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Stratification-induced hypoxia shapes greenhouse gas dynamics in ponds

Chiara Esposito*[1, 2,3], Joachim Audet[1, 2], Eti E. Levi[1], and Thomas A. Davidson[1, 2]

[1]Department of Ecoscience, Aarhus University, Denmark
[2]WATEC Aarhus University Centre for Water Technology, Aarhus, Denmark
[3]Department of Biology, Aarhus University, Denmark

*Corresponding author: Chiara Esposito, C. F. Møllers Alle 3, DK-8000 Aarhus C, Denmark, author e-mails: che@bio.au.dk, joau@ecos.au.dk, eel@ecos.au.dk, thd@ecos.au.dk

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Abstract

Fresh waters in general and ponds in particular are biogeochemical hot spots and as such are sources of greenhouse gas (GHG) emissions, especially methane (CH_4) and carbon dioxide (CO_2). Several studies have shown that thermal stratification of the water column is a common characteristic of ponds, but how this process influences GHG dynamics is less well studied. We tracked thermal stratification and the associated hypoxia in six ponds across the growing season and measured dissolved CH_4 and CO_2 in surface and bottom waters, along with CH_4 ebullition, every two weeks. The results showed that all ponds stratified during the growing season and that thermal stratification often, but not always, led to bottom-water hypoxia. Where hypoxia occurred, bottom-water CH_4 was approximately 16 times higher (median 3.05 vs 0.19 mg C L^{-1}), CO_2 about 3 times higher (11.6 vs 4.03 mg C L^{-1}) and CH_4 ebullition was about 3.4 times higher than in oxic conditions. In contrast, surface-water GHG concentrations showed no significant difference between periods with and without bottom-water hypoxia. Bottom-water temperatures were not significantly different between hypoxic and oxic states, indicating oxygen availability, rather than temperature, dominated GHG buildup and ebullition. Stratification and the resulting hypoxia enhance CH_4 ebullition and promote high concentrations of GHGs in bottom waters, which may be released in small or larger pulses as the water column mixes partially or fully. In summary this study demonstrates that thermal stratification, by inducing bottom-water hypoxia, strongly influences the GHG dynamics across a range of timescales in ponds.

1. Introduction

Freshwater ecosystems are major sources of greenhouse gases (GHGs), particularly methane (CH_4) and carbon dioxide (CO_2) (Cole et al., 2007). Small lakes and ponds, characterized by shallow depth and low coverage of emergent vegetation (Richardson et al., 2022), may be disproportionately large sources of CH_4 (Bastviken and Johnson, 2025; Rosentreter et al., 2021a; Saunois et al., 2025). Although small ponds (< 0.001 km^2) cover only 9% of the global freshwater area, they emit about 40% of the total freshwater diffusive CH_4 (Holgerson and Raymond, 2016). In shallow lakes and ponds, CH_4 is produced through microbial methanogenesis, mainly by methanogenic archaea in the anoxic zone of the sediment (Bastviken et al., 2002). A portion of this CH_4 is oxidized by methane-oxidizing bacteria in the oxic layer of the sediment and the oxic water column (Bastviken et al., 2002,

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2008). Methane that escapes oxidation is emitted to the atmosphere through diffusion or direct release of CH_4-rich bubbles from the sediment, a process termed ebullition (Bastviken et al., 2008).

Thermal stratification (i.e. the formation of distinct thermal layers) is a well-studied phenomenon in lakes whereas little focus has historically been directed at ponds (Andersen et al., 2017; MacIntyre et al., 2018; Holgerson et al., 2022). However, several publications have shown that ponds frequently stratify due to their small size and sheltered nature, limiting wind mixing (Chimney et al., 2006; Holgerson and Raymond, 2016; Markfort et al., 2010; Søndergaard et al., 2023b). Unlike deeper lakes, where stratification produces well-defined thermal layers, ponds more commonly exhibit continuous temperature gradients from surface to bottom. Nevertheless, even these gradients can suppress vertical mixing sufficiently to isolate bottom waters from atmospheric exchange, promoting oxygen depletion and the accumulation of CH_4 and CO_2 (Holgerson et al., 2022). These stratified periods may thus affect key ecosystem processes such as metabolism, nutrient cycling, oxygen availability and GHG production (Bartosiewicz et al., 2015; Davidson et al., 2024; Holgerson et al., 2022; Ray and Holgerson, 2023). Thermal stratification isolates bottom waters from the atmosphere, often leading to elevated GHG concentrations in the hypolimnion and lower concentrations in the epilimnion (Bartosiewicz et al., 2015, 2016; Rabaey et al., 2026). Whilst thermal stratification prevents free mixing of the water column, in shallow systems, CH_4 bubbles can bypass the density gradient and escape directly to the surface (Bastviken et al., 2008; DelSontro et al., 2016).

Thermal stratification limits mixing and prevents replenishment of oxygen in the hypolimnion resulting in hypoxia, creating favourable conditions for anaerobic organisms, including methanogenic archaea (Deppenmeier et al., 1996). Prolonged hypoxia may also limit CH_4 oxidation in the hypolimnion by methane-oxidizing bacteria (Bastviken et al., 2008). Furthermore, during stratification, factors such as shifts between primary producers and increased DOC may enhance GHG production (Bastviken et al., 2023; Davidson et al., 2015, 2018; Zhou et al., 2019). Mixing events, either partial or total, over the course of the summer or at the end of a stratification period, cause the upwelling of bottom waters which may be rich in CH_4 and CO_2, leading to relatively short-term emission events, or hot moments, of GHG emission (Davidson et al., 2024; Erkkilä et al. 2018; Rabaey & Cotner, 2024; Vachon et al., 2019).

In this study, we aimed to determine the effect of thermal stratification on GHG emissions from six ponds across the growing season by addressing the following two questions: 1) How do water temperature and bottom-water

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oxygen concentrations alter GHG dynamics? 2) How do prolonged periods of thermal stratification and hypoxia affect GHG concentrations and emissions?

2. Materials and methods

2.1 Study sites

Fieldwork was undertaken in Central Jutland, Denmark, between 27 April and 4 November 2021, over 192 days during the growing season. Six ponds located near the village of Ry were studied – Ry Hule (RH) 1–6 (Fig. 1 and Table 1). Ponds RH1 and RH2 were surrounded by pasture, RH3 by heathland and RH4 by heathland with a ring of shrubs and trees (mainly *Alnus glutinosa* and *Corylus avellana*). RH5 and RH6 were located in arable areas, RH6 being surrounded by a ring of trees (mainly *Alnus glutinosa* and *Salix*). All the ponds had an area < 0.4 ha and a depth < 2 m, and none dried out during the sampling period. RH2 had the highest concentrations of dissolved organic carbon (DOC, mean ± SD: 17.3 ± 2.3 mg L^{-1}) and the highest ratio of total nitrogen (TN) to total phosphorus (TP). RH5 and RH1 were the most eutrophic, exhibiting the highest concentrations of chlorophyll *a* (40.3 ± 6.0 and 53.3 ± 28.8 µg L^{-1} respectively, Table 1).

Table 1 The physical and chemical parameters analyzed: maximum depth (Max. Depth), dissolved organic carbon (DOC) and TN:TP, the ratio between total nitrogen (TN) and total phosphorus (TP). For DOC, chlorophyll *a* and TN:TP, mean and SD (mean ± SD) are shown.

Variable	RH1	RH2	RH3	RH4	RH5	RH6
Longitude (°)	9.65645	9.65095	9.64648	9.64876	9.60359	9.61469
Latitude (°)	56.04352	56.04286	56.04741	56.04919	56.02103	56.02286
Max depth (cm)	197	120	158	165	174	161
Mean depth (cm)	169	94	124	111	136	127
Area (m^2)	3197	506	753	523	491	244
DOC (mg L^{-1})	9.4 ± 2.7	17.3 ± 2.3	13.3 ± 1.7	13.2 ± 1.0	11.4 ± 1.8	10.4 ± 1.3
Chlorophyll *a* (µg L^{-1})	53.3 ± 28.8	32.0 ± 11.6	9.4 ± 4.3	23.8 ± 14.0	40.3 ± 6.0	28.7 ± 8.1
TN (mg L^{-1})	0.86 ± 0.34	0.87 ± 0.42	0.85 ± 0.38	1.1 ± 0.48	0.86 ± 0.41	0.71 ± 0.41
TP (mg L^{-1})	0.14 ± 0.06	0.15 ± 0.14	0.21 ± 0.03	0.23 ± 0.09	0.25 ± 0.17	0.23 ± 0.11

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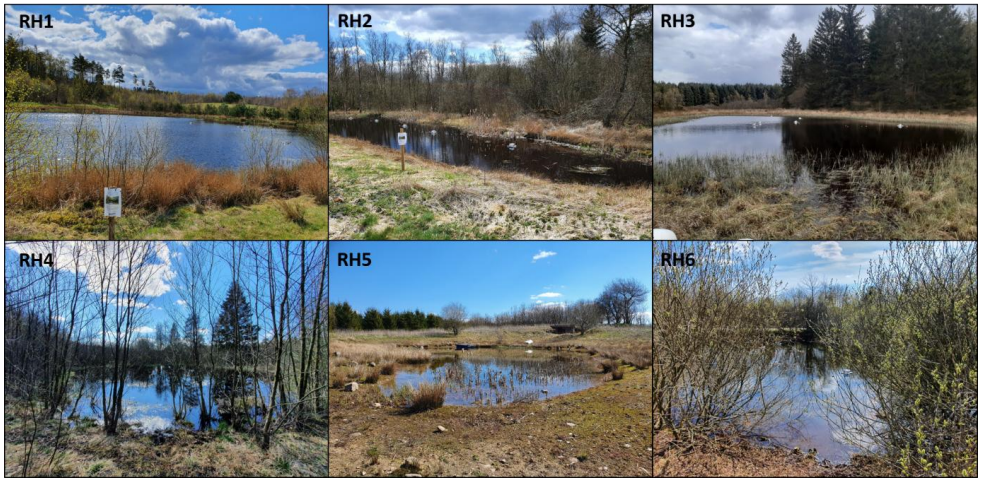

Fig. 1 Photos of the six studied ponds, RH1–6. First row, from left to right: RH1, RH2 andRH3; second row: RH4, RH5 and RH6 (Photos: C. Esposito).

2.2 Temperature, oxygen profiling and water chemistry

The ponds were sampled every two weeks between 9:00 and 16:00 from April to November 2021. Dissolved oxygen and temperature profiles were measured at 10-20 cm depth intervals at the deepest point of each pond with an Aqua TROLL 500 (In-Situ, Fort Collins, CO, USA) sensor on each of the 15 sampling occasions. In addition, from 28 June to 4 November, temperature loggers (Hobo MX2201) measuring the temperature every 30 min in the surface and bottom water (and in the middle of the water column, for RH1 that was the deepest pond) were added to each pond. The temperature logger results (accuracy ± 0.5 °C) were validated against the Aqua TROLL temperature profiles (accuracy ± 0.1 °C) by matching the profile sampling time with the closest corresponding measurements from both the top and bottom temperature loggers in each pond (see the Supplement). This approach provided continuous temperature records between the sampling dates (Figs. S1, S2). Thermal stratification was defined as minimum 1°C difference at 1 m depth (Wetzel, 2001). Stratification was evidenced based on the biweekly highly accurate Aqua TROLL profiles, and the continuous logger data provided additional information on potential mixing between sampling dates. High-frequency temperature logger data were used to calculate the duration of stratification – and by extension hypoxia – based on the assumption that thermal stratification prevented re-oxygenation of bottom waters. Periods of bottom-water hypoxia were confirmed during

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field measurements of dissolved oxygen with the Aqua TROLL sensor. In RH2, where depth fell below 1 m during summer, stratification was defined as a ≥1 °C temperature difference between surface and bottom waters. Bottom waters were classified as hypoxic when dissolved oxygen concentrations were ≤ 0.5 mg L^{-1}.

Water chemistry samples were collected on three occasions from the entire water column during the period April–November 2021 and analyzed for total phosphorus (TP), total nitrogen (TN), DOC and chlorophyll *a* according to Danish standard procedures (Søndergaard et al., 2005).

2.3 Greenhouse gas sampling

2.3.1 Dissolved concentrations and diffusive fluxes

Samples for determination of dissolved CH_4 and CO_2 concentrations were collected every two weeks from 27 April to 4 November 2021 from surface and bottom water using headspace equilibration (Hope et al., 2004). Forty ml of water was collected and 10 ml air was introduced as a headspace in a 60-ml syringe, after which the sample was shaken vigorously for one minute. The headspace was then transferred to a 5.9 ml pre-evacuated glass vial (exetainer, Labco). Additionally, 10 ml ambient air from above the pond was collected and sealed in a vial.

Gas concentrations were determined on an Agilent 8890 GC system (USA) interfaced with a PAL RSI 120 autosampler (CTC Analytics, Switzerland) (Petersen et al., 2012). Aqueous concentrations of CH_4 and CO_2 were calculated from the headspace gas concentrations according to Henry's law and using Henry's constant corrected for temperature and salinity (Weiss, 1974; Weiss and Price, 1980; Wiesenburg and Guinasso, 1979).

CH_4 and CO_2 fluxes between the water and the overlying atmosphere were estimated as

$$f_g = k_g\left(C_{wat,g} - C_{eq,g}\right)$$

where $f_g$is the flux of a specific gas g, k_g is the piston velocity of the gas, and $C_{wat,g} - C_{eq,g}$ is the gradient of concentration between the concentration of gas dissolved in the water ($C_{wat,g}$) and the concentration of gas that the water would have when in equilibrium with the atmosphere ($C_{eq,g}$), using concentrations measured in ambient air samples.

$$k_g = k_{600}\left(\frac{Sc_g}{600}\right)^x$$

Sc_g is the Schmidt number (Wanninkhof, 1992). We estimated the gas transfer velocity (k_{600}) based on wind speed and pond size using model B in Vachon and Prairie (2013).

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We chose x =-2/3 as this factor is used for smooth liquid surfaces (Deacon, 1981). Wind speed was obtained from the Danish Meteorological Institute (Supplement).

2.3.2 Ebullition

Methane ebullition was estimated using floating chambers adapted from Bastviken et al., (2015) with a circular base (chamber area 0.075 m^2) and a total volume of 8 L. Each chamber was covered in silver foil to reflect sunlight and minimise warming. In each pond, 4 to 6 chambers were deployed along a depth gradient on 13 April 2021 and subsequently sampled every two weeks. A 10 ml gas sample was extracted from each chamber and injected into a pre-evacuated 5.9 ml vial for analysis using GC. After each sampling event, atmospheric concentrations were restored, and the chamber was then repositioned on the water surface.

The ebullitive flux of CH_4 (CH_{4bub}) was estimated as:

$$CH_{4bub} = \frac{p_{gas} \times Vol_{bub}}{t \times A}$$

where p_{gas} is the concentration of CH_4 in the trapped gas, Vol_{bub} is the chamber volume, t is the duration the chamber was deployed (number of days between two sampling events), and A is the chamber area.

However, these ebullition estimates are biased due to diffusion of CH_4 back into the water, resulting in underestimation of fluxes. In a recent study, average ebullition in static chambers was underestimated by between 16 and 34% (Davidson et al., 2024).

On each sampling occasion, ebullitive fluxes per pond were expressed as mean of the fluxes recorded by all chambers deployed in the pond.

The total ebullitive flux over the sampling period was calculated without interpolation as emissions were continuous. A mean daily emission rate was calculated by averaging fluxes from the daily emissions rates from the 14 evenly spaced sampling occasions. Total emissions per pond were then calculated by multiplying the mean daily rate by the total number of deployment days (192).

The results were converted to CO_2 equivalents using a global warming potential (GWP) factor of 28 for CH_4 based on its 100-year impact (Global Methane Tracker, 2022).

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2.4 Statistical analysis

Pearson correlation was used to examine the associations among dissolved GHG concentrations and bubble fluxes and oxygen concentrations using R version 4.2.1 (R Development Core Team, 2022). For each result, r, *p* values, and degrees of freedom (df) between the two variables are reported. Logistic regression was used to determine how the absolute difference in temperature between surface and bottom water was related to bottom water hypoxia. To determine the effects of the hypoxia in the bottom waters on the concentrations of dissolved GHGs and CH_4 ebullition, we used linear mixed-effects models in the package *nlme* (Pinheiro et al., 2026) and for each result, *p* values and df are reported. The fixed effect was bottom-water hypoxia, with the pond as random effect. Residual patterns were examined, and a logarithmic transformation was applied for all GHG measures except surface water CO_2. A linear model was then used to determine the effects of chlorophyll *a* on GHG concentrations and CH_4 ebullition and for each result, r^2, *p* values and df are also reported.

3. Results

3.1 Temperature, stratification and controls on GHGs

All six ponds exhibited periods of thermal stratification alternating with mixing during the growing season, ranging from a few days to several months. At the end of April, manual temperature profiles showed that all ponds were fully mixed (Fig. S3). From late May to September, stratification occurred in all ponds, with varying duration. RH1, RH4 and RH6 were stably stratified from late May until the end of August, followed by total mixing with intermittent short-lived (< 1 week) stratification events until early October. RH3, RH2 and RH5 alternated more frequently between stratified and mixed states throughout summer. After August, a longer period of mixing was observed in most ponds, with only occasional stratification events until November.

Logistic regression indicated a weak but significant relationship between stratification status and the likelihood of hypoxia (Fig. S4a), demonstrating that while stratification increases the probability of oxygen depletion, it does not guarantee it. A stronger relationship emerged when considering the temperature difference between surface and bottom waters and the probability of hypoxia (Fig. S4b).

Bottom-water temperatures did not differ significantly between oxic and hypoxic states (mean [min–max]: 12.9 [6.6-19.3] °C for oxic and 14.4 [7.3-19.1] °C for hypoxic; $p = 0.83$, df = 81; Fig. 2). In contrast, surface-water temperatures were significantly higher during hypoxia (18.3 [7.3-22.5] °C) than during oxic conditions (13.6 [6.7-23.7] °C; $p = 0.026$, df = 81; Fig. 2), which was also consistent with the warmer months (Fig. S5). Despite these

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differences, evidence that water temperature directly influenced CH_4 or CO_2 concentrations is limited. Bottom-water gas concentrations showed weak positive correlations with temperature, but the explained variance was low (Fig. S6), and no relationships were detected in surface waters (Fig. S6).

Chlorophyll *a* concentrations were poor predictors of dissolved gas dynamics. No correlations were observed in surface waters, and only two ponds (RH1 and RH6) showed significant positive relationships between chlorophyll *a* and bottom-water CH_4 (RH1: $r^2 = 0.71$, $p = 0.018$; RH6: $r^2 = 0.77$, $p = 0.022$) and CO_2 concentrations (RH1: $r^2 = 0.65$, $p = 0.024$; RH6: $r^2 = 0.72$, $p = 0.013$; Fig. S7). However, no consistent pattern could be discerned across the ponds.

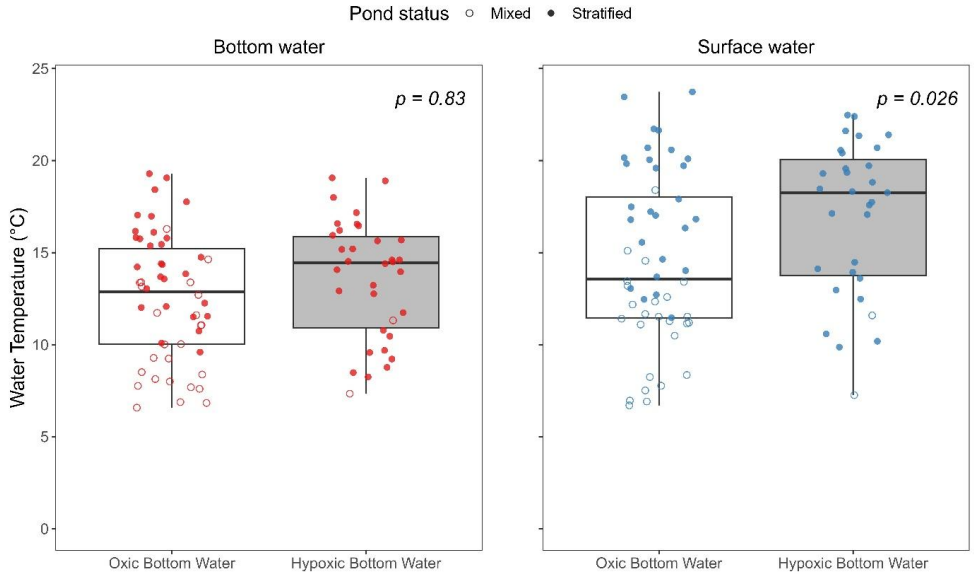

Fig. 2 Boxplots showing bottom and surface water temperatures (°C) in all six ponds. The data include all six ponds and indicate bottom-water oxygen concentrations under hypoxic (≤ 0.5 mg L^{-1}) and oxic (> 0.5 mg L^{-1}) conditions. The boxplots display the median and interquartile range (IQR), with whiskers indicating minimum and maximum values, excluding outliers (calculated as values outside box limits ±1.5 x IQR). Dots represent individual data points – filled coloured dots represent thermally stratified conditions (≥1°C difference at 1 m depth), while empty dots represent mixed conditions (< 1°C difference at 1 m depth d). The *p*-values correspond to the results from the linear mixed effect model between the response variable (left y-axis) and bottom-water oxygen concentrations (hypoxic-oxic).

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3.2 Seasonal patterns of dissolved GHGs

Hypoxia in bottom waters occurred in all ponds at points over the summer, but with varying duration. There was likely continuous hypoxia in RH4, RH1 and RH6 for 44, 57 and 126 days, respectively (Figs. S3, S5). In RH2, RH5 and RH3, the periods of bottom-water hypoxia were fewer and shorter in duration, lasting 14, 14 and 30 consecutive days, respectively. This pattern aligned with the number of samples with anoxic bottom water per pond based on the profiles collected every two weeks. Out of 15 sampling days, RH4, RH1 and RH6 showed hypoxia on 7, 8 and 11 occasions, respectively, while RH2, RH3 and RH5 exhibited hypoxia on 2, 3 and 2 occasions, respectively (Fig. S3).

RH1, RH4 and RH6 had high bottom-water concentrations of CH_4 (up to 8.5 mg CH_4 – C L^{-1} in RH4, Fig. 3) and CO_2 (up to 37.5 mg CO_2 – C L^{-1} in RH1, Fig. 4), whereas RH2, RH3 and RH5 had lower CH_4 and CO_2 in the bottom waters compared with the other ponds (up to 2.69 mg CH_4 – C L^{-1} in RH5 and 15.3 CO_2 – C L^{-1} in RH2, Figs. 3, 4).

In ponds RH1-5, bottom-water oxygen concentrations increased by early September. In contrast, RH6 remained hypoxic until the end of September and experienced an additional hypoxia event in late October, coinciding with a warm-weather period that triggered renewed stratification. Similar late-October hypoxia events were observed in RH2 and RH4 (Fig. S3), demonstrating that even brief warm periods late in the season can re-establish stratification and oxygen depletion.

Surface-water concentrations of CH_4 and CO_2 peaked in late summer (September-October) in RH1, RH3 and RH4 during periods with mixed water columns. In contrast, RH2 and RH5 showed elevated GHG concentrations earlier in the year (May-August), also during mixed periods. The surface-water concentrations of CO_2 were about 10-50 times higher than those of CH_4. Our results indicate that surface water GHG concentrations generally increased during mixing, following reductions of GHG concentrations in deep water (Figs. 3, 4).

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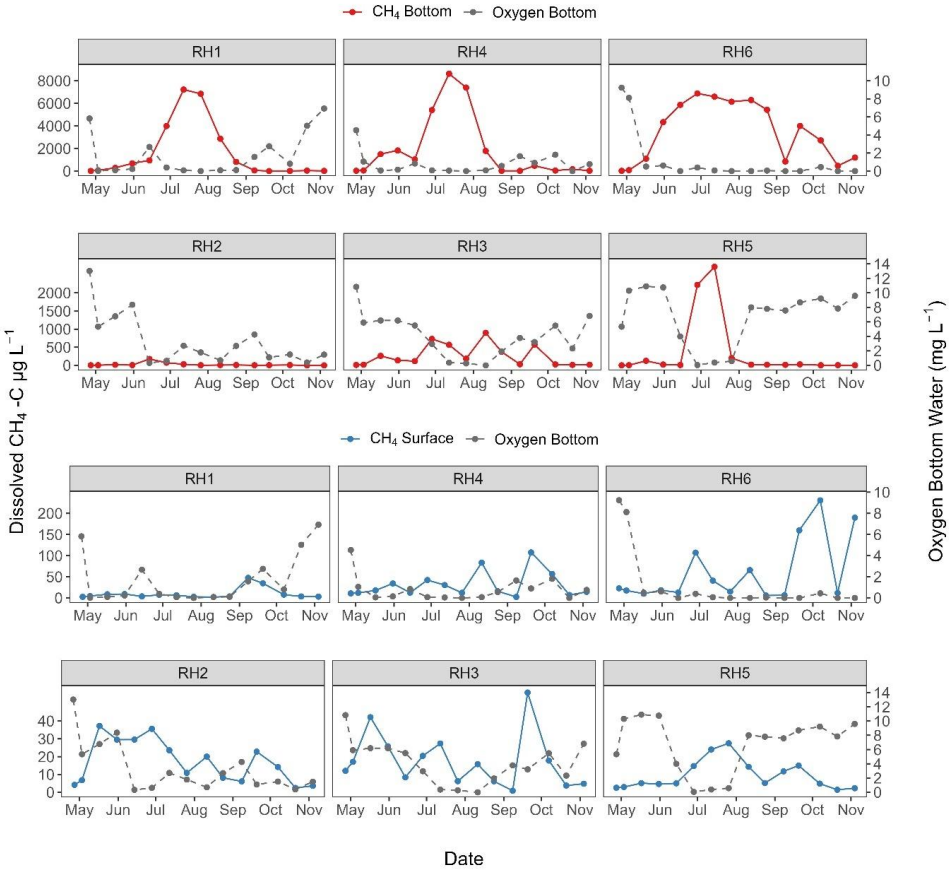

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Fig. 3 Boxplots showing CH_4 – C µg L^{-1} concentrations in the bottom (red line) and surface (blue line) waters for each pond (RH1-6) from 27 April to 4 November 2021 the left y-axis and bottom-water dissolved oxygen concentrations (mg L^{-1}, dashed lines) on the right y-axis. Note the different scales used on the y-axes.

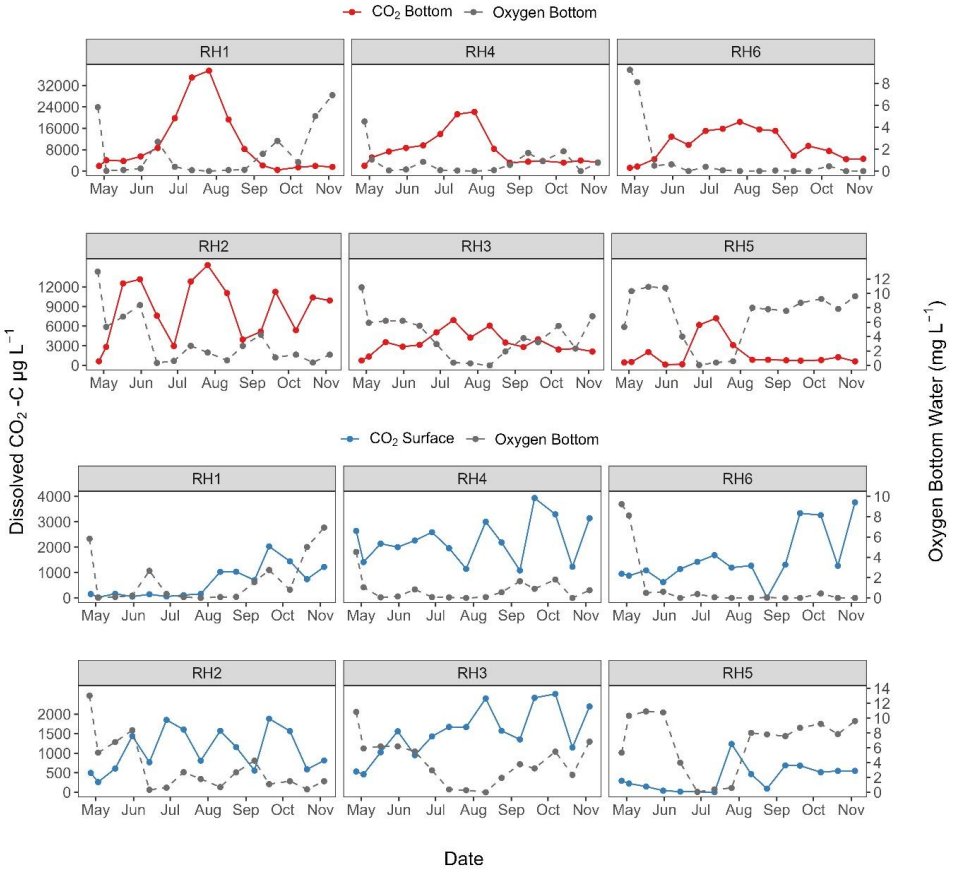

Fig. 4 Boxplots showing CO_2 – C µg L^{-1} concentrations in bottom (red line) and surface (blue line) waters in each pond (RH1-6) from 27 April to 4 November 2021 on the left y-axis and bottom-water dissolved oxygen concentrations (mg L^{-1}, dashed lines) on the right y-axis. Note the different scales used on the y-axes.

Dissolved gas concentrations varied widely across ponds and sampling dates (surface water: 24.3 [0.89-230.7] CH_4 – C µg L^{-1}, 1230 [3.1-3936] CO_2 – C µg L^{-1} bottom water: 1266 [1.2-8543] CH_4 - C µg L^{-1}, 6816 [125-37520] CO_2 – C µg L^{-1}). Despite this variability, all ponds exhibited consistent patterns with higher concentrations in bottom than in surface waters (Fig. 5). Bottom-water concentrations further increased under hypoxic conditions,

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reaching on average 3053 [1.56–8630] CH_4 – C µg L^{-1} compared with 189 [1.21–4339] CH_4 – C µg L^{-1} under oxic conditions ($p < 0.001$, df = 81, Fig. 5a). A similar trend was observed for CO_2, with hypoxic bottom waters averaging 11561 [3767-37587] CO_2 – C µg L^{-1} versus 4033 [125–15316] CO_2 – C µg L^{-1} in oxic bottom waters ($p < 0.001$, df = 81, Fig. 5c).

In contrast, surface-water concentrations of both CH_4 (38.8 [2.04-230] vs. 17.4 [1.43-108] CH_4 – C µg L^{-1}; p = 0.57, Fig. 5b) and CO_2 (1436 [24.5-3765] vs. 1235 [42.3–3936] CO_2 – C µg L^{-1}; $p = 0.18$, Fig. 5d) did not differ significantly between hypoxic and oxic periods.

Bottom-water hypoxia strongly influenced the balance between CO_2 and CH_4. The bottom water CO_2:CH_4 ratio (C mass) was significantly higher under oxic conditions (349 [2.97-4953]) than during hypoxic periods (203 [1.67-6640]; $p < 0.001$, df = 81, Fig. 5e). In contrast, surface waters showed no significant difference in CO_2:CH_4 ratios between the oxic (145 [9.08-458]) and hypoxic (95.9 [5.37-504]) states ($p = 0.10$, df = 81, Fig. 5f).

Dissolved gas concentrations in both surface and bottom waters showed a negative relationship with hypolimnetic oxygen concentrations. This relationship was particularly strong and statistically significant for bottom-water CH_4 and CO_2 ($p < 0.001$, r = -0.73, df = 86 and $p < 0.001$, r = -0.68, df = 86, respectively, for CH_4 and CO_2, Fig. S8b, d). In contrast, surface-water concentrations did not exhibit a similar tight coupling with hypolimnetic oxygen. Surface CH_4 showed a significant, but weak negative relationship with hypolimnetic oxygen ($p = 0.02$, r = -0.26, df = 81), but no significant relationship occurred for surface CO_2 ($p = 0.12$, r = -0.17, df = 81, Fig. S8a, c).

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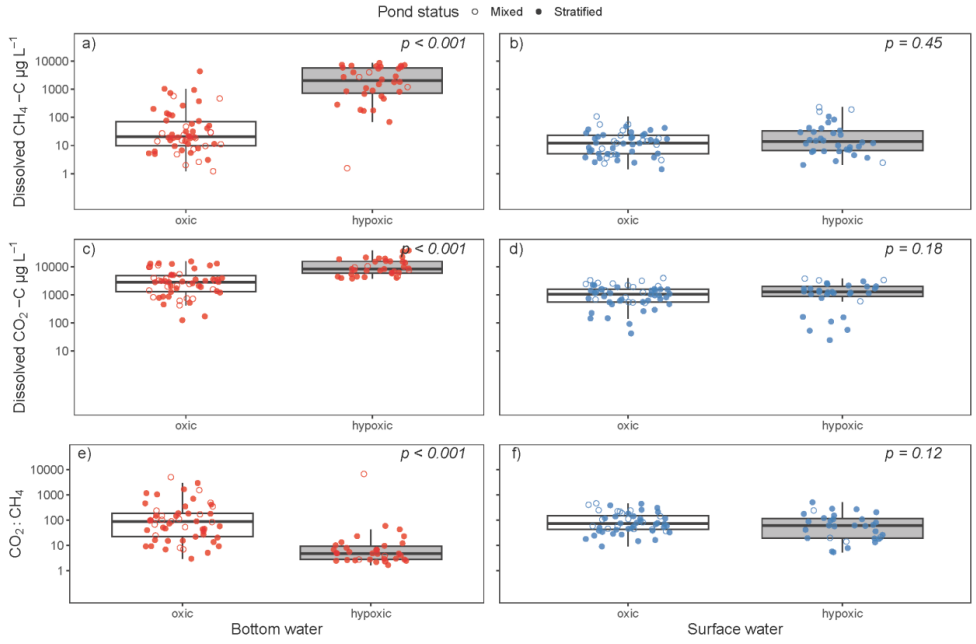

Fig. 5 Boxplots showing bottom and surface water concentrations of dissolved CH_4 (a, b), dissolved CO_2 (c, d) and the CO_2:CH_4 ratio (e, f) in all the ponds. The data were grouped according to bottom-water oxygen status with hypoxic conditions defined as ≤ 0.5 mg L^{-1} and oxic conditions as > 0.5 mg L^{-1}. The boxplots indicate the median and interquartile range (IQR), with whiskers representing minimum and maximum values, excluding outliers (calculated as box limits ±1.5 x IQR). Filled circles represent periods of thermal stratification (> 1°C difference at 1 m depth), while unfilled circles indicate mixed periods (< 1°C difference at 1 m depth). The *p*-values are the results derived from the linear mixed effect model between the response variable (left y axis) and the category of the presence or absence of hypoxia in the bottom water.

3.3 Seasonal patterns of ebullitive fluxes

Methane ebullition was highly variable among ponds and in time (Fig. S9). Ebullition was significantly higher under hypoxic conditions (6.73 [0.31 – 30.8] CH_4 (mg C m^{-2} d^{-1}) compared with oxic conditions (1.97 [0.22 – 13.5] CH_4 (mg C m^{-2} d^{-1}), $p = 0.004$, df = 77, Fig. 6). Ponds with long hypoxic periods (RH1, RH4, RH6) had the highest ebullition. RH2 and RH5, which were hypoxic less often, had lower fluxes overall but showed sharp increases when DO fell to lower levels (Fig. S9). RH3 was an exception as elevated ebullition preceded bottom-water hypoxia, and emissions decreased once hypoxia was established (Fig. S9).

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Both oxygen and temperature levels in the bottom waters significantly influenced ebullition (Fig. S10). Oxygen was negatively correlated with bubble flux ($p < 0.001$, r = –0.50, df = 82), whereas temperature showed a positive correlation ($p < 0.001$, r = 0.58, df = 82). We found no relationship between chlorophyll *a* and CH_4 bubble emissions for any of the ponds (Fig. S11).

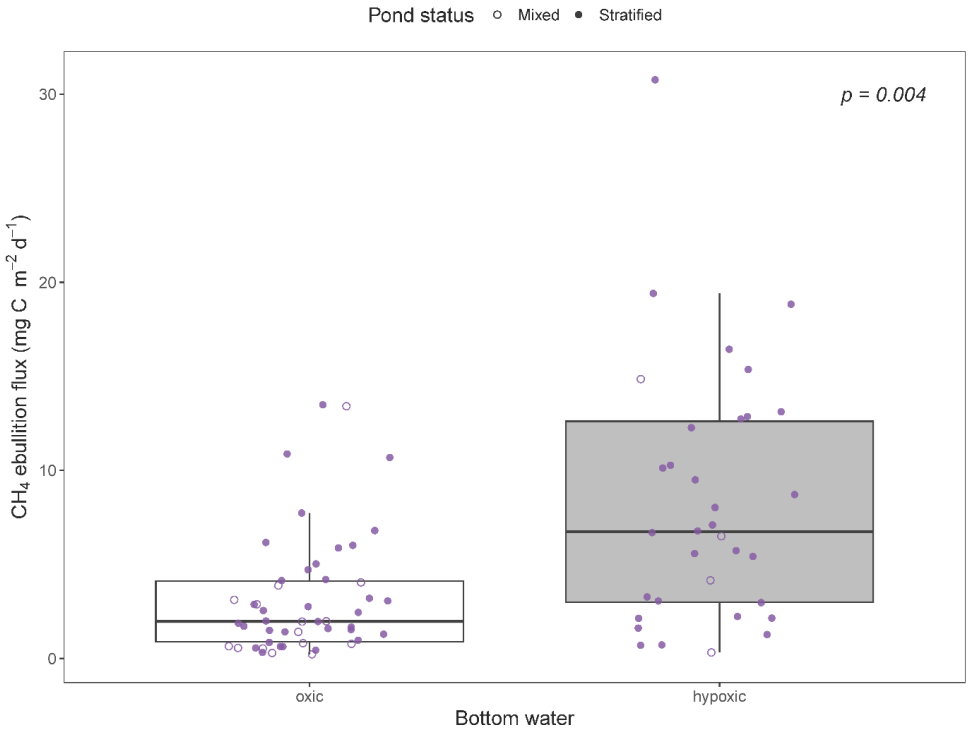

Fig. 6 Boxplots of CH_4 ebullition (mg C m^{-2} d^{-1}) grouped by bottom-water oxygen status: hypoxic (≤ 0.5 mg L^{-1}) or oxic (> 0.5 mg L^{-1}). The boxplots indicate the median and interquartile range (IQR), and whiskers mark minimum and maximum values excluding outliers (calculated as box limits ±1.5 x IQR). Circles represent the mean of the fluxes measured by the chambers deployed in each pond on each sampling occasion. Filled circles display the fluxes at thermal stratification (≥ 1°C difference at 1 m depth), and non-filled circles represent values from mixed periods (< 1°C difference at 1 m depth). The *p*-values are the results derived from the linear mixed effect model of the relationships between the response variable (left y-axis) and bottom-water oxygen concentrations (hypoxic-oxic).

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3.4 Prolonged periods of hypoxia and GHG emissions

Total CH_4 and CO_2 emissions were calculated as the sum of ebullitive and diffusive fluxes. Mean emissions were 946 [432–2075] mg CO_2-eq m^{-2} d^{-1} for CH_4 and 2924 [464–5723] mg m^{-2} d^{-1} for CO_2 (Fig. 7). Across the six ponds, CH_4 emissions were dominated by diffusive fluxes, which accounted on average for 74 % of the total, whereas ebullition contributed 26 %. Methane emissions were highest in pond RH6, while CO_2 emissions peaked in RH4. When expressed in CO_2 equivalents, GHG emissions were predominantly released as CO_2 (approximately 75 %), with CH_4 contributing a smaller fraction (approximately 25 %; Fig. 7). Ebullitive fluxes were moderately correlated with the number of hypoxic measurements ($p = 0.008$, $r = 0.82$, $df = 4$), whereas diffusive fluxes showed no significant relationship with the number of hypoxic measurements (CH_4: $p = 0.12$, $r = 0.50$, $df = 4$; CO_2: $p = 0.66$, $r = 0.05$, $df = 4$).

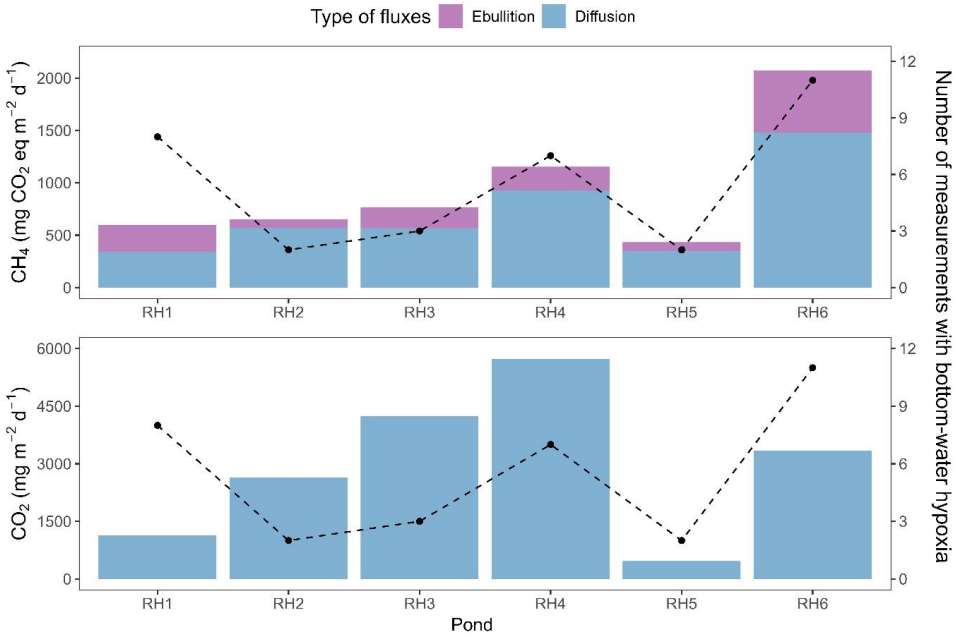

Fig. 7 Left y-axis displays CO_2 (lower panel) and CH_4 (upper panel) fluxes as CO_2 equivalents for each pond (noted RH1-6) per day (mg CO_2-eq m^{-2} d^{-1}). Total fluxes are the sum of diffusion (blue) and ebullition (purple) over the growing season. Right y-axis shows the number of bottom-water hypoxia measurements as a black dashed line.

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4. Discussion

4.1 Stratification, hypoxia and GHG dynamics and emissions

In agreement with recent work, all our ponds experienced periods of stratification, with varying frequency and intensity (Andersen et al., 2017; Holgerson et al., 2022; Søndergaard et al., 2023b). All ponds experienced bottom-water hypoxia which occurred almost exclusively during periods of thermal stratification, whereas oxygenated conditions were present under both stratified and mixed states (Fig. 2). Stratification did not always result in hypoxia, but hypoxia did not occur in its absence, which is consistent with studies highlighting the role of thermal stratification in regulating oxygen concentrations in the hypolimnion (Bartosiewicz et al., 2015; Holgerson et al., 2022; Søndergaard, et al., 2023a, b; Rabaey et al., 2026). Temperature and oxygen concentrations are among the factors shaping GHG emissions (Bartosiewicz et al., 2016; Schulz et al., 2006; Yvon-Durocher et al., 2010, 2014) and the fact that key microbial processes such as methanogenesis and respiration scale with temperature (Yvon-Durocher et al., 2014) has led to the notion that ecosystem scale emissions may respond in a similar way. There is, however, evidence that other factors, such as chlorophyll *a*, nutrients, and DOM can override the effects of temperature in shaping both CO_2 and CH_4 emissions (Davidson et al., 2015; Grasset et al., 2020; Zhou et al., 2019). Our data, with ponds experiencing different temperatures and non-correlated range of oxygen conditions over the course of the growing season, provides a novel opportunity to examine the relative importance of temperature versus oxygen concentrations in shaping GHG dynamics. We found that at similar hypolimnetic temperatures, CH_4 and CO_2 concentrations in the bottom waters were consistently higher during hypoxia, whereas temperature had a much weaker association with elevated GHG levels (Fig. S6). This demonstrates a stronger and more consistent relationship between oxygen availability and GHG accumulation (Fig. S8). Notably, both bottom-water dissolved gas concentrations and CH_4 ebullition rates increased under hypoxic conditions (Figs. 5, 6), supporting the hypothesis that in ponds oxygen depletion is the primary driver of GHG production and therefore accumulation in bottom waters. These results align with previous findings that link reduced oxygen availability to increased microbial activity and GHG emissions in shallow systems (Holgerson et al., 2022; Søndergaard et al., 2023b; Zhang et al., 2025). In contrast, the results suggest that in these ponds there was no real “shielding effect" as described by Bartosiewicz et al. (2019), where a cooler hypolimnion in stratified lakes suppressed methanogenesis by limiting the activity of temperature-dependent archaea. This effect may be more relevant in deeper, more thermally stable lakes, whereas ponds that tend to stratify and mix multiple times over a summer can behave differently (Richardson et al., 2026; Søndergaard et al., 2023b). Our results suggest that in shallow ponds, thermal stratification tends to enhance CH_4 production and emissions by facilitating bottom-water hypoxia,

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with frequent mixing also increasing temperatures at the sediment surface. An exception to this general pattern can shed light on the potential role of submerged plants which have been shown to have both positive and negative effects on emission (Kosten and Bodmer, 2024; Struik et al., 2022). In one pond RH6, hypoxia occurred on two occasions despite the absence of detectable thermal stratification. Here the dense beds of submerged macrophyte reduced water-column mixing and altered oxygen dynamics near the sediment, thereby promoting oxygen depletion and influencing GHG production as high densities of submerged plants have been shown to do(Theus et al., 2023).

In addition, there was a warm period in late October which resulted in stratification and hypoxia events in RH2, RH4 and RH6 which further illustrate the tight coupling between stratification dynamics and oxygen availability. These ponds had returned to oxic conditions following autumn mixing, yet a short warm-weather period was sufficient to re-establish stratification and trigger renewed bottom-water hypoxia. During these periods of autumn hypoxia, any effects on GHG concentrations in bottom waters or emissions were not recorded. This could be due to the very short hypoxic events or to the fact that the short-term increase in emissions was missed by our sampling frequency. Our results show that stratification in ponds is not confined to a single summer period but can occur episodically whenever meteorological conditions are suitable.

Beyond the effects of low oxygen in increasing GHG emissions, it also changed the relative balance between CO_2 and CH_4. The lower CO_2:CH_4 ratio (Fig. 5) demonstrates how periods of bottom-water hypoxia were characterized by a marked increase in CH_4 over CO_2 concentrations compared with the periods of oxic bottom waters (Deppenmeier et al., 1996). While CH_4 production may increase during hypoxic events, CH_4 oxidation is then limited. In contrast, a well oxygenated water column can promote the activity of methane oxidizing bacteria on plant surfaces (Esposito et al., 2023; Yoshida et al., 2014), on the sediment and in the water column (Bastviken et al., 2008; Nijman et al., 2021), contributing to reduce the emissions of CH_4. In RH2, where the bottom water only occasionally became hypoxic, the CO_2:CH_4 ratio was the highest across all the ponds. Moreover, RH2 had the highest TN:TP ratios, with relatively high concentrations of nitrate, which may inhibit methanogenesis (Scholten and Stams, 1995), limiting CH_4 production and promoting CH_4 oxidation (van Grinsven et al., 2020). In contrast to the pattern in the bottom waters, no differences were found in CH_4 or CO_2 concentrations in the surface water between hypoxic and oxic conditions (Fig. 5). Surface water concentrations, measured every two weeks, were highly variable but remained within a range as reported in previous studies. For example, median values observed in a study conducted in 40 Swedish ponds were 1350 µg C L^{-1} for CO_2 and 26 for CH_4 (Peacock et al., 2021),

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while urban ponds in Denmark showed median values of 1938 for CO_2 and 44 µg C L^{-1} CH_4 over a year (Audet et al., 2020). These values fall at the lower end of the range used in a global assessment of small natural and artificial ponds (< 0.1 ha) (Holgerson and Raymond, 2016).

In contrast with several studies (Davidson et al., 2015; Beaulieu et al., 2019; Colina et al., 2022;), differences in chlorophyll *a* concentration did not seem to drive the differences in GHG concentrations and emissions in most of the ponds. This could be due to the limited number of chlorophyll *a* measurements obtained during the sampling season. Another explanation could be that stratification, mixing dynamics, along with hypoxia might override the factors typically controlling GHG dynamics in larger water bodies, in part explaining the uncertainty associated with emissions from ponds and shallow lakes (Rosentreter et al., 2021).

4.2 Total emissions

Bottom-water hypoxia led to the accumulation of CH_4 and CO_2 in the hypolimnion, while surface waters remained isolated from the sediment, which is the main source of CH_4 and, to a lesser extent, CO_2. As a result, diffusive GHG fluxes from the pond surface likely reflect physical – either partial or full – mixing events between surface and bottom waters, in addition to ongoing biogeochemical activity in the surface layers (Bartosiewicz et al., 2015). These mixing events are often unpredictable and may lead to large differences in fluxes recorded only a few days apart under seemingly similar limnological conditions. Our findings confirm that GHG accumulation in hypoxic bottom waters is a common phenomenon. Partial and full mixing events likely transport these GHG-rich waters to the surface and this process can lead to large and unpredictable fluctuations in surface-water concentrations and emissions, a pattern typical of lakes and ponds (Davidson et al., 2024; Deemer et al., 2016; Peacock et al., 2021; Rabaey and Cotner, 2024; Ray and Holgerson, 2023; Rosentreter et al., 2021b).

The biweekly measurements of temperature and oxygen likely missed many short-lived mixing events, making it difficult to capture large emission events caused by full turnover of the water column. These fluxes could represent a substantial contribution to the total GHG budgets in ponds, but due to their high variability in time and space, we are unable to provide robust estimates. In addition, the unpredictability of when and where the bubbles rise makes it difficult to accurately estimate fluxes (Davidson et al., 2018). Nevertheless, our method has proven capable of providing reasonable emission estimates, despite some degree of uncertainty and underestimation of fluxes (Davidson et al., 2024). Only continuous,monitoring or high-frequency automatic flushing chambers

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(Bastviken et al., 2020; are capable of capturing these variations and give a better estimation of total GHG emissions.

When comparing CO_2 and CH_4 emissions expressed as CO_2 equivalents, CO_2 remained the dominant GHG emitted by the ponds, which aligns with previous findings (Peacock et al., 2021). Prolonged bottom-water hypoxia increased the concentrations of CH_4 and CO_2 stored in the bottom waters, which may subsequently be released to the atmosphere as turnover emissions (Rabaey and Cotner, 2024). This also enhanced CH_4 ebullition, turning the ponds into sources of GHGs rather than sinks.

5. Conclusions

Our study was based on biweekly measurements conducted during the growing season and on continuous measurements of ebullition. This represents a higher frequency and longer duration of GHG measurements in shallow waterbodies than in many previous studies, (Audet et al., 2020; Peacock et al., 2021; Sø et al., 2023). The increased measurement frequency revealed the key role of hypoxia induced by thermal stratification. It is also important to ensure a sufficiently long measurement period to capture the full range of stratification and mixing patterns in ponds and thereby provide a comprehensive estimation of GHG emissions from these dynamic systems. In conclusion, stratification impacted GHG emissions, but a key characteristic in increasing emissions was that stratification induced hypoxia. A warmer future with more frequent heatwaves due to climate change will alter the patterns and duration of thermal stratification (Aben et al., 2017; Audet et al., 2017; Christidis et al., 2015; Meehl and Tebaldi, 2004) , as well as mixing in ponds (Woolway and Merchant, 2019). For this reason, understanding the effect of thermal stratification – and how it interacts with other factors leading to hypoxia (e.g. organic content and nutrient enrichment) – is key to understanding pond ecosystem processes.

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Author Contribution Statement

TAD secured the funding for the research project. CE, TAD and JA conceptualised the study. CE, EEL and TAD collected the samples, and CE, EEL, JA and TAD analysed the data. CE wrote the paper and all authors commented, edited and approved the final draft.

Data Availability Statement

The data used in the study are derived from Zenodo: https://doi.org/10.5281/zenodo.21469976 During peer review, the dataset can be accessed by reviewers using the restricted preview link: https://zenodo.org/records/21469976?token=eyJhbGciOiJIUzUxMiJ9.eyJpZCI6IjU3NjI0MTRiLTg2Y2ItNGRiZS04ZDg1LTgzYTFjNjY5NjhjNyIsImRhdGEiOnt9LCJyYW5kb20iOiIyOTU1MjMzM2YyZDFiMzVlZTRiMTc2ZmUxNmU5NWIxOCJ9.VeUhlf5B5CM_RkxgBLUfd1oR0ijw4RanVc9a3v3UDhzPE7RpZyXklYr_yRLyXz-Z9PrO0TQhSealXAjQ0DICXg

The data can be also found on GitHub: https://github.com/thosdavidson/Stratification-induced-hypoxia-shapes-greenhouse-gas-emissions-in-ponds

- The particular datasets used are:
    - dissolved_gas_concs_2026.csv: Dissolved concentration and diffusive flux data and temperature and oxygen data.
    - CH4_ebullition.csv: Ebullitive flux data and temperature and dissolved oxygen data.
    - Meta_data.csv: Metadata describing the data and the units in the data files.

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