Coupled CH₄ production and oxidation support CO₂ supersaturation in a tropical flood pulse lake (Tonle Sap Lake, Cambodia)

Benjamin Lloyd Miller a,b,1, Gordon William Holtgrieve a, Mauricio Eduardo Arias c, Sophorn Uy d,e, and Phen Chheng f

aSchool of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98105; bSchool of Environmental and Forest Sciences, University of Washington, Seattle, WA 98195; cDepartment of Civil and Environmental Engineering, University of South Florida, Tampa, FL 33620; dInland Fisheries Research and Development Institute, Phnom Penh, Cambodia; eFaculty of Fisheries, Royal University of Agriculture, Phnom Penh, Cambodia; fFisheries Administration, Phnom Penh, Cambodia

Edited by Peter Raymond, Yale University, New Haven, CT; received April 22, 2021; accepted December 8, 2021 by Editorial Board Member Mary E. Power

Carbon dioxide (CO₂) supersaturation in lakes and rivers worldwide is commonly attributed to terrestrial-aquatic transfers of organic and inorganic carbon (C) and subsequent, in situ aerobic respiration. Methane (CH₄) production and oxidation also contribute CO₂ to freshwaters, yet this remains largely unquantified. Flood pulse lakes and rivers in the tropics are hypothesized to receive large inputs of dissolved CO₂ and CH₄ from floodplains characterized by hypoxia and reducing conditions. We measured stable C isotopes of CO₂ and CH₄, aerobic respiration, and CH₄ production and oxidation during two flood stages in Tonle Sap Lake (Cambodia) to determine whether dissolved CO₂ in this tropical flood pulse ecosystem has a methanogenic origin. Mean CO₂ supersaturation of 11,000 ± 9,000 μatm could not be explained by aerobic respiration alone. ¹³C depletion of dissolved CO₂ relative to other sources of organic and inorganic C, together with corresponding ¹³C enrichment of CH₄, suggested extensive CH₄ oxidation. A stable isotope-mixing model shows that the oxidation of ¹³C depleted CH₄ to CO₂ contributes between 47 and 67% of dissolved CO₂ in Tonle Sap Lake. ¹³C depletion of dissolved CO₂ was correlated to independently measured rates of CH₄ production and oxidation within the water column and underlying lake sediments. However, mass balance indicates that most of this CH₄ production and oxidation occurs elsewhere, within inundated soils and other floodplain habitats. Seasonal inundation of floodplains is a common feature of tropical freshwaters, where high reported CO₂ supersaturation and atmospheric emissions may be explained in part by coupled CH₄ production and oxidation.

Significance

Freshwaters inextricably link flows of carbon between the land, oceans, and atmosphere. Resulting carbon dioxide supersaturation relative to the atmosphere in most of the world’s lakes and rivers has long been assumed to come from aerobic respiration. Although carbon dioxide also comes from the oxidation of anaerobically produced methane, this has been largely ignored within freshwaters. Here, we use stable carbon isotopes of carbon dioxide and methane to show that a nontrivial proportion of the total dissolved carbon dioxide in a tropical flood pulse lake comes from methane oxidation. Seasonal pulses of flooding are common in the tropics, suggesting that coupled methane production and oxidation likely contribute more broadly to flows of carbon between the land, understudied tropical freshwaters, and atmosphere.

Author contributions: B.L.M., G.W.H., M.E.A., and P.C. designed research; B.L.M. and S.U. performed research; B.L.M., G.W.H., and M.E.A. contributed new analytic tools; B.L.M. analyzed data; and B.L.M. and G.W.H. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission. P.R. is a guest editor invited by the Editorial Board.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

1 To whom correspondence may be addressed. Email: blm8@uw.edu.

This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2107667119/-/DCSupplemental.

Published February 14, 2022.
Congo (22), Pantanal (23), and Amazon watersheds (4). CH₄ production and oxidation occur along such redox gradients (4, 16, 19, 23). CH₄ is produced by acetate fermentation (Eq. 1) and carbonate reduction (Eq. 2) within freshwaters (24, 25). CH₄ production coupled with aerobic oxidation results in CO₂ (Eq. 3 and ref. 25), yet no studies have quantified the relative contribution of coupled CH₄ production and oxidation to CO₂ supersaturation within tropical freshwaters. During this course reversal, the Tonle Sap River floods TSL. Monsoonal rains and Himalayan snowmelt increase discharge to a large fishery (21, 22, 27). Each May through October, floodplain environments of TSL during the high-water and falling-water stages of the flood pulse results in CH₄ that is depleted in ¹³C (24–26). By contrast, pH₂O was as low as 1/1 O₂:CO₂ ratio as one micro mole of dissolved O₂ is consumed for each µ mole of dissolved CH₄ produced. Instead, ratios of ~0.1/1 were observed during both the high-water and falling-water stages. During the high-water stage, the greatest CO₂ supersaturation occurred under the most hypoxic conditions (Fig. L4).

The relative contribution of coupled CH₄ production and oxidation to CO₂ supersaturation within floodwaters can be traced with stable C isotopes of CO₂ and CH₄. Methanogenesis results in CH₄ that is depleted in ¹³C (δ¹³C = −65 to −50‰ from acetate fermentation and −110 to −60‰ from carbonate reduction) compared to other potential sources of organic and inorganic C (δ¹³C = −37 to −7.7‰; see Materials and Methods) (24–26). The oxidation of this ¹³C-depleted CH₄ results in ¹³C-depleted CO₂ (24–26). At the same time, CH₄ oxidation enriches the ¹³C/¹²C ratio of residual CH₄ as bacteria and archaea preferentially oxidize ¹²C-CH₄ (25). This means that the ¹³C/¹²C of CO₂ and CH₄ can serve as powerful tools to determine the source of CO₂ supersaturation within freshwater.

Tonle Sap Lake (TSL) is Southeast Asia’s largest lake and an understudied flood pulse ecosystem that supports a regionally important fishery (21, 22, 27). Each May through October, monsoonal rains and Himalayan snowmelt increase discharge in the Mekong River and cause one of its tributaries, the Tonle Sap River, to reverse course from southeast to northwest (21). During this course reversal, the Tonle Sap River floods TSL. The TSL flood pulse increases lake volume from 1.6 to 60 km³ and inundates 12,000 km² of floodplain for 3 to 6 mo per year (21, 27). Holtgrieve et al. (22) have shown that aerobic respiration is consistently greater than primary production in TSL (i.e., net heterotrophy), with the expectation of constant CO₂ supersaturation. But, the partial pressures, C isotopic compositions, and ultimately the source of dissolved CO₂ in TSL remain unquantified.

To quantify CO₂ supersaturation and its origins in TSL, we measured the partial pressures of CO₂ and CH₄ and compared their C isotopic composition to other potential sources of organic and inorganic C. We carried out these measurements in distinct lake environments during the high-water and falling-water stages of the flood pulse, hypothesizing that CH₄ production and oxidation on the TSL floodplain would support CO₂ supersaturation during the high-water stage. We found that coupled CH₄ production and oxidation accounted for a nontrivial proportion of the total dissolved CO₂ in all TSL environments and during both flood stages, showing that anaerobic degradation of organic C at aquatic–terrestrial transitions can support CO₂ supersaturation within tropical freshwaters.

### Results

pCO₂ and pCH₄ in TSL were consistently supersaturated relative to atmospheric equilibrium. pCO₂ averaged 13,000 ± 6,000 µatm (mean ± 1 SD) across sites during the high-water stage and 13,000 ± 12,000 µatm during the falling-water stage (Table 1). pCH₄ was significantly greater during the high-water stage (11,000 ± 2,000 µ atm) than during the falling-water stage (600 ± 300 µ atm) (P < 0.001, d = 1.8). By contrast, pCO₂ and pCH₄ at sea level are ~400 and 1.8 µ atm, respectively.

CO₂ supersaturation exceeded dissolved O₂ deficits, indicating sources of dissolved CO₂ other than aerobic respiration (Fig. 1A and B). CO₂ supersaturation is expected to vary with dissolved O₂ deficits (Fig. 1A and B). CO₂ supersaturation was as low as 1/1 O₂:CO₂ ratio as one µ mole of dissolved O₂ is consumed for each µ mole of dissolved CO₂ produced. Instead, ratios of ~0.1/1 were observed during both the high-water and falling-water stages. During the high-water stage, the greatest CO₂ supersaturation occurred under the most hypoxic conditions (Fig. L4).

A two-source, stable isotope-mixing model for CO₂ (Keeling Intercepts; SI Appendix, Table S1) (28, 29). In TSL, the inverse of pCO₂ was strongly correlated with ¹³C depletion of CO₂. The intercept of δ¹³C-CO₂ was as low as −51‰ during the high-water stage and −43‰ during the falling-water stage. This indicates a ¹³C-depleted source of dissolved CO₂ relative to the other potential sources of organic and inorganic C measured, which ranged from −37 to −7.7‰ (Fig. 2A). Observed ¹³C depletion of dissolved CO₂ coincided with ¹³C enrichment of dissolved CH₄ (Table 1 and Fig. 3A and B). Acetate fermentation produces δ¹³C-CH₄ ranging from −65 to −50‰ and carbonate reduction produces δ¹³C-CH₄ ranging from −110 to −60‰ (24–26). By contrast, δ¹³C-CH₄ averaged −36 ± 2‰ during the high-water stage. During this flood stage, dissolved CO₂ became more ¹³C enriched from open water environments (δ¹³C-CO₂ = −37 ± 4‰, δ¹³C-CH₄ = −45 ± 9‰) to edge environments (δ¹³C-CO₂ = −39 ± 6‰, δ¹³C-CH₄ = −38 ± 6‰) to floodplain environments (δ¹³C-CO₂ = −41 ± 7‰, δ¹³C-CH₄ = −34 ± 2‰). Net fractionation between δ¹³C-CO₂ and δ¹³C-CH₄ of typically <10‰ in TSL indicates substantial CH₄ oxidation (25) (Fig. 3A and B).

Two-source, stable isotope-mixing model for δ¹³C-CO₂ was used to estimate fractional contributions to dissolved CO₂ by CH₄ oxidation, stable with other potential sources of organic and inorganic C (Fig. 2A). Assuming oxidation of CH₄...
produced by acetate fermentation only, the fractional contributions by CH$_4$ oxidation to dissolved CO$_2$ range from 63 to 85% across the distinct lake environments and flood stages of TSL (SI Appendix, Table S3). Assuming oxidation of CH$_4$ produced by both acetate fermentation and carbonate reduction, these contributions by CH$_4$ oxidation fall to a more conservative 47 to 67%. Apparent fractionation between $\delta^{13}$C-CO$_2$ and $\delta^{13}$C-CH$_4$ (simply, $\delta^{13}$C-CO$_2$/$\delta^{13}$C-CH$_4$) of typically <1.055 in TSL indicate substantial CH$_4$ production by acetate fermentation with some carbonate reduction (24, 25) (Fig. 2B).

$\delta^{13}$C-CO$_2$ was strongly correlated to independent measurements of net CH$_4$ oxidation in the water column during the high-water stage (Fig. 3 C and D). The same significant relationship was observed between $\delta^{13}$C-CO$_2$ and gross CH$_4$ production within the sediments (Fig. 3 E and F). Despite these relationships, CO$_2$ mass balance indicates that CH$_4$ production and oxidation within the water column and underlying sediments contribute at most 9% to dissolved CO$_2$ in TSL (SI Appendix, Table S4). Of these two processes, CH$_4$ production contributes one to two orders of magnitude more CO$_2$ than CH$_4$ oxidation. Other processing of C within the water column and underlying sediments, such as aerobic respiration, also contribute a relatively small share of total dissolved CO$_2$ (13 ± 8%).

**Discussion**

Contributions of CH$_4$ production and oxidation to CO$_2$ supersaturation are understudied within tropical freshwaters, where extensive flooding, dissolved O$_2$ deficits, and reducing conditions at aquatic–terrestrial transitions make such contributions likely. The subtropics and tropics are home to many high-order flood pulse rivers, such as the Amazon, Orinoco, Congo, Zambezi, and Mekong, which are collectively responsible for over 30% of global mean annual discharge (30). Along this tropical “active pipe” lays 52% of the world’s floodplains, transferring and transforming C at relatively high rates (20, 31). Using a combination of isotopic tracers and mass balance, we show that a substantial fraction this transfer and transformation of C occurs through coupled CH$_4$ production and oxidation in TSL.

A majority of our measured $\delta^{13}$C-CO$_2$ fell between the $^{13}$C-depleted CO$_2$ known to result from CH$_4$ oxidation and

---

**Fig. 1.** Dissolved O$_2$ deficit and CO$_2$ supersaturation, relative to atmospheric equilibrium in open water, edge, and floodplain environments of TSL (A) during the high-water and falling-water stages of the flood pulse (B). Dissolved O$_2$ deficit and CO$_2$ supersaturation are calculated as the difference between atmospheric equilibrium, according to Henry’s Law. Orange lines show atmospheric equilibrium at a dissolved O$_2$ deficit and CO$_2$ supersaturation of 0 μmol L$^{-1}$. A slope (m) of −1.0 represents the equimolar consumption of dissolved O$_2$ and production of dissolved CO$_2$ expected during aerobic respiration (black dashed line). Instead, a slope of −0.1 was observed during both the high-water and falling-water stages. O$_2$ deficits were strongly correlated to CO$_2$ supersaturation during the high-water stage, but there was no such correlation during the falling-water stage.

**Fig. 2.** (A) Measured $\delta^{13}$C-CO$_2$ (blue) relative to other potential sources of organic and inorganic C during the high-water and falling-water stages of the flood pulse. “Other” potential sources of organic and inorganic C measured by this study in TSL include macrophytes, terrestrial C3 vegetation, periphyton, phytoplankton, and DIC. Emergent aquatic C4 grasses, measured by Hedges et al. (48) in the Amazon, and atmospheric CO$_2$ in equilibrium with water (47) are also included. Isotopic values quantified by this study are in blue and white, and those quantified by other studies (24-26, 47, 48) are in gray. (B) Apparent fractionation between $\delta^{13}$C-CO$_2$ and $\delta^{13}$C-CH$_4$ ($\varepsilon_{app}$) indicated substantial CH$_4$ production through acetate fermentation with some carbonate reduction in TSL (24, 25). Therefore, a two-source, isotope-mixing model was created using 1) a continuous uniform distribution of $\delta^{13}$C-CO$_2$ known to result from the oxidation of CH$_4$ produced by both pathways (gray boxes) and 2) a continuous, uniform distribution of $\delta^{13}$C-CO$_2$ from DIC and the aerobic respiration of potential organic C sources (white box).
the relatively more $^{13}$C-enriched phytoplankton, periphyton, macrophytes, and terrestrial C3 vegetation measured in TSL (Fig. 24). Because there is little fractionation during aerobic respiration of organic C, measured $^{13}$C-activities in lakes can be expected to fall within the range of $^{13}$C observed for commonly considered sources of organic and inorganic C (31, 32). Instead, our observed $^{13}$C-activities fell outside of this range. Potential sources of organic and inorganic C in TSL ranged from $^{13}$C = −37‰ to $^{13}$C = −40‰ in equilibrium with water. De Kluiver and others (33, 34) have reported relatively $^{13}$C-depleted phytoplankton ($^{13}$C = −41‰ $^{13}$C-CO2, 13C-CO2, and 13C-CH4) are shaded in gray. $^{13}$C depletion of CO2 was strongly correlated to independent measurements of net CH4 oxidation in the water column during the high-water stage (C), though not during the falling-water stage (D). $^{13}$C depletion of CO2 was also strongly correlated to gross CH4 production within sediments during the high-water stage (E), though not during the falling-water stage (F).

**Fig. 3.** $^{13}$C-CO2 and $^{13}$C-CH4 in open-water, edge, and floodplain environments of TSL during the high-water (A) and falling-water stages of the flood pulse, modified from Whiticar (25) (B). Zones of CH4 production by acetate fermentation, CH4 production by carbonate reduction, and CH4 oxidation based on apparent fractionation between $^{13}$C-CO2 and $^{13}$C-CH4 ($\epsilon_2$) are shaded in gray. $^{13}$C depletion of CO2 was strongly correlated to independent measurements of net CH4 oxidation in the water column during the high-water stage (C), though not during the falling-water stage (D). $^{13}$C depletion of CO2 was also strongly correlated to gross CH4 production within sediments during the high-water stage (E), though not during the falling-water stage (F).

$^{13}$C depletion of dissolved CO2 in flood pulse lake (Tonle Sap Lake, Cambodia).
vegetation in the Amazon could export fully half of its primary production on an annual basis. Data from TSL supports a more nuanced interpretation. The most 13C-depleted source of organic C in TSL was an individual macrophyte (δ13C = −37.0‰, mean δ13C = −33 ± 4‰). Even so, 70% of our dissolved CO2 measurements were depleted in 13C below −37‰. As confirmed by our stable isotope-mixing model, this means that aerobic respiration of macrophytes can contribute to but not explain the C isotopic depletion of dissolved CO2 observed in TSL.

Corresponding 13C enrichment of dissolved CH4 indicated a fractionating loss process, further supporting the interpretation that CH4 oxidation supports CO2 supersaturation in TSL. Acetate fermentation within tropical lake sediments from the Amazon and Pantanal has been shown to produce δ13C-CH4 values ranging from −86 to −61‰ (36, 37). The same studies showed concurrent carbonate reduction producing CH4 even more depleted in 13C (<−61‰). The same studies showed concurrent carbonate reduction producing CH4 even more depleted in 13C (36, 37). By contrast, we measured an overall mean δ13C-CH4 of −43 ± 9‰ in TSL, with some values as high as −11‰ (Table 1 and Fig. 3A and B). Similar values were measured by Barbosa et al. (38) on Amazon River floodplains (δ13C-CH4 = −70.1 to −14.8‰). Independently measured rates of CH4 production and oxidation in TSL support this conclusion. Both net CH4 oxidation in the water column of TSL (Fig. 3B and C) and gross CH4 production within the sediments (Fig. 3D and E) were strongly correlated to δ13C-CO2.

It is important to note that the presence of other floodplain habitats, and infer that this is a far greater contributor to CO2 supersaturation. This was initially hypothesized by Junk et al. (18) and later combined with the “active pipe” by Abril and Borges (20). Yet it has been empirically tested using dissolved CO2 and CH4 in only two other locations (15, 17) and never with the C isotopic composition of these dissolved gases.

The 13C depletion of CO2, 13C enrichment of CH4, and their correlations to independently measured rates of CH4 production and oxidation suggest that these coupled processes support CO2 supersaturation in TSL. By extension, coupled CH4 production and oxidation are disproportionately responsible for CO2 emissions from TSL. Lauerwald et al. (13) estimate that >50% of global riverine CO2 emissions occur in the tropics, emphasizing the importance of tropical “active pipes.” Data on the stable C isotopes of both CO2 and CH4 are rarely reported for freshwater. However, 13C-enriched dissolved CH4 gas (δ13C = 0‰) reported in tropical and temperate lakes, wetlands, peatlands, and the Amazon River implies widespread oxidation of CH4 to CO2 (SI Appendix, Table S5). Coupled CH4 production and oxidation have thus been understudied but may support CO2 supersaturation and CH4 emissions from other tropical freshwaters with large amounts of seasonally or perennially flooded land. The extent of this flooding will most likely change under the twin stressors of hydropower development and climate change in the tropics (21), impacting the future role of floodplains in the transfer and transformation of C from terrestrial to aquatic ecosystems.

**Materials and Methods**

**Field Sampling.** Field sampling was conducted during the high-water and falling-water stages of the annual flood pulse in October 2015 and March 2016, respectively, representing the typical hydrological range in TSL. Flood stages were assessed using historical data from a gauging station at Kampong Luong (SI Appendix, Fig. S1) (21). Sampling focused on these locations in the southeast (Kampong Preah), central (Anlang Reang), and northwest (Prek Koteil) basins of TSL. Transects designed to capture horizontal gradients in dissolved O2 and reducing conditions were established at each location. These transects consisted of six points extending through the distinct open water (Transect Point 1), edge (Transect Point 2), and floodplain environments of TSL (Transect Points 4 to 6). The edge environments were characterized by a transition from open water environments to emergent, permanently rooted floodplain vegetation.

**Partial Pressures of CO2 and CH4.** Partial pressures of CO2 and CH4 at each transect point and flood stage in TSL were quantified as the average of three duplicates collected at 0.1 m below the water surface and at 0.5 m above the lake bottom where water depth exceeded 0.5 m (n = 143 duplicates, n = 47 replicates). Water was collected into 74-mL gas-tight serum bottles using a van Dorn sampler, preserved in the field with 74 μL of 50% mass/volume zinc chloride solution, and placed on ice for transport to the Royal University of Phnom Penh, where they were stored at 4°C until analysis. For analysis, samples were displaced with helium to roughly equal parts headspace and water, left to equilibrate for 12 h, and analyzed for headspace pCO2 and pCH4 using gas chromatography (SRI 8610CX) by referencing to certified standards of known concentrations.

**Stable C Isotopes of CO2 and CH4.** Following analysis for partial pressures, samples were resealed with Apiezon grease, inverted, placed on ice, and transported to the University of Washington for C isotopic analysis (n = 47). A 20-mL headspace sample was analyzed for the δ13C-CO2 of CO2 and CH4 simultaneously using a cavity ring-down spectrometer (Picarro G2201) with a small sample introduction module (Picarro A0314 SSIM). Following Malowany et al. (39), a column of reduced copper shavings was installed on the small sample introduction module to eliminate interference by hydrogen sulfide with isotopic measurements. Samples exceeding 300 μatm CH4 were diluted with ultra-high purity nitrogen to further eliminate interference by high concentrations of this gas in isotopic measurements. Stable C isotopes of CO2 and CH4 are each expressed in delta (δ) notation relative to Vienna Pee Dee Belemnite by referencing to certified CO2 and CH4 standards of known concentrations and 13C/13C.

**Stable C Isotopes of Organic and Inorganic C.** Grab samples of floating macrophytes (Eichhornia species), terrestrial C3 vegetation, periphyton, and phytoplankton were collected from three distinct lake environments and considered a single, lake-wide sample with a minimum of four replicates. Phytoplankton were collected using a Wisconsin net sampler (Wildco 40-A50), and periphyton was scraped from the benthos and the surfaces of floating macrophytes and emergent, aquatic vegetation. Macrophytes (δ13C = −33 ± 4‰, n = 4), terrestrial C3 vegetation (δ13C = −29 ± 2‰, n = 7), periphyton (δ13C = −28 ± 4‰, n = 18), and phytoplankton (δ13C = −24 ± 4‰, n = 6) in TSL were freeze dried, ground, and analyzed for bulk δ13C/13C using an elemental analyzer (CE Instruments 2500 NA) interfaced with an isotope ratio mass spectrometer (DeltaV IRMS). Laboratory working standards were glutamic acid 1 (δ13C = −28.3‰, versus VPDB), glutamic acid 2 (δ13C = −13.7‰), and sockeye salmon (δ13C = −21.3‰). DIC (δ13C = −13.8 ± 0.4‰) was from samples from another sampling effort across the same lake environments and flood stages were acidified, displaced with a helium headspace, analyzed on a DeltaV IRMS, and considered a lake-wide sample, as described previously.

**Depth-Integrated Gross Primary Production and Aerobic Respiration.** Gross primary production (GPP) and aerobic respiration were modeled across the distinct lake environments and flood stages of TSL using diel-dissolved O2 data in the “LakeMetabolizer” R package (n = 16) (40, 41). Model inputs include hourly dissolved oxygen (millimoles per liter), hourly water temperature (degrees Celsius), and hourly photon flux for photosynthetically active radiation (PAR; microeinstiens per second · square meter). Continuously logging dissolved O2 and water temperature sensors were deployed for a minimum of 20 h (Precision Measurement Engineering miniDO T Logger, accuracy ±0.16 mg O2 L-1, and ±0.1°C). Accuracy of dissolved O2 sensors was verified prior to field deployment using the Winkler titration method. PAR was not measured directly but calculated from full-spectrum irradiance based on latitude, longitude, aspect, slope, transmissivity data, and the “astrococlar” function in the “fishmethods” R package (42). GPP and aerobic respiration were converted to millimoles of CO2 per cubic meter per day using an assimilation efficiency of 1.2 for photosynthesis (43, 44) and a conversion efficiency of 1.0 for respiration.

Volumetric rates were multiplied by mixing depths to obtain areal rates in terms of millimoles of CO2 per square meter per day. Mixing depths were evaluated using dissolved O2 profiles at each site using a multiparameter sonde calibrated just prior to deployment with water-saturated air (YSI 6920). Dissolved O2 data were plotted over depth (m), smoothed using a loess-smoothing function of 0.2, and interrogated for inflection points in R (41). The depth of these inflection points at each transect was considered the mixing depth.
Depth-Integrated CH4 Production and Oxidation. Gross CH4 production within lake sediments was quantified as the average of three duplicate sediment incubations. At each transect point and flood stage in TSL, sediment cores were taken with a stainless-steel corer. The upper 1 cm² of each core was sealed inside a 74-ml gas-tight serum bottle (n = 72 duplicates, n = 24 replicates). The remaining volume of the bottle was filled with bottom water collected 0.5 m above the sediments. Three additional bottles were filled with bottom water only and three with water collected 0.1 m below the water surface. All bottles were incubated at ambient air temperatures (25 ± 3.4°C), which were typically <4°C different from water temperatures in TSL and sampled daily from a helium headspace for 7 d. pCH4 was analyzed as described previously and corrected for progressively decreasing headspace:water ratios. Net CH4 oxidation in surface waters was multiplied by mixing depths to obtain areal rates as in Depth Integrated Gross Primary Production and Aerobic Respiration. Net oxidation in bottom waters was added to net CH4 production measured in the bottles containing a combination of sediment cores and bottom water and considered gross CH4 production. Following incubation, each sediment core was dried at 100°C for 3 h and weighed. Gross CH4 production rates were then corrected for sediment core weight and scaled to nanomoles of CH4 per cubic meter per day. Previously published studies of CH4 production in lake sediment cores show that rates measured at the sediment–water interface are consistent to a sediment depth of 0.1 m (25, 45). Volumetric rates of CH4 production were thus multiplied by 0.1 m to obtain areal rates in terms of nanomoles of CH4 per square meter per day. Because one mole of CH4 is produced for each mole of CH4 produced during acetate fermentation, presumed to be dominant in TSL (Fig. 2B) and within freshwaters more broadly (24, 25), rates were also considered in terms of nanomoles of CO2 per square meter per day. Each transect sample included negative control incubations amended with a 74 μL of 50% mass-volume zinc chloride solution.

Mass Balance. A mass balance for dissolved CO2 in TSL was created from processes resulting in a gain or loss of CO2:

$$\text{CO}_2^\text{Measured} = \text{CO}_2^\text{Mesulf} + \text{CO}_2^\text{Respiration} + \text{CO}_2^\text{GPP} + \text{CO}_2^\text{MProd} + \text{CO}_2^\text{Diffusion}$$

where CO2 measured is the CO2 gained from modeled aerobic respiration, CO2 GPP is the CO2 lost from modeled GPP, CO2 MProd is the CO2 gained from measured net CH4 oxidation in the water column, each in millimoles per square meter per day. CO2 Respiration is the CO2 measured within the water column of TSL and multiplied by a temperature dependent Henry’s constant and mixing depth to yield millimoles of dissolved CO2 per square meter on the day of sampling. Diffusion of CO2 from TSL to the atmosphere was modeled using CO2 Measured following Cole and Caraco (46). CO2 Diffusion reflects an atmospheric loss subsequent to CO2 Measured and was ultimately excluded from the mass balance. CO2 Mesulf is the remaining CO2 in the mass balance assumed to result from aerobic respiration and anaerobic degradation of organic C elsewhere, within inundated soils and other floodplain habitats under steady-state conditions (millimoles per square meter per day). Mean daily CO2 Measured ± 1 SE was quantified using normal distributions—based on sample size, mean, and SD—of other terms in the mass balance over 10,000 Monte Carlo simulations in R (41).

Stable, Isotope-Mixing Model. The C isotopic composition of CO2 measured in TSL fell between the 13C-CO2 produced by 1) the oxidation of 13C-depleted CH4 to CO2 (−110 to −50‰) and 2) that of other potential organic and inorganic sources of CO2 (−37 to −7.7‰). Here, the sole concern is the fraction of CO2 derived from CH4 oxidation. Thus, a two-source (“Methane” versus “Other”), stable, isotope-mixing model was deemed appropriate. The model also accounted for CO2 losses from primary production and atmospheric diffusion and took the form:

$$\delta^{13}C_{\text{CH}_4} = \left( \delta^{13}C_{\text{Me}} \frac{f_{\text{Me}}}{f_{\text{Me}} + f_{\text{Oth}}} + \delta^{13}C_{\text{Oth}} \frac{f_{\text{Oth}}}{f_{\text{Me}} + f_{\text{Oth}}} \right) - \left( \delta^{13}C_{\text{Me}}^\text{Measured} + \frac{f_{\text{Me}}}{f_{\text{Me}} + f_{\text{Oth}}} \delta^{13}C_{\text{Oth}}^\text{Diffusion} + \frac{f_{\text{Oth}}}{f_{\text{Me}} + f_{\text{Oth}}} \delta^{13}C_{\text{Me}}^\text{Diffusion} \right)$$

where: fMe = 1.00. Δ13CMe was modeled as a continuous, uniform distribution of δ13C-CH4 values produced by methanogenesis, ranging from −110 to −50‰ (Fig. 2A) (24–26). fOth is the fraction of CO2 resulting from CH4 oxidation. Because Δ13CMe encompasses the range of Δ13C values produced by both acetate fermentation (−65 to −50‰)—presumed to be dominant in TSL and within freshwaters more broadly (24, 25)—and carbonate reduction (−110 to −60‰), the model results in a conservative estimate of fMe, for this freshwater lake (Fig. 2B).

δ13CMe was also modeled as a continuous, uniform distribution of δ13C values encompassing other potential sources of organic and inorganic C (SI Appendix, Fig. S2). This distribution ranges from the most 13C-depleted source of organic C measured in TSL, macrophytes (δ13C = −37‰), to the most 13C-enriched source of inorganic C, atmospheric CO2 in equilibrium with water (δ13C = −7.7‰) (47). δ13CMe therefore encompasses the δ13C of terrestrial C3 vegetation, periphyton, phytoplankton, and DIC measured in TSL and the δ13C of emergent, aquatic C4 grasses (δ13C = −12.2 ± 0.3‰) measured by Hedges et al. (48) in the Amazon. With multiple sources of organic and inorganic C that overlap in δ13C and no prior information on the relative importance of each, the most parsimonious option was to treat them as a group with equal probability across the full range of δ13C values. However, multiple alternative models were also tested (SI Appendix, Table S3).

Data Availability. All data are freely available and can be accessed at the publically accessible repository GitHub, https://github.com/blm8/PNAS_Tonle-Sap–Carbon-Dioxide-Supersaturation. Code data have been deposited in GitHub, https://github.com/blm8/PNAS_Tonle-Sap-Carbon-Dioxide-Supersaturation. All other study data are included in the article and/or SI Appendix.

Acknowledgments. We gratefully acknowledge assistance from the Cambodian Inland Fisheries Research and Development Institute, Cambodian Fisheries Administration, and inhabitants of the floating villages in TSL. We also thank Vittoria Elliott of World Fish and David Ford of Royal University of Phnom Penh for further in-country support. David Butman, Jeffrey Richey, Dangvan Southee, and two anonymous reviewers provided helpful comments during the preparation of this manuscript. This research was supported by the NSF and Margaret A. Cargill Foundation. B.L.M. was additionally supported by an NSF Graduate Research Fellowship and a National Security Education Program Boren Fellowship. M.E.A. was additionally supported by a Florida Education Fund Knight Junior Faculty Fellowship. Portions of this paper were developed from the doctoral thesis of B.L.M.

1. J. J. Cole, N. F. Caraco, G. W. Kling, T. K. Kratz, Carbon dioxide supersaturation in the surface waters of lakes. Science 265, 1568–1570 (1994).
2. P. A. Raymond et al., Global carbon dioxide emissions from inland waters. Nature 503, 355–359 (2013). Correction in: Nature 507, 387 (2014).
3. G. W. Kling, C. M. Miller, A. B. McManus, The chemical and biological characteristics of late Pleistocene and Holocene streams as gas conduits to the atmosphere: Implications for tundra carbon budgets. Science 251, 298–301 (1991).
4. J. E. Richey, A. H. Devol, S. C. Wofsy, R. Victoria, M. N. G. Rivero, Biogenic gases and the oxidation and reduction of carbon in Amazon River and floodplain waters. Limnol. Oceanogr. 33, 551–561 (1988).
5. J. B. Jones Jr., P. J. Mulholland, Carbon dioxide variation in a hardwood forest stream: Integrative measure of whole catchment soil respiration. Ecosystems 1, 183–196 (1998).
6. P. A. Raymond, J. E. Saiers, W. V. Sebczas, Hydrological and biogeochemical controls on watershed dissolved organic matter transport: Pulse-shunt concept. Ecology 87, 5–16 (2016).

7. P. Lopez, R. Marce, J. Armengol, Net heterotrophy and CO₂ evasion from a productive calcareous reservoir—Adding complexity to the metabolism-CO₂ evasion issue. J. Geophys. Res. 116, G02021 (2011).

8. R. Marze et al., Carbonate weathering as a driver of CO₂ supersaturation in lakes. Nat. Geosci. 8, 107–110 (2015).

9. P. A. Del Giorgio, J. J. Cole, N. F. Caraco, R. H. Peters, Linking planktonic biomass and metabolism to net gas fluxes in northern temperate lakes. Ecology 80, 1422–1431 (1999).

10. J. J. Cole, N. F. Caraco, Carbon in catchments—Connecting terrestrial carbon losses with aquatic metabolism. Mar. Freshw. Res. 52, 101–110 (2001).

11. J. J. Cole et al., Plumbing the global carbon cycle—Integrating inland waters into the terrestrial carbon budget. Ecosystems 10, 171–185 (2007).

12. R. Lauterwald, G. G. Lanuelle, J. Hartmann, P. Clai, P. A. G. Regnier, Spatial patterns in CO₂ evasion from the global river network. Global Biogeochem. Cycles 29, 534–554 (2015).

13. J. E. Richey, J. M. Melack, A. K. Aufdenkampe, V. M. Ballester, L. L. Hess, Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO₂. Nature 416, 617–620 (2002).

14. G. Abril et al., Amazon River carbon dioxide outgassing fuelled by wetlands. Nature 505, 395–398 (2014).

15. J. H. F. Amaral et al., Seasonal and spatial variability of CO₂ in aquatic environments of the central lowland Amazon basin. Biogeosciences 143, 133–149 (2019).

16. W. J. Junk, P. B. Baylery, R. E. Sparks, “The flood pulse concept in river-floodplain systems” in Proceedings of the International Large River Symposium, D. P. Dodge, Ed. (Canadian Special Publication of Fisheries and Aquatic Sciences, Ottawa, Canada, 1989), pp. 110–127.

17. A. Zuidgeest, S. Baumgartner, B. Wehrli, Hysteresis effects in organic matter turn-over in a tropical floodplain during a flood cycle. Biogeochimistry 131, 49–63 (2016).

18. A. V. Borges et al., Divergent biological controls of aquatic CO₂ and CH₄ in the World’s two largest rivers. Sci. Rep. 5, 15614 (2015).

19. G. Abril, A. V. Borges, Ideas and perspectives—Carbon leaks from flooded land—Do we need to replumb the inland water active pipe? Biogeosciences 16, 769–784 (2019).

20. M. E. Arias et al., Quantifying changes in flooding and habitats in the Tonle Sap Lake (Cambodia) caused by water infrastructure development and climate change in the Mekong Basin. J. Environ. Manage. 112, 53–66 (2012).

21. G. W. Holtgrieve et al., Quantifying ecosystem metabolism in the Tonle Sap Lake, Cambodia with links to capture fisheries. PLoS One 8, e71395 (2013).

22. A. V. Borges et al., Variations in dissolved greenhouse gases (CO₂, CH₄, N₂O) in the Congo River overwhelmingly driven by fluvial wetland connectivity. Biogeosciences 16, 3801–3834 (2019).

23. S. K. Hamilton, S. J. Sippel, D. F. Calheiros, J. M. Melack, An anoxic event and other biogeochemical effects of the Pantanal wetland on the Paraguay River. Limnol. Oceanogr. 42, 257–272 (1997).

24. M. J. Whiticar, C. Faber, Methane oxidation in sediment and water column environments—Isotopic evidence. Org. Geochem. 10, 759–768 (1986).

25. M. J. Whiticar, Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. Chem. Geol. 161, 291–314 (1999).

26. E. R. Hornibrook, J. F. Longstaffe, W. S. Fyfe, Evolution of stable C isotope compositions for methane and carbon dioxide in freshwater wetlands and other anaerobic environments. Geochim. Cosmochim. Acta 64, 1013–1027 (2000).

27. W. Burnett et al., Groundwater discharge and phosphorus dynamics in a flood-pulse system—Tonle Sap Lake, Cambodia. J. Hydrod. 549, 79–91 (2017).

28. C. D. Keeling, The concentration and isotopic abundance of carbon dioxide in rural areas. Geochim. Cosmochim. Acta 13, 322–334 (1958).

29. C. D. Keeling, The concentration and isotopic abundance of carbon dioxide in rural and marine air. Geochim. Cosmochim. Acta 24, 277–298 (1961).

30. A. Dai, K. E. Trenberth, Estimates of freshwater discharge from continents—Latitudinal and seasonal variations. J. Hydrometeorol. 3, 660–687 (2002).

31. J. F. Pekel, A. Gottom, N. Gorelick, A. S. Belward, High-resolution mapping of global surface water and its long-term changes. Nature 540, 418–422 (2016).

32. B. J. Peterson, B. Fry, Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18, 293–320 (1987).

33. A. De Kluijver, P. L. Schoon, J. A. Downing, S. Schouten, J. J. Middleburg, Stable carbon isotope biogeochemistry of lakes along a trophic gradient. Biogeosciences 11, 6265–6276 (2014).

34. S. L. McCallister, P. A. Del Giorgio, Direct measurement of the d13C signature of carbon respired by bacteria in lakes—Linkages to potential carbon sources, ecosystem baseline metabolism, and CO₂ fluxes. Limnol. Oceanogr. 53, 1204–1216 (2008).

35. J. M. Melack, D. L. Engle, An organic carbon budget for an Amazon floodplain lake. Verh. Int. Ver. Theor. Angew. Limnol. 30, 1179–1182 (2009).

36. R. Conrad, M. Klose, P. Claus, A. Enrich-Prast, Methanogenic pathway, 13C isotope fractionation, and archaean community composition in the sediment of two clearwater lakes of Amazonia. Limnol. Oceanogr. 55, 689–702 (2010).

37. R. Conrad et al., Stable carbon isotope discrimination and microbiology of methane formation in tropical anoxic lake sediments. Biogeosciences 8, 795–814 (2011).

38. P. M. Barbosa et al., High rates of methane oxidation in an Amazon floodplain lake. Biogeochimistry 137, 351–365 (2018).

39. K. Malowany, J. Stix, A. Ven Pelt, G. Luiz, H2S interference on CO₂ isotopic measurements using a PicoG1101+ cavity-ring down spectrometer. Atmos. Meas. Tech. 8, 5651–5675 (2015).

40. L. A. Winslow et al., LakeMetabolizer—An R package for estimating lake metabolism from free-water oxygen using diverse statistical models. Inland Waters 6, 622–636 (2016).

41. R. Core Team, R—A Language and Environment for Statistical Computing (Version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria, 2019).

42. G. A. Nelson, Fishmethods—Fishery science methods and models (R package Version 11.1-1, 2019). https://cran.r-project.org/web/packages/fishmethods/index.html. Accessed 1 May 2020.

43. G. E. Likens, “Primary production of inland aquatic ecosystems” in Primary Productivity of the Biosphere, H. Leith, R. H. Whittaker, Eds. (Springer, 1975), pp. 185–202.

44. J. M. Melack, Primary productivity and fish yields in tropical lakes. Trans. Am. Fish. Soc. 105, 575–580 (1976).

45. B. Thebrath, F. Rothfuss, M. J. Whiticar, R. Conrad, Methane production in littoral sediment of Lake Constance. FEMS Microbiol. Lett. 11, 279–283 (1993).

46. J. J. Cole, N. F. Caraco, Atmospheric exchange of carbon dioxide in a low wind oligotrophic lake measured by the addition of SF₆. Limnol. Oceanogr. 43, 647–656 (1998).

47. M. H. O’Leary, Carbon isotope fractionation in plants. Phytochemistry 20, 553–567 (1981).

48. J. J. Hedges, A. C. Clark, P. D. Quay, J. E. Richey, A. H. Devol, Compositions and fluxes of particulate organic material in the Amazon River. Limnol. Oceanogr. 31, 717–738 (1986).

49. D. L. Phillips, S. D. Newsome, J. W. Gregg, Combining sources in stable isotope mixing models: Alternative methods. Oecologia 144, 520–527 (2000).

50. J. Cohen, Statistical Power Analysis for the Behavioral Sciences (Lawrence Erlbaum Associates, 1988).