# Topsoil dominance in $CO_2$ fluxes from agricultural peat soils across moisture and temperature gradients: evidence from an incubation study

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## SUMMARY

Drainage of peatlands for agriculture reverses their natural carbon sink function and leads to substantial $CO_2$ emissions. However, the relative contributions of topsoil and subsoil layers to these emissions remain poorly quantified, limiting the accuracy of $CO_2$ flux models in national greenhouse gas inventories. In this incubation study, we quantified $CO_2$, $N_2O$ and $CH_4$ fluxes from intact peat cores collected at 5–13 cm (topsoil) and 25–33 cm (subsoil) depth from arable and permanent grassland sites at a cultivated Danish bog. Fluxes were measured at five temperatures (2.5–23.4 °C) and across four soil water tensions ranging from full rewetting to pF 2.5. Topsoil consistently showed higher $CO_2$ fluxes than subsoil, averaging a threefold difference. Temperature exerted a strong exponential control on emissions, with $Q_{10}$ values ranging from 1.7 to 4.0 depending on depth, site and moisture status. Rewetting reduced $CO_2$ fluxes from the topsoil at elevated temperatures but had little effect on the subsoil, which showed weaker temperature sensitivity and lower microbial activity. These findings align with asymptotic depth–flux relationships indicating that topsoil dominates the short-term response of $CO_2$ fluxes to rewetting. Fluxes of $N_2O$ were negligible except in rewetted arable subsoil, where high fluxes suggested incomplete denitrification under micro-oxic and acidic conditions. Fluxes of $CH_4$ were also negligible, indicating insufficiently anaerobic conditions for methanogenesis during the short-term incubation. Our results emphasise the importance of incorporating depth-specific $CO_2$ dynamics in emission models and indicate that rewetting strategies must target the near-surface peat to optimise mitigation outcomes.

**KEY WORDS:** drained bog, greenhouse gas fluxes, organic soil, soil profile, soil respiration

## INTRODUCTION

Pristine peatlands act as net carbon (C) sinks due to the accumulation of plant biomass under water-saturated and often acidic conditions that delay microbial decomposition (Robinson *et al*. 2023). Over millennia, peat soils have sequestered about 600 Gt of organic carbon (OC) derived from photosynthesis, which highlights their effect on atmospheric carbon dioxide ($CO_2$) levels (Yu *et al*. 2010, Leifeld & Menichetti 2018). When peatlands are drained for agriculture, the OC sink function is reversed. Drainage exposes the peat to oxygen ($O_2$), accelerating aerobic microbial respiration, which releases stored OC as $CO_2$ (Karki *et al*. 2016). Moreover, nitrogen (N) surplus from peat decomposition and crop fertilisation may promote emissions of nitrous oxide ($N_2O$), a greenhouse gas (GHG) with a global warming potential 273 times greater than that of $CO_2$ (IPCC 2023). Globally, GHG emissions from drained peat soils amount to ~2 Gt of $CO_2$ equivalents per year (UNEP 2022), which is a major contribution to agricultural GHGs (Maljanen *et al*. 2010, Martin & Couwenberg 2021).

To reduce $CO_2$ emissions and meet national targets for agricultural GHG reductions, peatland rewetting is practised as a management strategy in several European countries (Jensbye & Yu 2024, Wichmann & Nordt 2024). In Denmark, for example, the government will financially support the rewetting of at least 100,000 hectares of peat soils by 2030 (Koch *et al*. 2023). Rewetting aims to restore water-saturated conditions that limit $O_2$ diffusion into the soil (Moldrup *et al*. 2000), thereby reducing aerobic peat oxidation. Although increased anaerobic methane ($CH_4$) production represents a trade-off (Hemes *et al*. 2018), rewetting remains a key climate mitigation strategy (Wilson *et al.* 2016, Günther *et al.* 2020).

The accountable climate benefit of rewetting depends on the GHG (notably $CO_2$) emissions before and after the intervention. The $CO_2$ emissions have traditionally been estimated using IPCC or national emission factors (Wilson *et al*. 2016). Dynamic models linking $CO_2$ emissions to mean annual water table depth (WTD) may provide more accurate estimates than fixed emission factors (Tiemeyer *et al*. 2020, Evans *et al*. 2021, Koch *et al*. 2023); however, the relationship between WTD and net $CO_2$ emissions is still under debate (Nijman *et al.* 2024). Some studies report a linear increase in $CO_2$ efflux with increasing WTD (Evans *et al*. 2021), suggesting comparable contributions from drained topsoil and subsoil. Other studies suggest an asymptotic relationship, where drained subsoil contributes progressively less to the total $CO_2$ efflux (Tiemeyer *et al*. 2020). Although some studies found the asymptotic model to provide a better fit (Koch *et al*. 2023, Leifeld & Torrús Castillo 2024), the evidence remains limited. Empirical measurements that directly compare GHG fluxes between topsoil and subsoil remain scarce, leaving a critical gap in our ability to model depth-integrated $CO_2$ emissions based on environmental drivers.

Here we report on GHG fluxes from intact topsoil (depth below ground surface 5–13 cm) and subsoil (25–33 cm) of a drained bog under both arable cropping and permanent grassland. Fluxes of $CO_2$, $N_2O$ and $CH_4$ were measured across a range of temperature and moisture gradients to capture topsoil and subsoil responses to these environmental drivers. The focus was on $CO_2$ from peat mineralisation, for which we hypothesised that fluxes from the subsoil would be consistently lower than from the topsoil due to lower management effects such as influence from amendments (e.g., inputs of animal excreta, fertilisers, lime), tillage operations, root exudates and return of plant residues. We discuss the results with reference to their implications for reporting of GHG emissions from organic soils in national inventories.

## METHODS

### Study site and soil sampling

The study site was the *Sphagnum* bog Store Vildmose (5,500 ha), located in the northern part of Jutland, Denmark. This area has a mean annual temperature of 7.9 °C and mean annual rainfall of 740 mm $yr^{-1}$ (Kandel *et al.* 2018). The bog was drained in the 1920s and subsequently used as grassland for cattle grazing (Pedersen 1978). Arable crops were introduced after a few decades. The main cash crop was potato (*Solanum tuberosum* L.), grown in rotation with cereals such as spring barley (*Hordeum vulgare* L.). The pristine bog was 4.5–5.3 m deep in the central parts with peat characterised by 41.5 % OC, 0.8 % total N, 5.1 % ash and a $pH_{KCl}$ of 3.4 (Pedersen 1978). Drainage and agricultural use have reduced the peat depth to less than 1–2 m (Regina *et al*. 2016, Adetsu *et al*. 2024), even exposing the underlying sand in some fields.

Soil cores for the experiment were collected from an arable (AR) site (57° 13′ 35″ N, 9° 46′ 59″ E) and from a permanent grassland (PG) site (57° 13′ 38″ N, 9° 47′ 08″ E). The sites were separated by a distance of ~100 m and were previously described in detail by Kandel *et al*. (2018). The AR site was in long-term arable rotation with potato and spring barley. At the time of sampling (12 November 2014) the soil was fallow after a potato harvest in late September. The potato crop had received mineral fertiliser including N at 121 kg $ha^{-1}$ in the spring (15 April) (Kandel *et al.* 2018). The PG site was used for cattle grazing without fertilisation and had not been ploughed for more than 40 years. The dominant grasses were *Alopecurus geniculatus* L. and *Poa pratensis* L. with minor presence of *Holcus lanatus* L. and senescent dicotylendonous (dicot) species such as *Trifolium repens* L. and *Ranunculus repens* L. At this site, soil cores were collected from an area that had been fenced with electric wire for six months to eliminate recent cattle excreta.

At both sites, the uppermost 0–5 cm layer of soil was removed from a 0.5 × 0.5 m quadrat prior to soil sampling. This was done to eliminate vegetation and larger roots at the PG site, and more generally to enable sampling of bare soil cores - thereby avoiding any introduction of artefacts from living and germinating plants during the measurement of $CO_2$ fluxes arising from peat decomposition (Säurich *et al*. 2019a, Blondeau *et al*. 2024). At each site, 12 intact soil cores for GHG flux measurements were collected from the topsoil (5–13 cm) and 12 from the subsoil (25–33 cm) using stainless steel cylinders (height 8 cm, diameter 10 cm) with sharpened edges (48 soil cores in total). Extra soil cores were taken for physical and chemical analyses and for temperature monitoring during the incubation experiment. All cores were trimmed at both ends and sealed with plastic caps. The soil cores for GHG flux measurements were used after two days of storage at 2 °C, while those for physical and chemical analysis were frozen at -18 °C.

### Greenhouse gas fluxes at controlled moisture and temperature

Soil cores from each combination of site and depth were equilibrated at four moisture regimes (0, -100, -200 and -300 cm soil water tension; rewetted and pF 2.0, 2.3, 2.5, respectively) in three replicates. The soil cores at 0 cm tension were saturated with deionised water from above (rewetted) at 21 °C, while those at -100, -200 and -300 cm tension were moisture-adjusted in vacuum pots for 14 days at 21 °C (Dane & Hopmans 2002).

After moisture adjustment, the cores were transferred to a climate chamber with temperature control and 100 % relative humidity. A small (0.5 cm diameter) hole in each plastic top cap allowed gas exchange without moisture loss. GHG fluxes from the soils were measured first at a temperature setting of 23 °C, followed by 18 °C, 13 °C, 8 °C and 3 °C. Measurements were taken after an equilibration period of 48 h at each target temperature (Figure A1 in the Appendix). Soil cores were weighed before and after the 23 °C incubation, confirming that no moisture loss had occurred.

GHG fluxes were measured using a closed chamber method. The flux chambers consisted of steel cylinders like those used for soil sampling, with one end sealed and fitted with a butyl rubber septum for gas sampling. During measurements, the flux chamber (628 mL) was placed on top of the soil core with the cap removed and with a rubber gasket ensuring a gas-tight seal between the two cylinders. Air samples (10 mL) were collected from the climate chamber using a syringe with a hypodermic needle just before closing the flux chambers to establish a common starting ($t_0$) gas concentration. Subsequent samples were taken from each chamber headspace after approximately 20 min ($t_1$) and 40 min ($t_2$) of closure. The gas samples were stored in evacuated 6 mL Exetainer vials and analysed using an Agilent 7890 GC system with a CTC combiPal autosampler (Agilent, Nærum, Denmark). The GC was equipped with TCD, ECD and FID detectors for quantification of $CO_2$, $N_2O$ and $CH_4$, respectively, and was operated as previously described by Petersen *et al*. (2012).

**Physical and chemical soil properties**

Five extra AR soil cores were used to monitor soil temperature during the incubation period by placing a HOBO Pendant logger (Onset Computer Corporation, USA) in each core. Two of these cores were water saturated, while three were kept at field moisture level. Air temperature in the climate chamber was also monitored using a HOBO data logger. Temperatures recorded hourly by the six loggers were similar, with averages of 23.4, 18.2, 13.1, 7.8 and 2.5 °C for the five target temperatures (Figure A1).

Redox potential was measured in all 48 GHG flux soil cores after the final gas sampling, using a pH meter (PHM220, Radiometer) and a double-junction calomel electrode (REF251, Hach Lange). Standard hydrogen electrode potentials (Eh) were calculated by adding +245 mV to the measured values. The soil cores were then dried in an oven at 80 °C to determine the volumetric water content (VWC) and soil bulk density (BD). Ash content was determined from dry subsamples after combustion in a muffle furnace (525 °C, 4 h). Organic C and total N were measured in subsamples from the 12 soil cores at pF 2.0 (i.e., three soil cores from each site-and-depth combination) using a Thermo Flash 2000 NC Analyser (Thermo Fisher Scientific).

The degree of peat decomposition, soil pH, and nitrate ($NO_3^-$) and ammonium ($NH_4^+$) concentrations were determined for each site-depth combination. Degree of decomposition was determined using the von Post scale (von Post 1922). Soil pH was measured by glass electrode after extraction of soil with 0.01 M $CaCl_2$ (1:10, wt/wt), and $NO_3^-$ and $NH_4^+$ were analysed with an auto-analyser (Bran+Luebbe, Norderstedt, Germany) after extraction of soil with 1 M KCl (1:4, wt/wt) and filtration of the supernatant.

**Calculations and statistics**

Flux rates ($F$) of $CO_2$, $N_2O$ and $CH_4$ (µg $m^{-2}$ $h^{-1}$) were calculated as:

$$F = dc/dt \times M \times P \times V_{\mathrm{fc}} \times R^{-1} \times T^{-1} \times A_{\mathrm{fc}}^{-1} \qquad [1]$$

where $dc/dt$ is the linear rate of change in gas concentration during the time interval $t_0$ to $t_2$ (µL $L^{-1}$ $h^{-1}$), $M$ is the molecular weight of the gas (g $mol^{-1}$), $P$ is the atmospheric pressure (atm), $V_{\mathrm{fc}}$ is the volume of the flux chamber (L), $R$ is the gas constant (L atm $K^{-1}$ $mol^{-1}$), $T$ is the temperature during the flux measurement (K) and $A_{\mathrm{fc}}$ is the area of the flux chamber ($m^2$). Due to fluctuating $CO_2$ levels in the climate chamber at $t_0$, the common starting $CO_2$ concentration was uncertain and $CO_2$ fluxes were calculated using the actual chamber headspace concentrations at $t_1$ and $t_2$. Fluxes of $N_2O$ and $CH_4$ were calculated using all three time points ($t_0$, $t_1$, $t_2$).

Temperature effects on gas fluxes were analysed using the linearised Arrhenius model:

$$\ln F = \ln A - E_a \times R^{-1} \times T^{-1} \qquad [2]$$

where $F$ is the flux rate, $A$ is a pre-exponential factor, $E_a$ is the activation energy (J $mol^{-1}$), $R$ is the gas constant (J $K^{-1}$ $mol^{-1}$) and $T$ is the temperature (K) during the flux measurement. The coefficient of determination ($r^2$) was used as a measure of the goodness-of-fit of the linear regressions to determine $E_a$. The temperature sensitivity $Q_{10}$ (i.e., the flux increase factor per 10 °C) was calculated from $E_a$ for the 5–15 °C interval:

$$\ln Q_{10} = 10 \times E_a \times R^{-1} \times (T + 10)^{-1} \times T^{-1} \qquad [3]$$

where $T$ is 278.15 K ($E_a$ and $R$ as in Equation 2).

Statistical analysis of the main and interactive effects of soil depth (topsoil, subsoil), moisture and temperature on gas fluxes was conducted using the *glmmTMB* package in R version 4.4.2 (Brooks *et al*. 2017, R Core Team 2024). Simpler linear (*lm*) and generalised linear (*glm*) models were tested but departed from assumptions of homogeneity of variance and normality as evaluated through visual inspection of residuals (residual vs. fitted plots, histograms and Q–Q plots). Nonetheless, all three modelling approaches produced consistent results. When ANOVA indicated significant effects ($p < 0.05$), post hoc pairwise comparisons were performed using the *emmeans* package, with Tukey adjustments applied to control for multiple testing (Zar 2010, Lenth 2016). Measures of dispersion are reported as standard error (SE), standard deviation (SD) or 95 % confidence intervals (CI), as indicated.

## RESULTS

### Soil properties

At the AR site the topsoil was more decomposed (H7) than the subsoil (H3), whereas at the PG site both soil layers were relatively undecomposed with a von Post index of H3–H4 (Table 1). Nevertheless, the topsoil at PG had lower OC and higher BD, pH and ash content than the subsoil, reflecting the long-term use as cattle pasture. The peat at AR was typically less than 50 cm deep and mixed with sand from below, resulting in high BD and low OC of the AR subsoil compared to the PG subsoil (Table 1). Total N was higher in the topsoil than in the subsoil at both sites, while PG subsoil had the lowest mineral N content and the highest C/N value (Table 1). All site-depth combinations were acidic with pH($CaCl_2$) of 3.6–4.1.

### Soil moisture and redox potentials

Volumetric water content in the rewetted soils was 74–85 % across all site-depth combinations, and significantly higher than the VWC of 42–67 % observed in drained soils at pF 2.0–2.5 (Figure 1). Among the drained soils, however, VWC did not differ systematically across the pF range 2.0–2.5 within each site-depth combination. Across all moisture levels, topsoil consistently had significantly lower VWC than subsoil at identical soil water tension ($p < 0.05$).

Rewetted topsoil samples had lower redox potentials (Eh, 341–379 mV) than their drained counterparts (Figure 2). However, among the drained topsoils, the redox potentials were similar across pF 2.0–2.5, ranging from 529 mV to 602 mV (Figure 2). In subsoils, Eh under rewetted conditions ranged from 520 mV to 523 mV and was not consistently lower than Eh under drained conditions (501–672 mV; Figure 2).

### Fluxes of $CO_2$

The range of topsoil $CO_2$ fluxes at AR was 13–178 mg m$^{-2}$ h$^{-1}$, while that of subsoil fluxes was 0–50 mg m$^{-2}$ h$^{-1}$ (Figure 3). Likewise, topsoil $CO_2$ fluxes at PG (16–187 mg m$^{-2}$ h$^{-1}$) were consistently higher than

Table 1. Characteristics of the topsoil (5–13 cm) and subsoil (25–33 cm) at the arable rotation (AR) and the permanent grassland (PG) sites in the Store Vildmose drained bog. Data are presented as means and standard errors with $n = 3$ for organic carbon (C), total nitrogen (N), C/N and ash content; and $n = 12$ for bulk density. Soil pH, nitrate ($NO_3$-N) and ammonium ($NH_4$-N) concentrations were determined from a single soil core for each site-depth combination.

| Characteristic | AR topsoil | AR subsoil | PG topsoil | PG subsoil |
|---|---|---|---|---|
| Decomposition (von Post index) | H7 | H3 | H4 | H3 |
| Organic carbon (%) | 31.9 ± 0.5 | 16.7 ± 2.5 | 37.7 ± 0.4 | 48.4 ± 0.3 |
| Total nitrogen (%) | 1.27 ± 0.02 | 0.76 ± 0.08 | 1.68 ± 0.04 | 1.27 ± 0.05 |
| C/N | 25.2 ± 0.2 | 21.8 ± 1.0 | 22.5 ± 0.7 | 38.2 ± 1.3 |
| Bulk density (g cm$^{-3}$) | 0.34 ± 0.01 | 0.33 ± 0.02 | 0.29 ± 0.01 | 0.13 ± 0.00 |
| Ash (%) | 36.3 ± 0.0 | 61.6 ± 2.2 | 25.1 ± 2.2 | 4.2 ± 0.3 |
| pH($CaCl_2$) | 4.0 | 3.7 | 4.1 | 3.6 |
| $NO_3$-N (mg kg$^{-1}$) | 27 | 12 | 9 | 1 |
| $NH_4$-N (mg kg$^{-1}$) | 4 | 1 | 22 | 2 |

those from subsoil (5–59 mg m$^{-2}$ h$^{-1}$). The average topsoil-to-subsoil $CO_2$ flux ratio (evaluated as a quotient) was 3.0 (95 % CI: 2.6–3.4) across all depth, moisture and temperature combinations (Figure 4).

Soil moisture ($p < 0.001$–$0.002$) and temperature ($p < 0.001$) had significant effects on $CO_2$ fluxes at both sites (Tables A1 and A2 in the Appendix). Interactions between soil depth and both moisture and temperature indicated differing responses in topsoil and subsoil layers. Specifically, the effect of soil moisture on $CO_2$ fluxes depended on soil depth at both sites with $p = 0.081$ for AR and $p < 0.001$ for

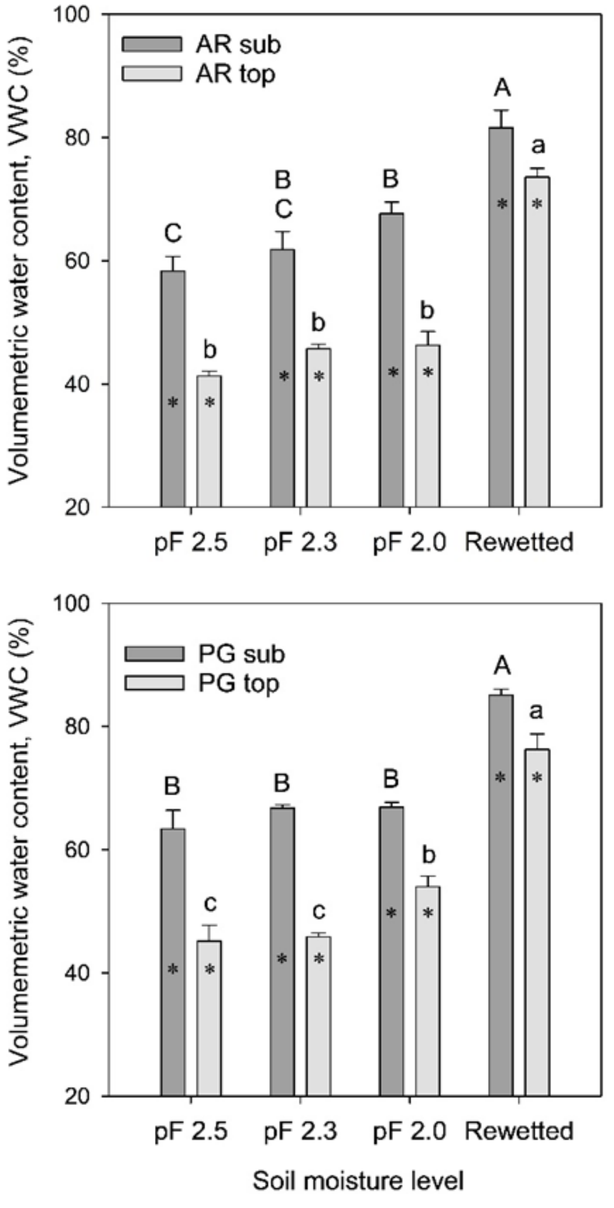


Figure 1. Volumetric water content (VWC) of subsoil (25–33 cm) and topsoil (5–13 cm) peat cores incubated under drained (pF 2.5, 2.3 and 2.0) and rewetted conditions. Intact cores were sampled from a drained agricultural bog managed as arable rotation (AR) and permanent grassland (PG). Values are means ($n = 3$) and error bars show standard error. Capital and lowercase letters indicate significant differences amongst subsoil and topsoil treatments, respectively. Asterisks mark significant differences between paired subsoil and topsoil samples ($p < 0.05$).

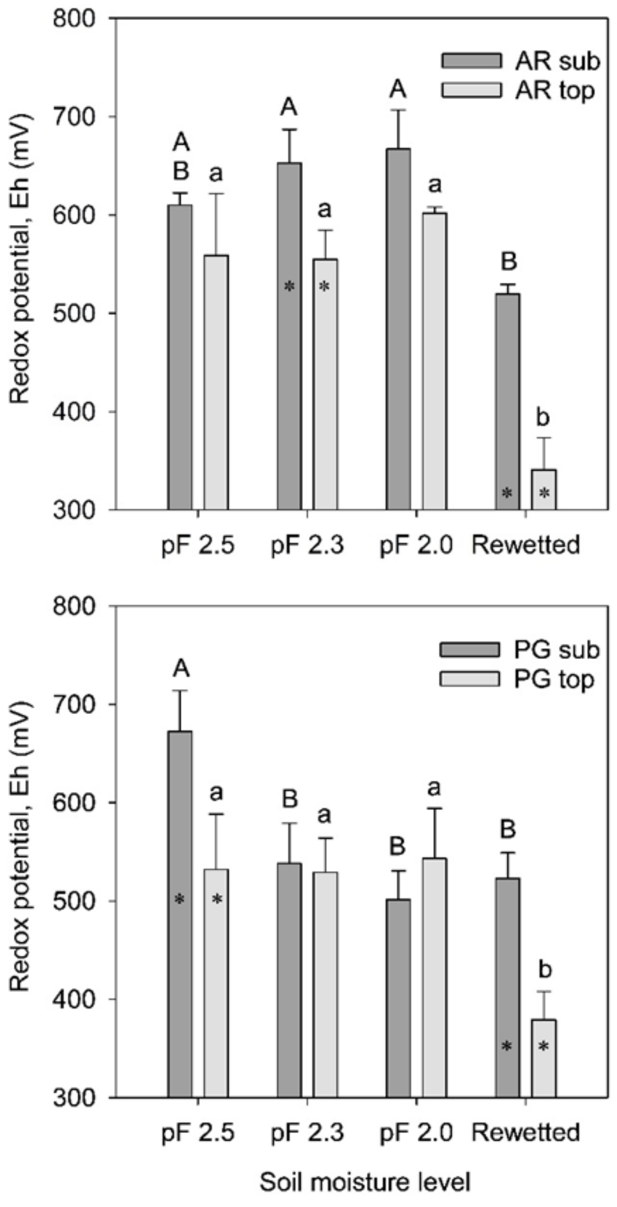


Figure 2. Redox potential (Eh) of subsoil (25–33 cm) and topsoil (5–13 cm) peat cores incubated under drained (pF 2.5, 2.3 and 2.0) and rewetted conditions. Intact cores were sampled from a drained agricultural bog managed as arable rotation (AR) and permanent grassland (PG). Values are means ($n = 3$) and error bars show standard error. Capital and lowercase letters indicate significant differences amongst subsoil and topsoil treatments, respectively. Asterisks mark significant differences between paired subsoil and topsoil samples ($p < 0.05$).

PG. Thus, at both sites, rewetting of topsoil reduced the $CO_2$ emission at 18.2 °C and 23.4 °C but not at 2.5 °C and 7.8 °C (Figure 3). For subsoil samples, $CO_2$ fluxes were similar under rewetted and drained conditions at all temperatures.

The interaction between soil depth and temperature ($p < 0.001$; Tables A1, A2) indicated that rewetted AR subsoil had higher temperature sensitivity ($Q_{10}$ 4.0) than rewetted AR topsoil ($Q_{10}$ 2.2) (Figure 5, Table A3), while the opposite was found in drained AR soil with $Q_{10}$ values of 1.7–2.0 in subsoil and 3.0–3.4 in topsoil. For PG, topsoil and subsoil showed similar $Q_{10}$ (2.2–3.6) across both rewetted and drained treatments (Figure 5, Table A3).

**Fluxes of $N_2O$ and $CH_4$**

Nitrous oxide fluxes were negligible (-6 to 16 µg $m^{-2}$ $h^{-1}$) across all depth, moisture and temperature combinations, except in rewetted AR soil (Figure 6). In rewetted AR soil, $N_2O$ fluxes ranged from 53–88 µg $m^{-2}$ $h^{-1}$ in the topsoil and from 653–1753 µg $m^{-2}$ $h^{-1}$ in the subsoil over the temperature range 2.5–23.4 °C. Temperature sensitivity in the topsoil was particularly low ($Q_{10}$ 1.0–1.5) with poor fits to the Arrhenius model ($r^2 < 0.11$), partly due to a declining trend at 23.4 °C (Table A4). Excluding the fluxes at 23.4 °C improved the model fits but the resulting $Q_{10}$ values remained low (1.2–1.7; Table A4).

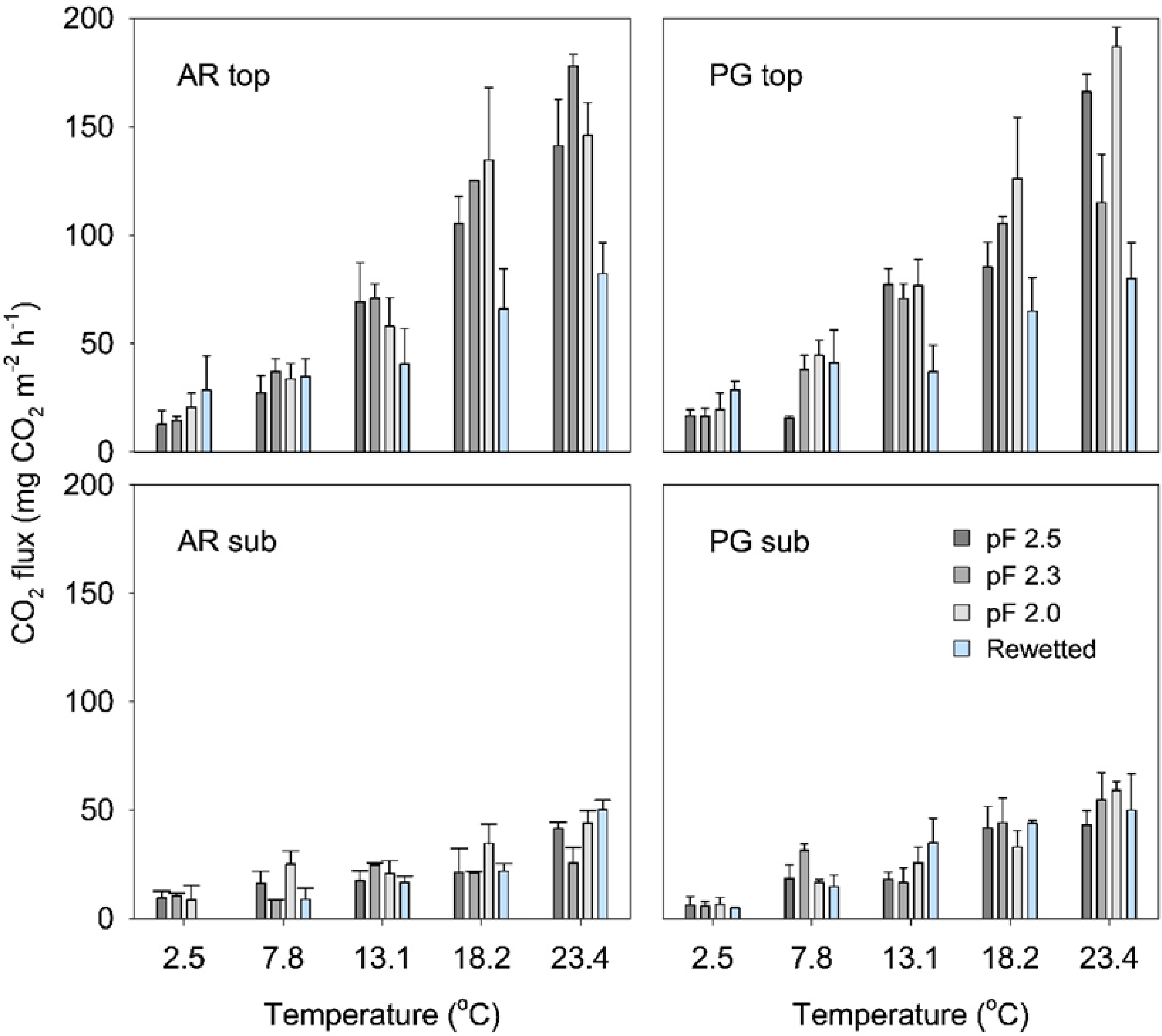


Figure 3. Temperature response of $CO_2$ fluxes from subsoil (25–33 cm) and topsoil (5–13 cm) peat cores incubated under drained (pF 2.5, 2.3 and 2.0) and rewetted conditions. Intact peat cores were sampled from drained agricultural bog sites, managed as arable rotation (AR) and permanent grassland (PG). Data are means and standard errors ($n$ = 3). One observation (rewetted AR subsoil at 2.5 ºC) showed zero flux.

Methane fluxes were within the range -18 to 10 $\mu g\ m^{-2}\ h^{-1}$ across all depth, moisture and temperature combinations (Figure 7). These low fluxes fall within the 95 % probability range of apparent $CH_4$ fluxes ($\pm 26\ \mu g\ m^{-2}\ h^{-1}$) that could result from random variation in GC measurements of atmospheric $CH_4$ background concentrations (Figure A2, Table A5).

## DISCUSSION

This study examined how GHG fluxes from peat soils vary with depth, temperature, and moisture. While based on a short-term incubation, the results offer insight into the relative contributions of topsoil and subsoil layers to emissions. Below, we discuss the

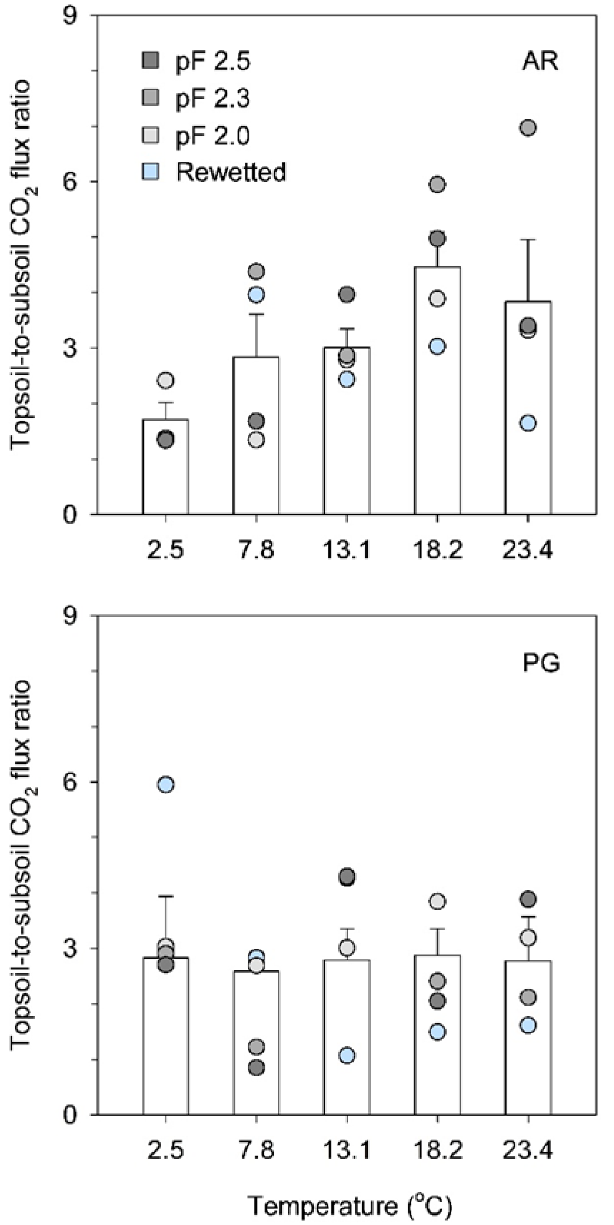


Figure 4. Ratios (evaluated as quotients) of $CO_2$ flux between topsoil (5–13 cm) and subsoil (25–33 cm) peat cores incubated at different temperatures under drained (pF 2.5, 2.3 and 2.0) and rewetted conditions. Coloured circles show ratios at individual moisture levels; bars show means and standard errors ($n$ = 4). Intact cores were sampled from drained agricultural bog sites, managed as arable rotation (AR, upper panel) and permanent grassland (PG, lower panel).

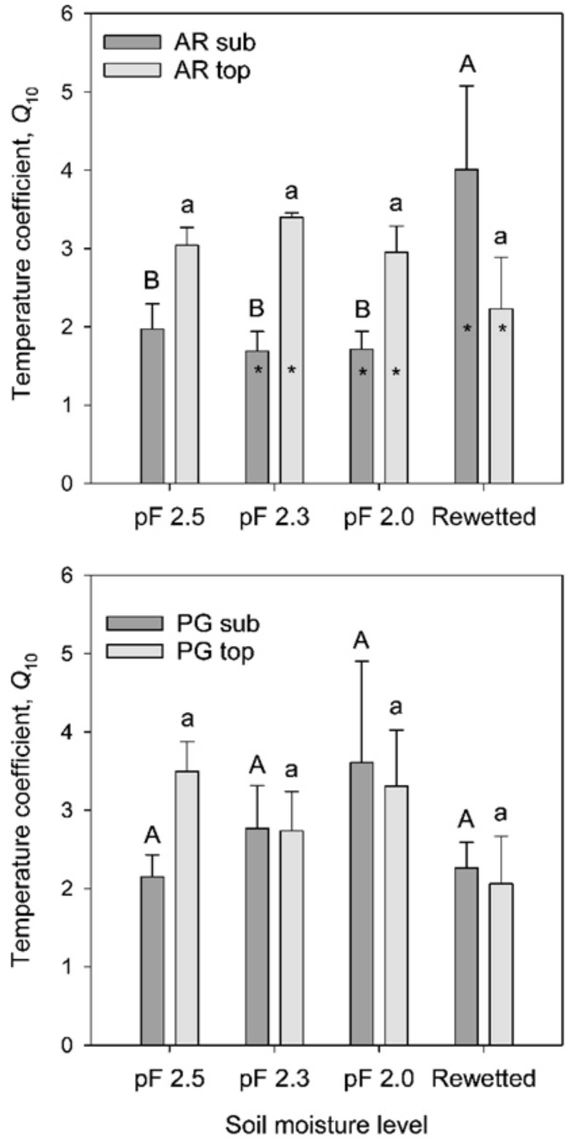


Figure 5. Temperature sensitivity ($Q_{10}$) of $CO_2$ flux in subsoil (25–33 cm) and topsoil (5–13 cm) peat cores incubated under drained (pF 2.5, 2.3 and 2.0) and rewetted conditions. Intact peat cores were sampled from drained agricultural bog sites, managed as arable rotation (AR) and permanent grassland (PG). Data are means and standard errors ($n$ = 3), except for AR topsoil at pF 2.3 ($n$ = 2). Capital and lowercase letters indicate significant differences amongst subsoil and topsoil treatments, respectively. Asterisks mark significant differences between paired subsoil and topsoil samples ($p < 0.05$).

observed patterns and their relevance for national emission modelling and rewetting strategies.

**Fluxes of $CO_2$**

The topsoil $CO_2$ fluxes of 58–77 mg m$^{-2}$ h$^{-1}$ measured at pF 2.0 and 13.1 °C (Figure 3) were comparable to those from a national study of 103 Danish organic soils incubated under similar, though not identical, conditions (Liang *et al*. 2024). That study reported $CO_2$ fluxes within a 95 % CI of 34–52 mg m$^{-2}$ h$^{-1}$ at pF 2.0 and 15 °C based on cumulative emissions from intact peat cores of 3.5 cm height incubated for 60 days (Liang *et al.* 2024), whereas our flux measurements were obtained from intact peat cores of 8 cm height during a short (< 1 h) incubation period. Thus, while a one-to-one comparison is not possible, the approximately two-fold thicker soil layer would be expected to produce higher $CO_2$ fluxes (as was observed) even at the slightly lower incubation temperature. Most importantly, however, the agreement in flux magnitude across the studies despite the large difference in incubation period, supports the relevance of the present short-term incubation study.

Overall, soils from the AR and PG sites showed similar $CO_2$ fluxes. Although land use was not replicated, this observation aligns with the result of a systematic review of 28 paired studies from temperate and boreal regions that found no consistent difference in $CO_2$ emissions between cropland and grassland on peat soils (Holzknecht *et al*. 2025). Default IPCC emission factors for peat soils are

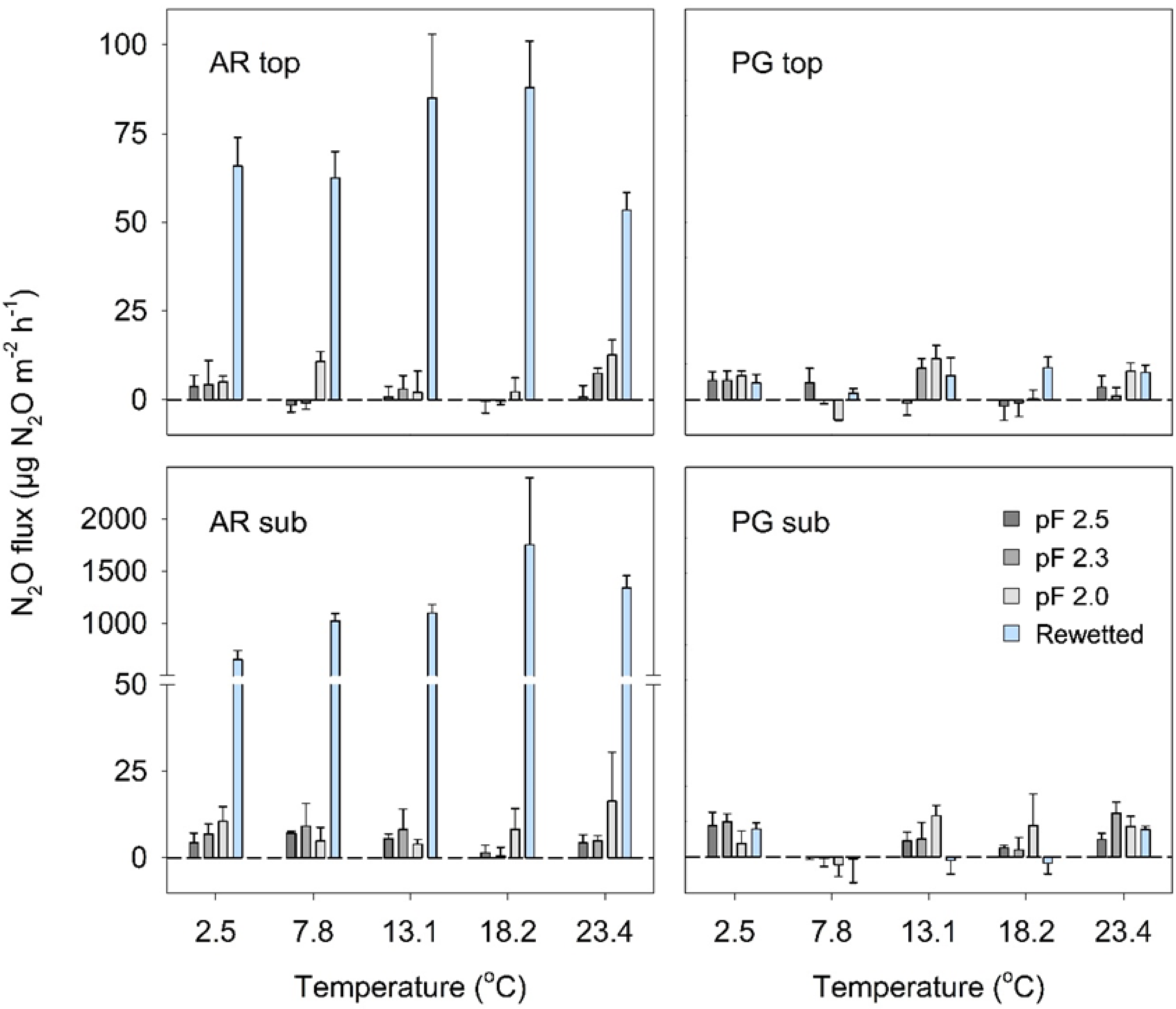


Figure 6. Nitrous oxide ($N_2O$) fluxes from subsoil (25–33 cm) and topsoil (5–13 cm) peat cores incubated at different temperatures under drained (pF 2.5, 2.3 and 2.0) and rewetted conditions. Intact peat cores were sampled from drained agricultural bog sites, managed as arable rotation (AR) and permanent grassland (PG). Data are means and standard errors ($n$ = 3). Note the scale break in the panel for AR subsoil.

30–49 % higher for drained temperate cropland than for grassland (IPCC 2014), but this is based on sparse data with high variability and including unpaired observations. Differences in site-specific factors such as soil properties, drainage intensity and nutrient status often override land use category as the primary control of $CO_2$ emissions (Norberg *et al*. 2016). Therefore, conversion from arable cropping to grassland may not lead to large benefits in terms of GHG emissions unless combined with other changes in management (Holzknecht *et al*. 2025).

*Topsoil dominance in $CO_2$ emissions*

Topsoil emitted $CO_2$ at rates that were on average three times higher than those of subsoils across all temperature and moisture conditions (Figure 4). In an incubation study of soil cores 10 cm long from ten German peatland sites, $CO_2$ fluxes from topsoil were nine times higher than from subsoil, on average (Säurich *et al*. 2019a). This result was derived for subsoils sampled at an average depth of 84 cm (Säurich *et al*. 2019a, 2021). The present study supports a marked decrease in microbial $CO_2$ production already below the uppermost 25 cm of the peat profile. Our observations agree with those from other studies reporting gradients of substrate availability, microbial activity and respiration in the upper peat profile, highlighting the disproportionately large contribution of topsoils to net emissions (Reddy *et al*. 2006, Berglund & Berglund 2011). The

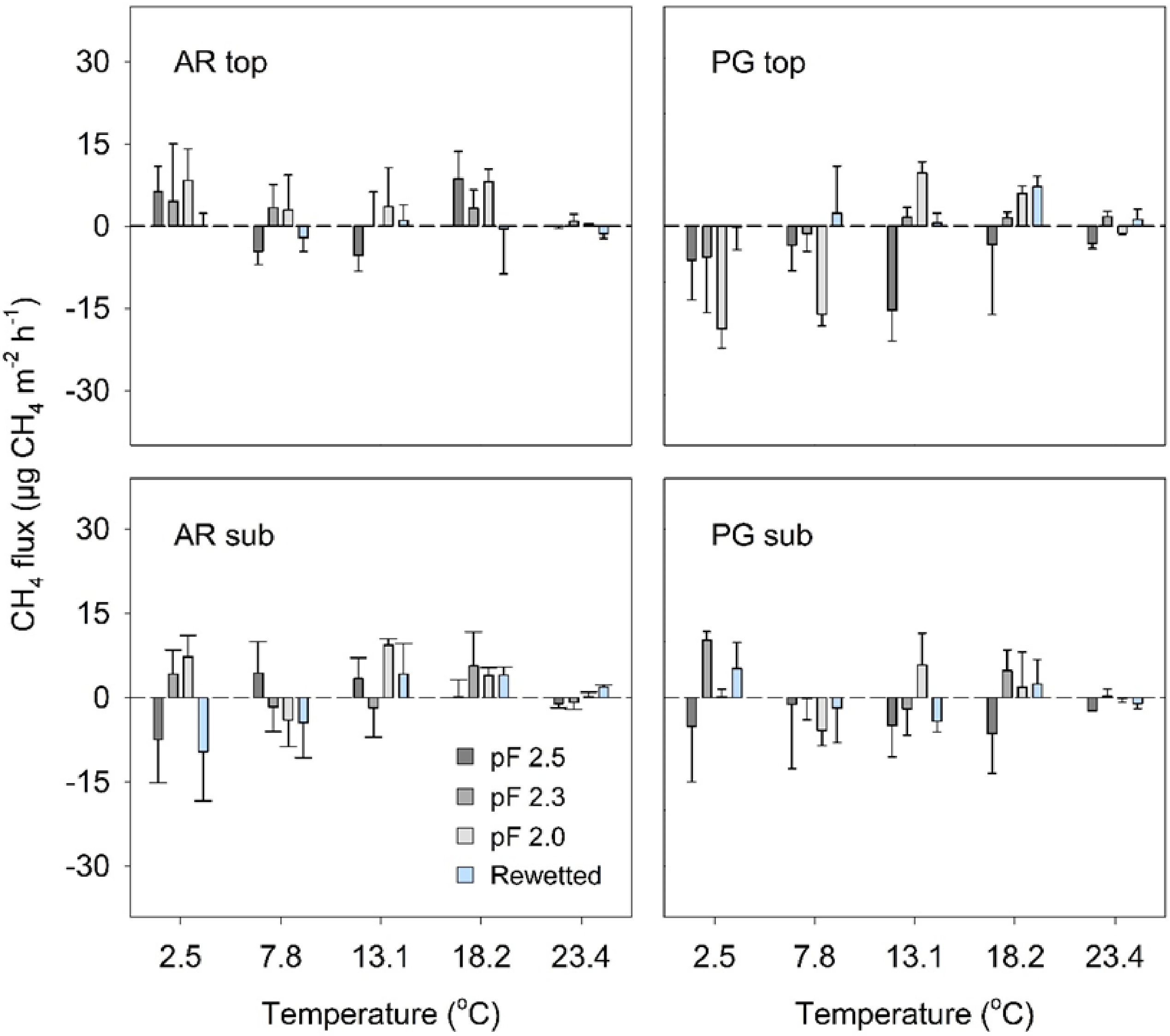


Figure 7. Methane ($CH_4$) fluxes from subsoil (25–33 cm) and topsoil (5–13 cm) peat cores incubated at different temperatures under drained (pF 2.5, 2.3 and 2.0) and rewetted conditions. Intact peat cores were sampled from drained agricultural bog sites, managed as arable rotation (AR) and permanent grassland (PG). Data are means and standard errors ($n$ = 3).

dominant role of topsoil in driving $CO_2$ emissions is also supported by studies showing that WTD manipulations in 100 cm deep peat cores primarily affected emissions within the top 40 cm of peat and that only near-surface saturation (0 cm WTD) significantly suppressed respiration (Blondeau *et al*. 2024). Likewise, a four-year study of subsurface irrigation in an intensively managed Dutch fen grassland, which reduced WTD from 38 cm to 25 cm, showed only marginally reduced $CO_2$ emissions (Heller *et al*. 2025), indicating that more complete topsoil rewetting is needed to achieve the intended climate benefit.

In our experiment, the uppermost 5 cm layer of peat was removed prior to incubation, but it is still likely that the remaining topsoil contained residual recent root material that could contribute to the higher $CO_2$ fluxes observed in topsoil relative to subsoil. However, Kandel *et al*. (2018) estimated that such sources could account for 20 % of total soil respiration at maximum, indicating a relatively minor contribution. Previous studies have indicated that higher respiration in topsoil compared with subsoil is plausibly explained by stronger $O_2$ limitation, greater OC recalcitrance and lower microbial biomass in deeper peat layers (Berglund & Berglund 2011). In this context, a limitation of the present study is that the subsoil in the incubation cores was exposed directly to atmospheric $O_2$ (i.e., without overlying topsoil), potentially enhancing subsoil peat mineralisation relative to in situ conditions. This effect would act to underestimate the true topsoil-to-subsoil $CO_2$ flux ratio.

*Rewetting reduced $CO_2$ only in topsoil at elevated temperatures*

At 18.2 and 23.4 °C, rewetting significantly reduced $CO_2$ emissions from the topsoil (Figure 3), probably due to soluble $O_2$ limitation under saturated and relatively high-temperature conditions. However, this effect was smaller or absent at lower temperatures (2.5–13.1 °C). Subsoil $CO_2$ fluxes showed little response to rewetting across all temperatures, possibly reflecting greater inherent adaptation to anaerobic conditions (Norberg *et al*. 2018) or a slower depletion of $O_2$ due to lower microbial biomass and the presence of more recalcitrant OC at depth (Säurich *et al*. 2019a).

While our study confirms that $CO_2$ emissions from drained peat soils are predominantly driven by topsoil processes, we found that rewetting reduced the topsoil $CO_2$ emissions only at the elevated temperatures, and rewetting of subsoil had minimal effects on $CO_2$ fluxes. We acknowledge the limitations of a short-term incubation study; however, if such patterns persist over time in the field, they may have important implications for the climate-change mitigation potential of rewetting in colder regions such as Denmark.

*Temperature effects on $CO_2$ fluxes*

Temperature had a strong influence on $CO_2$ emissions from both topsoil and subsoil, with fluxes increasing exponentially across the tested range of 2.5–23.4 °C (Table A3). The corresponding $Q_{10}$ values ranged from 1.7 to 4.0 (mean 2.7), aligning well with those (1.4–3.7, mean 2.2) reported for organic soils in a synthesis of eleven globally distributed incubation studies (Bader *et al.* 2018).

Data syntheses indicate that $Q_{10}$ for peat $CO_2$ emissions varies with depth and moisture status, reflecting complex interactions between hydrology, substrate quality and microbial activity (Liu *et al*. 2024). Thus, different studies show that low soil moisture can lead to either an increase (Hardie *et al*. 2011) or a decrease in $Q_{10}$ (Hirano *et al*. 2014). Surface peat often has a higher $Q_{10}$ value than deeper layers (Liu *et al*. 2016), although the opposite has also been reported (Li *et al*. 2021). In our incubation study, $Q_{10}$ responses differed between topsoil and subsoil at the AR site but not at the PG site (Figure 5). Also, a noticeable response (increase) to rewetting was found only in the AR subsoil. While these contrasting patterns within the present data cannot be fully explained, they reflect the broader variability reported in the literature. Overall, our results suggest that rewetting does not significantly alter the temperature sensitivity of topsoil $CO_2$ emissions.

**High $N_2O$ fluxes from rewetted AR soil**

$N_2O$ fluxes were consistently low except for rewetted AR soils, which showed high $N_2O$ emissions particularly from the subsoil (Figure 6). Similar observations of high subsoil $N_2O$ production at 0.3–0.5 m depth have been reported by Goldberg *et al*. (2010) for a minerotrophic acidic fen. The high subsoil fluxes were within a comparable range to on-site $N_2O$ emissions of around 2.2 mg $m^{-2}$ $h^{-1}$ measured at the AR site in October 2014 (Kandel *et al*. 2018) and to $N_2O$-N peak emissions of 67 mg $m^{-2}$ $day^{-1}$ reported for intact bare peat soil cores from a Dutch grassland (Blondeau *et al*. 2024). Our high fluxes may reflect pre-adapted denitrifier communities stimulated by residual N availability after the potato harvest (Kandel *et al*. 2018), while the acidic pH may have increased the product ratio of $N_2O$ to $N_2$ in denitrification (Pfülb *et al*. 2025). Redox potentials in rewetted AR topsoil (341 mV) and subsoil

(520 mV) were within the range indicative of $NO_3^-$ reduction via denitrification (Boonman *et al*. 2024, Mansfeldt 2004). However, the higher redox potential in the subsoil indicates a lower level of $O_2$ depletion, which may favour incomplete denitrification with resulting high emission of $N_2O$ (Rohe *et al*. 2021, Wang *et al*. 2023). Therefore, more incomplete denitrification in the subsoil (rather than more gross denitrification) might have contributed to the high $N_2O$ fluxes.

The temperature effect on $N_2O$ fluxes was modest ($Q_{10}$ 1.0–1.5) and influenced by a decreasing trend at the highest incubation temperature (23.4 °C; Table A4). While increasing temperatures generally enhance microbial metabolism (as seen also for the $CO_2$ fluxes), the product ratio of $N_2O$ to $N_2$ in denitrification may decrease or increase with increasing temperature (Saggar *et al*. 2013, Phillips *et al*. 2015). This can result in subdued effects on $N_2O$ fluxes. In conclusion, however, the high AR subsoil $N_2O$ emissions and weak temperature response support the idea that rewetting intensively managed peat soils, such as those used for potato cropping, can potentially trigger substantial $N_2O$ emissions in the early stages also at low temperatures (Goldberg *et al*. 2010, Blondeau *et al*. 2024).

**Negligible $CH_4$ fluxes from short-term incubation**

Although $CH_4$ fluxes were measured (Figure 7), they remained near zero throughout the short-term incubation. It is likely that this is due to insufficient time for methanogenic communities to become active (Moore & Dalva 1993, Taft *et al*. 2018). The energy metabolism of methanogens thermodynamically depends on the depletion of $O_2$ and alternative terminal electron acceptors such as $NO_3^-$ and iron Fe(III) oxyhydroxides (Thauer *et al*. 1977, Boonman *et al*. 2024). This was not achieved in the present study where the range of redox potentials in the rewetted peat soils was 341–523 mV, well above the expected threshold of -140 mV for methanogenesis (Boonman *et al*. 2024). Addressing the trade-off between reduced $CO_2$ emissions and increased $CH_4$ emissions following rewetting requires long-term studies that can capture the delayed onset and sustained dynamics of hydrogenotrophic and acetoclastic methanogenesis after rewetting (Hemes *et al*. 2018, Kandel *et al*. 2019, Kalhori *et al*. 2024). While such studies are essential for capturing long-term $CH_4$ dynamics and assessing rewetting success from a climate perspective, this was not the main focus here, where the primary aim was to understand the role of topsoil and subsoil dynamics in $CO_2$ emissions from drained peat soils.

**Implication for $CO_2$ modelling in national inventories**

Denmark's National Inventory Document 2025, submitted under the United Nations Framework Convention on Climate Change and the Paris Agreement (Nielsen *et al*. 2025), introduces a WTD-based $CO_2$ response model to estimate $CO_2$ emissions from cultivated organic soils (Gyldenkærne & Callisen 2025). This model calculates emissions at a 10 × 10 m pixel scale, using a sigmoid response function (Gompertz type) driven by annual mean WTD and peat thickness (Gyldenkærne & Callisen 2025). While this approach improves spatial resolution and better reflects drainage conditions (as compared to fixed emission factors), it also introduces uncertainties related to the accuracy of the modelled annual WTD map (Koch *et al*. 2023) and the peat depth map (Beucher *et al*. 2023).

Moreover, the relationship between annual mean WTD and net $CO_2$ fluxes remain uncertain, and both linear and sigmoid functions have been suggested (Tiemeyer *et al*. 2020, Evans *et al*. 2021). The sigmoidal model implies that microbial activity and $CO_2$ production decline with increasing soil depth under drained conditions. Although high-resolution field data are missing, the present study supports a pronounced reduction in soil microbial $CO_2$ production already below the uppermost 25 cm of the peat profile, consistent with earlier reports of depth-dependent emission declines (Säurich *et al.* 2019a). However, the explanatory power of the relationship between WTD and net $CO_2$ fluxes remains limited ($r^2$ = 0.48) in comprehensive data syntheses (Leifeld & Torrús Castillo 2024), highlighting the need to refine current models to improve $CO_2$ emission estimates in national GHG inventories. The need for refinement and improved data availability applies to both the predictor (WTD) and the response variable (net $CO_2$ flux). Here, it should be acknowledged that the relationship between annual mean WTD and $CO_2$ fluxes may differ depending on whether emissions are quantified as soil respiration, ecosystem respiration, net ecosystem exchange or net ecosystem carbon balance (Nijman *et al.* 2024), with the latter representing the analogue to the emission factors used in national GHG inventories for organic soils (Elsgaard *et al.* 2012).

Also relevant for refinement is improved characterisation of peat hydraulic properties and aeration dynamics, as $O_2$ availability, rather than WTD, is the direct control of aerobic peat mineralisation (Iiyama *et al*. 2012, Dickopp *et al.* 2018). In addition, the amount of peat exposed to oxidation, as well as the quality or recalcitrance of the organic matter, may further influence $CO_2$ emissions, although the roles of OC content, density and quality remain complex (Liang *et al.* 2024, Wüst-Galley *et al.* 2025). Incorporating site-specific indicators of nutrient availability (e.g., N and P) may also improve model performance (Säurich *et al.* 2019a,b). However, for national-scale application, such refinements must strike a balance between model complexity and data availability, prioritising input variables that can be mapped reliably at high spatial resolution.

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## AUTHOR CONTRIBUTIONS

TK and LE conceived the study. TK developed the methodology and carried out the investigation. ZL performed the formal analysis. Data curation was conducted by TK and ZL. Writing of the original draft, review and editing was done by TK, ZL, PEL and LE. LE prepared the visualisations. LE and PEL supervised the project, with project administration and funding acquisition handled by LE.

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# Appendix

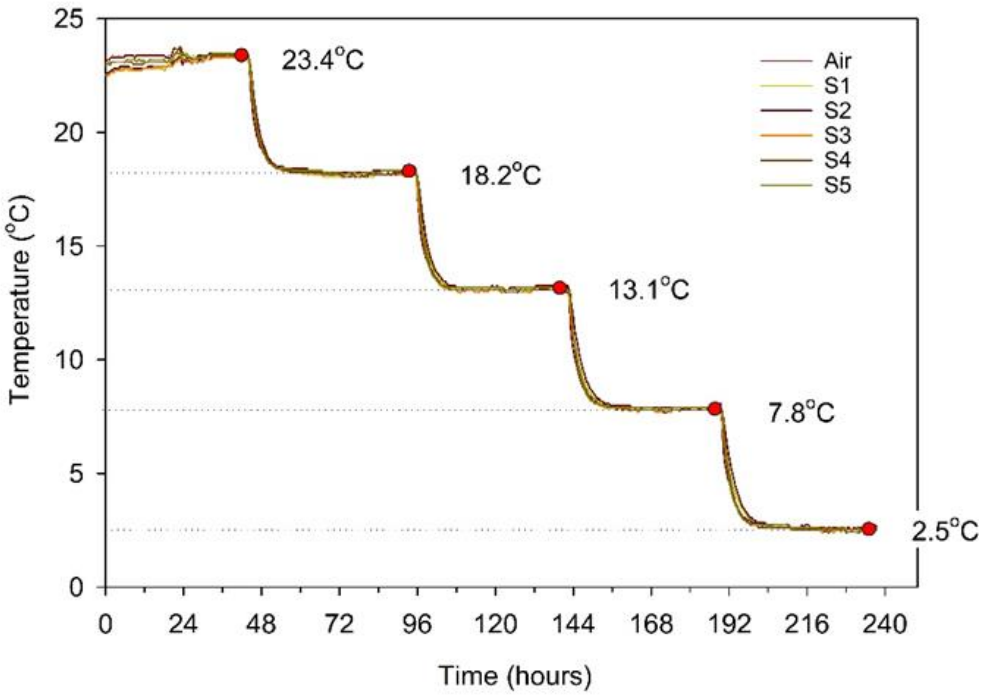


Figure A1. Hourly temperature recordings in the air and five peat soil cores (S1–S5) in the climate chamber during the incubation experiment. Greenhouse gas fluxes were measured over a 40-min period (red circles) following a 48-h equilibration period at each target temperature.

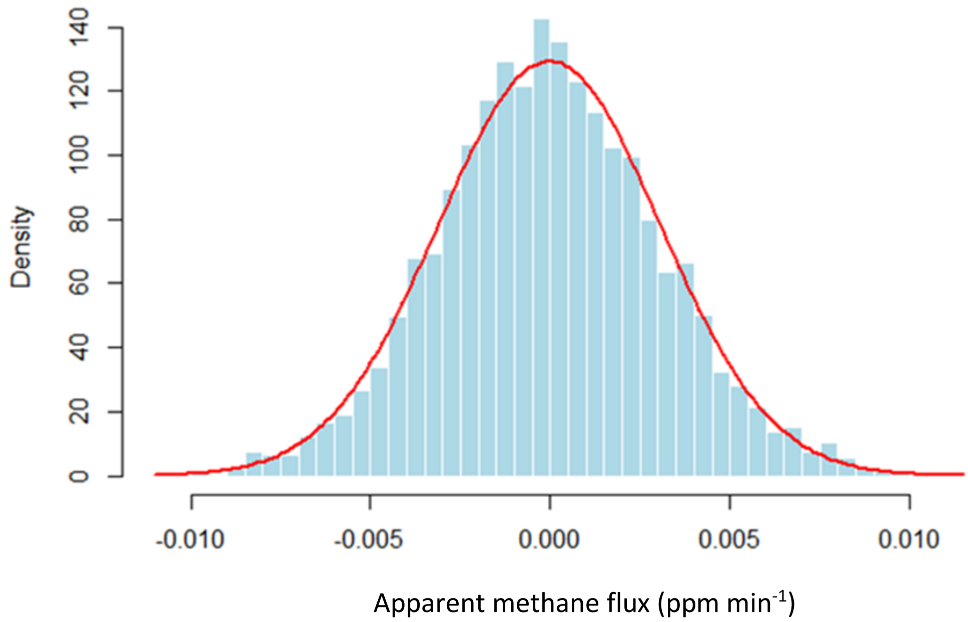


Figure A2. Frequency distribution of apparent methane ($CH_4$) fluxes (ppm min$^{-1}$) that can result from random noise in GC measurements of atmospheric background $CH_4$ concentrations according to the sampling frequency applied in the study (i.e., time 0, 20 and 40 min) and the precision of the GC system (see Table A5). The Gaussian distribution has a mean ($\mu$) of -0.0000237 ppm min$^{-1}$ and a standard deviation ($\sigma$) of 0.003085 ppm min$^{-1}$, which means that 95 % of the fluxes lie within ± 0.0076 ppm min$^{-1}$ ($\mu \pm 1.96\sigma$). As calculated from Equation 1 in the main article, these $CH_4$ fluxes correspond to ± 24 µg m$^{-2}$ h$^{-1}$ for the highest incubation temperature (23.4 °C) and ± 26 µg m$^{-2}$ h$^{-1}$ for the lowest incubation temperature (2.5 °C).

Table A1. Main and interactive effects of soil depth (topsoil, subsoil), moisture (volumetric water content) and temperature on $CO_2$ fluxes at the arable rotation (AR) site in the Store Vildmose drained bog.

| Variable | Df | Chisq | Pr(>Chisq) |
|---|---|---|---|
| Depth | 1 | 283.4 | <0.001 |
| Moisture | 3 | 22.9 | <0.001 |
| Temperature | 4 | 372.4 | <0.001 |
| Depth:moisture | 3 | 6.7 | 0.081 |
| Depth:temperature | 4 | 49.8 | <0.001 |
| Moisture:temperature | 12 | 8.1 | 0.774 |

Table A2. Main and interactive effects of soil depth (topsoil, subsoil), moisture (volumetric water content) and temperature on $CO_2$ fluxes at the permanent grassland (PG) site in the Store Vildmose drained bog.

| Variable | Df | Chisq | Pr(>Chisq) |
|---|---|---|---|
| Depth | 1 | 242.2 | <0.001 |
| Moisture | 3 | 15.1 | 0.002 |
| Temperature | 4 | 507.0 | <0.001 |
| Depth:moisture | 3 | 16.7 | <0.001 |
| Depth:temperature | 4 | 31.9 | <0.001 |
| Moisture:temperature | 12 | 29.4 | 0.003 |

Table A3. Temperature sensitivity of $CO_2$ fluxes from topsoil (5–13 cm) and subsoil (25–33 cm) peat cores incubated under drained (pF 2.5, 2.3 and 2.0) and rewetted conditions. Data are shown as activation energy ($-E_a$) and $Q_{10}$ at 5–15 °C (mean ± standard error), where $E_a$ was calculated from linear Arrhenius plots. Goodness-of-fit is shown as $r^2$ values for the individual soil cores at each treatment. Intact peat soil cores were sampled from drained agricultural bog sites, managed as arable rotation (AR) and permanent grassland (PG). n.a. : not available.

| Land use | Soil depth | Moisture | $-E_a$ (kJ mol$^{-1}$) | $r^2$ | $Q_{10}$ |
|---|---|---|---|---|---|
| AR | topsoil | pF 2.5 | 73.8 ± 4.8 | 0.93; 0.85; 1.00 | 3.04 ± 0.23 |
| AR | topsoil | pF 2.3 | 81.5 ± 0.9$^{\dagger}$ | 0.99; 0.94; n.a. | 3.40 ± 0.06 |
| AR | topsoil | pF 2.0 | 81.5 ± 0.9 | 0.96; 0.85; 0.90 | 2.95 ± 0.34 |
| AR | topsoil | rewetted | 48.1 ± 18.1 | 0.70; 0.77;0.84 | 2.23 ± 0.66 |
| AR | subsoil | pF 2.5 | 43.4 ± 11.4 | 0.67; 0.94; 0.11 | 1.97 ± 0.32 |
| AR | subsoil | pF 2.3 | 33.7 ± 9.3 | 0.51; 0.32; 0.86 | 1.69 ± 0.25 |
| AR | subsoil | pF 2.0 | 34.7 ± 8.6 | 0.31; 0.66; 0.57 | 1.71 ± 0.23 |
| AR | subsoil | rewetted | 87.4 ± 19.0 | 0.95; 0.65; 0.80 | 4.01 ± 1.07 |
| PG | topsoil | pF 2.5 | 82.6 ± 7.7 | 0.90; 0.91; 0.81 | 3.50 ± 0.38 |
| PG | topsoil | pF 2.3 | 64.9 ± 11.6 | 0.76; 0.94; 0.96 | 2.73 ± 0.51 |
| PG | topsoil | pF 2.0 | 76.9 ± 13.4 | 0.94; 0.90; 0.98 | 3.31 ± 0.71 |
| PG | topsoil | rewetted | 41.5 ± 21.8 | 0.80; 0.00; 0.26 | 2.06 ± 0.61 |
| PG | subsoil | pF 2.5 | 49.7 ± 9.3 | 0.66; 0.26; 0.70 | 2.15 ± 0.28 |
| PG | subsoil | pF 2.3 | 64.7 ± 14.9 | 0.33; 0.84; 0.66 | 2.77 ± 0.55 |
| PG | subsoil | pF 2.0 | 77.2 ± 22.3 | 0.73; 0.80; 0.88 | 3.61 ± 1.29 |
| PG | subsoil | rewetted | 53.2 ± 9.12 | 0.43; 0.71; 0.92 | 2.26 ± 0.32 |

Table A4. Temperature sensitivity of $N_2O$ fluxes from rewetted topsoil (5–13 cm) and subsoil (25–33 cm) peat cores from the arable rotation (AR) site. Data are shown as activation energy ($-E_a$) and $Q_{10}$ at 5–15 °C (means ± standard errors, $n$ = 3), where $E_a$ was calculated from linear Arrhenius plots. Goodness-of-fit is shown as $r^2$ values for the three individual soil cores at each treatment. Temperature sensitivity was calculated for the full dataset and for a reduced dataset excluding the fluxes at 23.4 °C, which showed a decreasing trend.

| Land use | Soil depth | Dataset | $-E_a$ (kJ mol$^{-1}$) | $r^2$ | $Q_{10}$ |
|---|---|---|---|---|---|
| AR | Topsoil | Full | -0.6 ± 4.4 | 0.05; 0.11; 0.04 | 1.0 |
| AR | Topsoil | Reduced | 14.3 ± 1.6 | 0.58; 0.22; 0.40 | 1.2 |
| AR | Subsoil | Full | 24.8 ± 7.0 | 0.87; 0.57; 0.59 | 1.5 |
| AR | Subsoil | Reduced | 34.8 ± 11.5 | 0.80; 0.75; 0.86 | 1.7 |

Table A5. R code to generate fluxes (ppm min$^{-1}$) that could result from random noise in GC measurements of atmospheric background $CH_4$ concentrations according to the sampling frequency applied in the study (i.e., time 0, 20 and 40 min) and the precision of the GC system. The GC results showed a mean and standard deviation of 1.752 ± 0.086 ppm $CH_4$ for analysis of 289 samples of atmospheric $CH_4$. For more details, see Karki *et al*. (2015).

```
library(openxlsx)
t0=rnorm(100, mean = 1.752, sd = 0.086)
t20=rnorm(100, mean = 1.752, sd = 0.086)
t40=rnorm(100, mean = 1.752, sd = 0.086)
#x-values
b=c(0,20,40)
#y-values
results=data.frame( 'Estimate'=0,'StdError'=0,'t.value'=0,'pr.of.t'=0)
for(i in 1:10000){
a=c(sample(t0,1), sample(t20,1), sample(t40,1))
lin=data.frame(a=a, b=b)
res=summary(lm(a ~ b), data = lin)
k=coef(res)
r=data.frame(k)
names(r)=c('Estimate','StdError','t.value','pr.of.t')
results=rbind(results,r)}
results=results[-1,]
write.xlsx(results,file='simulation_flux.xlsx',sheetName='results')
```