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Livestock-induced N_2O emissions may limit the benefits of converting cropland to grazed grassland as a greenhouse gas mitigation strategy for agricultural peatlands

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Abstract

Drained peatlands support highly profitable agriculture, but also represent a globally important source of greenhouse gas (GHG) emissions. Grasslands can typically be maintained at higher water levels than croplands, so conversion of cropland to grassland represents a potential CO_2 mitigation strategy that allows for continued agricultural production. However, the presence of high water levels and livestock on grasslands risks generating high emissions of N_2O, particularly associated with livestock urine patches. In the present study, a controlled mesocosm experiment was carried out to quantify the interactive impacts of groundwater level (10 cm, 30 cm and 50 cm water table depth, WTD) and sheep urine deposition on GHG emissions from peat soils. Our results showed that N_2O emissions were significantly higher at 30 cm for both urine-treated and control mesocosms, due to the conditions favouring the interplay of nitrification and incomplete denitrification. The urine N_2O emission factor was 0.25±0.17% at the 30 cm WTD and 0.20±0.07% at 50 cm WTD, lower than typical values for grasslands. No significant difference was observed in ecosystem respiration or methane flux between 30 cm and 50 cm WTDs. Overall, we conclude that strategies to raise water levels in drained peatlands through conversion of cropland to grassland need to account for the potential impacts of N_2O emissions when seeking to minimise overall GHG emissions. Shifting from cropland to grassland management on peatlands for climate change mitigation also requires consideration of the effects of livestock methane emissions, and displaced emissions resulting from increased land demand for

crop production elsewhere.

Key words: greenhouse gas (GHG) mitigation, hydrological regime, land use management, soil fertility, sustainable agriculture

1. Introduction

Peat is a precious natural resource ecologically and economically. Global peatlands store >600 Gt of carbon (C) but are highly vulnerable to degradation following drainage for productive uses (Joosten, 2010; Yu *et al*., 2010). Drainage aerates peat soils, increasing rates of soil organic matter (SOM) mineralisation and resulting in estimated greenhouse gas (GHG) emissions of ~1.9 Gt CO_2-eq annually from degraded peatlands (Leifeld and Menichetti, 2018). Peatlands are also highly productive and profitable for agriculture, creating a challenge for policy makers balancing climate, economic and food security concerns. As cessation of agriculture could have negative effects on the local economy and communities reliant upon it, there is interest in land use options (e.g. conversion of cropland to grazed grassland) that can retain some economic productivity whilst reducing GHG emissions.

The relationship between increasing CO_2 emissions and deeper drainage depths in peat soils is well established (Couwenberg *et al.*, 2011; Evans *et al*., 2016). Raising the water table depth (WTD) nearer to the surface has been shown to reduce CO_2 emissions from agricultural peatlands and represents an important potential mitigation option (Wen *et al*., 2020a, b). Grasslands can be managed for shallower WTDs than cropland due to differences in vegetation traits and vehicle access requirements. Moreover, pasture plants are less prone to damage by anaerobic conditions than arable crops and do not need to be cultivated annually. The presence of year-round vegetation cover also provides protection against wind erosion losses which can be substantial on peat

cropland (2.3 - 12.8 t ha^{-1} yr^{-1}; Cumming, 2018). There is evidence that the GHG balance (soil CO_2 and soil CH_4) of extensively grazed (12.4 t CO_2-eq ha^{-1} yr^{-1}) and intensively grazed (16.7 t CO_2-eq ha^{-1} yr^{-1}) grassland sites can be substantially lower than for cropland (25.3 – 28.5 t CO_2-eq ha^{-1} yr^{-1}) on UK lowland peatlands (Evans *et al*., 2016). This is also reflected in lower 'Tier 1' emission factors (EFs) for grassland versus cropland in all climate zones reported by the Intergovernmental Panel on Climate Change (IPCC, 2014). Conversion of croplands to extensively grazed grasslands is thus a candidate strategy for responsible management of peatlands under agricultural use.

N_2O emissions from peat soils are more variable than CO_2 emissions, and the factors driving them are less well understood (Liimatainen *et al*., 2018). Fluctuating WTD can influence both nitrification which occurs under aerobic conditions and denitrification which is promoted under anaerobic conditions (Firestone and Davidson, 1989; Tiemeyer et al., 2016). The optimal range of water-filled pore space (WFPS) for N_2O emissions from agricultural peat soils is 78-95% (Säurich *et al*., 2019), with the majority of emissions due to denitrification (Pihlatie *et al*., 2004). This is supported by large pulses of N_2O emissions observed following application of N rich, cover crop residue to UK lowland peat soils moistened by a shallow water table (Wen *et al*., 2019a). However, N_2O emissions will decline under water-saturated environment as the terminal step of denitrification can reduce N_2O to N_2 (Firestone and Davidson, 1989). Overall, it remains unclear how water table elevation and livestock presence will

influence N_2O emissions under land use change.

The capacity of agricultural peat soils to produce substantial N_2O emissions under moist conditions clearly has the potential to offset some of the CO_2 mitigation benefits associated with raising water tables. This is an important consideration for grassland conversion, where raising water tables is a key driver and where inputs of N from both livestock excreta and fertilizer can be substantial on more intensively managed sites. Urine deposited by livestock produces a spatially concentrated, bioavailable source of both N and C (e.g. urea, purine derivatives, hippuric acid and amino acids; Marsden *et al*., 2020), whilst simultaneously increasing soil moisture content. Boon *et al*. (2014) recorded cumulative N_2O emissions of 3.26 kg N_2O ha^{-1} over eight weeks following application of urine to a peat grassland in the Somerset Levels, UK. A substantial proportion of these N_2O emissions occurred as a pulse following heavy rain and an associated rise in the WTD from approximately 50 cm to 15 cm (Boon *et al*., 2014). This indicates that interaction of the WTD with urine-derived N may exacerbate N_2O emissions from urine patches on peat grasslands by creating conditions favoring incomplete denitrification of any nitrate (NO_3^-) produced to N_2O. High N_2O emissions from urine patches under raised WTD management could offset some of the benefits of converting cropland to grassland. Quantification of these effects is, therefore, an important step in assessing the overall potential of converting cropland to grassland for GHG mitigation on lowland peat soils.

The East Anglian Fens in the UK have been extensively drained and now include

~50% of England's grade 1 agricultural land, produce ~33% of England's vegetables and support a local agricultural economy worth approximately £3 billion (GBP; NFU, 2019). However, under arable management, East Anglian fen peat soils produce an estimated 26.1 – 38.8 t CO_2-eq ha^{-1} yr^{-1} of GHG emissions (including N_2O; Taft *et al*., 2017) and probably represent one of the largest source of land use GHG emissions in Europe per unit area (Evans *et al*., 2017). Partially rewetted cropland converted to seasonally inundated grassland has been found to have GHG emissions (soil CO_2 and soil CH_4) ~80% lower than cropland in the region (Peacock *et al*., 2019). However, there is currently only limited evidence available with which to assess the effects of grassland conversion in the region on N_2O emissions, which could have important implications for its effectiveness.

This study represents the first controlled experiment examining the interaction between the impacts of WTD and urine deposition following grassland establishment on a former arable soil. We aim to provide insights into the N dynamics of peat under grassland and allow a better understanding of the potential for grassland establishment as a responsible GHG management strategy for temperate eutrophic peatlands. We hypothesised that: 1) Urine deposition will increase N_2O emissions due to substantial N substrate added and 2) this effect will be more pronounced in shallow than deep drained soils because soil moisture conditions in shallow drained soils will be more favourable for denitrification.

2. Materials and methods

2.1. Study site and experimental design

We conducted an indoor mesocosm experiment to elucidate the interactions between soil WTD and urine deposition. Soil cores were sampled from a site under intensive arable management in East Anglia, UK (52°31'N, 0°23'E). The field had been used to produce vegetables and wheat over the past 80 years (Taft *et al*., 2017). The soil is classified as an Earthy Sapric Fen Soil (Avery, 1990) or Typic Haplosaprist (USDA-NRCS, 2006). The soil properties were organic matter 78.5%, total C 50.7%, total N 2.71%, pH 6.45, and bulk density 0.32 g cm^{-3} (Wen *et al*., 2019a). We collected 20 intact soil cores by driving PVC pipes (16 cm inner diameter and 55 cm height) into the soil and then transported these to Bangor University where they were prepared for use as mesocosms. The mesocosms were placed in a greenhouse (average ca. 20 ℃, simulating the mean temperature during May-Sep. in East Anglia) throughout the study. The mesocosms were placed into outer plastic containers, which were manually filled every two days throughout the study, in order to allow bottom-up control of WTD at the experimentally defined levels (50 cm, 30 cm, and 10 cm). This method ensured that the WTD would not be affected by differing evapotranspiration rates between treatments, as water addition rates would track losses. We chose these depths as 50 cm is current practice during the growing season on cropped soils (although average drainage depth was 1.5 m deep). A 30 cm WTD has been reported to supress GHG emissions (excluding N_2O) whilst maintaining vegetation productivity (Musarika *et al*.,

2017). The 10 cm WTD was selected to simulate a restoration situation, which would be expected to further reduce GHG emissions but have little/no capacity for livestock grazing. After a 3-day acclimation period, we randomly imposed experimental WTDs on eight cores each for 50 cm and 30 cm treatments and four cores for the 10 cm treatment.

Thirty seeds of ryegrass (*Lolium perenne* L.) were sown in each core. One week after germination, we applied 200 mL of sheep urine (4.3 g N L^{-1} and 8.2 g C L^{-1}, equal to 1.63 g C and 0.87 g N per core) to half of the cores in both the 50 cm and 30 cm WTD treatments. No sheep urine was applied on 10 cm WTD cores, as its load bearing capacity for grazing approaches zero, so such a treatment was considered unrealistic. A 200 mL urination event represents a typical volume produced by a lowland ewe, and the area of the mesocosm (201 cm^2) is within the range of urine patch wetted areas reported for sheep (Marsden *et al*., 2018). We applied 200 mL of distilled water to the remaining cores to act as a control. The sheep urine was collected from sheep fed on *Lolium perenne* L. (Marsden *et al*., 2017), which has been approved by Bangor University (Ethics approval code CNS2016DC01). The urine application resulted in an equivalent total N loading rate of ca. 435 kg N ha^{-1}. No fertilizer was applied to mesocosms during the experiment. This study formed five treatments (i.e. 50 cm WTD, 50 cm WTD + Urine, 30 cm WTD, 30 cm WTD + Urine, and 10 cm WTD). Each treatment had four replicates (in total 20 mesocosms).

173 *2.2. GHG measurements and calculations*

174 We conducted intensive gas sampling using cylindrical opaque chambers (16.5 cm
175 inner diameter and 12 cm height). Chambers were fitted with a Suba-Seal® (Sigma, UK)
176 to enable gas sampling. On each sampling occasion (at days 1, 2, 3, 5, 7, 9, 13, 17, 22,
177 27, 33, 41 after urine application), three headspace samples were taken using a syringe
178 at 1, 11, and 21 mins following chamber closure. Gas samples were placed in pre-
179 evacuated 20 mL glass vials (QUMA Electronik & Analytik GmbH, Wuppertal,
180 Germany) for storage. Gas samples were analysed using a gas chromatograph
181 (PerkinElmer, CT, USA) with a TurboMatrix 110 auto sampler. Gaseous fluxes were
182 calculated from the linear changes of gas concentrations in the headspace, adjusting
183 with atmospheric pressure and air temperature (Wen *et al*., 2017). Cumulative fluxes of
184 CO_2 (i.e. ecosystem respiration), N_2O and methane (CH_4) were calculated by linear
185 interpolation of measured flux rates (Wen *et al*., 2017). The 6-week N_2O emission factor
186 (EF) for sheep urine addition was calculated as follows:

187 $$EF = \frac{N_2O\_N_{treatment} - N_2O\_N_{control}}{Total\ \ N\ \ applied} \times 100\%$$

188

189 *2.3. Soil solution measurements*

190 Soil solution samples were taken using Rhizon suction samplers (Rhizosphere
191 Research Products, Wageningen, The Netherlands), which were vertically installed in
192 the cores at a depth of 5 cm. Sterile vacutainer tubes were used to recover soil water
193 over a 24 h period. The samples were kept frozen until analysis for NO_3^-, ammonium

194 (NH_4^+), dissolved organic C (DOC), pH and electrical conductivity (EC). Colorimetric
195 methods were used to quantify NH_4^+-N (Mulvaney, 1996) and NO_3^--N (Miranda et al.,
196 2001) contents. DOC was determined using a Multi N/C 2100/2100 analyzer
197 (AnalytikJena AG, Jena, Germany). Soil pH was determined using a pH meter (Hanna
198 Instrument Ltd., Leighton Buzzard, UK), and EC was analysed using a standard Pt
199 electrode.
200
201 *2.4. Soil microbial community structure measurement*
202 After plant harvest, 10 g of soil were collected from each mesocosm at 0−10 cm
203 depth and stored at −80 °C until analysis. The microbial community structure was
204 determined by phospholipid fatty acid (PLFA) analysis, based on the method of Bartelt-
205 Ryser *et al*. (2005). The Sherlock® PLFA Method and Tools Package (PLFAD1;
206 Microbial ID Inc., Newark, USA) was used to disentangle taxonomic groups. The
207 PLFAs, which are higher than 0.5% of the total PLFA amount, were selected for
208 biomarker and taxonomic group annotation. The fatty acids used to identify different
209 taxonomic groups are shown in Table S1.
210
211 *2.5. Biomass-C and -N measurements*
212 Grass was harvested at the end of the experimental period (at 41 d after
213 treatment application) to allow quantification of aboveground biomass. Fresh shoot
214 biomass was measured immediately. Dry shoot biomass was measured by oven-drying
215 at 60 ℃ for 72 h. Biomass-C and biomass-N were determined from ground dry samples

with a TruSpec® CN Analyzer (Leco Corp., St. Joseph, MI, USA).

2.6. Statistical analysis

Data was tested for normality and homogeneity of variance using the Shapiro-Wilk test and Levene's test, respectively. Parameters with non-normal distributions or unequal variances were transformed as required. Effects of urine deposition and water table depth were analyzed using two-way analysis of variance (ANOVA) without interactions. Tukey's post hoc test with correction for multiple testing (SPSS Statistics 24, IBM Corp, NY, USA) was used to compare treatment means. The proportions of total PLFA biomass associated with specific taxonomic groups were used as PLFA fingerprints to assess variation of microbial communities under different treatments. PLFA data were analyzed by principal component analysis (PCA) and redundancy analysis (RDA) with CANOCO 5.0 (Microcomputer Power, Ithaca, NY, USA). Statistical evaluation of differences in the soil properties between groups of samples was performed by applying an analysis of similarity (ANOSIM) with 999 permutations using R v.4.0.2.

3. Results

3.1. GHG fluxes under sheep urine deposition and water table depth treatments

A large N_2O emission pulse occurred 1-5 days after urine application regardless of water table level (Fig. 1a). This pulse dominated the cumulative N_2O fluxes and

accounted for 53±5% and 54±5% of the cumulative N_2O emissions from the 50 cm and 30 cm WTD treatments respectively. N_2O fluxes decreased following this pulse and were indistinguishable from the control (no urine addition) two weeks later. Urine addition significantly increased cumulative soil N_2O emissions compared to the controls ($P < 0.01$). At the end of the study, 104 - 204 mg N m^{-2} were lost through N_2O emissions from urine application treatments (Fig. 2a). The EFs of sheep urine for the 41 day measurement period were 0.20±0.07% (50 cm WTD treatment) and 0.25±0.17% (30 cm WTD treatments; $P > 0.05$). Regardless of sheep urine application, cumulative N_2O flux from the 30 cm WTD treatments was highest and the flux from 10 cm WTD treatments was lowest, whilst the flux from the 50 cm WTD treatments was intermediate ($P < 0.01$; Fig. 2a).

Ecosystem respiration, which consists of heterotrophic respiration (microbial metabolism) and autotrophic respiration (grass root and shoot metabolism), was significantly affected by both WTD and sheep urine application ($P < 0.01$; Fig. 1b and Fig. 2b). Raising the WTD decreased ecosystem respiration, with emissions lower at a WTD of 10 cm than in the 30 cm and 50 cm treatments ($P < 0.01$). Urine application treatments had higher ecosystem respiration rates than the controls ($P < 0.01$).

Soil CH_4 fluxes ranged from -55 to 97 μg CH_4-C m^{-2} h^{-1} over the whole measurement period. No CH_4 emission peaks were observed during the measurement period (Fig. 1c). Neither sheep urine application nor WTD significantly influenced CH_4 fluxes ($P > 0.05$; Fig. 1c and Fig. 2c). Cumulative CH_4 fluxes ranged from -1±6 mg C

m^{-2} (50 cm WTD + Urine) to 25±11 mg C m^{-2} (50 cm WTD) over the whole measurement period.

3.2. Temporal dynamics of soil solution concentrations

Soil NH_4^+ concentrations in the mesocosms without urine application were close to zero throughout the study and no significant differences were found between WTD treatments ($P = 0.13$; Fig. 3a). Urine application significantly increased soil NH_4^+ concentration ($P < 0.01$), with 138 mg N L^{-1} and 54 mg N L^{-1} under the 50 cm and 30 cm WTD treatments at the first sampling point. Thereafter, NH_4^+ concentrations in the soil solution gradually decreased, and were effectively zero by day 21, remaining as such for the remainder of the measurement period (Fig. 3a). As with NH_4^+ concentrations, soil solution NO_3^- concentrations in mesocosms without urine application were close to zero throughout the study (Fig. 3b). However, the mesocosms with urine application significantly influenced soil solution NO_3^- concentrations ($P < 0.01$), displaying higher NO_3^- concentrations and a clear pattern of temporal variation. Soil NO_3^- concentrations were similar in the 30 cm and 50 cm WTD urine treatments and increased from 9 mg N L^{-1} on day 1 to 383 mg N L^{-1} by day 16, before decreasing to 5 mg N L^{-1} by day 41.

Soil DOC concentrations in the mesocosm without urine application were low throughout the experiment (range from 58-126 mg C L^{-1}), and the concentration was significantly lower at 10 cm and 30 cm WTD compared to 50 cm WTD treatments (*P*

279 = 0.04; Fig. 4). The concentration of DOC in the mesocosms with sheep urine addition
280 decreased substantially between the first and second sampling events and remained low
281 until the end of the measurement period. No significant difference was observed in
282 average DOC concentration between with and without urine applied mesocosms (P =
283 0.08).

284 Soil pH ranged from 6.0 to 7.4 over the whole measurement period (Fig. 5a). No
285 significant differences in pH were observed between WTD treatments (P = 0.32),
286 whereas sheep urine application significantly decreased mean pH value during the
287 measurement period (P = 0.03). Soil EC was not affected by water table depth, but was
288 significantly increased by sheep urine application (P < 0.01; Fig. 5b). With urine
289 addition, mean EC increased from 2.5 mS cm^{-1} on day 1 to 5.0 mS cm^{-1} on day 22, and
290 then decreased to 0.6 mS cm^{-1} by day 41.

291

292 *3.3. Soil microbial community structure*

293 Total soil microbial PLFA biomass was not affected by urine application (P = 0.06),
294 but was significantly increased with raised WTD (P < 0.01; Table 1). The proportion of
295 PLFA biomass associated with Gram-negative bacteria increased with shallower WTDs
296 (P < 0.01), whereas the proportion of arbuscular mycorrhizal (AM) fungi decreased
297 with shallower WTDs (P < 0.01). No effect of WTD treatment was found on the
298 proportions of Gram-positive bacteria, total fungi, actinomycetes, or protozoa (P =
299 0.06-0.44). The ratio of bacteria to fungi increased with shallower WTDs (P < 0.01)

300 and the ratio of Gram-positive to Gram-negative bacteria decreased ($P < 0.01$). Sheep
301 urine deposition significantly decreased the proportion of AM fungi ($P < 0.01$), but
302 increased the ratio of bacteria to fungi ($P = 0.03$).

303 PCA carried out on the PLFA data showed microbial community shifts in response
304 to different moisture regimes ($P < 0.01$; Fig. 6a). The first two principal components
305 derived from the PLFA fingerprints explained 72.5% of the total variance. When points
306 were grouped by treatment, there was clear separation between the three WTDs. The
307 effects of urine application on microbial community structure were not statistically
308 significant ($P = 0.35$). The RDA showed that the abiotic environmental variables
309 measured explained 87.6% of the variance in the soil microbial community composition
310 (Fig. 6b). The RDA supports the relationship of WTD with Gram-negative bacteria and
311 AM fungi.

312

313 *3.4. Aboveground biomass and biomass-C and -N*

314 Grass biomass was significantly affected by WTD, as raising water table levels
315 decreased both fresh and dry biomass ($P < 0.05$; Table 2). Aboveground grass biomass
316 was significantly higher with sheep urine application treatments ($P < 0.01$), with mean
317 biomass three times higher in urine treated cores. Sheep urine application significantly
318 increased biomass-N, and decreased both biomass-C and C:N ratio ($P < 0.01$) but there
319 was no effect of WTD on these variables (P =0.35-0.63).

320

4. Discussion

4.1. Nitrogen cycling and nitrous oxide emissions

Soil N_2O emissions were significantly affected by WTD, although the relationship was not linear. Elevated N_2O emissions were observed in both lower WTD treatments compared to the 10 cm WTD. This likely resulted from both (1) increased rates of peat mineralisation providing substrate for nitrification and denitrification and (2) soil redox conditions favourable for production of N_2O through nitrification and denitrification (Koops *et al*., 1997). However, cumulative N_2O emission at 30 cm WTD was five times higher than 50 cm WTD, indicating that the production of N_2O (as a product of incomplete denitrification) has an intermediate moisture optimum (Butterbach-Bahl *et al*., 2013). Very low N_2O emissions in the 10 cm WTD treatment would be explained by low soil redox potential (Wen *et al*., 2019b) inhibiting SOM mineralisation and presenting a bottleneck for N cycling. Also, the water-saturated and mostly anaerobic conditions promoted the last step of denitrification that reduces N_2O to N_2 before it escapes from the soil surface (Firestone and Davidson, 1989).

Cumulative N_2O emissions were higher in the urine deposition treatments, which would mostly be derived from N in sheep urine (Fig. 2a). Cumulative N_2O emissions across the measurement period were dominated by a large initial peak in the urine treatments (Fig. 1a, Fig. 2a). Urine application increases the bioavailable organic and inorganic N pool (ca. 866 mg N for each mesocosm; high NH_4^+ and NO_3^- contents showed in Fig. 3a, b), creates short-term wet soil conditions, and lowers soil redox

potential (Marsden *et al*., 2016), whilst also providing a supply of labile C (higher DOC contents showed in Fig. 4). Under these conditions, denitrification may have co-occurred with nitrification, explaining the high N_2O emissions (Yamulki *et al*., 2000; Carter, 2007; Surey *et al*., 2020). Equilibration of urine (i.e. downward percolation) in the soil profile and plant-derived water loss via evapotranspiration would rapidly reduce moisture content and increase redox potential. As N_2O emissions are highest in the narrow range of redox potentials between 120-250 mV (Yu *et al*., 2001), we hypothesise that optimal conditions for N_2O emissions are only short-lived. Higher peak N_2O emissions and longer peak duration in the 30 cm WTD treatment support this moisture-driven interpretation (Fig. 1a).

4.2. Urine patch N_2O emission factors

The urine N_2O EFs obtained in this study (50 cm WTD, 0.20±0.07%; 30 cm WTD, 0.25±0.17%) were comparable with the IPCC sheep urine default value of 0.39% in wet climates and 0.31% in dry climates (IPCC, 2019). They were lower than a UK nationwide estimate of 0.69% for cattle urine (Chadwick *et al*., 2018) and an estimate of 0.63% for sheep urine (Marsden *et al*., 2017). It might result from the absence of rainfall in this greenhouse study, reflecting conditions which frequently occur in the study location (SE England; Dodd *et al*., 2020). Urine patch N_2O emissions have been observed to be higher in wetter months (Allen *et al*., 1996), with rainfall a key driver of seasonal differences (Bell *et al*., 2015). Marsden *et al*. (2019) obtained an EF of 0.01%

for sheep urine on an upland, extensively grazed grassland with peat soil. The low emissions were attributed to inhibition of nitrification by low soil pH (4.5-5.1), below the optimum range of 6.5-8.0 (Šimek and Cooper, 2002). Low pH reduces biological demand for nitrite and allows N loss through the abiotic NO transformation pathway (Khan *et al*., 2011). pH would be unlikely to limit nitrification in the agricultural fen soil studied here (pH = 6.7) where both the minerotrophic nature of the peatland and agricultural liming combine to raise the pH.

4.3. Carbon cycling: carbon dioxide and methane

The observed effects of WTD on ecosystem respiration agree with previous evidence that raised water levels suppress ecosystem respiration rates in agricultural peatlands (Wen *et al*., 2020a, 2020b). The reduction in ecosystem respiration under the 10 cm WTD treatment corresponds with (1) a reduction in the volume of the oxic soil layer, which constrains rates of aerobic decomposition; and (2) the lowest grass biomass, resulting in reduced autotrophic contributions to total ecosystem respiration. Raised WTDs, at levels intersecting the rhizosphere, could submerge roots and create anoxic conditions, limiting grass growth (Armstrong and Drew, 2002). Additionally, suppression of peat mineralisation could reduce the available nutrient supply and constrain plant growth (Wen et al., 2020a, 2020b). However, no significant differences in ecosystem respiration and grass biomass were found between the 30 cm and 50 cm WTD treatments. This is in contrast with previous findings on lettuce, which showed

384 significantly lower biomass under 30 cm WTD compared to 50 cm WTD (Wen et al.,
385 2020a), suggesting that ryegrass is less sensitive to WTD effects in this range.
386 Urine application resulted in increased ecosystem respiration rates in both 30 cm
387 and 50 cm WTD treatments (Fig. 1b). Urine addition supplied nutrients, enhancing
388 primary production and thus increasing autotrophic respiration rates, which is
389 supported by grass biomass being two times higher on urine-treated mesocosms (Table
390 2). Whilst stimulation of plant growth is clearly the predominate cause of raised
391 ecosystem respiration on urine-treated cores, higher initial CO_2 emissions were likely
392 driven by (1) mineralisation of highly labile low-molecular-weight organic compounds
393 in the urine (e.g. urea, allantoin, hippuric acid and creatinine; Dijkstra *et al*., 2013;
394 Marsden *et al*., 2020) and (2) urea hydrolysis that can release CO_2 directly and rapidly
395 ($CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2$). Addition of urine caused a transient spike in DOC
396 (Fig. 4) but our data suggests it was rapidly mineralized as DOC concentrations
397 declined to background levels by day 3. In this study we could not disentangle
398 autotrophic respiration and heterotrophic respiration, or account for primary
399 productivity. Future studies, which address these limitations will be necessary to
400 improve our understanding of the balance between N_2O emissions from livestock urine
401 and reduced CO_2 emissions under elevated water table following grassland conversion.
402 The low CH_4 emissions observed are in agreement with both mesocosm (Wen *et*
403 *al*., 2020a, 2020b) and field studies (Evans *et al*., 2016; Tiemeyer *et al*., 2016; Taft *et*
404 *al*., 2017) on agricultural peat soils, where topsoil is unsaturated. This indicates that

405 either methanotrophy during CH_4 transport from deep soil to top soil, or the presence
406 of compaction layer (acting as a physical barrier for upward diffusion) limited CH_4
407 emissions (Dinsmore *et al*., 2009). CH_4 emissions from shallow-drained peat grasslands
408 can be high but it is likely this is caused predominately by inundation of easily
409 decomposed biomass (Tiemeyer *et al*., 2016). Flooding did not occur during this study,
410 even in the 10 cm WTD cores but shallower WTDs may be associated with CH_4
411 emissions under more variable field conditions (e.g. Couwenberg *et al*., 2011; Turetsky
412 *et al.*, 2014).
413
414 *4.4. Impacts on soil microbial community structure*
415 Total microbial biomass was increased with elevated water levels, indicating
416 greater moisture stress in more deeply drained peat soils (Mäkiranta *et al*., 2009).
417 Raised WTDs were also associated with a decreased Gram-positive to Gram-negative
418 ratio (Table 1), which is an indicator of microbial stress (Bertram, 2009). However, the
419 difference is likely attributable to utilization of different C sources. Gram-negative
420 bacteria utilize more plant-derived C and Gram-positive bacteria utilize more SOM-
421 derived C (Kramer and Gleixner, 2008). Abundance was thus related to SOM
422 mineralisation rates under different WTDs. Addition of urine to pasture causes short-
423 lived (3-8 days) increases in microbial biomass in response to labile C and N
424 availability (Petersen *et al*., 2004; Bertram, 2009). However, after depletion of labile
425 nutrients, biomass decreases and the microbial community might show signs of salt

426 stress due to raised EC (Fig. 5b; Bertram, 2009). In this study, sampling after harvest
427 did not show any lasting impacts of urine deposition on the microbial community
428 structure (Fig. 6).
429
430 *4.5. Implications for peatland management*
431 Land use conversion from cropland to grassland, with associated raising of the
432 water table, represents an important option to mitigate soil loss and GHG emissions
433 whilst retaining productive use of lowland peatlands. The shallower WTDs achievable
434 under grassland are associated with lower CO_2 emissions (Evans *et al*., 2016) and lower
435 rates of subsidence (Berglund and Berglund, 2010), whilst the improved vegetation
436 cover can reduce vulnerability to wind erosion (Warburton, 2003).
437 The wider evidence base from in-situ studies is clear that raising the WTD closer
438 to the ground surface reduces terrestrial CO_2 emissions from agricultural peatlands
439 (Evans *et al*., 2016, 2017; Tiemeyer *et al*., 2016, 2020). Our finding of higher N_2O
440 emissions and urine patch EFs at a WTD of 30 cm than 50 cm suggest that N_2O may
441 make an important contribution to the GHG balance of grassland following conversion
442 from cropland on peat. Urine patch EFs may be higher on more shallow drained
443 grassland but the load bearing capacity of wetter land will be lower, necessitating
444 reduced stocking rates (Schothorst, 1982). Urine patch coverage is highly dependent on
445 stocking rates. Therefore, increases in urine patch EF may be offset by reductions in
446 total urine-N loading rate on more extensively managed sites. Urine patches on wetter

447 sites may also be less prone to NH_3 volatilization (Saarijärvi *et al*., 2006). Whilst
448 livestock-induced N_2O emissions may therefore not differ substantially between WTDs
449 of 30 cm and 50 cm in practice, the effects of WTD alone would increase background
450 soil N_2O emissions on shallow drained sites. This suggests the need for consideration
451 of N_2O emissions when balancing GHG emissions against economic productivity to
452 assess the optimal WTD for management of peat cropland converted to grassland.
453 Bog peat may be better suited to grassland conversion for grazing use due to its
454 lower pH, whilst fen peat may be better suited to mowing, minimizing excreta inputs,
455 especially under raised WTDs. Nitrification inhibitors (e.g. dicyandiamide) have shown
456 promise for mitigating urine patch N_2O emissions on mineral soils and may be an option
457 on grazed fen peat pasture (Chadwick *et al*., 2018). Administering soil N-process
458 inhibitors directly to ruminant animals via drinking water or infusion is likely a viable
459 way to selectively deliver N cycling inhibitors to the urine patch and thus reduce N
460 losses from grazed grassland (Ledgard *et al*., 2008; Welten *et al*., 2014). However, this
461 practice may create food safety challenges, due to the potential for inhibitors to appear
462 as a residual contaminant in dairy products and enter the food chain (Byrne et al., 2020).
463 Excreta and fertilizer inputs appear to contribute additively to N_2O emissions from peat
464 grassland (Velthof and Oenema, 1995) and there is evidence that N surplus to
465 vegetation requirements can result in substantial emissions (Eickenscheidt *et al*., 2014;
466 Poyda *et al*., 2016). Therefore, urine patch inputs must be considered in the wider
467 framework of total N inputs, vegetation requirements and mitigation options when

assessing N_2O emissions impacts for a specific site (Cardenas *et al*., 2019).

Peat derived GHG emissions are on average lower from grassland than cropland sites (Evans *et al*., 2016, 2017; Tiemeyer *et al*., 2016, 2020). This is largely driven by less intensive drainage requirements under grass swards than crops, leading to lower terrestrial CO_2 emissions (Evans *et al*., 2016). However, it is important to note that the partial WTD reductions associated with grassland conversion, will leave part of the peat layer aerated. This will slow SOM mineralisation but not prevent it completely, and so eventual peat loss remains inevitable under this strategy. Conversion of cropland to grazed grassland on agricultural peatland will also have wider indirect effects beyond the direct effects on soil nutrient cycling processes identified in this study. Most notably, if livestock production on converted croplands increases the total area under livestock production, then any gains made in mitigating emissions from peat decomposition would be offset by additional livestock derived emissions overall (e.g. CH_4 from enteric fermentation; Hopkins and Lobley, 2009). There may also be indirect emissions associated with meeting the different infrastructure requirements associated with the land use change (e.g. cattle sheds). In addition, the loss of highly productive cropland may displace production elsewhere, potentially resulting in habitat destruction, deforestation and indirect GHG emissions (Searchinger *et al*., 2008). Any strategy aiming to increase the area under grassland, with resultant increases in livestock production could also have wider societal effects (Springmann *et* al., 2018; Tilman and Clark, 2014). These outcomes lie outside the scope of this study and would only be

evident over the full life cycle of production but they represent important considerations for researchers and policy makers addressing this issue.

5. Conclusions

Converting cropland on peat soils to grassland is currently being considered as an important option to mitigate soil GHG emissions, whilst retaining productive use of agricultural peatlands (HM Government, 2018). We found that N_2O emissions from livestock urine patches may negatively affect the GHG balance of grazed grassland established on drained peatlands. N_2O emissions were elevated at a WTD of 30 cm compared to 50 cm for both urine-treated and control cores suggesting N_2O emissions may be higher on more extensively managed grasslands, where soil moisture conditions are favourable for N_2O production through the interplay of nitrification and incomplete denitrification. As a result, our findings suggest that N_2O emissions should be considered when attempting to optimize WTD for management of grazed grassland on drained peatlands. However, urine patch EFs were low compared to IPCC defaults and other findings for mineral soils suggesting that overall, CO_2 emissions and economic productivity may be more important considerations at a site level. Further investigations measuring net ecosystem exchange or net ecosystem carbon balance would be necessary to make a quantitative assessment of the effects of site management on the GHG balance. Whilst beyond the scope of this study, it is clear that indirect GHG emissions from livestock, along with other societal and environmental impacts across

the full production cycle would be important to consider when developing policy recommendations regarding grassland establishment to mitigate GHG emissions from croplands on drained peat.

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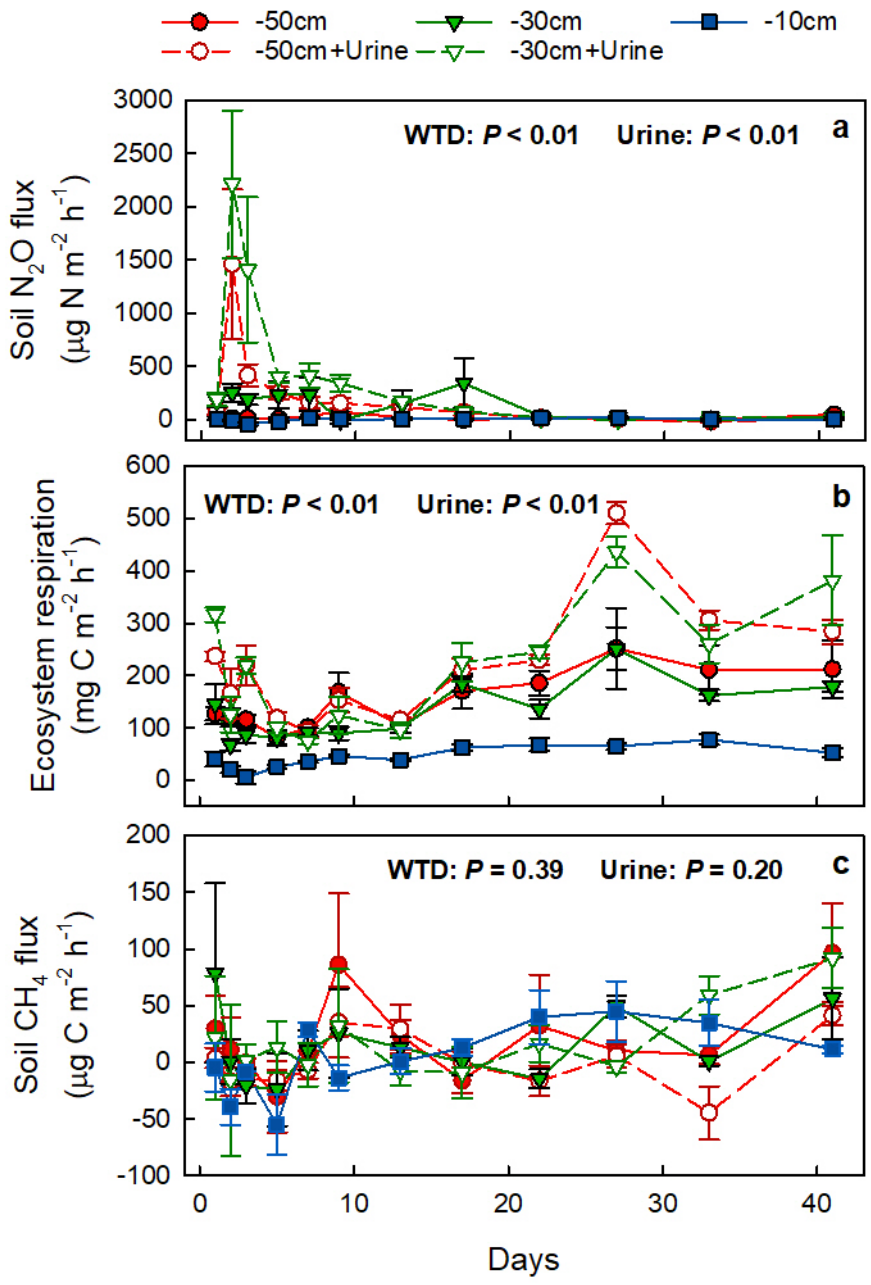

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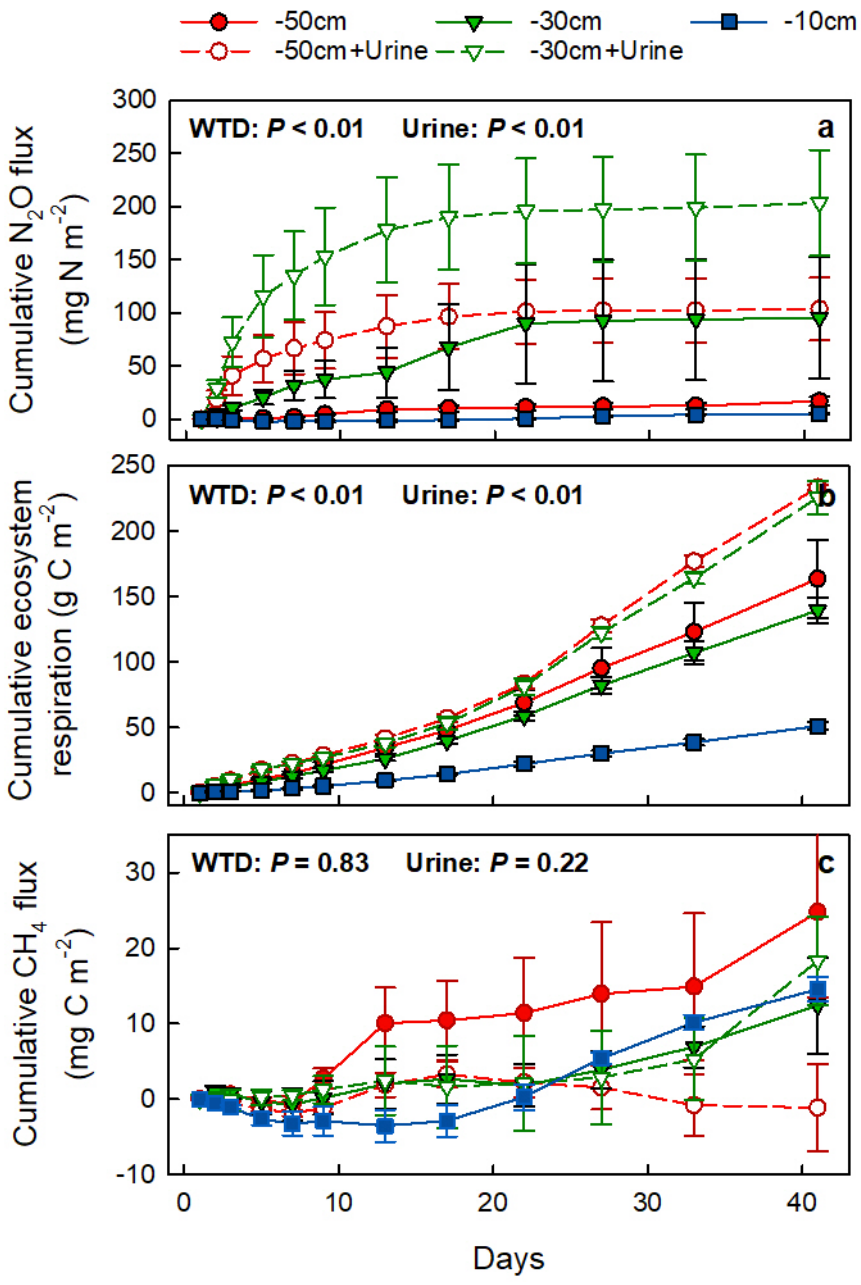
816 **Fig. 1** Temporal variation of soil N_2O (a), ecosystem respiration (b), and soil CH_4 (c)

817 fluxes from peat mesocosms (means ± standard errors, $n = 4$). 50 cm, 30 cm and 10 cm

818 indicate water table depth relative to the soil surface. Urine indicates mesocosms treated

819 with sheep urine.



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821 **Fig. 2** Cumulative N_2O (a), ecosystem respiration (b), and CH_4 (c) fluxes from the peat

822 cores (means ± standard errors, n = 4). 50 cm, 30 cm and 10 cm indicate water table

823 depth relative to the soil surface. Urine indicates mesocosms treated with sheep urine.

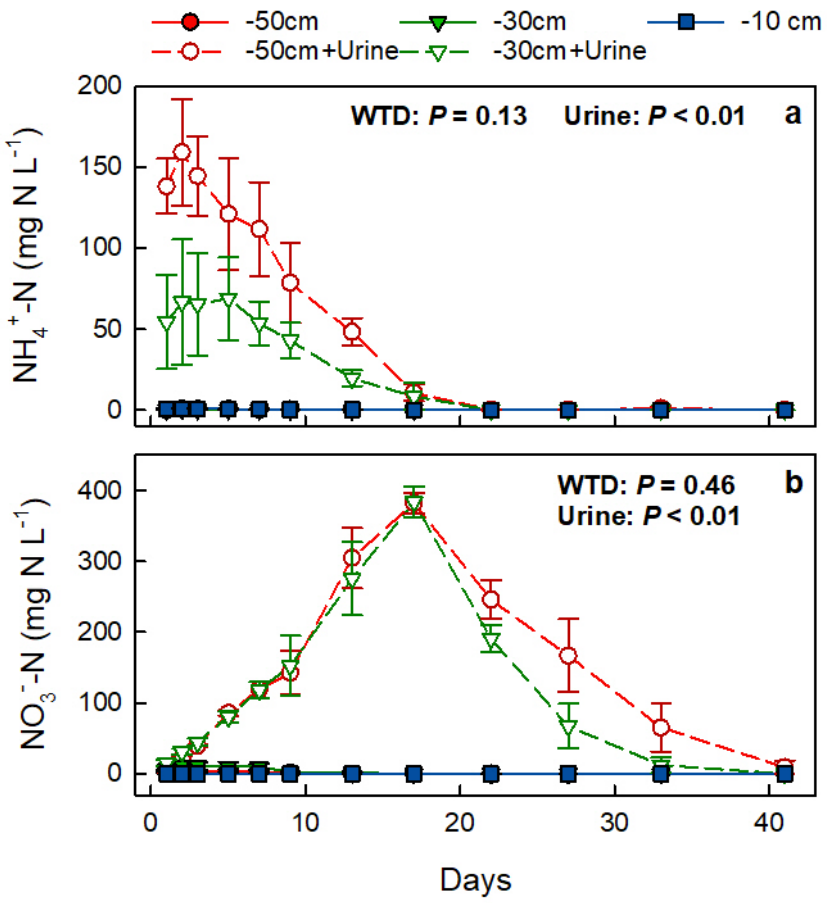

Fig. 3 Temporal variation of soil solution ammonium (NH_4^+-N) (a) and nitrate (NO_3^--N) (b) concentrations at 10 cm depth in mesocosms (means ± standard errors, n = 4). 50 cm, 30 cm and 10 cm indicate water table depth relative to the soil surface. Urine indicates mesocosms treated with sheep urine.

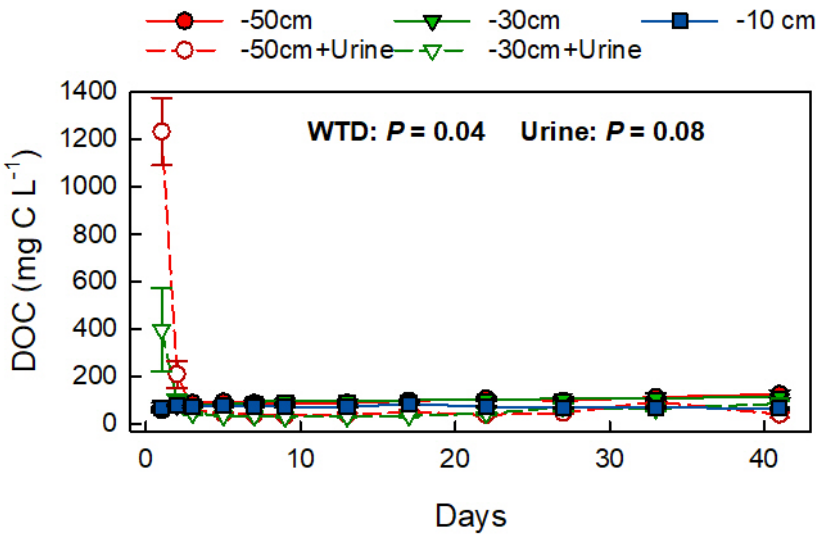

Fig. 4 Temporal variation of soil solution dissolved organic carbon (DOC) concentrations at 10 cm depth in mesocosms (means ± standard errors, n = 4). 50 cm, 30 cm and 10 cm indicate water table depth relative to the soil surface. Urine indicates mesocosms treated with sheep urine.

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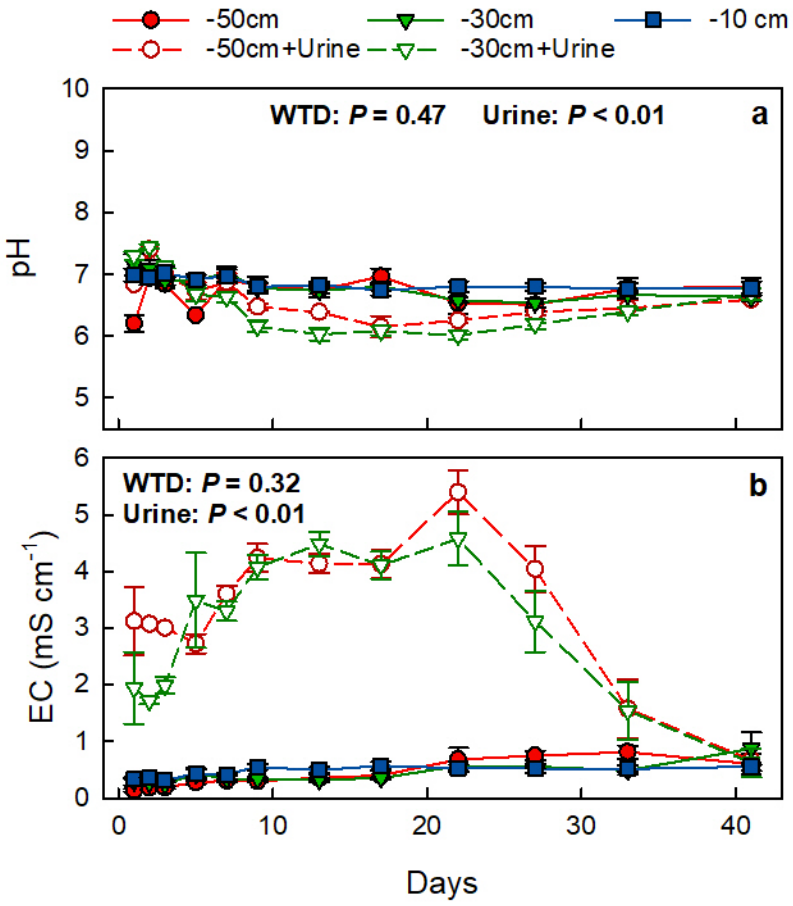

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840 **Fig. 5** Temporal variation of pH (a) and electrical conductivity (EC; b) at 10 cm depth

841 in mesocosms (means ± standard errors, $n = 4$). 50 cm, 30 cm and 10 cm indicate water

842 table depth relative to the soil surface. Urine indicates mesocosms treated with sheep

843 urine.

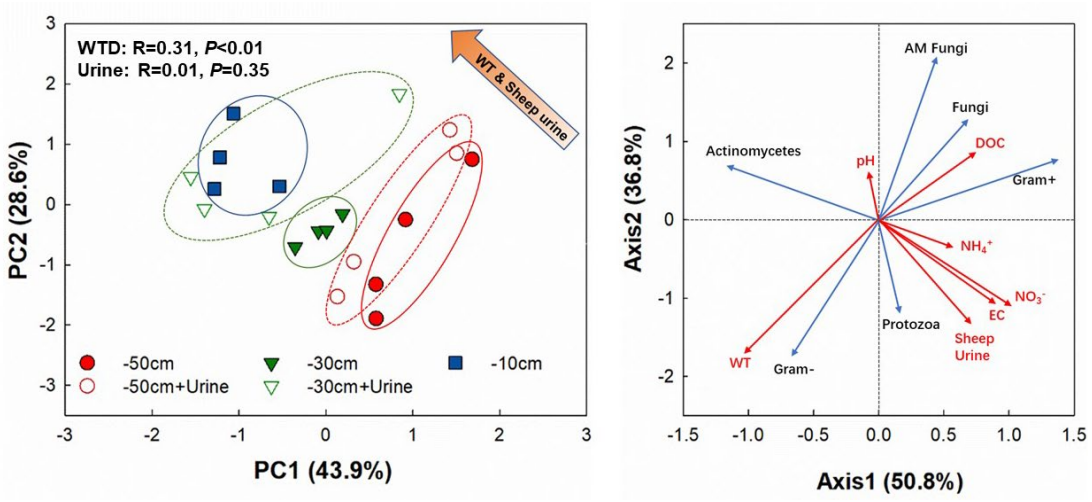

Fig. 6 Left: Principal component analysis (PCA) of soil microbial phospholipid fatty acid (PLFA) fingerprints for: Gram-negative bacteria, Gram-positive bacteria, total fungi, putative arbuscular mycorrhizal fungi, protozoa, and actinomycetes. 50 cm, 30 cm and 10 cm indicate water table depth relative to the soil surface. Urine indicates mesocosms treated with sheep urine. Ellipses show within-group variance. PC1 and PC2 explained 43.9% and 28.6% of the variation respectively. The arrow illustrates the effects of shallower water table depths and sheep urine application. **Right:** Redundancy analysis (RDA) of soil microbial PLFA fingerprints and abiotic environmental factors. pH, soil pH value; DOC, dissolved organic carbon; NH_4^+, ammonium; NO_3^-, nitrate; EC, electrical conductivity; WT, water table depth; Sheep urine, application of sheep urine.

857 **Table 1** Soil microbial PLFA biomass and fingerprints from 0-10 cm depth within the mesocosms.

	50 cm WTD	50 cm WTD + Urine	30 cm	30 cm WTD + Urine	10 cm WTD	WTD effect	Urine effect
Total PLFA biomass (nmol g^{-1})	111±2	116±3	119±4	129±6	138±1	*P*<0.01	*P*=0.06
Gram+ bacteria (%)	37±2	39±2	35±0.1	35±2	34±1	*P*=0.06	*P*=0.54
Gram- bacteria (%)	42±0.3	42±0.1	43±0.2	45±1	47±1	*P*<0.01	*P*=0.10
Fungi (%)	2.1±0.2	1.7±0.2	1.7±0.2	1.3±0.3	1.6±0.2	*P*=0.41	*P*=1.00
AM Fungi (%)	3.5±0.1	3.4±0.1	3.2±0.1	3.0±0.1	2.8±0.1	*P*<0.01	*P*=0.01
Actinomycetes (%)	13±2	12±2	15±0.1	12±1	12±1	*P*=0.44	*P*=0.22
Protozoa (%)	2.3±0.3	2.5±0.2	2.7±0.1	2.9±0.2	2.6±0.2	*P*=0.20	*P*=0.38
Bacteria : Fungi	14±1	15±1	16±1	19±1	18±1	*P*<0.01	*P*=0.03
Gram+ : Gram-	0.9±<0.1	0.9±<0.1	0.8±<0.1	0.8±<0.1	0.7±<0.1	*P*<0.01	*P*=0.22

858 Values represent means ± standard errors (n = 4). 50 cm, 30 cm and 10 cm indicate water table depth relative to the soil surface. Urine indicates

859 mesocosms treated with sheep urine.

Table 2 Grass fresh biomass, dry biomass, biomass-C, biomass-N and biomass C:N ratio within peat mesocosms under different water table depth and sheep urine treatments (means ± standard errors, n = 4).

	50 cm WTD	50 cm WTD + Urine	30 cm WTD	30 cm WTD + Urine	10 cm WTD	WTD effect	Urine effect
Fresh biomass (g m^{-2})	2025±583	7015±36	2160±381	6826±539	500±55	P=0.01	P<0.01
Dry biomass (g m^{-2})	323±68	780±19	329±40	787±75	92±7	P<0.01	P<0.01
Biomass-C (%)	44.0±0.4	41.2±0.2	44.4±0.3	41.5±0.6	44.7±0.2	P=0.35	P<0.01
Biomass-N (%)	2.1±0.3	3.9±0.2	1.9±0.2	3.8±0.4	1.8±0.2	P=0.63	P<0.01
Biomass C:N ratio	21±3	11±1	24±3	11±1	26±4	P=0.48	P<0.01

Values represent means ± standard errors (n = 4). 50 cm, 30 cm and 10 cm indicate water table depth relative to the soil surface. Urine indicates mesocosms treated with sheep urine.

Supplementary Information

Table S1 Phospholipid fatty acids (PLFAs) representing >0.5% of total PLFA biomass used as microbial biomarkers to generate PLFA fingerprints for taxonomic groups.

Taxonomic group	Biomarker
Gram-positive bacteria	14:0 iso, 15:0 iso, 15:0 anteiso, 15:1 iso w6c, 15:1 iso w9c, 16:0 iso, 17:0 iso, 17:0 anteiso, 17:1 iso w9c
Gram-negative bacteria	16:1w7c, 16:1w9c, 17:1w8c, 17:0 cyclo w7c, 18:1w5c, 18:1w9c, 18:1w7c, 19:0 cyclo w7c
Actinomycetes	16:0 10 methyl, 17:0 10 methyl, 17:1w7c 10 methyl, 18:0 10 methyl, 18:1w7c 10 methyl
Fungi	18:2w6c
Arbuscular mycorrhizal (AM) fungi	16:1w5c
Protozoa	20:4w6
Anaerobic bacteria	15:0 DMA
Not assigned to a taxonomic group	14:0, 15:0. 16:0, 17:0, 18:0, 20:0, 22:0, 24:0

Taxonomy based on Ratledge and Wilkinson (1988), Kieft et al. (1994), Paul and Clark (1996), Olsson et al. (1999), Zelles (1999), Madan et al. (2002), Niklaus et al. (2003), and Bartelt-Ryser et al. (2005).

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