

## ESSAY

**A rationale for higher ratios of CH<sub>4</sub> to CO<sub>2</sub> production in warmer anoxic freshwater sediments and soils**Yizhu Zhu<sup>1\*</sup>, Kevin J Purdy<sup>2</sup>, Ana Martínez Rodríguez<sup>1</sup>, Mark Trimmer<sup>1\*</sup><sup>1</sup>School of Biological and Behavioural Sciences, Queen Mary University of London, London, UK; <sup>2</sup>School of Life Sciences, University of Warwick, Coventry, UK**Scientific Significance Statement**

Freshwaters emit significant amounts of CH<sub>4</sub> and CO<sub>2</sub> and, as CH<sub>4</sub> is the stronger greenhouse gas, understanding how carbon gets mineralized to either gas is important. In theory, under anoxia, methanogenesis coupled to fermentation should produce CH<sub>4</sub> and CO<sub>2</sub> in a 1 : 1 ratio. Here, we find that this 1 : 1 ratio is rare, with lower ratios of 0.1 : 1 being typical which confounds understanding CH<sub>4</sub> in freshwaters. First, using a simple mathematical model we rationalize low ratios as poor methanogenic substrate utilization, including loss to nonmethanogenic processes. Second, we find substrate utilization improves at higher temperatures, especially for hydrogen. This increases CH<sub>4</sub> to CO<sub>2</sub> production ratios exponentially which could drive higher CH<sub>4</sub> to CO<sub>2</sub> emission ratios. Hence, we rationalize how warmer freshwaters may emit more methane.

**Inland freshwaters and mineralization of organic carbon to CH<sub>4</sub> and CO<sub>2</sub>**

Inland freshwaters (e.g., wetlands, rivers, lakes, and rice paddies) are significant players in the carbon cycle (Cole et al. 2007), sequestering large amounts of fixed carbon (0.5 Pg yr<sup>-1</sup>) in their sediments and soils and mineralizing approximately twice as much (1.2 Pg yr<sup>-1</sup>) back to CO<sub>2</sub> and CH<sub>4</sub> (Regnier et al. 2013). In turn, inland freshwaters are responsible for nearly 50% of global CH<sub>4</sub> emissions. With CH<sub>4</sub> having approximately 80 times the global warming potential of CO<sub>2</sub> over 20 yr, there is a clear need to understand the processes and conditions that determine the relative production of CH<sub>4</sub> vs. CO<sub>2</sub> in aquatic sediments and water-logged soils (Balcombe et al. 2018; Rosentreter et al. 2021).

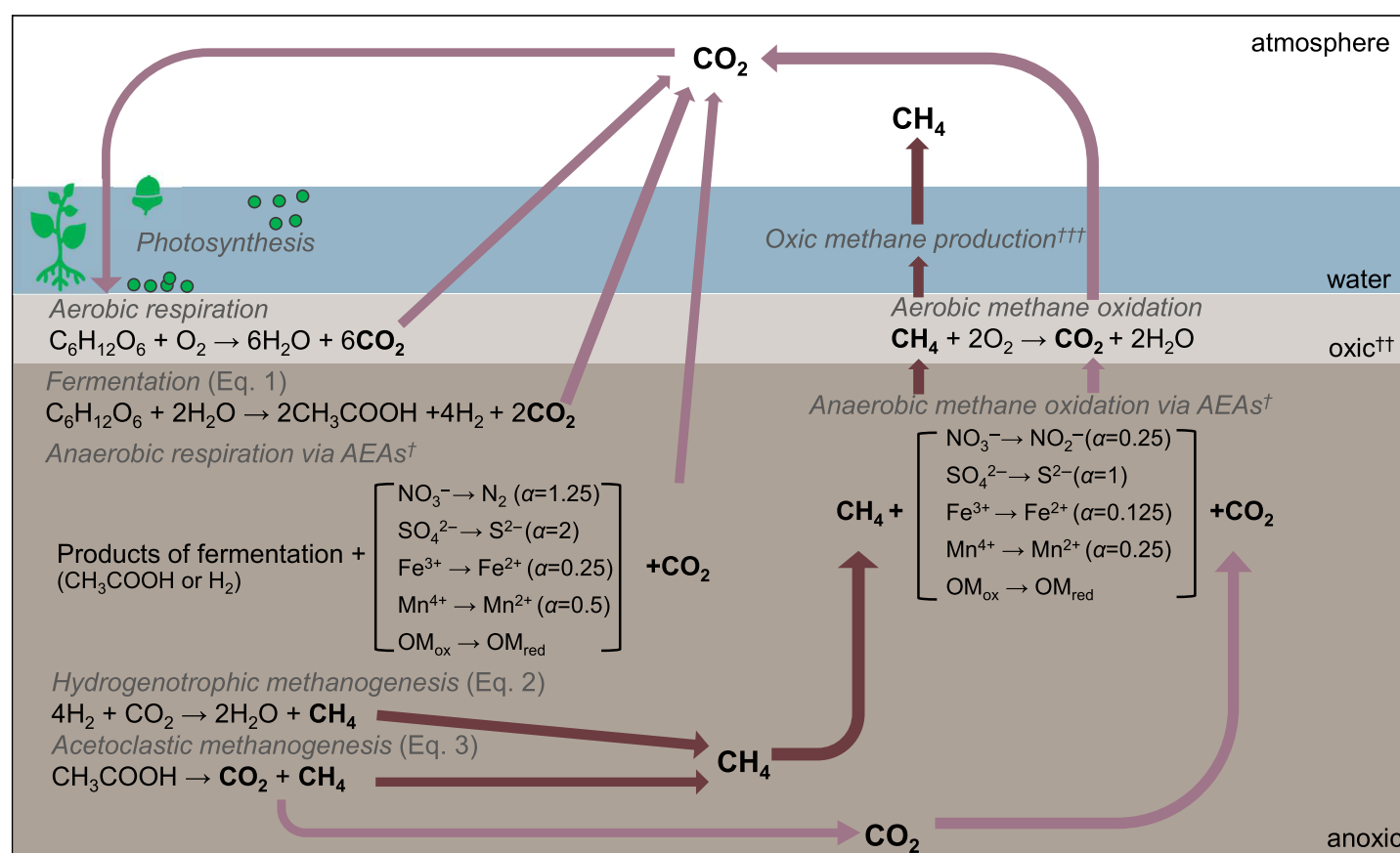
In the top layers of sediments and water-logged soils, and most overlying waters, organic carbon can be respired

aerobically to CO<sub>2</sub> or, deeper in, where O<sub>2</sub> is limiting, also anaerobically to CO<sub>2</sub> via any available alternative electron acceptors (e.g., inorganic ions such as Fe<sup>3+</sup>, NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> and/or oxidized organic matter; see Fig. 1). Eventually, when these aerobic and anaerobic electron acceptors are depleted the remineralization of any remaining organic carbon to CO<sub>2</sub> and/or CH<sub>4</sub> is completed via a combination of fermentation and methanogenesis. First, fermentation produces acetate, CO<sub>2</sub> and H<sub>2</sub> (Eq. 1) and then methanogenesis utilizes CO<sub>2</sub> and H<sub>2</sub> (hydrogenotrophic, Eq. 2) to make CH<sub>4</sub> and/or acetate (acetoclastic, Eq. 3) to produce CH<sub>4</sub> and CO<sub>2</sub> (Fig. 1) (Thauer et al. 2008). In addition to acetate and CO<sub>2</sub> and H<sub>2</sub>, a wide variety of simple organics, including methyl-compounds, can fuel methanogenesis, but in anoxic freshwaters for example, anoxic lake sediment or water-logged soils, methanogenesis is dominated by the acetoclastic and hydrogenotrophic pathways (Liu and Whitman 2008).

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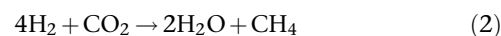
**Author Contribution Statement:** YZ, MT, and KJP conceived the original study and YZ developed the mathematical models. AMR collected the ratio data and YZ analyzed the data. MT and YZ wrote the paper and all the authors commented on and edited the manuscript.**Data Availability Statement:** Data and metadata are available at the Zenodo depository (<https://doi.org/10.5281/zenodo.7612326>).This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.



**Fig. 1.** Simplified illustration of carbon cycling in freshwaters (modified from Bridgman et al. 2013). †Instead of O<sub>2</sub>, anaerobic respiration or anaerobic methane oxidation (in [brackets]) can proceed via various inorganic, for example, Fe<sup>3+</sup>, NO<sub>3</sub><sup>-</sup> or organic (OM<sub>ox</sub>) alternative electron acceptors (AEAs) to produce CO<sub>2</sub>, where  $\alpha$  is the equivalence factor, that is, mole CO<sub>2</sub> produced per mole of each AEA reduced, respectively (Canfield et al. 1993; Smemo and Yavitt 2011). Note, we assume a nominal oxidation state of zero for organic carbon and there is no balanced stoichiometry or equivalence for OM<sub>ox</sub> (see Gao et al. 2019 for a fuller discussion). For simplicity, photosynthesis (green coloration) can occur in both the water column and surficial sediments but we have not included anaerobic phototrophic purple and green sulfur bacteria, for example. ††In stratified lakes and reservoirs where the hypolimnion can also be anoxic, aerobic respiration and aerobic methane oxidation would be restricted to shallower oxic water, rather than surficial sediments. †††Significant oxic water column methane production—“the methane paradox”—may also occur in stratified lakes, especially those with relatively small littoral zones, but we do not consider it further here (Günthel et al. 2019; Peeters and Hofmann 2021; Bartosiewicz et al. 2022).

Despite acetoclastic and hydrogenotrophic methanogenesis both producing CH<sub>4</sub>, they influence the ratio of CH<sub>4</sub> to CO<sub>2</sub> produced in anoxic sediments in distinct ways. The acetoclastic pathway (Eq. 3) makes both CO<sub>2</sub> and CH<sub>4</sub> while, in contrast, the hydrogenotrophic pathway (Eq. 2) consumes CO<sub>2</sub> to produce CH<sub>4</sub> (Fig. 1). Although, in general, methanogenesis is known to be highly sensitive to temperature (e.g., apparent activation energy  $E = 0.96$  eV vs. 0.65 eV for respiration; Yvon-Durocher et al. 2014), the hydrogenotrophic pathway has been shown to cease in cold lake sediments (Schulz 1996) and we have characterized it as being more sensitive to warming (apparent activation energy  $E = 1.4$  eV) than the acetoclastic pathway ( $E = 1.1$  eV; Zhu et al. 2020). At first, therefore, it may appear that by favoring hydrogenotrophic over acetoclastic methanogenesis, climate

warming could increase the ratio of CH<sub>4</sub> to CO<sub>2</sub> produced in anoxic sediments and water-logged soils.



### Conrad's idealized 1 : 1 CH<sub>4</sub> to CO<sub>2</sub> production ratio

In 1999, however, Conrad proposed that by coupling the complete fermentation of glucose to methanogenesis, CH<sub>4</sub> and CO<sub>2</sub> would be produced in a 1 : 1 ratio (Eq. 4, which is

the sum of Eqs. 1–3 (Conrad 1999). This expectation is based on the idea that one-third of the total CH<sub>4</sub> would be produced using H<sub>2</sub> derived from the original fermentation, with the remaining two-third coming from the acetate. Hence, in an idealized methanogenic system, both deplete in electron acceptors and where *all of the acetate and H<sub>2</sub>* produced by fermentation are subsequently used in methanogenesis, the ratio of CH<sub>4</sub> to CO<sub>2</sub> leaving the anoxic sediment and soil layers would be fixed at 1 : 1—regardless of temperature. Despite this broadly accepted 1 : 1 assumption even being used to model CH<sub>4</sub> emissions from global wetlands (Chadburn et al. 2020), published ratios from incubations of anoxic sediments and soils often appear far lower (*see* Amaral and Knowles 1994; Conrad et al. 2011; Kolton et al. 2019 as examples). Furthermore, while we acknowledge that ratios lower than 1 : 1 are recognized (Wilson et al. 2017; Gao et al. 2019), there has been no systematic characterization of how often it occurs or, more importantly, how the CH<sub>4</sub> to CO<sub>2</sub> ratio might increase toward 1 : 1 in response to warming.

### Realized CH<sub>4</sub> to CO<sub>2</sub> production ratios in the literature

To explore this, we compiled data from the Web of Science for CH<sub>4</sub> and CO<sub>2</sub> production in typical microcosm incubations of sediments or water-logged soils—examples of our search terms are included as metadata with the source data at <https://doi.org/10.5281/zenodo.7612326>. As we knew this search to be incomplete—for example, omitting the terms “oxidation” and “marine” missed Roden and Wetzel (1996) and Shelley et al. (2015) that both report CH<sub>4</sub> and CO<sub>2</sub> production, we supplemented our search using the compilations in Yvon-Durocher et al. (2014) and Wilson et al. (2017) or those related to our previous publications (Zhu et al. 2020, 2022). Note, we further excluded any publications where the authors had not stated explicitly that they had accounted for all of the CO<sub>2</sub> produced in their incubations, that is, CO<sub>2</sub> gas in the headspace plus that dissolved in the water phase (ΣDIC: CO<sub>2</sub>, HCO<sub>3</sub><sup>2-</sup>, CO<sub>3</sub><sup>-</sup>). Whereas only measuring the incubation headspace would typically capture >95% of any CH<sub>4</sub> produced, the same is not true for CO<sub>2</sub> which is approximately 100 times more soluble than CH<sub>4</sub>. Our search yielded 64 publications and a total of 512 measurements of CH<sub>4</sub> and CO<sub>2</sub> production including: lakes (*n* = 109), wetlands (*n* = 276), rice paddies (*n* = 64), a reservoir (*n* = 28), rivers (*n* = 4) and our own incubations of streambed sediments (*n* = 31, Zhu et al. 2022) for which we previously published just the CH<sub>4</sub> data. We extracted the data using WebPlotDigitizer (<https://automeris.io/WebPlotDigitizer/>) and calculated CH<sub>4</sub> to CO<sub>2</sub> production ratios. Our compilation (Fig. 2a) clearly shows that Conrad’s idealized 1:1 ratio is seldom realized in practice, with ratios >0.95:1 only appearing in 2% of the 512 incubations and 50% of all incubations producing CH<sub>4</sub> to CO<sub>2</sub> at a ratio of only 0.1:1.

### Rationalization of ratios deviating from Conrad’s idealized 1 : 1 ratio

As outlined above, we would expect any truly anoxic, methanogenic system to be depleted in alternative electron acceptors (e.g., porewater NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Fe<sup>3+</sup>, or Mn<sup>4+</sup>). To exclude the possibility, however, of such low CH<sub>4</sub> to CO<sub>2</sub> ratios being due the production of CO<sub>2</sub> from AEAs-induced CO<sub>2</sub> here we have estimated their contribution through anaerobic methane oxidation (AMO) or anaerobic respiration (*R*<sub>ana</sub>):

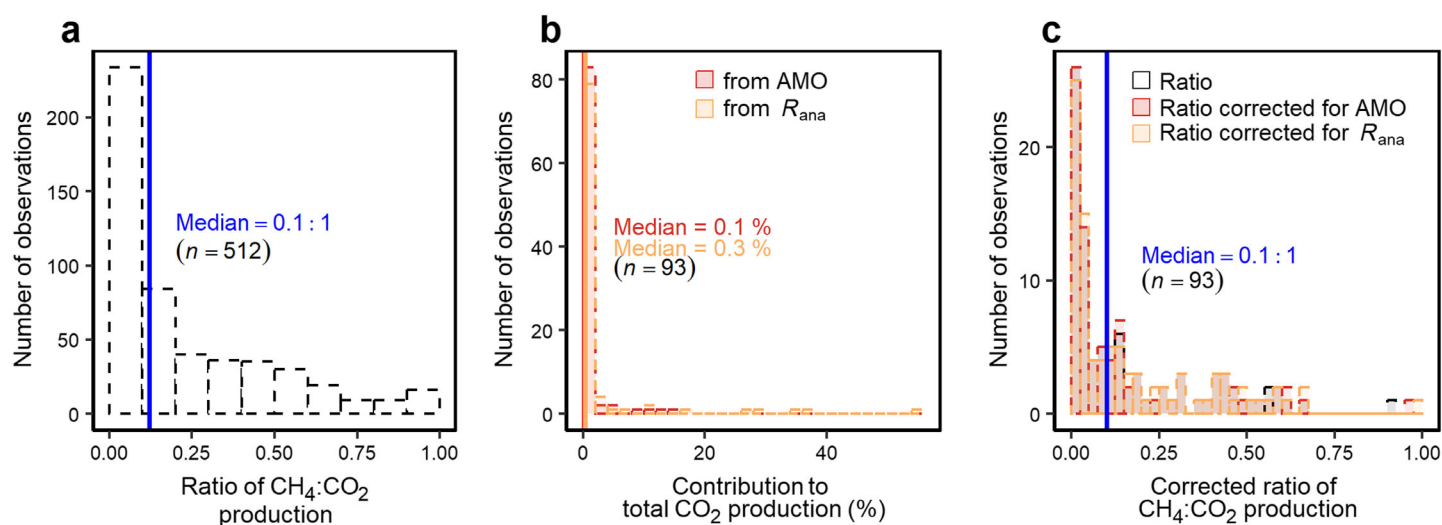
$$F_{\text{CO}_2\text{-AEA}} = \frac{C_{\text{AEA}} \times \alpha}{\rho} \quad (5)$$

$$P_{\text{CO}_2\text{-AEA}} = \frac{F_{\text{CO}_2\text{-AEA}}}{F_{\text{CO}_2\text{-tot}}} = \frac{F_{\text{CO}_2\text{-AEA}}}{R_{\text{CO}_2} \times D_{\text{incubation}}} \times 100\% \quad (6)$$

where *F*<sub>CO<sub>2</sub>-AEA</sub> is the AEA-induced production of CO<sub>2</sub> in an incubation in μmol g<sup>-1</sup>, *C*<sub>AEA</sub> is the concentration of alternative electron acceptors (μmol L<sup>-1</sup>), *ρ* is the density of wet soil (1550 g L<sup>-1</sup>) (Faoziah et al. 2019), and *α* the equivalence factor for CO<sub>2</sub> produced per mole AEA reduced for either AMO or *R*<sub>ana</sub>, respectively (see Fig. 1). *P*<sub>CO<sub>2</sub>-AEA</sub> (%) is the fraction of AEA-induced CO<sub>2</sub> to total CO<sub>2</sub> production (*F*<sub>CO<sub>2</sub>-tot</sub>) and *F*<sub>CO<sub>2</sub>-tot</sub> the product of the rate of CO<sub>2</sub> production (*R*<sub>CO<sub>2</sub></sub>, μmol g<sup>-1</sup> d<sup>-1</sup>) and the duration of the incubation (*D*<sub>incubation</sub>, d).

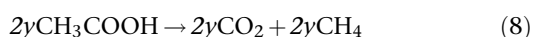
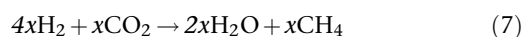
Of the 64 publications included here, 47 had very low CH<sub>4</sub> to CO<sub>2</sub> production ratios (<0.1 : 1) and, of those, 13 provided data for the concentration of any alternative electron acceptors (NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Fe<sup>3+</sup>, or Mn<sup>4+</sup>). Using these 13 datasets we estimated *P*<sub>CO<sub>2</sub>-AEA</sub> to be only 0.1% or 0.3% (median) through AMO and *R*<sub>ana</sub>, respectively, in lake sediment and peatland soil (*n* = 93, Fig. 2b). Note, varying the density of wet soil from 1250 to 1850 g L<sup>-1</sup> would alter *P*<sub>CO<sub>2</sub>-AEA</sub> for AMO, from 0.09% to 0.14%, and for *R*<sub>ana</sub>, from 0.25% to 0.37%, for the median concentration of the most abundant AEA, that is, 12 μmol SO<sub>4</sub><sup>2-</sup> L<sup>-1</sup>. Our minor contribution from AEA-induced CO<sub>2</sub> agrees with estimates of 0.1–11% through *R*<sub>ana</sub> in other peatland soils (Gao et al. 2019) and would unlikely explain the typically low CH<sub>4</sub> to CO<sub>2</sub> production ratio of 0.1:1 reported here (Fig. 2c).

Having discounted inorganic alternative electron acceptors, the other possible explanation for low CH<sub>4</sub> to CO<sub>2</sub> ratios is that a significant proportion of any potential methanogenic substrates (e.g., products of Eq. 1: acetate, CO<sub>2</sub> and H<sub>2</sub>) suffer a nonmethanogenic fate. This could be due to loss of substrates by diffusion away from the methanogenic zone (Schütz et al. 1988); hydrogenation of unsaturated organic carbon compounds (Wilson et al. 2017); and homoacetogenesis (Fey and Conrad 2000). A significant fraction (6–39%) of CH<sub>4</sub> precursors could also potentially be oxidized to CO<sub>2</sub> by alternative organic electron acceptors, for example, reduction of quinone to hydroquinone (Walpen et al. 2018;

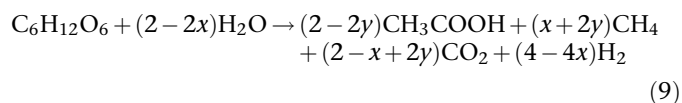


**Fig. 2.** Compilation of anoxic incubation measurements of CH<sub>4</sub> to CO<sub>2</sub> production ratios, contribution of anaerobic CH<sub>4</sub> oxidation and anaerobic respiration to total CO<sub>2</sub> production and ratios of CH<sub>4</sub> to CO<sub>2</sub> production after correction for anaerobic CH<sub>4</sub> oxidation or anaerobic respiration. **(a)** The median of all 512 production ratios at 0.1 : 1 is 10-fold lower than the idealized 1 : 1. **(b)** Potential CO<sub>2</sub> production from anaerobic CH<sub>4</sub> oxidation (AMO) or anaerobic respiration ( $R_{\text{ana}}$ ) contributes only minor fractions of total CO<sub>2</sub> production, that is, of 0.1% and 0.3%, respectively. **(c)** The median ratio for CH<sub>4</sub> to CO<sub>2</sub> production after correction for CO<sub>2</sub> production from AMO or  $R_{\text{ana}}$  is still 0.1 ( $p = 0.86$  or  $p = 0.84$  for paired Wilcoxon tests to original ratios).

Gao et al. 2019). Any of the processes described above could prevent some fraction of the methanogenic substrates in Eq. 1 from being used for methanogenesis in Eqs. 2 and 3 to drive down the ratio of CH<sub>4</sub> to CO<sub>2</sub>. We explore this further by modeling the fraction of available acetate or H<sub>2</sub> being consumed through either hydrogenotrophic (Eq. 2) or acetoclastic (Eq. 3) methanogenesis by revising Eqs. 2 and 3:



Here,  $x$  ( $0 \leq x \leq 1$ ) is the proportion of H<sub>2</sub> utilized by hydrogenotrophic methanogenesis and  $y$  ( $0 \leq y \leq 1$ ) is the proportion of acetate utilized by acetoclastic methanogenesis. Subsequently, the sum of Eqs. 1, 7, and 8 is:

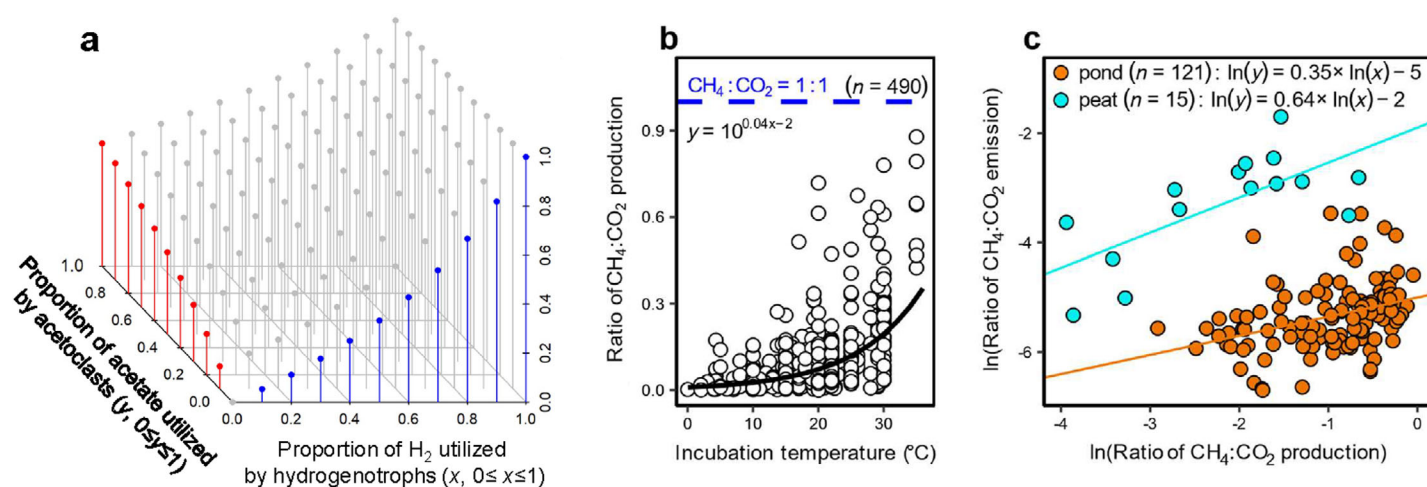


Equation 9 shows that the CH<sub>4</sub> to CO<sub>2</sub> production ratio is  $(x + 2y)/(2 - x + 2y)$  and, as proposed by Conrad, if all of the H<sub>2</sub> and acetate produced by fermentation is used to make CH<sub>4</sub> (i.e.,  $x = 1$  and  $y = 1$ ), the ratio of CH<sub>4</sub> to CO<sub>2</sub> will be 1 : 1 (Conrad 1999). Conversely, if either substrate escapes or gets used in a nonmethanogenic pathway (i.e.,  $0 \leq x < 1$  or  $0 \leq y < 1$ ), the resulting ratio will always be less than 1 : 1 (Fig. 3a).

Eq. 9 provides a tool to help us rationalize the typically low CH<sub>4</sub> to CO<sub>2</sub> production ratios by quantifying the effect of variable utilization of acetate and H<sub>2</sub> by methanogens. Acetate and H<sub>2</sub> are both direct precursors to CH<sub>4</sub> and at the median CH<sub>4</sub> to CO<sub>2</sub> ratios of 0.1 : 1 reported here (Fig. 2a), it appears that a maximum of 11% of any acetate (no hydrogenotrophy and  $x = 0$ , Fig. 3a) or 18% of any available H<sub>2</sub> (no acetoclasty and  $y = 0$ , Fig. 3a) was used to produce CH<sub>4</sub>. However, whereas we can use the literature values to account for any potential CO<sub>2</sub> production through anaerobic respiration of inorganic  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-/\text{NO}_2^-$ ,  $\text{Fe}^{3+}$  (as detailed above), we cannot account for anaerobic respiration with organic compounds, hydrogenation, homoacetogenesis, growth, or loss by diffusion (Schütz et al. 1988; Greening et al. 2016; Wilson et al. 2017; Gao et al. 2019). No matter what the non-methanogenic fate, it appears that only minor proportions of either acetate or H<sub>2</sub> are used to produce CH<sub>4</sub> constraining the ratios below Conrad's idealized prediction of 1 : 1.

### Driving up CH<sub>4</sub> to CO<sub>2</sub> production ratios toward Conrad's idealized 1 : 1 ratio

As non-methanogenic utilization of acetate and H<sub>2</sub>, be it anaerobic respiration, hydrogenation etc., would only lower the CH<sub>4</sub> to CO<sub>2</sub> production ratio, we continue to use Eq. 9 to explore how CH<sub>4</sub> to CO<sub>2</sub> production ratios would increase with better utilization of acetate or H<sub>2</sub>. Starting from the typical nonidealized CH<sub>4</sub> to CO<sub>2</sub> production ratio of 0.1 : 1 with poor substrate utilization (11% for acetate and 18% for H<sub>2</sub>), if



**Fig. 3a.** A 3D plot depicting CH<sub>4</sub> to CO<sub>2</sub> production ratios (i.e.,  $(x + 2y)/(2 - x + 2y)$  in Eq. 9) as a function of the proportion of either H<sub>2</sub> utilized by hydrogenotrophs ( $x$ ) or the proportion of acetate utilized by acetoclasts ( $y$ ). The colored dots demonstrate that ratios of CH<sub>4</sub> to CO<sub>2</sub> increase exponentially with increasing utilization of H<sub>2</sub> (blue) or subexponentially with increasing utilization of acetate (red). **(b)** Ratios of CH<sub>4</sub> to CO<sub>2</sub>, measured in each incubation in Fig. 2a, increase exponentially with increasing incubation temperature ( $p < 0.001$ , log-likelihood ratio test). **(c)** Ratios of CH<sub>4</sub> to CO<sub>2</sub> emitted to atmosphere increase as ratios of CH<sub>4</sub> to CO<sub>2</sub> produced in sediments increase (both  $p < 0.05$ , log-likelihood ratio test and  $F$ -statistic for pond and peatland data, respectively). Note, in **(b)**, we excluded 22 ratios from the total of 512 where the incubation temperatures were either not reported or where methanogenesis was clearly declining in incubations done at  $>40^{\circ}\text{C}$ , that is, in excess of the optimal temperature generally reported for freshwaters (Schulz et al. 1997). **(c)** The emission ratios are either those measured with dynamic-chambers on our experimental ponds ( $n = 121$ ; Zhu et al. 2020) or static-chambers on peatlands ( $n = 15$ ) (Roden and Wetzel 1996; Blodau and Moore 2003; Rinnan et al. 2003; Hopple et al. 2020). **(b,c)** The ratios on the  $y$ -axis are presented as partial residuals from a mixed-effect model including either the individual study or measurement month as random effects and holding the  $x$ -axis constant (Bates et al. 2015; Breheny and Burchett 2017). All statistical analyses were carried out using R software (version 4.0.4) (R Core Team 2021).

we increase the utilization of acetate (i.e., increasing  $y$  from 0.11 to 1), the ratio increases subexponentially to a maximum of 0.5 : 1 (Fig. 3a), that is, Conrad's idealized ratio can never be realized through acetoclastic methanogenesis alone. In contrast, if we increase the utilization of H<sub>2</sub> (i.e., increasing  $x$  from 0.18 to 1), then the ratio increases exponentially to the maximum idealized ratio of 1 : 1 (Fig. 3a), that is, by using H<sub>2</sub> to reduce CO<sub>2</sub> Conrad's idealized ratio of 1 : 1 can be realized.

Recently we reported that long-term (11 yr) experimental warming of freshwater ponds disproportionately enhanced methane emissions along with an increase in CH<sub>4</sub> to CO<sub>2</sub> production ratios (Zhu et al. 2020). We argued how such an increase in CH<sub>4</sub> to CO<sub>2</sub> production ratio could be rationalized by a greater proportion of the available H<sub>2</sub> being used to make CH<sub>4</sub> and here we have extended that analysis to include acetate (Eqs. 3, 8, and 9). Although the CH<sub>4</sub> to CO<sub>2</sub> production ratio would increase with greater utilization of either H<sub>2</sub> or acetate, the fact that the ratio only increases exponentially with greater H<sub>2</sub> utilization provides a clue to answering how CH<sub>4</sub> to CO<sub>2</sub> production ratios would increase with warming. We would argue that given the higher sensitivity of the hydrogenotrophic over the acetoclastic pathway to temperature (1.4 vs. 1.1 eV; Zhu et al. 2020), the hydrogenotrophic methanogens would respond more rapidly to warming. This is corroborated by the dominance of hydrogenotrophic over

acetoclastic methanogenesis observed in warmer lake sediments and peat soils (Conrad et al. 2014; Kolton et al. 2019) and a general selection for hydrogenotrophic genera in naturally warmer environments (Wen et al. 2017). In turn, warming increases the fraction of H<sub>2</sub> used to reduce CO<sub>2</sub> to CH<sub>4</sub> and, as implied in Fig. 3a, increases the CH<sub>4</sub> to CO<sub>2</sub> production ratio exponentially toward the idealized 1 : 1 ratio. Indeed, if we plot the data in Fig. 2a as a function of each individual incubation temperature ( $n = 490$  for incubations at  $<40^{\circ}\text{C}$ , that is, the generally accepted optimal methanogenic temperature in freshwaters; Schulz et al. 1997), the CH<sub>4</sub> to CO<sub>2</sub> production ratio increases exponentially—regardless of each initial ratio—toward the idealized ratio of 1 : 1, as hypothesized (Fig. 3b,  $p < 0.001$ , log-likelihood ratio test).

### Coupling production ratios to emission ratios

Any such increase in CH<sub>4</sub> to CO<sub>2</sub> production ratios in warmer sediments or water-logged soils would only be of relevance to Earth's climate if they ultimately drive higher emission ratios to the atmosphere (Fig. 1). Although we compiled 512 production ratios and there are several thousand emission ratios available through FLUXNET (Pastorello et al. 2017; Delwiche et al. 2021), for example, there are—to the best of our knowledge—few paired measurements with which to establish a relationship between the two (Roden and

Wetzel 1996; Blodau and Moore 2003; Rinnan et al. 2003; Zhu et al. 2020; Hopple et al. 2020). We used our frequent measurements of CH<sub>4</sub> to CO<sub>2</sub> emission ratios from our experimental ponds (three times per day) and parallel monthly laboratory incubations for sediment production ratios (Zhu et al. 2020) to look for any correlation between the two. Note, to present any such correlation at in situ temperature, the laboratory sediment production ratios were standardized to the same emission temperature using known temperature sensitivities ( $E = 0.7$  and  $E = 0.58$  eV for apparent activation energies of CH<sub>4</sub> and CO<sub>2</sub> production, respectively) (Zhu et al. 2020). We also used a further 15 paired measurements available for peatland soils (Roden and Wetzel 1996; Blodau and Moore 2003; Rinnan et al. 2003; Hopple et al. 2020). The positive relationship between production and emission ratios is very clear in Hopple et al. (2020) for peatland soils, as is the increase in CH<sub>4</sub> to CO<sub>2</sub> emission ratios at higher temperatures. Overall, CH<sub>4</sub> to CO<sub>2</sub> emission ratios increase at higher production ratios in both our ponds and peatland soils (Fig. 3c, both  $p < 0.05$ , log-likelihood ratio test and  $F$ -statistic for pond and peatland measurements, respectively). Furthermore, CH<sub>4</sub> to CO<sub>2</sub> emission ratios are lower than production ratios which is both expected and relatively easy to explain. For example, CH<sub>4</sub> can both be consumed before emission due to aerobic and/or anaerobic oxidation of CH<sub>4</sub> to CO<sub>2</sub> and further diluted by additional production of CO<sub>2</sub> (King et al. 1990; Nedwell and Watson 1995) outside of the methanogenic zone (Fig. 1). We acknowledge that the non-linear correlation between emission and production ratios (i.e., the ln-ln plot in Fig. 3c) is complex, but a full discussion lies beyond the scope of our essay. In brief, possible combinations of respiration, anaerobic or aerobic methane oxidation, dissolved oxygen in sediments (Zhu et al. 2020) and water column (Jane et al. 2021) and water table depth (in peatland soils) (Evans et al. 2021) may explain the increase in attenuation of emission ratios at higher production ratios. Nevertheless, we would argue that emission ratios will likely increase as production ratios increase under climate warming and for that connection between production and emission ratios to be far stronger in soils than aquatic sediments as indicated by the 20-fold offset between the two lines in Fig. 3c.

### Conclusions for a warmer Earth

Conrad's idealized 1 : 1 CH<sub>4</sub> to CO<sub>2</sub> production ratio is seldom realized in anoxic sediments and far lower ratios are typical (0.1 : 1). Here, using a simple mathematical model we rationalize the prevalence of these low ratios as only small fractions of any available acetate and H<sub>2</sub> being used for methanogenesis, with the remainder suffering a multitude of nonmethanogenic fates. Furthermore, despite the idealized ratio seldom being realized, CH<sub>4</sub> to CO<sub>2</sub> production ratios increase exponentially at higher temperatures through greater utilization of H<sub>2</sub> for CH<sub>4</sub> production. Higher CH<sub>4</sub> to CO<sub>2</sub>

production ratios ultimately appear to drive higher CH<sub>4</sub> to CO<sub>2</sub> emission ratios. As Earth continues to warm, therefore, we expect this mechanism to contribute to higher ratios of CH<sub>4</sub> to CO<sub>2</sub> emitted to the atmosphere from freshwaters with the potential to further accelerate climate warming.

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### Conflict of interest

None declared.

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