

The plant community on the experimental site is classified as MG8 according to the National Vegetation Classification (Rodwell, 1992). The MG8 vegetation community is described as a species-rich, varied water meadow with no particular dominant species but grasses accounting for most of the cover.

2.2. Experimental approach

Cattle urine was supplied by the Centre for Dairy Research (CEDAR) dairy unit (University of Reading) and stored unacidified for two weeks at −5 °C before application. The urine was thawed over a period of 48 h prior to application. The control application was water collected from one of the ditches surrounding the field. Prior to application, urine and ditch water samples were analysed for Total N and Total C using a Skalar 5000-02 Autoanalyser for N analyses and a Skalar Formacs^{HT} TOC Analyser for C analyses. Urine total N was 6.7 ± 1.5 g L^{-1} and total C was 13.9 ± 0.5 g L^{-1}. Total N and C in the ditchwater were negligible in comparison (2.5 ± 0.0 and 66.0 ± 0.1 mg L^{-1} respectively).

Two rows of five 2 m^2 replicated plots, each 5 m apart with 2 m between each row, were set up in the field on 15/09/2010. These were placed approximately 5m from the east ditch, which is managed for high water-table during the summer by preventing drainage into a wider channel leading to the river. An area of relatively low water-table fluctuation was chosen based upon results from a previous field study in order to improve the replication of the treatment and control plots. Each plot comprised a static chamber (described below) and three soil atmosphere samplers inserted at 10, 20 and 30 cm depth in the profile. The static chamber and soil atmosphere samplers were offset by approximately 0.75 m within each plot to ensure soil disturbance did not affect the chamber measurements. Treatment and control plots were placed 2 m apart and each adjacent pair of plots shared a dipwell, placed one metre from each plot.

One week following installation of the chambers and soil atmosphere samplers, treatments were applied to the plots (22/09/10). The 5 L m^{-2} treatments were applied in marked quadrants of the 2 m^2 area using a watering can with a sprinkler. The full area of the plots was covered with urine in order to ensure the comparability of soil under the chamber, soil surrounding the subsurface samplers and the area of soil that was taken for analysis throughout the experimental period. The urine application rate was approximately 49.8 g N m^{-2} and 65.2 g organic C m^{-2} (equivalent to an N loading of 498 kg ha^{-1} and a C loading of 652 kg ha^{-1}), appropriate to the average N contents reported in Oenema et al. (1997) and Leterme et al. (2003). The loading is lower than the expected figure given by Haynes and Williams (1993); however, it is within the range of typical values expressed by Oenema et al. (1997) and Whitehead (1986).

Analysis of the N and C content of the ditch water used on the control plots gave an application rate of 12.4 mg N m^{-2} and 105 mg C m^{-2} (equivalent to an N loading of 0.124 kg ha^{-1} and a C loading of 1.05 kg ha^{-1}).

During the first two weeks following application, the plots were monitored for water-table depth and sampled for CO_2, CH_4 and N_2O emissions (chambers) and subsurface concentrations (soil atmosphere samplers) on six occasions (three times per week). Subsequently, monitoring and sampling took place every two weeks for two months. Samples were taken from static chambers to determine the surface fluxes of N_2O, CO_2 and CH_4, and from soil atmosphere samplers to determine the below ground concentrations of the gases. Soil was periodically sampled for WSOC, NH_4^+ and Total Oxidised Nitrogen (TON). Meteorological data (maximum and minimum daily air temperature and rainfall) were collected three to four times a week from a meteorological station located on West Sedgemoor, within 2 km of the field site.

2.3. Gas sampling and analysis

The static chamber method (Mosier, 1989; Hutchinson and Livingston, 2002) was used to measure fluxes. Chambers were 0.4 m × 0.4 m × 0.25 m (internal dimensions) white plastic boxes with gas tight lids (Cardenas et al., 2010). A specialised cutting tool was used for chamber installation which prepared slots for the chamber to be pushed approximately 5 cm into the soil. To ensure a good seal between the chamber and the soil it was essential that all sides of the chamber were fully inserted, so this was checked thoroughly. On each sampling date lids were placed on the chambers at time 0. Following this, 60 ml samples were taken from the chamber headspace using a plastic syringe after 0, 30 and 60 min. The samples were flushed through pre-evacuated, airtight, 20 ml vials using a needle. In between sampling dates, the lids were removed from the chambers in order to re-expose the soil and vegetation inside the chamber to ambient conditions of light and rainfall. Fluxes were calculated based on the rate of change in gas concentration inside the chamber after 30 min for CO_2 and CH_4 and 60 min for N_2O. Accumulation of the gases was shown to be linear during these closed periods during a previous trial (data not shown) and a linear increase was assumed when calculating fluxes from all chambers.

Subsurface gas samplers were based on the design of Clark et al. (2001). The key component is a 10 cm length of gas permeable silicone rubber tubing (11.5 mm diameter Tygon® 3350 sanitary tubing). Jacinthe and Dick (1996) and DeSutter et al. (2006) showed that an equilibration period of less than 6 h is required for the target gases to closely match soil atmospheric concentrations in an unflooded soil. The body of the sampler was a 60 ml syringe which served as a headspace container (140 mm × 25mm) with a septum to allow manual needle sampling. The connection between the syringe unit and the silicone rubber tube was a length of gas impermeable, flexible Tygon® fuel and lubricant tubing allowing a horizontal alignment of the silicone tubing at a single depth, rather than a profile (vertical) alignment (Clark et al., 2001). All connections within the unit were sealed with bungs and silicone sealant to ensure gas and water-tight seals.

The samplers were installed two weeks prior to the commencement of the experiment. A 35 cm trench was dug and the soil and vegetation carefully removed. A tool consisting of a long handle and a pointed extrusion to the diameter of the Tygon tubing was inserted into intact soil at the side of the trench at 10 cm, 20 cm and 30 cm depth and then the sampler tubes inserted. The displaced soil and vegetation was then carefully replaced around the sampler, staying as close as possible to the original layering and bulk density. Vegetation regrew around the samplers within the space of one month. On each sampling date a single 30 ml sample was taken from each sampler and flushed through a 20 ml pre-evacuated vial

for storage and transport. Immediately following sampling, ambient air was allowed back into the sampler to regain equal pressure between the sampler and the atmosphere.

All gas samples were analysed using a PerkinElmer Clarus 500 gas chromatograph (GC) with a Flame Ionisation Detector (FID) at 350 °C for CO_2 and CH_4 detection and a $_{63}$Ni Electron Capture Detector (ECD) at 300 °C for the detection of N_2O. A Turbo Matrix 110 auto-sampler extracted a 0.03 μL min^{-1} sample from each vial and injected it through two 30m × 0.53 mm Elite Plot Q columns. The GC system had a minimum detectable amount (MDA) of 0.33, 0.15 and 0.004 ppm for CO_2, CH_4 and N_2O respectively. Samples were analysed within two weeks of collection.

2.4. Soil sampling and analysis

Two soil cores were taken from each plot on each sampling date using a 5 cm × 15 cm Dutch auger. These were split into three depths (0–10 cm, 10–20 cm and 20–30 cm) and bulked together by depth within each plot, therefore 30 samples were collected on each occasion providing five replicates of each depth for each treatment. Upon return to the laboratory soil samples were stored at 4 °C prior to analysis (within one week of their collection).

Soil was sieved to 4 mm to remove roots and other debris. A 50 g subsample was weighed and then placed in an oven overnight at 105 °C for gravimetric moisture determination. Soil was then analysed for NH_4^+–N and Total Oxidised Nitrogen (TON) using the KCl extraction technique (Bremner and Keeney, 1966). TON is the sum of NO_3^- and NO_2^- and for the purposes of this study is assumed approximately equivalent to NO_3^- content as NO_2^- is generally short-lived in the soil and accumulation is negligible. All analyses for TON and NH_4^+ were performed using an Aquakem 250 or Skalar 5000-02 Autoanalyser.

Soil was analysed for water soluble (available) carbon (WSOC) using a cold water extraction technique with a ratio of one part soil to five parts deionised water agitated for 2 h in an orbital shaker (e.g. McGill et al., 1986; Lu et al., 2011). WSOC analysis was performed using a Skalar Formacs^{HT} TOC Analyser. WSOC was defined as the difference between total C and inorganic C in the solution.

2.5. Statistical analyses

All statistical processing was carried out using Genstat 13th edition (2010). Student's *t*-tests were used to compare greenhouse gas fluxes and subsurface concentrations and soil NH_4^+, TON and WSOC concentrations between treated and control plots. For each soil depth (0–10 cm, 10–20 cm and 20–30 cm), the significance of the treatment over time on subsurface greenhouse gas concentrations, NH_4^+, TON and WSOC was assessed using a two-way repeated measures ANOVA where the degrees of freedom for the *F*-test were scaled by the Greenhouse–Geisser epsilon coefficient.

Daily CO_2, CH_4 and N_2O fluxes were calculated on each measurement occasion. The area under the curve (trapezoidal) method (e.g. Cardenas et al., 2010) was used to calculate cumulative fluxes for each gas across the entire sampling period. These were calculated from the adjusted predictions from general linear regression models of date and location. The proportion of the added N that was emitted from the soil surface was calculated using the average cumulative flux from control plots subtracted from the average cumulative flux from treated plots. The trace N content of the ditch-water (control) applications was assumed to be negligible.

The attribution of factors to GHG fluxes or concentrations was achieved using multiple regression models. Forward selection all-subsets regression was used in the first instance to identify contributing factors to GHG production at each depth and to emissions from the surface. WSOC, NH_4^+, TON, WTD and ambient temperature were included as factors. Although time (days after application) was initially also included as a factor, analysis showed that it followed the same trends as WTD therefore the decision was taken to remove it from the regression models. Following the identification of the most significant contributing factors, a stepwise generalised linear regression was used to fit these terms.

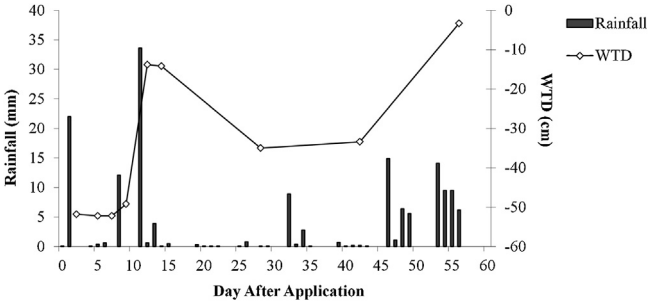

Fig. 1. Rainfall (mm) and average water-table depth (cm ± SE, $N = 5$) throughout the experimental period.

3. Results

3.1. Environmental Variables

During the experimental period, maximum air temperatures fluctuated between 8 and 22 °C and minimum temperatures between −3 and 10 °C with a general downward trend. The minimum temperature dropped to below freezing overnight on occasions. There were several episodes of rainfall throughout the experiment, notably rainfall exceeded 10 mm on Days 1, 8, 11, 32 and from Day 46 (after urine application) onwards (Fig. 1). For the first week after application the water-table was steady, at approximately 55 cm depth below the ground surface. Following the first week the water-table fluctuated in response to rainfall (Fig. 1). By the final sampling date 56 days after urine application, the water-table had risen to approximately 3 cm below the surface. Gravimetric soil moisture varied between 61 and 81% with the majority of the variation as a result of the profile depth of soil sampling ($P < 0.001$) and no significance as a result of date ($P = 0.985$) or treatment ($P = 0.926$). This is likely to be due to the soil type; peat is known to retain moisture due to the high organic matter content and there were incidences of rainfall and decreases in WTD after the first week of the experiment (Fig. 1). The gravimetric methodology does not allow finely accurate quantifications of soil moisture and therefore WTD is considered to be a stronger indicator of soil hydrological conditions than measured water content for the purposes of this study. An in-situ dielectric soil moisture probe calibrated specifically for organic soils may provide the depth of information required to use soil moisture as an explanatory variable.

Soil NH_4^+, TON and WSOC in the 0–10 cm surface layer (the layer showing the most substantial temporal and treatment variation throughout the experimental period) are summarised in Table 2.

Soil NH_4^+ concentrations were between 15 and 300 mg N kg dry soil^{-1} throughout the sampling period (Table 2). Lower concentrations (0–50 mg N kg dry soil^{-1}) were observed at 10–30 cm soil depth. Soil NH_4^+ concentrations were significantly higher ($P < 0.001$) in the plots treated with cattle urine than in the control plots at 0–10 cm and 10–20 cm soil depth but not significant ($P = 0.165$) at 20–30 cm soil depth. In the urine treated plots, NH_4^+ concentrations at 0–10 cm depth increased substantially between 12 and 28 and again between 28 and 42 days after urine application (Table 2). There was a significant effect of sampling date on soil NH_4^+ concentrations at 0–10 cm ($P = 0.019$) but not at 10–20 cm or 20–30 cm. NH_4^+ concentrations in the control soil remained below 20 mg N kg dry soil^{-1} with a generally decreasing trend towards the end of the experimental period.

Soil TON concentrations peaked at 284 mg N kg dry soil^{-1} on Day 12 in the 0–10 cm soil layer of the treated plots and decreased from this point forward (Table 2). As for NH_4^+, lower concentrations (0–150 mg N kg dry soil^{-1}) were observed at 10 to 30 cm soil depth. There was a significant effect of the cattle urine treatment at all depths ($P < 0.001$ in all cases). There was no significance of sampling date at 0–10 cm ($P = 0.165$) but there was evidence of significant temporal variation at 10–20 and 20–30 cm depth. Until the final sampling date, TON concentrations were always significantly higher at 0–10 cm than at 10–20 or 20–30 cm depths in urine treated plots. TON concentration in the control soil remained below 30 mg N kg dry soil^{-1} throughout the experimental period.

Soil WSOC in both urine treated and control plots were within the range of 29–50 mg C kg dry soil^{-1} at 0–10 cm soil depth (Table 2) and 10–20 cm soil depth. WSOC concentrations were consistently higher at 20–30 cm soil depth, but were within the range 30–70 mg C kg dry soil^{-1} at 20–30 cm due to the higher organic matter content of the peat layer. For all sampled depths, WSOC concentrations were not significantly different between urine treated and control plots ($P = 0.121$, 0.373 and 0.222 for 0–10, 10–20 and 20–30 cm respectively). There was a significant effect

Table 2
Average TON, NH_4^+ and WSOC at 0–10 cm soil depth on selected dates throughout the experimental period in treated (cattle urine) and control (ditch water) plots (±standard error). $N = 5$.

Day after application	Cattle urine treated			Ditch water treated		
	TON (mg N kg dry soil^{-1})	NH_4^+ (mg N kg dry soil^{-1})	WSOC (mg C kg dry soil^{-1})	TON (mg N kg dry soil^{-1})	NH_4^+ (mg N kg dry soil^{-1})	WSOC (mg C kg dry soil^{-1})
2	197.4 ± 72.7	15.3 ± 1.1	32.3 ± 0.5	11.6 ± 1.2	9.6 ± 2.7	29.6 ± 0.6
5	172.4 ± 74.0	26.9 ± 4.3	36.3 ± 0.7	8.0 ± 1.6	19.2 ± 6.0	35.8 ± 0.6
12	284.5 ± 88.1	25.4 ± 5.3	35.5 ± 0.5	8.1 ± 0.7	13.4 ± 2.1	37.1 ± 0.9
28	185.2 ± 7.4	185.2 ± 50.7	41.2 ± 0.8	22.9 ± 2.1	3.2 ± 1.0	40.5 ± 0.5
42	68.5 ± 5.0	292.0 ± 93.7	32.2 ± 0.5	16.8 ± 1.5	0.0 ± 0.0	49.3 ± 0.6

of time after application on WSOC concentrations at all sampling depths ($P = 0.028$, 0.002 and 0.002 for 0–10, 10–20 and 20–30 cm respectively).

3.2. Gaseous emissions

The CO_2 fluxes peaked at 5262 mg CO_2 m^{-2} d^{-1} initially a few hours following urine application to the soil, exceeding baseline fluxes by approximately 4000 mg CO_2 m^{-2} d^{-1}. This peak was smaller in the control plots, suggesting that wetting of the soil alone did not prompt this CO_2 release (Fig. 2a). CO_2 fluxes were significantly higher from the urine treated plots than the control plots ($P = 0.010$) largely as a result of this substantial peak. A week after application, CO_2 emissions from the urine treated plots followed the same trend as the control. Cumulative CO_2 emissions over the full 56-day experimental period were higher from the urine treated plots than the control plots (42,014 and 29,462 mg CO_2 m^{-2} respectively).

The CH_4 fluxes initially responded negatively to urine addition, with a mean negative flux after two days (Fig. 2b). There were no clear outliers in the treated chambers, with negative fluxes between −500 and −3000 μg CH_4 m^{-2} d^{-1} for all chambers; however, for the control plots there were three chambers within the range of −2000 to −3400 μg CH_4 m^{-2} d^{-1} and two giving positive fluxes of 186 and 3649 μg CH_4 m^{-2} d^{-1}. Following this date, CH_4 from the urine treated plots was consistently higher than from the control plots with a peak (up to 19 mg CH_4 m^{-2} d^{-1}) evident on Day 12. There was no significant difference between CH_4 fluxes from treated and control plots ($P = 0.111$). Despite the early CH_4 uptake flux, cumulative CH_4 emissions showed net emission from the urine treated plots, whereas the control plots remained an overall CH_4 sink (540 and −13,696 μg CH_4 m^{-2} from the treated and control plots respectively).

Two peaks of N_2O emission were observed during the experimental period (Fig. 2c). The first (20 mg N_2O m^{-2} d^{-1}) was on Day 7 and the second and most pronounced peak (up to 88 mg N_2O m^{-2} d^{-1}) was measured on Day 12 following heavy rain (Fig. 1). Emissions from treated plots were always significantly higher than the control ($P < 0.001$) and had not returned to background levels by the end of the study. Cumulative emissions showed the clear increase in N_2O fluxes following urine application as total emissions from the control site were several orders of magnitude lower (326 mg N_2O m^{-2} and 916 μg N_2O m^{-2} from the treated and control plots respectively). Over the study period, cumulative emissions from the urine treated plots were 356 times higher than those from the control plots. The total emitted N_2O during the 8-week measurement period (urine treated minus control) represented 0.65% of the added N from the urine.

3.3. Subsurface gas concentrations

Concentrations of CO_2 in the subsurface samplers were within a similar range for treated and control plots with clear increases by depth (Fig. 3). There was no significant difference in subsurface CO_2 concentrations between treated and control plots for any depth. There was a significant effect of date after application ($P < 0.001$ at all depths). In both urine treated and control plots, CO_2 concentrations at depth increased as the water-table moved towards the surface. The highest CO_2 concentrations were measured 14 and 56 days after application, corresponding to shallow WTD (Fig. 1).

Subsurface CH_4 concentration was significantly higher in the urine treated plots than in the control plots at 20–30 cm ($P = 0.010$) but not at the shallower soil depths (Fig. 4). Day after application was a significant driver of variation in CH_4 concentrations at 0–10 ($P < 0.001$) and 10–20 cm depth ($P = 0.005$) but not at 20–30 cm ($P = 0.064$). This suggests that the CH_4 concentrations in all plots were more subject to relatively natural variations such as water-table change than the treatment.

Average subsurface concentrations of N_2O were significantly higher in the urine treated plots than in the control plots at all soil depths ($P = 0.005$ for 0–10 cm, 0.002 for 10–20 cm and 0.034 at 20–30 cm, Fig. 5). Day after application was a significant factor controlling subsurface N_2O concentrations in the surface 20 cm ($P = 0.017$ for 0–10 cm and $P < 0.001$ for 10–20 cm) but not significant at 20–30 cm soil depth. Variation of N_2O over time for both control and treated plots tracked WTD variation, especially at 20 cm soil depth (Fig. 5). As for CO_2, soil N_2O concentration peaked on Day 14 and Day 56 for the urine treated plots (Fig. 5a) and this corresponded with shallower WTD (Fig. 1). By the second day following urine addition, there was already a significant difference between control and treated plots for all depths (Fig. 5a). On Day 12, there was an increase in N_2O concentration at 20 cm depth of

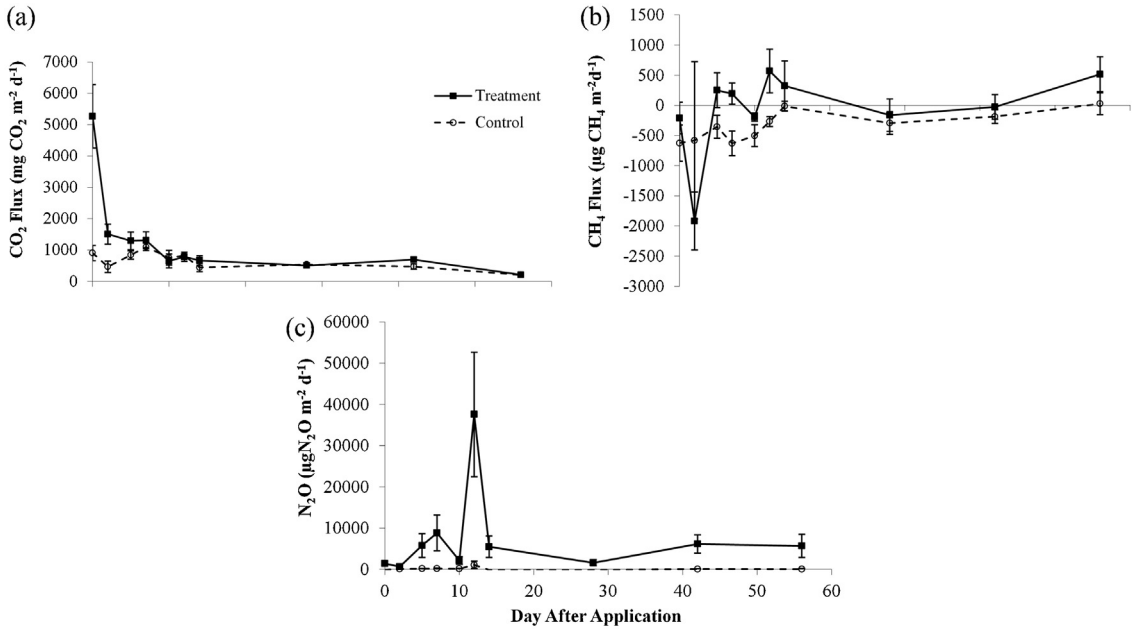

Fig. 2. Average flux of (a) CO_2, (b) CH_4 and (c) N_2O for urine-amended and ditch water amended control plots by day after treatment application. Error bars reflect ± standard error of the mean. $N = 5$.

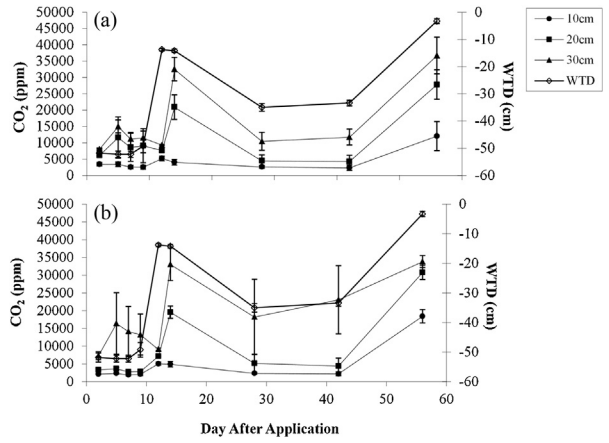

Fig. 3. Average subsurface CO_2 concentrations in (a) the urine treated plots and (b) the ditch water amended control plots. Error bars reflect ± standard error of the mean. $N = 5$.

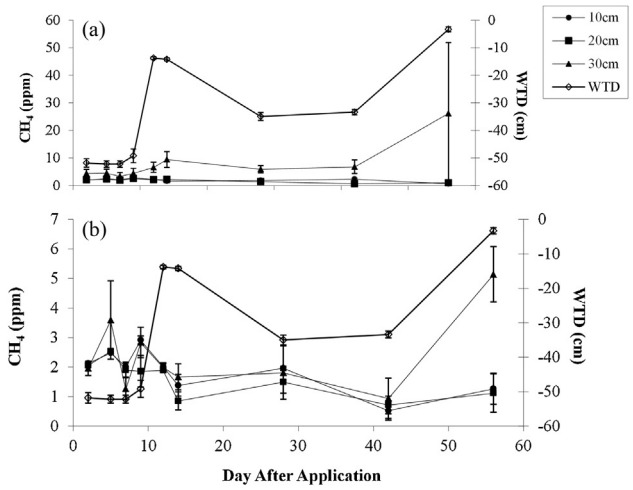

Fig. 4. Average subsurface CH_4 concentrations in (a) the urine treated plots and (b) the ditch water amended control plots. Error bars reflect ± standard error of the mean. $N = 5$.

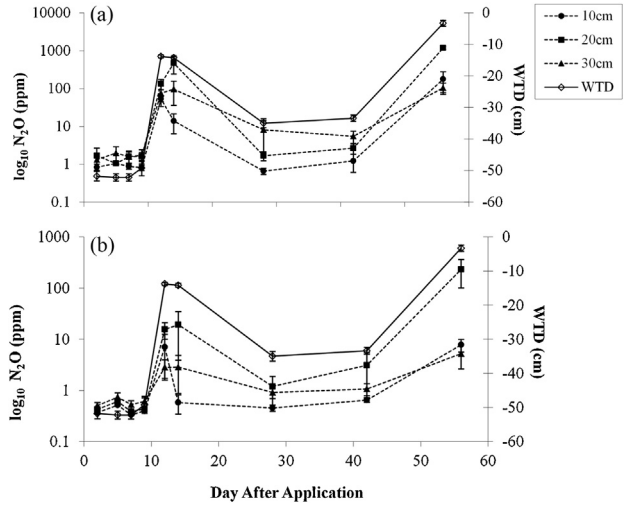

Fig. 5. Average subsurface $\log_{10} N_2O$ concentrations in (a) the urine treated plots and (b) the ditch water amended control plots. Error bars reflect ± standard error of the mean. $N = 5$.

over 30 times the level recorded on in the urine treated plots and the control plot (Fig. 5a and b). By Day 14 the subsurface N_2O in control plots had increased by a factor of up to 4.5 at 20 cm, although there was a concentration decrease at 10 cm in the urine treated plots (Fig. 5a). On the final sampling day, there was another large rise in production at 20 cm in both the urine treated and control plots, with some values at over double those recorded on Day 14. For both urine treated and control plots the highest N_2O concentrations were detected at 20 cm depth during these peak production events.

3.4. Controls on GHG fluxes and subsurface concentrations

Regression analysis indicated WTD was the key control on CO_2 flux, although this explained only 12% of variation. WSOC at any depth contributed a negligible improvement to the model fit. WTD was also the only significant control on CO_2 concentrations at depth, explaining 24.0%, 32.5% and 14.6% of variation in CO_2 at 10 cm, 20 cm and 30 cm depth respectively.

Variation in CH_4 fluxes was controlled by WTD and WSOC measured at 10–20 cm soil depth; however, the variation explained by this model was very low (6.2%). These results suggested that no measured variables were significant controlling factors on CH_4 emission from this site. Subsurface CH_4 concentrations were explained by combinations of WTD, WSOC and NH_4^+. WTD and NH_4^+ in the surface 20 cm of soil explained the greater part of the variation of CH_4 concentrations at 10 cm depth (44.2%). Stepwise linear regression for the identified terms and CH_4 at 20 cm showed WTD accounted for 9.7% of the variation. Addition of NH_4^+ measured in soil taken from 0–10 cm to 10–20 cm depth improved the model fit to 24.0%. The WSOC measured at 0–10 cm provided small increases to the model fit. The CH_4 concentrations at 30 cm were only explained by variation in NH_4^+ in the surface 20 cm of soil (48.2% of variance explained). Inclusion of WTD did not improve the model.

The N_2O fluxes were explained by WTD and surface TON and NH_4^+. WTD alone accounted for 21.0% of the variation. Adding NO_3^- content at 0–10 cm improved this to 26.6%. Therefore WTD, followed by TON at 0–10 cm, followed by NH_4^+ at 0–10 cm was the order of importance of these controlling factors. Subsurface N_2O concentrations were explained by WTD, WSOC and TON. Addition of NH_4^+ did not improve the fit of models of subsurface N_2O concentrations.

4. Discussion

4.1. Effect of urine addition on soil NH_4^+, TON and WSOC

Addition of cattle urine increased concentrations of NH_4^+ and TON in the soil relative to a control. There was a substantial increase in NH_4^+ in the 0–10 cm soil layer between 12 and 28 days after urine application and further increases to the end of the experimental period. A smaller increase was observed in the 10–20 cm soil layer and little increase was observed at 20–30 cm. This accumulation of NH_4^+ in the surface layer may be due to mineralisation of the urine (Allen et al., 1996), suggested also by the decrease in WSOC between days 28 and 42 in the treated plots (but not the controls); however, this is difficult to confirm with low temporal resolution data as changes in WSOC between 12 and 28 days corresponding to mineralisation of organic carbon could not be detected. However, the key control of the observed accumulation is likely to be the sustained shallow WTD during this period. Although there was a variation of around 20 cm, the water-table was observed to remain around 30 cm below the surface and soil moisture was likely to be maintained due to rainfall (Fig. 1). This would have maintained anoxic conditions that are not well suited to nitrification; a mechanism that may have been preventing significant accumulation of NH_4^+. Accumulation of NH4+ is known to be an indicator of denitrification because reduced WTD creates anoxic conditions better suited to denitrification processes than nitrification processes (Nieder and Benbi, 2008) and has previously been shown to be higher and more variable in peat and clay soils than sandy soils after urine addition (Clough et al., 1998).

Soil TON (approximately equal to NO_3^- concentrations as NO_2^- was anticipated to be limited) concentration also peaked 12 days after application in the 0–10 cm soil layer but *decreased* in the surface 10 cm of soil from this point forward. This increase of TON during the first half of the study (corresponding to deep WTD) suggests that during this time nitrification was the key N_2O producing process; however following this (corresponding to shallow WTD), there was a switch to denitrification. This is supported by

the increasing NH_4^+ and decreasing TON. There was no evidence of leaching of NO_3^- to lower soil layers, suggesting either the low temporal resolution of soil sampling could not capture NO_3^- movement through the profile or there was substantial consumption of the NO_3^- in the topsoil. The overall consumption of NO_3^- in the West Sedgemoor topsoil following urine application agrees with studies suggesting denitrification is the key N transformation process in peatland soils (e.g. Aerts and Ludwig, 1997; Nieder and Benbi, 2008); however, a large proportion of this NO_3^- may have been taken up by the vegetation (Urban et al., 1988). Similarly to this study, Li and Kelliher (2005) found that 2 months after urine application, the NO_3^- content in both soils remained greater than that of the controls, whereas Allen et al. (1996) found increased NH_4^+ concentrations throughout a 70 day period after application but no change in NO_3^- concentration.

Addition of cattle urine did not have a significant effect on soil WSOC, suggesting substantial loss of the urine organic carbon pool through hydrolysis within the first two days after application (Li and Kelliher, 2007; Lin et al., 2009). Very similar variation in WSOC over time was shown in both treated and control soils. This is similar to the observations of Kelliher et al. (2005) who showed an increase in soil carbon at 0–10 cm immediately following urine application to soil samples from a dairy farm which began to fall to background levels by two days after application. However, they also found that following urine addition to samples from an ungrazed grassland soil, WSOC in the topsoil remained elevated for eleven days. In their study, no vegetation was present which may account for the rapid loss of the available carbon pool in the West Sedgemoor field shortly after application. There was no evidence of increased WSOC in the treated plots and therefore the remaining C was probably rapidly leached from the top 30 cm of soil or taken up by vegetation. For future work, isotopic labelling of urine C may be used to accurately determine the movement of C through the soil following application to determine the fate of added C in peat soil (Bol et al., 2004; Lambie et al., 2012, 2013). A higher frequency of soil sampling would also be useful in future studies to examine the rate of the loss of this pool from the soil, particularly with reference to high resolution CO_2 flux measurements.

4.2. Nitrous oxide

Cumulative N_2O emission during this study was 3.26 kg N_2O ha^{-1} from the treated plots and emission from the control plots was negligible in comparison (0.009 kg N_2O ha^{-1}). Very low emission of N_2O was expected in this field as peatlands often have low amounts of soil N and there has not been substantial N addition to the field site for an extended period of time. This was supported by the low amounts of TON and NH_4^+ consistently measured in the control plots throughout the experiment. The temporal variation in N_2O emission following cattle urine application found in this study is consistent with others in the literature (Koops et al., 1997; Anger et al., 2003; Di and Cameron, 2012). Li and Kelliher (2005), Maljanen et al. (2007) and Lin et al. (2009) found a peak in N_2O fluxes on the day of application, which was not detected at West Sedgemoor. Anger et al. (2003) suggested that a delay in N_2O emission following urine addition is due to an inactive nitrifier population on swards that do not receive regular N addition. On fertilised swards, they found more rapid and much greater initial N_2O release as a result of the nitrifier community having been primed for N addition. Delay in the emission of NH_4^+ is likely to be due to a combination of gradual mineralisation of urea to NH_4^+, slow response of nitrifier communities to the NH_4^+ increase and possibly competition with plants. The largest emissions were recorded on Day 12, following heavy rain and a rise in the water table by 20 cm. Rainfall has been widely shown to trigger substantial N_2O release following urine application (Allen et al., 1996; Li and Kelliher, 2005; Di and Cameron, 2012). Research has shown that nitrification and denitrification can occur in soils simultaneously, particularly in short periods of high moisture, wherein nitrifying bacteria can turn to short-term denitrification (nitrifier denitrification) of NO_2^- to N_2 via N_2O (Wrage et al., 2011). Regression analysis identified WTD and NO_3^- concentration at 0–10 cm as the key controls on surface flux although these did not explain a great deal of the variation (28.6%). This may be due to higher importance of other factors such as moisture content and inorganic N contents in the surface 1–2 cm of soil, as suggested by Neftel et al. (2007).

Cumulative N_2O emissions data showed increased fluxes with respect to the control plots. The total emitted N_2O (treated minus control) represented 0.65% of the added N from the urine. This figure lies within the expected ranges of values available from other short-term experimental studies (Li and Kelliher, 2005; Hoeft et al., 2012; Smith et al., 2012). Li and Kelliher (2005) found between 0.4 and 1.3% of added N was then emitted to the atmosphere over a 4-month period, with the higher values found in poorly drained soils. Total emitted N_2O appears to be higher for peat soils (between 2 and 4%), as a result of this anticipated waterlogging (Koops et al., 1997; van Beek et al., 2011).

The N_2O concentrations at depth initially corresponded to surface N_2O release, increasing substantially at 10 cm depth between Day 10 and Day 12. This increase was even more substantial at 20 cm depth with concentrations as high as 200 ppm in some plots. By Day 14 N_2O production in the treated plots had increased yet further at 20 cm (values up to 1300 ppm) but had decreased at 10 cm and no peak in surface N_2O fluxes was detected.

The N_2O accumulation at 20 cm depth is likely to be a result of the physical changes in porosity and diffusion coefficient between soil horizons. Boon et al. (2013) showed a decrease in porosity and gaseous diffusion between the peat subsoil and the clay layer at this field site at lower airfilled porosities. The subsurface samplers were below or close to the water-table for much of the experimental period; therefore diffusion of the produced N_2O is likely to have been restricted at 20 cm, leading to the observed accumulation. The strong likelihood that the release of N_2O produced in the soil was controlled by the diffusion coefficient supports the findings of Balaine et al. (2013). Balaine et al. (2013) also showed that the production of N_2O is sensitive to changes in the diffusion coefficient and therefore finer scale monitoring or modelling of the diffusion coefficient in the surface soil may have aided explanation of N_2O production at 20 cm. There may also have been chemical changes between the peat and clay horizons which influenced N_2O production (Clough et al., 1998; Clough et al., 2003b); however, this study did not focus on variation in soil chemistry as a significant source of variation. Future work considering the combination of soil chemical variation and addition of cattle urine in peat soils may provide additional perspectives on the observed variability of N_2O production within the soil profile.

Although the highest N_2O concentrations were detected at 20 cm both for urine treated and control plots, N_2O emissions were only influenced strongly by changes detected at 10 cm. This is probably due to the low diffusion coefficient of N_2O through water (2.04×10^{-5} cm^2 s^{-1}; Grabble, 1966) reducing its ability to pass through the waterlogged soil. In addition, denitrification of N_2O to N_2 also depletes N_2O in an anaerobic soil (Terry et al., 1981; Arah et al., 1991). Maljanen et al. (2003) likewise suggested that when a soil is waterlogged only concentrations at 5 cm depth can be indicative of flux; however, when the soil is dry, N_2O at 20 cm correlated well with the surface flux. Neftel et al. (2007) state that N_2O fluxes are only influenced by the first 1–2 cm of soil, particularly where uptake fluxes are concerned; therefore surface fluxes cannot be easily predicted from N_2O concentrations below this depth. Surface measured N_2O fluxes are unlikely to be indicative of the

concentrations of N_2O at depth within the soil and likewise, fluxes calculated from subsurface concentrations may overestimate the amount of N_2O that actually reaches the surface. N_2O may also be carried from depth by mass movement events and episodic fluxes may occur that are not captured by low resolution studies.

Finally, ambient temperature was not shown to have a significant effect on greenhouse gas fluxes or subsurface concentrations by the regression analyses used to study these data. Air and soil temperature is known to have a positive correlation with greenhouse gas emissions due to stimulation of microbial metabolisms (Smith et al., 2003). The effect is complex for N_2O fluxes however, since temperature also controls the functionality of microorganisms facilitating CH_4 oxidation and N volatisation as NH_3 gas (Lockyer and Whitehead, 1990; Sugimoto et al., 1993).

4.3. Methane

The finding of an apparent increase in CH_4 oxidation (negative fluxes) following application is contrary to expectation as most other studies found CH_4 peaked shortly after application (Li and Kelliher, 2005; Lin et al., 2009) or did not influence on continual uptake fluxes (Liebig et al., 2008). Despite this negative peak, cumulative CH_4 fluxes showed net emission of CH_4 from the treatment plots compared with net uptake on the control plots. Regression modelling could not explain a high amount of variation in CH_4 fluxes. Further study would be required to determine whether the negative fluxes of CH_4 following urine application can be repeated and a higher temporal resolution would be beneficial to determine the duration of the negative response.

It has been shown that presence of NH_4^+ can inhibit CH_4 oxidising bacteria and promote CH_4 production (Dobbie and Smith, 1996; Li and Kelliher, 2007; Lin et al., 2009), although quantifying this effect separately from the effect of water addition requires further research. This is the key mechanism that should increase CH_4 emission from urine spots, simply by preventing its oxidation. However, the results of this experiment also suggest enhanced production of CH_4, or perhaps enhanced storage of CH_4 at depth. However, closer examination of the data reveals a number of 'hotspots' that may be unrelated to the urine addition and rather associated with zones of anaerobicity in the soil (Blodau and Moore, 2003). CH_4 concentrations of 0 (or close to 0) were much more frequent in the soil atmosphere samplers located in control plots than the urine treated plots, once more supporting the hypothesis that NH_4^+ inhibition rather than enhanced CH_4 production is the main cause of increased CH_4 emission from the urine treated plots. The regression models for CH_4 concentrations at depth also showed that NH_4^+ was a significant control on CH_4 concentrations; however, the nature of its influence varied between a positive and negative contribution. Further research at a higher temporal and spatial resolution, under a controlled environment would be useful to examine the importance of NH_4^+ on subsurface CH_4 concentrations in this soil.

4.4. Carbon dioxide

The range of CO_2 fluxes observed during this experiment supports findings of other studies on temperate peat soils (Carter et al., 2012; Danevčič et al., 2010; Maljanen et al., 2010), including an earlier study carried out at the same field site (Kechavarzi et al., 2007). The large peak in CO_2 fluxes a few hours after urine application was also shown following yak urine application to a meadow soil in China (Lin et al., 2009). This is likely to be due to carbon release from hydrolysis of urea or promotion of microbial respiration (Kelliher et al., 2007; Lin et al., 2009). This may be related to the possible rapid loss of urine C from the topsoil discussed in Section 4.1. The CO_2 peak was not evident in the control plots, suggesting that this was not a wetting effect. Lin et al. (2009) indicated that temperature was the key control on CO_2 emissions rather than soil WFPS, however the water table in their study field (an alpine environment) did not vary as substantially as found during the autumn rewetting at West Sedgemoor. Conversely, Uchida et al. (2011) found no temperature effect (varied between 11 and 23 °C) on the significant increase of CO_2 following urine addition in a sub-tropical environment. Further research on controls on the effect of urine on CO_2 emissions from temperate peatland soils would be beneficial in order to disentangle these effects. Subsurface CO_2 concentrations recorded within the top 10 cm of soil would also be useful to determine the zones of production of this microbial response.

Cumulative emissions of CO_2 showed that over the experimental period approximately 13 g of C was lost as CO_2 during the experimental period (subtracting the cumulative CO_2 from the control plots from that of the treated plots). This is 20% of the total added C, slightly higher than the 11% loss estimated by Bol et al. (2004) and 15% by Petersen et al. (2004), although these studies were shorter in duration. It is likely that there was not full capture of the CO_2 loss from hydrolysis during the first day after application and therefore the cumulative emissions from the treated plots may be underestimated. There was little evidence of increased CO_2 emission from the priming of soil C by the urine (Clough et al., 2003a; Lambie et al., 2013); however as discussed previously, a large proportion of urine-C may have been leached from the surface soil, leading to the limited change in WSOC observed in the surface soil layers.

Subsurface CO_2 concentrations were within a similar range for treated and control plots and supported by the concentrations recorded in other studies on peatland soils (Jungkunst et al., 2008; Elberling et al., 2011). Regression models for subsurface concentrations of CO_2 following urine addition showed WTD was the only measured factor that controlled variation for this gas. These results suggest no impact of cattle urine application on CO_2 concentrations in the soil profile.

5. Conclusion

This study showed that there was a significant effect of cattle urine addition on both subsurface concentrations and emissions of CH_4 and N_2O but urine addition had little long-term impact on CO_2 fluxes. Cumulative emissions clearly showed the potential for considerable N_2O fluxes from this field site during periods of grazing. Regression analysis on the field data showed that only the inorganic N concentrations in the first 10 cm of soil have a significant relationship with the surface fluxes of N_2O. This analysis also identified water-table depth as the dominant control on N_2O production and emission. An accurate estimate of soil moisture would be beneficial in future studies to further examine the influence of soil hydrology on production and emission of greenhouse gases. Accumulation of NH_4^+ and depletion of NO_3^- suggested denitrification as the major N_2O producing process. Regression analysis suggested NH_4^+ to be a significant control of CH_4 concentrations, supporting other studies that demonstrate the inhibitory effect of NH_4^+ on methane oxidation. This research also found a significant increase in CH_4 oxidation in the treated plots two days following application. Further research is required to understand the mechanisms behind this apparent initial increase in CH_4 oxidation.

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