Long-term warming amplifies shifts in the carbon cycle of experimental ponds

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Lakes and ponds cover only about 4% of the Earth's non-glaciated surface[1], yet they represent disproportionately large sources of methane and carbon dioxide[2-4]. Indeed, very small ponds (e.g. < 0.001 km^2) may account for approximately 40% of all CH_4 emissions from inland waters[5]. Understanding how greenhouse gas (GHG) emissions from aquatic ecosystems will respond to global warming is therefore vital for forecasting biosphere-carbon cycle feedbacks. Here, we present findings on the long-term effects of warming on the fluxes of GHGs and rates of ecosystem metabolism in experimental ponds. We show that shifts in CH_4 and CO_2 fluxes, and rates of gross primary production (GPP) and ecosystem respiration (R_{eco}), observed in the first year became amplified over seven years of warming. The capacity to absorb CO_2 was nearly halved after seven years of warmer conditions. The phenology of GHG fluxes were also altered, with CO_2 drawdown and CH_4 emissions peaking one month earlier in the warmed treatments. These findings show that warming can fundamentally alter the carbon balance of small ponds over a number of years, reducing their capacity to sequester CO_2 and increasing emissions of CH_4; such positive feedbacks could ultimately accelerate climate change.

Respiration and methanogenesis respond more strongly to temperature change than photosynthesis[6-9]. Consequently, warming has been shown to increase CO_2 and CH_4 emissions and reduce carbon sequestration in experimental ponds[10-13]. However, these experiments have either been restricted to relatively short-term responses to warming (e.g. 1 year or less)[10,11] or have not investigated how the effects of warming change over time[13,14]. Therefore, a key unanswered question for understanding how greenhouse gas dynamics in freshwater ponds will respond to global warming is: *do the high temperature sensitivities of methanogenesis and respiration result in increased emissions of CH_4 and CO_2, and reduced carbon sequestration under warming that is sustained over the long-term, and which could potentially accelerate the rate of climate change*?

Unlike work in aquatic ecosystems, the long-term effects of experimental warming have been assessed in terrestrial ecosystems, and tend to show elevated emissions of CO_2 in the short-

term, driven by the exponential temperature dependence of respiration, followed by a damped effect of warming later[15-17]. These diminishing long-term responses have been attributed to loss of labile carbon substrates[15], physiological acclimation[16], evolutionary adaptation[18] or community turnover through ecological dynamics[19]. These findings are important because they imply carbon cycle responses to global warming should be more complex than the simple exponential effect of temperature on respiration rates alone[17].

Small freshwater ponds contribute disproportionately to greenhouse gas emissions budgets from inland waters[5]. However, whether shifts in the fluxes of CH_4 and CO_2 observed in short-term warming experiments[10,11], are sustained in the long-term (e.g. > 1 year to decadal timescales) in these ecosystems is unknown, which severely limits our ability to predict whether future changes in greenhouse gas fluxes[4,5] will contribute to accelerating or slowing global warming. We tackled this fundamental knowledge gap using an array of experimental mesocosms that were designed to mimic mid-latitude ponds, to investigate the effects of long-term warming on the ecosystem-level exchange of CO_2 and CH_4 with the atmosphere. We present a detailed analysis of the seasonal dynamics of the key metabolic and GHG fluxes in the carbon cycle in the sixth (2012) and seventh (2013) year of the experiment, which we contrast with our initial findings from the first year (2007) of warming[10,11] to explore whether, like terrestrial ecosystems[15-17], the effects of warming on the carbon cycle in freshwater ponds are dampened in the long-term.

CH_4 emissions were elevated in the warmed treatments in both 2007 and 2013. However, the magnitude of the effect size increased over the seven years of the experiment (Fig. 1). Annual rates of CH_4 emissions were 1.5-fold higher in the warmed treatments after one year of experimental warming[11], but after seven years this effect size had increased to 2.5-fold (Fig. 1; Table 1; Table 2). Consequently, a generalised additive mixed effects model (GAMM) that included a ‘treatment’ by ‘year’ interaction on the intercept provided the best fit to the data (Table 1), demonstrating that the effects of warming on median CH_4 emissions were larger in 2013 than 2007.

We also saw a comparable amplification of the effects of warming on total ecosystem-level

carbon metabolism. The effect size of warming increased from 1.2- to 1.8-fold for gross primary production (GPP) and 1.4- to 2-fold for ecosystem respiration (R_{eco}) between 2007 and 2012 (Fig. 2a-d; Table 1). Consequently, GAMMs fitted to the seasonal distributions of GPP and R_{eco} and including a 'treatment' by 'year' interaction on the intercept, provided the best fit to the data. Because rates of R_{eco} increased more with warming than those of GPP, the R_{eco}/GPP ratio was 1.15-fold higher in the warmed treatments, indicating reduced capacity for carbon sequestration in both 2007 and 2012 (Fig 2e & f). High frequency measurements of CO_2 exchange between the ponds and the atmosphere in 2013 confirmed these findings, with annual net CO_2 uptake reduced by 50% in the warmed mesocosms (Fig. 3; Table 1; Table 2). Together these results demonstrate that the effects of warming on the key fluxes in the carbon cycle became amplified over the seven years of the experiment, in stark contrast to the damped effects of long-term warming reported for terrestrial systems[15-17]. So what mechanisms might be responsible for the amplified effects of warming in freshwater ponds?

The experimental mesocosms were seeded in 2005 with organisms and organic matter (see Methods) and have since been on a trajectory of ecosystem development. Succession theory proposes that in the early stages of ecosystem development, as organic matter and biomass accumulate, rates of GPP exceed R_{eco} and the ratio of R_{eco}/GPP < 1[20]. As ecosystems develop towards later successional stages, energy fixed by GPP tends to be balanced by energy consumed through R_{eco} (i.e. the ratio of R_{eco}/GPP ≈ 1) and biomass production is maximised[20]. Consistent with ecosystem succession theory, the R_{eco}/GPP ratio and annual totals for GPP and R_{eco} all increased substantially over the course of the experiment in both the warmed and ambient treatments, with increases in GPP and R_{eco} much larger in the warmed mesocosms (Fig. 2a-d). In line with the data on total carbon metabolism, we also observed consistently higher biomass of macrophytes (Fig S5; Table S3), phytoplankton and zooplankton in the warmed treatments in the long term[21]. Together, these data show that warming enhanced rates of ecosystem development, community succession and biomass accumulation, amplifying the divergence between treatments in GHG emissions and

97 metabolic fluxes. These results demonstrate that warming can fundamentally alter the energetic
98 balance at the ecosystem level: firstly, because in the short term, rates of respiration rise more
99 sharply with temperature than photosynthesis (increasing R_{eco}/GPP); and secondly, over the long
100 term, because higher rates of metabolism drive more rapid ecosystem development[22], magnifying
101 energetic imbalances and shifts in the carbon cycle. Natural ecosystems are typified by far from
102 equilibrium dynamics[23], and thus, because warming can act both as a stressor and a driver of
103 physiology, understanding the long-term impacts of warming on ecosystem properties requires both
104 an appreciation of the acute effects of temperature change on organism metabolism and subsequent
105 impacts on the successional dynamics of ecosystems.

106 Focusing on the fluxes of CO_2 and CH_4 measured in 2013 at a high temporal resolution
107 reveals that, in addition to driving shifts in the annual budgets, long-term warming also profoundly
108 altered the seasonality of CH_4 emissions (Fig. 1) and net daily exchange of CO_2 (Fig. 3). Rates of
109 net daily CO_2 emission (i.e. days where total CO_2 emissions > absorption) peaked in October in the
110 warmed treatments, whilst, on average, ambient ponds were net sinks for CO_2 over the entire year.
111 By contrast, rates of net daily CO_2 absorption (i.e. days where total CO_2 absorption > emissions)
112 peaked in the warmed treatments in June, while they peaked in July in the ambient ponds (Fig. 3).
113 These respective peaks in net CO_2 absorption coincided with peak CH_4 emissions (Fig. 1), implying
114 a strong coupling between CO_2 drawdown by photosynthesis and substrate supply for
115 methanogenesis, which is a well-known characteristic of many natural aquatic ecosystems[24]. Indeed,
116 the most marked effects of warming on CH_4 emissions occurred during the spring and early summer
117 (Fig. 1). Later in the year, however, the effects were negligible (Fig. 1) when rates of respiration
118 exceeded those of photosynthesis (e.g. CO_2 production > CO_2 consumption leading to net CO_2
119 emissions; cf Figs 1 & 3) in the warmed treatments and rates of methanogenesis may have been
120 limited by photosynthetically derived carbon. These results suggest that the effects of global
121 warming could shift the seasonal timing of carbon fluxes, which, in turn, affect the supply and
122 demand of substrates that support aquatic ecosystem productivity.

Overall, our findings provide the first experimental evidence that the annual balance of greenhouse gas fluxes from freshwater ponds remain profoundly altered at inter-annual timescales, with substantially elevated CH_4 emissions and lower CO_2 absorption. The extent to which these results are important for understanding how carbon fluxes from globally important pond ecosystems[5] respond to warming depends on whether carbon dynamics in our experimental mesocosms are broadly representative of those in natural systems. One important distinction between the mesocosms and natural shallow lakes is that they are not embedded within a watershed, and consequently, receive little terrestrially derived organic carbon, which is often an important carbon flux in lakes and ponds[25]. In consequence, carbon cycle dynamics in the mesocosms are driven predominantly by autochthonous production, which could alter sediment characteristics, coupling between photosynthesis and respiration, and GHG emissions compared with natural ponds that receive allochthonous carbon subsidies. To investigate this and assess the relevance of our findings for natural ponds, we measured the carbon (C), nitrogen (N) and C:N ratios of the mesocosm sediments (Table 2) and compared them with values from natural lakes and ponds spanning the dystrophic to oligotrophic spectrum[25]. The sediment characteristics of the mesocosms are similar to those from natural oligotrophic lakes[26]. Mean annual rates of gross primary production and ecosystem respiration (Table 2) are also comparable to those from natural lakes[5,27], indicating that rates of total carbon metabolism reflect those of natural systems. Finally, recent work has shown that the size of lakes and ponds (in terms of surface area) are critical for determining their greenhouse gases (GHG) emissions, with CH_4 flux per unit area increasing as a power function of decreasing lake surface area[5,28,29]. Our mesocosms, with a surface area of 3.14×10^{-4} ha, fall within the smallest category of ponds analysed in a global synthesis of GHG emissions[5] and have average CH_4 concentrations that are indistinguishable from natural ponds of a similar size (Fig. S4). Taken together, this evidence demonstrates results from our mesocosm experiment are of direct relevance for understanding carbon cycle responses to warming in freshwater ponds. Our results suggest that profound shifts in the carbon fluxes of small ponds should be expected over the

long term in a warming world, which is of particular concern in light of the fact that we are only just beginning to appreciate the importance of such small water bodies in global budgets of GHG emissions from inland waters[5,28,29].

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Author contributions

MT and G. Y-D conceived the study. CJH & G. Y-D conducted the experiments. G. Y-D, CJH and MT analysed the data. G. Y-D. wrote the manuscript and all authors contributed to revisions. The authors declare no conflict of interest.

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References

1. Verpoorter, C., Kutser, T., Seekell, D. A. & Tranvik, L. J. A global inventory of lakes based on high-resolution satellite imagery. *Geophys. Res. Lett.* **41,** 6396–6402 (2014).
2. Bastviken, D., Tranvik, L. J., Downing, J. A., Crill, P. M. & Enrich-Prast, A. Freshwater Methane Emissions Offset the Continental Carbon Sink. *Science* **331,** 50–50 (2011).
3. Cole, J. J. *et al.* Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* **10,** 171–184 (2007).
4. Wik, M., Varner, R. K., Anthony, K. W., MacIntyre, S. & Bastviken, D. Climate-sensitive northern lakes and ponds are critical components of methane release. *Nature Geoscience* **9,** 99– (2016).
5. Holgerson, M. A. & Raymond, P. A. Large contribution to inland water CO2 and CH4 emissions from very small ponds. *Nature Geosci* **9,** 222–226
6. Yvon-Durocher, G. *et al.* Reconciling the temperature dependence of respiration across timescales and ecosystem types. *Nature* **487,** 472–476 (2012).
7. Yvon-Durocher, G. *et al.* Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. *Nature* **507,** 488– (2014).
8. Allen, A. P., Gillooly, J. F. & Brown, J. H. Linking the global carbon cycle to individual

metabolism. *Functional Ecology* **19,** 202–213 (2005).
9. Marotta, H. *et al.* Greenhouse gas production in low-latitude lake sediments responds strongly to warming. *Nature Climate Change* **4,** 467–470 (2014).
10. Yvon-Durocher, G., Jones, J. I., Trimmer, M., Woodward, G. & Montoya, J. M. Warming alters the metabolic balance of ecosystems. *Philosophical Transactions of the Royal Society B - Biological Scienes* **365,** 2117–2126 (2010).
11. Yvon-Durocher, G., Montoya, J. M., Woodward, G., Jones, J. I. & Trimmer, M. Warming increases the proportion of primary production emitted as methane from freshwater mesocosms. *Global Change Biology* **17,** 1225–1234 (2011).
12. Atwood, T. B. *et al.* Warming alters food web-driven changes in the CO2 flux of experimental pond ecosystems. *Biology Letters* **11,** (2015).
13. Davidson, T. A. *et al.* Eutrophication effects on greenhouse gas fluxes from shallow-lake mesocosms override those of climate warming. *Global Change Biology* **21,** 4449–4463 (2015).
14. Liboriussen, L. *et al.* Effects of warming and nutrients on sediment community respiration in shallow lakes: an outdoor mesocosm experiment. *Freshwater Biology* **56,** 437–447 (2010).
15. Melillo, J. M. Soil Warming and Carbon-Cycle Feedbacks to the Climate System. *Science* **298,** 2173–2176 (2002).
16. Luo, Y., Wan, S., Hui, D. & Wallace, L. Acclimatization of soil respiration to warming in a tall grass prairie. *Nature* **413,** 622–625 (2001).
17. Luo, Y. Terrestrial carbon-cycle feedback to climate warming. *Annu. Rev. Ecol. Evol. Syst.* (2007).
18. Bradford, M. A., Watts, B. W. & Davies, C. A. Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Global Change Biology* **16,** 1576–1588 (2010).
19. Karhu, K. *et al.* Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature* **513,** 81– (2014).
20. Odum, E. P. The strategy of ecosystem development. *Science* **164,** 262–270 (1969).
21. Yvon-Durocher, G. *et al.* Five Years of Experimental Warming Increases the Biodiversity and Productivity of Phytoplankton. *Plos Biol* **13,** (2015).
22. Anderson-Teixeira, K. J., Vitousek, P. M. & Brown, J. H. Amplified temperature dependence in ecosystems developing on the lava flows of Mauna Loa, Hawai'i. *P Natl Acad Sci Usa* **105,** 228–233 (2008).
23. Levin, S. A. Ecosystems and the biosphere as complex adaptive systems. *Ecosystems* **1,** 431–436 (1998).
24. Whiting, G. J. & Chanton, J. P. Primary production control of methane emission from wetlands. *Nature* **364,** 794–795 (1993).
25. Tranvik, L. J. *et al.* Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol. Oceangr.* **54,** 2298–2314 (2009).
26. Dean, W. E. The carbon cycle and biogeochemical dynamics in lake sediments. *Journal of Paeleolimnology* **21,** 375–393 (1999).
27. Likens, G. E. in *Primary Productivity of the Biosphere* **14,** 185–202 (Springer Berlin Heidelberg, 1975).
28. Kankaala, P., Huotari, J., Tulonen, T. & Ojala, A. Lake-size dependent physical forcing drives carbon dioxide and methane effluxes from lakes in a boreal landscape. *Limnol. Oceangr.* **58,** 1915–1930 (2013).
29. Bastviken, D., Cole, J., Pace, M. & Tranvik, L. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochem. Cycles* **18,** (2004).
30. Dossena, M. *et al.* Warming alters community size structure and ecosystem functioning. *Proceedings of the Royal Society B: Biological Sciences* **279,** 3011–3019 (2012).

Table 1| Multi-model selection on generalised additive mixed effects models fitted to the seasonal CH_4, CO_2 and metabolic flux data. A range of models testing hypotheses on the effects of the warming treatment ('treat') and the 'year' of the experiment were fitted to the seasonal greenhouse gas and metabolic flux data; 'treat' and 'year' along with their interaction assess differences in median flux values, while comparisons between s(DOY) and s(DOY, by = treat) assess whether the seasonality of flux differs among treatments. Models were compared via the small sample size corrected Akaike Information Criterion (AICc), delta AICc is the difference in AICc score relative to the model with the lowest value (most parsimonious model) and AICc Weight (Wt) is the relative support for the model. The best fitting models were selected as those returning the lowest AICc score and the highest AICc weight and are highlighted in bold.

Model	df	*N*	AICc	Δ AICc	AICc Wt
CH_4 Emissions					
ME0 – fixed = treat * year + s(DOY)	**8**	**311**	**961.53**	**0.00**	**0.71**
ME1 – fixed = treat + year + s(DOY)	7	311	965.47	3.94	0.16
ME2 – fixed = treat * year + s(DOY, by = treat)	10	311	966.03	1.51	0.13
ME3 – fixed = year + s(DOY)	6	311	969.66	5.13	0.00
ME4 – fixed = treat + s(DOY)	6	311	996.13	31.60	0.00
ME3 – fixed = s(DOY)	5	311	999.85	35.32	0.00
GPP					
GPP0 – fixed = treat * year + s(DOY)	**8**	**208**	**243.00**	**0.00**	**0.97**
GPP1 – fixed = treat + year + s(DOY)	7	208	250.58	7.59	0.02
GPP2 – fixed = treat * year + s(DOY, by = treat)	10	208	253.66	10.67	0.00
GPP3– fixed = year + s(DOY)	6	208	255.69	12.69	0.00
GPP4 – fixed = treat + s(DOY)	6	208	266.18	23.19	0.00
GPP5 – fixed = s(DOY)	5	208	271.54	28.55	0.00
R_{eco}					
R0 – fixed = treat * year + s(DOY)	**8**	**208**	**277.11**	**0.00**	**0.82**
R1 – fixed = treat + year + s(DOY)	7	208	280.34	3.23	0.16
R2 – fixed = treat * year + s(DOY, by = treat)	10	208	285.33	8.22	0.01
R3– fixed = year + s(DOY)	6	208	293.76	16.65	0.00
R4 – fixed = treat + s(DOY)	6	208	315.73	38.63	0.00
R5 – fixed = s(DOY)	5	208	327.21	50.11	0.00
R_{eco} / GPP					
RAT0 – fixed = treat + year + s(DOY)	**7**	**208**	**46.14**	**0.00**	**0.81**
RAT1 – fixed = year + s(DOY)	6	208	49.90	3.76	0.12
RAT2 – fixed = treat + year + s(DOY)	8	208	51.17	5.03	0.07
RAT3– fixed = treat * year + s(DOY, by = treat)	10	208	59.43	13.29	0.00
RAT4 – fixed = treat + s(DOY)	6	208	65.17	19.03	0.00
RAT5 – fixed = s(DOY)	5	208	67.37	21.23	0.00
Net CO_2 Flux - 2013					
NEE0 – fixed = treat + s(DOY, by = treat)	**8**	**4656**	**103884.7**	**0.00**	**1.00**
NEE1 – fixed = s(DOY, by = treat)	7	4656	103901.5	16.79	0.00
NEE2 – fixed = treat + s(DOY)	6	4656	103965.2	80.55	0.00
NEE3 – fixed = s(DOY)	5	4656	103982.0	97.34	0.00

245 **Table 2| Annual carbon fluxes and sediment characteristics of the mesocosms.**
246

	2007		2012		2013	
	Ambient	Heated	Ambient	Heated	Ambient	Heated
CH_4 emissions (g CH_4 m^{-2} y^{-1})	0.20 (0.016)	0.27 (0.013)	-	-	0.33 (0.012)	0.74 (0.04)
Net CO_2 flux (g C m^{-2} y^{-1})	-	-	-	-	-20.1 (6.4)	-10.9 (12.3)
GPP (g C m^{-2} y^{-1})	207.1 (1.1)	243.7 (1.6)	221.9 (1.1)	392.1 (1.8)	-	-
R_{eco} (g C m^{-2} y^{-1})	164.3 (1.4)	233.2 (1.7)	210.3 (1.3)	422.9 (1.9)	-	-
R_{eco}/GPP	0.83 (0.06)	0.97 (0.09)	1.03 (0.1)	1.16 (0.20)	-	-
Sediment % Carbon	-	-	-	-	2.9 (1.3)	3.7 (2.3)
Sediment % Nitrogen	-	-	-	-	0.3 (0.1)	0.4 (0.3)
Sediment C:N (molar)	-	-	-	-	11.8 (1.1)	11.1 (1.3)

247 Annual flux values were calculated by exponentiation of the median natural log-transformed daily fluxes estimated for each pond from the mixed effects model and multiplying by 365.
248 Values given in the table are averages of the annual fluxes across treatments and years. Numbers in parentheses are standard deviations. Positive fluxes indicate that the mesocosms
249 were sources of GHGs to the atmosphere, whilst negative fluxes denote they were sinks.

250

251

Figures

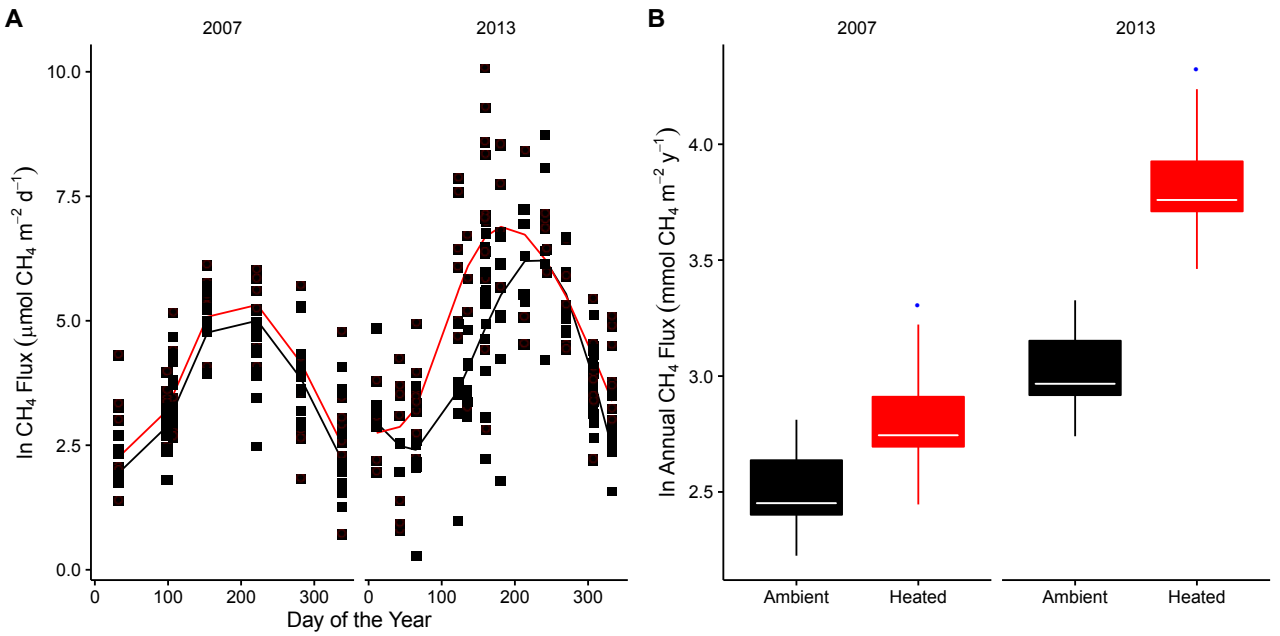


Figure 1| Long-term experimental warming amplifies shifts in CH_4 emissions. (a) The seasonal variation in CH_4 flux from 2007 and 2013 demonstrate that the effect of warming was larger in 2013 than 2007. (b) Box whisker plots of annual CH_4 flux from each pond calculated by integrating the seasonal data over time. These data show that the effect size of warming increased from 1.5 fold in 2007 to 2.5 fold in 2013 highlighting that the effects of warming became amplified in the long term. The solid lines denote the fixed effects from the best fitting GAMM model (see Table 1 for model selection). Red circles and lines denote warmed treatments, while the ambient treatments are in black. Tops and bottoms of boxes in box-whisker plots correspond to the 25th and 75th percentiles, horizontal white lines correspond to medians, whisker extents correspond to 1.5 x the interquartile range and blue points are outliers.

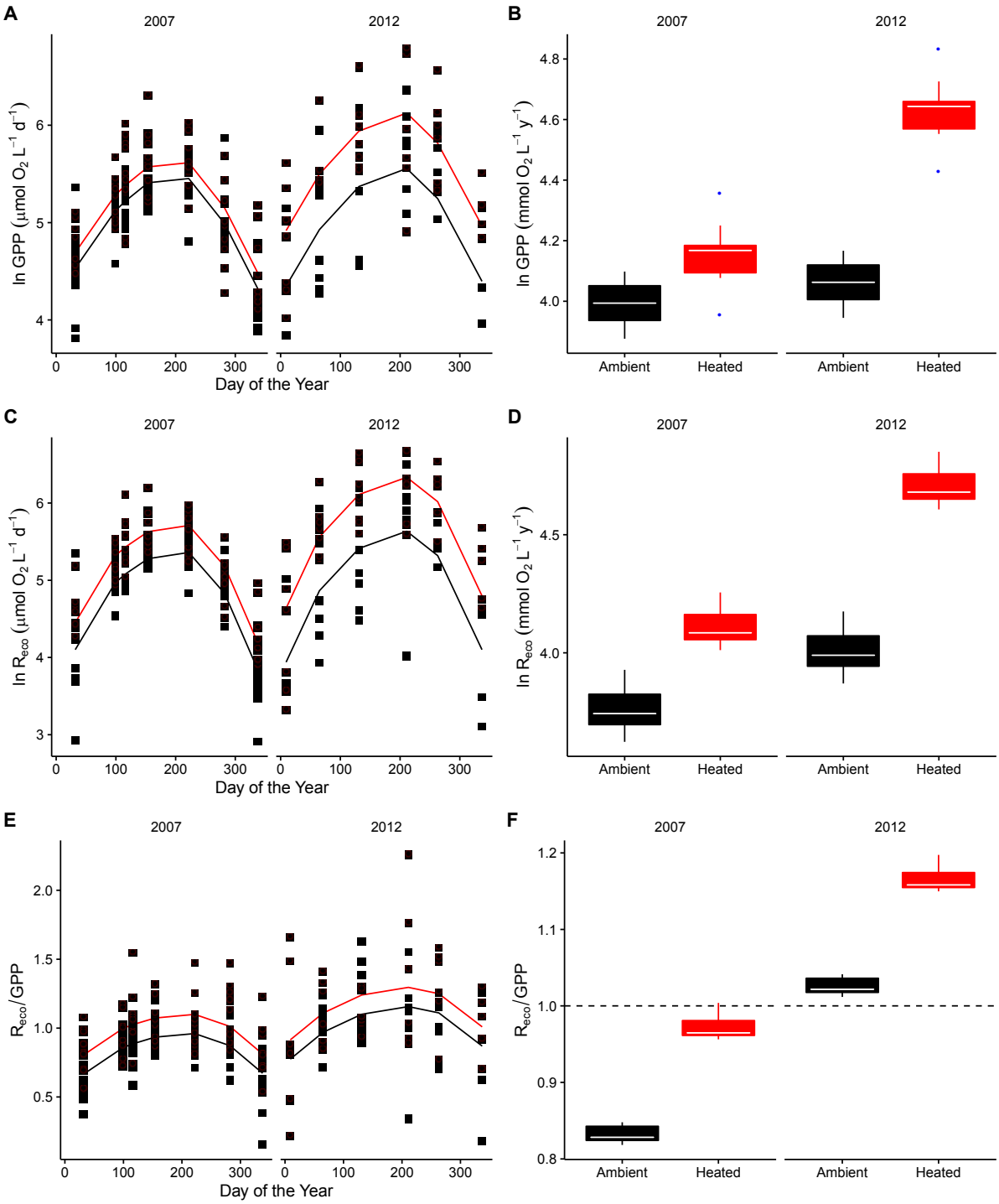
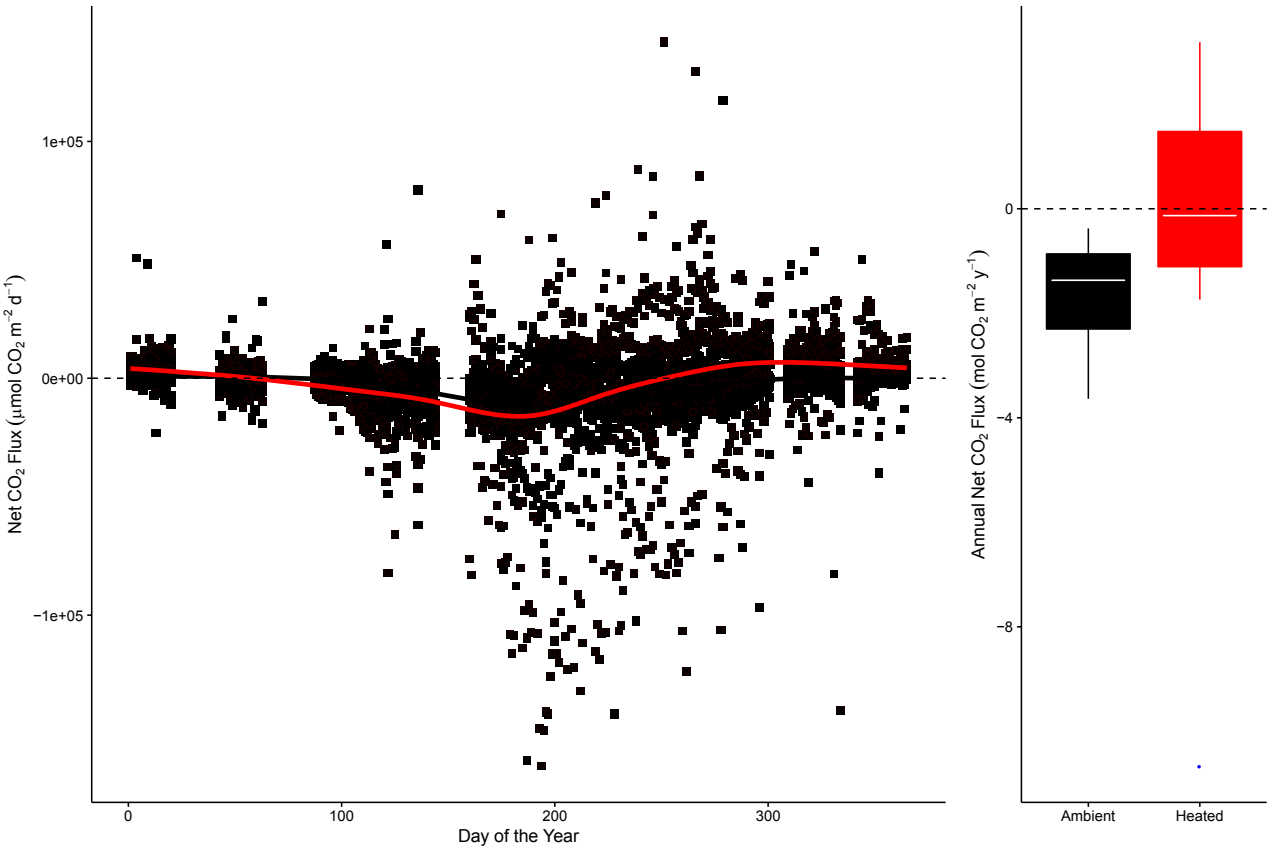


Figure 2| Long-term experimental warming amplifies shifts in ecosystem metabolism. Seasonal distributions of (a) Rates of gross primary production, GPP, (c) ecosystem respiration, R_{eco}, and (e) the R_{eco}/GPP ratio were fitted to generalised additive mixed effects models (see Methods). For GPP and R_{eco} the effects of warming on median rates of ecosystem metabolism were larger in 2012 than in 2007. The R_{eco}/GPP ratio was higher in the warmed ponds and increased between 2007 and 2012. The solid lines denote the fixed effects from the best fitting GAMM model (see Table 2 for model selection). Red circles and lines denote warmed treatments, while the ambient treatments are in black. Box whisker plots of annual rates of (b) GPP, (d) R_{eco}, and (f) R_{eco}/GPP ratio from each pond calculated by integrating the seasonal data over time. Tops and bottoms of boxes in box-whisker plots correspond to the 25th and 75th percentiles, horizontal white lines correspond to medians, whisker extents correspond to 1.5 x the interquartile range and blue points are outliers.



277
278 **Figure 3| Experimental warming alters the phenology and annual budget of net CO_2 flux.** (a)
279 Seasonal distribution of net CO_2 flux data collected in 2013 reveal differences in the seasonality of
280 net daily CO_2 fluxes with maximal rates of net CO_2 absorption peaking earlier in the year in the
281 warmed treatments. The solid lines denote the fixed effects from the best fitting GAMM model.
282 Red circles and lines denote warmed treatments, while the ambient treatments are in black. (b) Box
283 whisker plot of the annual net CO_2 fluxes calculated for each pond by integrating over the seasonal
284 data reveal higher net fluxes in the warmed treatments (indicating lower CO_2 absorption). Tops and
285 bottoms of boxes in box-whisker plots correspond to the 25th and 75th percentiles, horizontal white
286 lines correspond to medians, whisker extents correspond to 1.5 x the interquartile range and blue
287 points are outliers.

METHODS

Mesocosm pond facility

The facility was established in 2005 and consists of 20 artificial ponds of approximately $1m^3$ volume, 50cm depth, sited in southern England (Freshwater Biological Association Rivers Laboratory, East Stoke, 2°10`W, 50°13`N), designed to be broadly representative of mid-latitude shallow lakes[10]. Warming of 4-5°C above ambient began in half of the ponds in 2006 by maintaining a constant differential between thermocouples in a pair of warmed and ambient ponds (Fig. S1). The ponds contain well established benthic and pelagic communities including assemblages of macrophytes (Table S2), phytoplankton, algal biofilms and invertebrates; for a detailed description of the community composition see previous publications from this facility[10,21,30]. Sediments are comprised of 8-10 cm of fine sands with a developed organic layer of 1-3 cm (Table 2).

Methane flux measurements

CH_4 fluxes were measured using a static, polythene-dome chamber (0.5 L) fitted with a foam collar and butyl septum[31]. Gas samples (1 mL) were withdrawn from the chamber through the septum using a gas-tight syringe and transferred to a glass-vial (3 mL, Exetainer, Labco, UK) containing helium-purged water. A single flux measurement on each pond was made by collecting samples at five time-points over an hour just after sunrise. Samples of atmospheric air and water were also collected immediately after each flux measurement and stored in the same way. All water samples were fixed with mercuric chloride (50µL saturated solution) and stored at 4°C before analysis. Methane in the headspace of vials was measured by Gas Chromatography with a Flame Ionization Detector (GC-FID, Agilent Technologies, UK)[32]. The GC was calibrated using 0 and 100 ppm span gases. The concentration of methane in the sample headspace was converted to *in situ* concentration using the solubility coefficient of methane in water at analytical temperature[33]. CH_4 efflux (µmol m^{-2} d^{-1}) was calculated using linear regression of methane concentration in the chamber headspace *vs*

time, taking the respective chamber volume and surface-area into account. In 2007-8, after one year of experimental warming, CH_4 fluxes were measured on 7 occasions over a year[11]. In 2013-14, after seven years of warming, CH_4 fluxes were measured on 12 occasions (2-6 weeks apart).

Carbon dioxide flux measurements

Carbon dioxide fluxes were measured over the annual cycle of 2013-14 using sixteen multiplexed automatic gas flux chambers (LI8100 & LI8150, Li-Cor) mounted on floating rings (Fig. S1c). Because this system is limited to analysing 16 parallel ports, we deployed gas flux chambers on 8 of the 10 heated and 8 of the 10 ambient replicate ponds and all analyses of the CO_2 flux data focus on this subset of the experiment. Acquisition frequency was 1Hz and one flux measurement was recorded from each pond hourly. The optical bench of the infra-red gas analyser was calibrated using zero and 500 ppm span-gases (Spantech, UK). Fluxes of CO_2 were estimated by linear regression of the chamber CO_2 concentration over time using standard Li-Cor software. Carbon dioxide fluxes followed a regular diel pattern of emission during darkness followed by absorption during daylight (Fig. S3). Trapezoidal integration was used to find the daily area under the efflux and influx curves, and daily net exchange was calculated as the sum of daily influx and efflux.

Ecosystem metabolism

Rates of gross primary production (GPP) and ecosystem respiration (R_{eco}) were measured over a 24h diel cycle for each replicate mesocosm on alternate months in 2007-08 and 2012-13 using the free water dissolved oxygen (DO) change technique[34]. Measurements of DO and temperature were taken every 15 minutes for 24 hours at mid-depth (0.25 m) in the water column of each pond with YSI 600XLM multi-parameter Sondes, equipped with 6562 rapid pulse™ dissolved oxygen sensors. Prior to deployment, the Sondes were calibrated in water-saturated air with a correction for barometric pressure. Calibration accuracy was verified by monitoring the DO concentration of water-saturated air for 10 minutes and checking against 100% O_2 saturation for the measured

temperature and pressure. Measurements of DO, wind speed at 1.7m (Cole-Parmer, WS-821), and light intensity (Licor, LI-193) at mid-depth in the water column were used to calculate GPP and R_{eco} following the methods outlined in Staehr *et al*[34] and using the equations given in Table S1.

Temperature

Water temperature was recorded automatically every 15 minutes by a temperature probe (TMC6-HD Air/Soil/Water sensor, Onset, USA) and data-logger installed in each pond and additionally checked on each visit to the facility with a YSI thermometer (Model 550A, YSI, USA). The temperature of the warmed ponds averaged 4.8±1.0 °C higher than that of the ambient ponds over the entire experiment.

Sediment characteristics

Sediment samples were collected in August 2013 using a 100 mm diameter corer (area 78.5 cm^2). Carbon and Nitrogen analysis of fine benthic organic matter (<250 μm particle size) was conducted using homogenized sediment that was acidified (1M HCl) to remove carbonates[35]. The carbon and nitrogen content of oven-dried (60°C) samples was measured with an elemental analyzer (Integra, Sercon UK). In-house certified reference materials were EMA P2 and Casein (Elemental Microanalysis, UK). These materials were included during each batch of samples to check for accuracy and repeatability.

Statistical analysis

Rates of CH_4 emissions, GPP and R_{eco} exhibited strong right-skew (e.g. high frequency of low rates and few high rates) and were therefore natural-logarithm transformed prior to statistical analyses and plotting. Net CO_2 fluxes were normally distributed and were therefore left untransformed. We used generalised additive mixed effects models (GAMMs) to characterise the phenology and overall treatment effects on rates of greenhouse gas emissions (CH_4 and CO_2 fluxes) and ecosystem

366 metabolism (GPP and R_{eco}), to account for the hierarchical nature of our experimental data[36]. For
367 example, our experimental design yielded replicate seasonal responses for each rate in each
368 treatment over two sampling years (2007 and 2012 [GPP and Reco] or 2013 [CH_4 and CO_2 fluxes]).
369 This hierarchical structure meant that rate measurements were non-independent – e.g.
370 measurements from the same pond and sampling year are likely to be autocorrelated. We account
371 for this by treating sampling year nested within pond as a random effect on the intercept of the
372 model which models deviations among ponds and years from the fixed effects as normally
373 distributed with a mean of zero. The most complex models included a treatment by year interaction
374 on the intercept (which characterises the median value of the response variable) and allowed the
375 shape of the seasonal phenology, which was modelled using a cubic regression spline, to vary
376 among treatments. Treatment effects on the shape and intercept of the seasonal phenology and year
377 effects on the intercept were modeled as fixed effects in the GAMMs. Model selection entailed
378 fitting a range of models to the rate data, starting with the full model and then a series of reduced
379 models with interaction terms and main effects removed to test hypotheses about the potential
380 differences in rates among treatments and sampling years. For multi-model selection we computed
381 small sample-size corrected AIC scores (AICc) and then compared between models by calculating
382 delta AICc values and AIC weights using the 'MuMIn' package. GAMMs were fitted to the data
383 using the 'gamm4' package and all statistical analyses were conducted in R (v.3.23). We calculated
384 the annual rates of greenhouse gas emissions (CH_4 and CO_2 fluxes) and ecosystem metabolism
385 (GPP and R_{eco}) for each pond on each year by exponentiation of the median natural log-transformed
386 daily rate estimated from the mixed effects model and multiplying by 365.
387
388 **Data availability**
389 The data that support the findings of this study are available from the corresponding author upon
390 reasonable request.
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393 **References**

394 31. Bastviken, D., Ejlertsson, J. & Tranvik, L. Measurement of methane oxidation in lakes: A
395 comparison of methods. *Environ. Sci. Technol.* **36,** 3354–3361 (2002).
396 32. Sanders, I. A. *et al.* Emission of methane from chalk streams has potential implications for
397 agricultural practices. *Freshwater Biology* **52,** 1176–1186 (2007).
398 33. Wilhelm, E., Battino, R. & Wilcock, R. J. Low-pressure solubility of gases in liquid water.
399 *Chem. Rev.* **77,** 219–262 (1977).
400 34. Staehr, P. A. *et al.* Lake metabolism and the diel oxygen technique: State of the science.
401 *Limnol Oceanogr-Meth* **8,** 628–644 (2010).
402 35. Hedges, J. I. & Stern, J. H. Carbon and nitrogen determinations of carbonate-containing
403 solids. *Limnol. Oceangr.* **29,** 657–663 (1984).
404 36. Zuur, A., Ieno, E., Walker, N. & Saveliev, A. *Mixed effects models and extensions in ecology*
405 *with R*. (Springer Verlag, 2009)

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