Global Change Biology

Global Change Biology (2011) **17**, 1225–1234, doi: 10.1111/j.1365-2486.2010.02289.x

Warming increases the proportion of primary production emitted as methane from freshwater mesocosms

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Abstract

Methane (CH_4) and carbon dioxide (CO_2) are the dominant gaseous end products of the remineralization of organic carbon and also the two largest contributors to the anthropogenic greenhouse effect. We investigated whether warming altered the balance of CH_4 efflux relative to gross primary production (GPP) and ecosystem respiration (ER) in a freshwater mesocosm experiment. Whole ecosystem CH_4 efflux was strongly related to temperature with an apparent activation energy of 0.85 eV. Furthermore, CH_4 efflux increased faster than ER or GPP with temperature, with all three processes having sequentially lower activation energies. Warming of 4 °C increased the fraction of GPP effluxing as CH_4 by 20% and the fraction of ER as CH_4 by 9%, in line with the offset in their respective activation energies. Because CH_4 is 21 times more potent as a greenhouse gas, relative to CO_2, these results suggest freshwater ecosystems could drive a previously unknown positive feedback between warming and the carbon cycle.

Keywords: carbon cycle, ecosystem respiration, global warming, metabolic theory, methane, primary production

Received 26 January 2010 and accepted 9 June 2010

Introduction

The two most important gaseous end products of the remineralization of organic carbon, carbon dioxide (CO_2) and methane (CH_4), are also the two largest contributors to the anthropogenic greenhouse effect (IPCC, 2007). The net emission of greenhouse carbon gases from an ecosystem is the balance between the CO_2 absorbed by the ecosystem by gross primary production (GPP) and the carbon that is respired and released as CO_2 and/or CH_4 (Whiting & Chanton, 2001). Further, the fraction of fixed carbon that is respired and released as either CO_2 or CH_4 may be decisive for future global warming, as shifts in this balance will affect the greenhouse gas efflux potential of ecosystems, because CH_4 has 21 times the radiative forcing potential of CO_2 over periods of up to 20 years (Rodhe, 1990; Lelieveld *et al*., 1991; Whiting & Chanton, 2001).

Methanogenesis in freshwater ecosystems is the result of complex and often interrelated biotic and abiotic processes (Christensen *et al*., 2003a). CH_4 is produced under strictly anaerobic conditions during organic matter mineralization but the net efflux of CH_4 from ecosystems can be considerably reduced through oxidation by methanotrophs, which can consume significant quantities of the CH_4 produced in the sediments of lakes (Kuivila *et al*., 1988) and wetlands (Bartlett & Harriss, 1991; Segers, 1998). Primary production by plants also influences CH_4 production (Joabsson *et al*., 1998, 1999; Christensen *et al*., 2003b), and this has been attributed to the co-variability of organic carbon through root exudation (Chanton *et al*., 1995), the turnover of labile carbon, and/or litter production (Joabsson *et al*., 1999; Christensen *et al*., 2003b). These lines of evidence are supported by isotopic data which have shown that a large fraction of the organic material that fuels methanogenesis in wetlands is derived from recently synthesized carbon (Chanton *et al*., 1995; Joabsson *et al*., 1999). Vascular plants can also enhance emissions of CH_4 to the atmosphere via root aerenchyma that act as conduits across zones of potential CH_4 oxidation in soils and sediments (Kelker & Chanton, 1997; King *et al*., 1998).

Clearly the mechanisms that influence CH_4 efflux are diverse, however, when all other limiting factors (e.g. substrate limitation, water-table depth) are equal, temperature, via the physiological stimulation of microbial metabolism, has been shown to exert strong control on CH_4 efflux (Schutz *et al*., 1990; Christensen *et al*., 2003a; Gedney *et al*., 2004). Recently, significant focus has been given to the temperature dependence of the biotic components of the carbon cycle, and how the 'metabolic

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balance' of ecosystems, that is the balance between the gross sequestration and release of CO_2, may respond to future global warming (Allen *et al*., 2005; Lopez-Urrutia *et al*., 2006; Yvon-Durocher *et al*., 2010). Recent evidence suggests that autotrophic and heterotrophic metabolisms (e.g. photosynthesis and respiration) have different temperature dependencies (or activation energies when depicted in an Arrhenius plot) at the ecosystem level, such that respiration increases more rapidly with temperature than does photosynthesis (Allen *et al*., 2005; Lopez-Urrutia *et al*., 2006; Yvon-Durocher *et al*., 2010). We have recently shown in an aquatic mesocosm experiment, that the differential temperature dependence of these two processes reduced the ability of warmed systems to sequester CO_2 because more of the carbon fixed by primary production was respired (Yvon-Durocher *et al*., 2010). The greenhouse gas efflux potential of aquatic ecosystems is further complicated by considering the balance of CH_4 efflux in relation to carbon sequestration and CO_2 emission.

A substantial body of work over the last two decades has established the strong temperature dependence of methanogenesis in a wide range of ecosystems (i.e. from landfill sites to high latitude wetlands) and from pure cultures of methanogens to whole ecosystem-level production (Schutz *et al*., 1989; Westermann *et al*., 1989; Conrad & Wetter, 1990; Schutz *et al*., 1990; Walter & Heimann, 2000; Christensen *et al*., 2003a; Gedney *et al*., 2004). Studies of the temperature dependence of methanogenesis in pure cultures (under optimal conditions) have revealed that its activation energy is typically higher than for other forms of metabolism, due to the relatively large entropy change of the reaction (Westermann *et al*., 1989; Conrad & Wetter, 1990; Segers, 1998). Methanogenesis and potentially CH_4 efflux may, therefore, be especially sensitive to increases in temperature which raises a number of important unanswered questions. First, does the temperature dependence of CH_4 efflux at the ecosystem scale differ from that of GPP and ecosystem respiration (ER)? Second, how will the balance between carbon sequestration, ER and CH_4 efflux respond to warming?

Although our knowledge of CH_4 efflux and its regulation by temperature is extensive, it is largely based on seasonal field surveys (e.g. in wetlands, soils, lakes) and laboratory experiments (e.g. with peat monoliths and rice paddy-soil incubations) which cannot fully address these unanswered questions. We sort to extend this knowledge using a controlled freshwater mesocosm experiment where we compared the efflux of CH_4 with rates of GPP and ER in replicated mesocosms maintained at either ambient temperature or $\sim 4\,^{\circ}C$ above ambient, in line with warming scenarios predicted for temperate latitudes by the end of the 21st century (IPCC, 2007).

Materials and methods

Experimental design

The experiment was carried out between December 2005 and April 2008 at the Freshwater Biological Association River Laboratory (2°10′W, 50°13′N) East Stoke, Dorset, UK. The experiment consisted of 20 freshwater mesocosms ($\sim 1\,m^3$, 0.5 m water depth): 10 replicates remained at ambient temperature, while the other 10 were maintained at 3–5 °C (mean 4 °C) above ambient. Warming was achieved by an electronic heating element at the base of the mesocosm connected to a thermocouple which monitored the temperature in a given heated and unheated treatment pair. Treatments were arranged in five blocks of four mesocosms such that each block contained two replicates of each treatment. The mesocosms were seeded in December 2005 with organic substrates and a suite of organisms [species list in Yvon-Durocher *et al*. (2010)] to mimic the organismal composition and physical structure of shallow lake ecosystems [e.g., after McKee *et al*. (2003)]. The submerged macrophytes *Elodea canadensis* Michaux, *Myriophyllum spicatum* L. and *Ceratophyllum spicatum* L. were added to each pond in equal quantities (250 g wet weight) and *Chara contraria* A. Braun ex Kutz colonized all 20 ponds during the experiment. The biota was left to establish for 10 months before experimental warming, which commenced in September 2006.

Dissolved CH_4

The concentration of dissolved CH_4 was measured by removing a water sample (30 mL in a gas-tight syringe) and gently transferring it to a gas tight vial (12.5 mL Exetainers, Labco, High Wycombe, UK), allowing it to overflow, fixing it with a bactericide (100 μL 50% w/v $ZnCl_2$) and sealing it. Samples were collected at hourly time intervals (in total 6–10 h depending on the time of year) over a day for each replicate on alternate months for 1 year (April 2007–April 2008, $n = 1416$ individual measurements). Upon return to the laboratory, a headspace (2 mL analytical grade helium) was introduced to the gas tight vial and the sample was shaken vigorously for 0.5 min and then allowed to stand for a further 30 min to allow for headspace equilibration, before analysis of the headspace concentration of CH_4 using a gas chromatograph after (Sanders *et al*., 2007). Samples (50 μL) were withdrawn from the headspace of the sample vials and injected into a gas-chromatograph fitted with a flame ionizing detector (GC/FID; Agilent Technologies, UK). Headspace concentrations of CH_4 were calculated from peak areas calibrated against known standards (Scientific and Technical gases, Staffs, UK) and the total amount of CH_4 in the gas tight vial (water plus headspace) was calculated using the appropriate solubility coefficients (see S1 in Supporting Information and Yamamoto *et al*., 1976). Finally, the 1416 individual measurements were pooled

in each case to give an average daily pool of dissolved CH_4 for each pond (140 measures over the year for 70 heated and 70 ambient).

CH_4 efflux

Measurements of the efflux of CH_4 were made simultaneously to those of dissolved CH_4. A single gas chamber was positioned at the water surface of each mesocosm on each sampling occasion. The chambers were made of polycarbonate and enclosed a headspace (300 mL) of ambient air at the air–water interface of the mesocosm (see Figure S1 in Supporting Information). The lid of the gas chamber was equipped with a Teflon septum port, through which samples of gas (1 mL) were removed using a gas-tight syringe (2 mL VICI gas tight syringe) every 15 min for the first hour of the incubation, then hourly for up to 10 h thereafter. The samples were then transferred to water filled gas tight vials (3 mL, Exetainers; Labco, High Wycombe, UK) through a two way valve with venting through a narrow bore needle. The gas-tight vials were then stored upside down before analysis.

The concentration of CH_4 in the headspace of the sample was determined by gas chromatography as described above. The efflux of CH_4 across the water–air interface was calculated by regression analysis of the change in concentration of CH_4 in the chamber headspace over time. Subsequently, 1 h was used as an appropriate duration for accurately estimating the flux of CH_4 (Lambert & Frechette, 2005) (see Figure S2 in Supporting Information). As such, only data from the first hour of the incubation were used to estimate the efflux of CH_4 and we made a total of 140 measurements (70 heated and 70 ambient) over the annual cycle. Regression slopes with a significance of $P > 0.05$ and/or an R-squared of below 0.9 were considered non-significant and were excluded from further analyses (9 from the 140 individual flux measurements).

Determination of GPP and ER

GPP and ER were estimated simultaneously with the measurements of dissolved CH_4 and CH_4 efflux, by applying the well established single station dissolved oxygen (DO) change technique (Odum, 1956; Marzolf *et al*., 1994; Mulholland *et al*., 2001). This technique assumes that changes in DO concentration over a diel cycle represent the metabolic activity (photosynthetic and respiratory) of an aquatic ecosystem. YSI 600XLM multiparameter sondes equipped with 6562 rapid pulse™ DO sensors were deployed for 24 h in each heated and unheated treatment pair on each of the seven sampling occasions over the year. Measurements of DO and temperature were taken every 15 min for 24 h at the mid depth (0.25 m) in the water column of each pond. The daylight and nighttime analysis periods were delimited as follows: the total analysis period was defined from the minimum O_2 concentration on the first night and extended for 24 h to include the minimum O_2 concentration on the second night. Photosynthetic dawn was identified as the minimum O_2 concentration after which all subsequent values were greater than it. Photosynthetic dusk was defined as the maximum O_2 concentration after which all subsequent values were lower (Mulholland *et al*., 2001; Bales & Nardi, 2007). The change in dissolved oxygen (ΔDO) over each 15 min time interval was calculated as the difference in O_2 concentration between t_1 and t_2 (i.e., $t_2 - t_1$). Each ΔDO value was then assigned to a day or nighttime category. The metabolic parameters GPP and ER were calculated, in turn, by numerical integration according to:

$$GPP = \sum \Delta O_{2\mathrm{day}} + R_{\mathrm{day}}, \quad (1)$$

where R_{day} is day time respiration. Since it is impossible to measure R_{day} directly it was estimated, in keeping with the literature, by extrapolating the mean nighttime respiration value across the hours of daylight (Cole *et al*., 2000). Current biogeochemical techniques cannot discriminate between autotrophic and heterotrophic respiration at the ecosystem level (Mulholland *et al*., 2001) and preclude the estimation of photorespiration (Marzolf *et al*., 1994). Our measures of GPP using the DO change technique may, therefore, be slightly overestimated given the inclusion of heterotrophic respiration in calculation of R_{day}. ER was calculated as:

$$ER = R_{\mathrm{day}} + \sum \Delta O_{2\mathrm{night}}. \quad (2)$$

Estimated gas transfer velocity

Any systematic variability in the rate of gas transfer between the surface water and the atmosphere due to physical forcing (e.g. advection) induced by the experimental treatment (e.g. warming) may have biased the interpretation of our results.

The rate of exchange of a gas between the surface water and the atmosphere is dependent on two principal parameters: the concentration gradient of the gas between the water and the atmosphere, and the gas transfer velocity, k. The gas transfer velocity is frequently modelled as a function of wind speed, which controls the rate of gas transfer by determining turbulence in the surface water (Cole & Caraco, 1998). When wind speed drops below about 3 m s^{-1}, however, as was the case in >97% of our measurements (Yvon-Durocher *et al*., 2010), gas transfer becomes independent of wind speed, though it can remain substantial being governed by other physical (advective) processes besides wind (Cole & Caraco, 1998). To determine whether our experimental warming systematically altered the gas transfer velocity we estimated k from our measurements of the efflux of CH_4 and dissolved CH_4 from:

$$k = f/(C_{\mathrm{water}} - C_{\mathrm{eq}}), \quad (3)$$

where f is the measured efflux of CH_4 across the air–water interface, C_{water}–C_{eq} is the concentration gradient of the gas in the water and the concentration in the water at equilibrium with the atmosphere (C_{eq}). C_{eq} was calculated using the equations of Yamamoto *et al*. (1976) (see S1) and the measured mixing ratio for CH_4 in the air and temperature of the water on each occasion.

Statistical analyses

All data were checked for normality using the Shapiro Wilks test for normality and were natural log transformed before statistical analysis where necessary. The activation energy of any metabolism is given by the slope of the relationship of an Arrhenius plot between ln(x flux) and $1/kT$, where k is Boltzmann's constant and T is absolute temperature (K). The activation energy of ln(CH_4 efflux), ln(GPP) and ln(ER) was determined by ANCOVA to derive the most parsimonious model. For example, this was achieved by fitting the most complex model (i.e. different slopes for each treatment and sampling occasion) to test for statistical differences in the slopes of each of these relationships between treatments and sampling occasions, subsequently, nonsignificant terms were deleted to give the best model to describe the data. Model comparison was carried out using the Akaike Information Criterion (AIC). In the ANCOVA, temperature was delimited as a continuous variable and defined as $1/kT$. To account for temporal pseudoreplication in the statistical model pond identity ($n = 131$) was nested within sampling occasion. ANCOVA computations were carried out in R statistical software (R. Development. Core Team, 2006).

Between treatment differences in the overall mean annual values of CH_4 efflux, dissolved CH_4 pool, k gas transfer, CH_4 efflux/GPP and CH_4 efflux/ER were analysed with restricted maximum likelihood methods using the *lme* (linear mixed-effects model) function in R (R. Development. Core Team, 2006). In the model, treatment (heated or unheated) was treated as the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms seasonally over the year was accounted for by including mesocosm identity nested within sampling occasion as random effects. The repeated measures model was used to test for overall statistical differences between treatments in mean annual values of the above parameters.

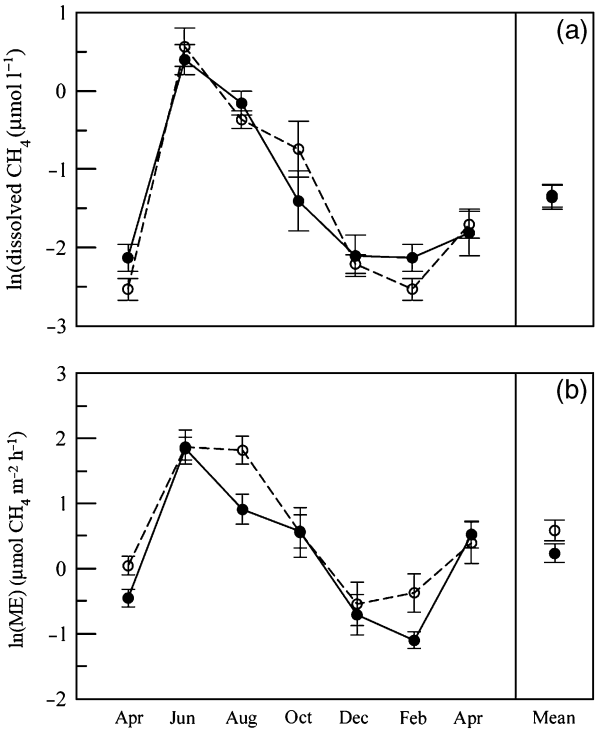

Fig. 1 (a) Differences in the pool of dissolved methane [ln(CH_4)] (± SE) between heated (dashed lines, open cricles) and unheated treatments (solid lines, filled circles). The pool of dissolved methane exhibited strong seasonal trends which were identical between treatments. Furthermore, the average annual pool of dissolved methane was identical between treatments (Table 1). (b) Differences in methane efflux, ln(ME) (± SE) between heated and unheated treatments. Methane efflux showed a strong seasonal pattern and was elevated, on average, over the annual cycle in warmed treatments reflecting its strong temperature dependence (Table 1).

Results

The concentration of CH_4 exhibited clear and near identical seasonal trends in the water of both the heated and ambient mesocosms and, on average, over the year, was not significantly different between treatments (Fig. 1a and Table 1). The concentration of CH_4 ranged from 0.04 to 6.8 μmol L^{-1}, though the distribution of CH_4 concentration exhibited strong positive skew (Shapiro Wilks test; $W = 0.63$; $P < 0.005$), such that 75% of all measurements were less than 0.66 μmol L^{-1} over the seasonal cycle. Using the 75th percentile for dissolved CH_4 as a conservative estimate, and the average concentration of CH_4 that would be at equilibrium with the atmosphere ($\sim 3.55 \times 10^{-3}$ μmol L^{-1}), we estimated that the mesocosms were about 128 times supersaturated with respect to the atmosphere.

Similarly, the rate of CH_4 efflux showed strong seasonal trends with peaks in early summer and lowest rates in winter (Fig. 1b) and was strongly positively

Table 1 Linear mixed effects model analysis

Variable	df	F-ratio	P-Value
ln(CH_4 Pool)	1123	0.0001	0.992 (ns)
ln(CH_4 efflux)	1123	6.22	**0.014**
ln (k)	1123	3.46	0.068 (ns)
ln(CH_4 efflux)/ln(GPP)	1123	6.33	**0.013**
ln(CH_4 efflux)/ln(ER)	1123	6.23	**0.0139**

Analysing differences between heated and unheated treatments in the annual means of the dissolve methane pool [ln(CH4 Pool)], methane efflux [ln(CH4 efflux)], gas transfer velocity [ln(k)], the ratio of methane efflux to GPP [ln(CH4 efflux)/ln(GPP)], and the ratio of methane efflux to ER [ln(CH4 efflux)/ln(ER)].
Significant P-values are given in bold.

correlated ($r = 0.94$; $P < 0.005$) with the concentration of CH_4 in the water column. The rate of CH_4 efflux ranged from 0.35 to 7.02 μmol $m^{-2} h^{-1}$ in the ambient mesocosms and from 0.96 to 8.14 μmol $m^{-2} h^{-1}$ in the warmed mesocosms. The distribution of CH_4 efflux between heated and ambient mesocosms was also strongly positively skewed (Shapiro Wilks test; $W = 0.74$; $P < 0.005$), with 75% of measurements falling below 3.6 μmol $m^{-2} h^{-1}$. Furthermore, the rate of CH_4 efflux was elevated in the warmed mesocosms over parts of the seasonal cycle (April, August and February), and, on average, the mean annual rate of CH_4 efflux was significantly greater in the warmed mesocosms (Table 1). The gas transfer velocity, k, exhibited no clear seasonal variability (Fig. 2) and was not significantly different between treatments (Fig. 2 and Table 1).

The rate of CH_4 efflux was strongly related to temperature ($1/kT$) (Table 2 and Fig. 3), with an apparent activation energy in the order of 0.85 eV (95% confidence interval: 0.64–1.02 eV). In addition, CH_4 efflux was also related to GPP, though much more weakly (Table 2 and Fig. 4), with temperature explaining 43% of the variance in rate of CH_4 efflux, while GPP explained only 23% (Figs 3 and 4).

GPP and ER were also strongly related to temperature, as has been described previously (Yvon-Durocher *et al.*, 2010), with apparent activation energies in the order of 0.45 eV (95% confidence interval 0.38–0.53 eV) and 0.62 eV (95% confidence interval 0.55–0.69 eV) for each, respectively. Importantly, here, the temperature dependence of CH_4 efflux was significantly higher than that for either GPP or ER (Table 2) and, correspondingly,

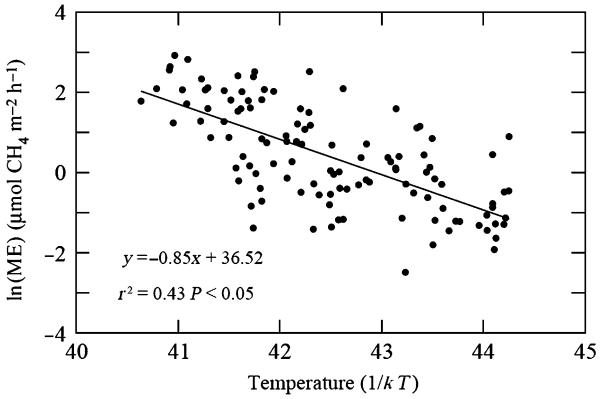

Fig. 3 Temperature dependence of whole ecosystem methane efflux. The slope of the temperature response of methane efflux in our experiment was equivalent to the activation energy of methanogenesis. Each data point corresponds to the CH_4 efflux from a single mesocosm on each of the seven sampling occasions ($n = 131$). There were no significant differences in the slopes of the temperature dependences of CH_4 efflux between heated and unheated mesocosms nor any effects due to repeatedly sampling individual ponds (Table 2): this facilitated the use of a single model with a common slope to characterize the activation energy.

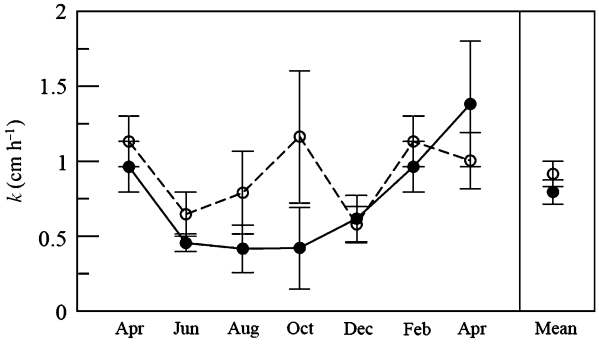

Fig. 2 Seasonal trends in the gas transfer velocity (k), between heated (dashed lines, open circles) and unheated treatments (solid lines, filled circles). The gas transfer exhibited little seasonal variability and was not systematically affected by the heating of the mesocosms (Table 1). Data were natural log transformed for statistical analysis but presented here untransformed for ease of interpretation.

Table 2 Analysis of Co-Variance table for the relationships between ln(CH_4 efflux), ln(GPP) and ln(ER) vs. $1/kT$

Relationship	df	F-ratio	P-Value
ln(CH_4 efflux) vs. $1/kT$	1127	97.98	**< 0.0001**
Difference in slope of ln(CH_4 efflux) vs. $1/kT$ between treatments	1127	1.23	0.23
Difference in intercept of ln(CH_4 efflux) vs. $1/kT$ between treatments	1127	2.29	0.132
ln(CH_4 efflux) vs. ln(GPP)	1127	38.61	**< 0.0001**
Difference in intercept of ln(CH_4 efflux) vs. ln(GPP) between treatments	1127	1.28	0.26
Difference in slope of ln(CH_4 efflux) vs. ln(GPP) between treatments	1127	0.38	0.54
Difference in slope between ln(CH_4 efflux) vs. $1/kT$ and ln(GPP) vs. $1/kT$	1258	21.61	**< 0.0001**
Difference in slope between ln(CH_4 efflux) vs. $1/kT$ and ln(ER) vs. $1/kT$	1258	8.36	**0.0042**

Significant P-values are given in bold.

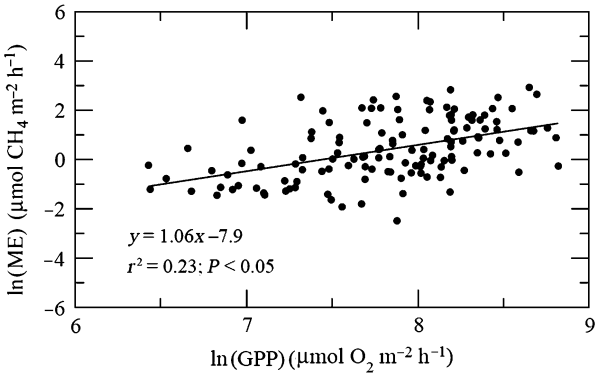

Fig. 4 Positive correlation between methane efflux [ln(ME)] and gross primary production and [ln(GPP)]. Each data point corresponds to the CH_4 efflux and GPP of a single mesocosm on each of the seven sampling occasions ($n = 131$). There were no significant differences in the slope or intercepts of the relationship between ln(ME) vs. ln(GPP) between heated and unheated mesocosms nor any effects due to repeatedly sampling individual ponds (Table 2), facilitating the use of a single model to characterize the relationship.

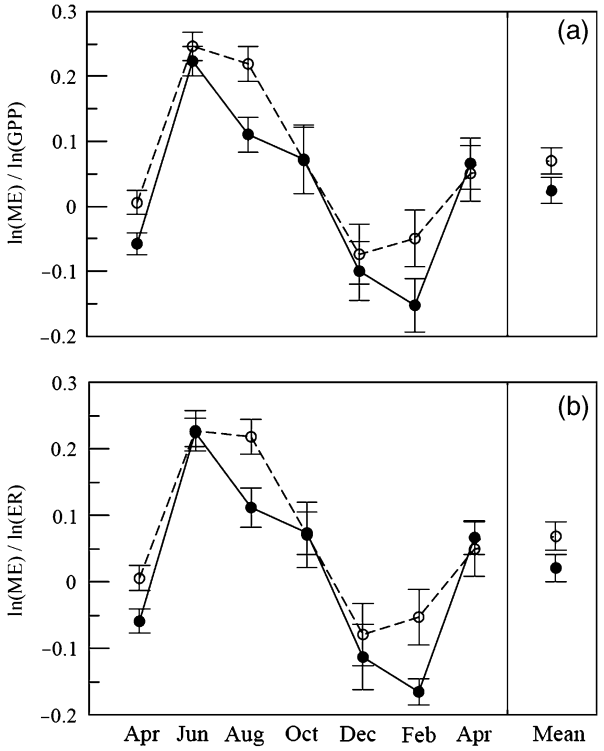

Fig. 5 (a) Differences in the ratio of methane efflux to GPP [ln(ME)/ln(GPP)] (± SE) and (b) methane efflux to ER ln(ME)/ln(ER) (± SE) between heated (dashed lines, open circles) and unheated treatments (solid lines, filled circles). Both ln(ME)/ln(GPP), and ln(ME)/ln(ER), were elevated, on average over the annual cycle in the warmed treatments. The magnitude of the increase in ln(ME)/ln(GPP) and ln(ME)/ln(ER) reflected the differences in activation energies of these three metabolic processes. Correspondingly, the fraction of GPP respired via the methanogenic pathway increased by 20% in the warmed mesocosms. Furthermore, the fraction of ER due to methanogenesis was 9% greater in the heated treatment.

CH_4 efflux increased more rapidly in response to warming than did either GPP or ER.

The balance between carbon absorption and CH_4 emission is given by the ratio of CH_4 efflux to GPP, which was found to be significantly elevated in the heated mesocosms, on average, over the year (Table 1 and Fig. 5a). The mean annual ratio of CH_4 efflux to GPP was elevated by 20% in response to the $\sim 4\,^{\circ}C$ experimental warming. Similarly, the ratio of CH_4 efflux to ER was significantly elevated in the heated mesocosms, on average, over the annual cycle (Table 1 and Fig. 5b), with warming elevating the mean annual ratio of CH_4 efflux to ER by 9%.

Discussion

Mesocosm experiments represent a compromise between the control and replication of laboratory studies and the realism of descriptive field surveys but, despite their limitations, can provide a fundamental tool for predicting how global change scenarios might affect ecosystem level processes (Benton *et al.*, 2007). Our measured rates of mesocosm CH_4 efflux (0.35–8.14 $\mu mol\, CH_4\, m^{-2}\, h^{-1}$) were comparable to those measured in natural shallow lakes (Rudd & Hamilton, 1978; Bastviken *et al.*, 2004), suggesting that our experimental scale was sufficient enough to reproduce some of the complex components of the biogeochemical cycling of carbon observed in natural ecosystems.

Experimental warming might have artificially stimulated advective processes resulting in elevated gas transfer at the air-water interface, and a detailed consideration of this potential artefact is fundamental before interpretation of our results. Our estimates of the gas transfer velocity, based on detailed measurement of dissolved CH_4 and rates of CH_4 efflux, however, suggest that this was not the case. If the gas transfer velocity was systematically enhanced by artificial warming, we would have expected to observe considerable differences in the concentration of dissolved CH_4 between treatments, but, again, this was not the case. At first glance, the consistency in both the pool size of dissolved CH_4 and the estimated gas transfer velocity between treatments appears at odds with the elevated efflux of CH_4 measured in the warmed mesocosms. According to Eqn (3), the gas transfer velocity and the concentration gradient (i.e. between the water and the atmosphere) drive the efflux of gas across the air–water

interface (Cole & Caraco, 1998). This discrepancy can be explained when the relative magnitudes of the respective processes and pool sizes are taken into consideration. Using the 75th percentiles for both CH_4 efflux and dissolved CH_4 (scaled to a whole mesocosm) of 11 and 662 μmol CH_4 mesocosm^{-1}, respectively, 75% of our measurements of the efflux of CH_4 represented $<1.7\%$ of the total pool of dissolved CH_4. The subtle differences detected in the efflux of CH_4 between treatments would have been masked when analysing for treatment effects at the level of the pool, because the overall magnitude of the pool size of dissolved CH_4 was vast compared with the efflux. Therefore, any error associated with the measurement of the CH_4 pool would likely overwhelm the detection of any subtle statistical differences between treatments. This evidence suggests that the physical influence of heating the mesocosms by $\sim 4\,^{\circ}C$ had little discernable effect on advective processes. We can therefore be confident that the biogeochemical patterns revealed by our experiment are due to the biological consequences of warming on aquatic communities.

Our experimental results have implications for understanding the mechanisms controlling CH_4 efflux from freshwater ecosystems, and how CH_4 dynamics in relation to carbon sequestration rates might be affected by future global warming. Our experiment revealed that temperature was the dominant driver of CH_4 efflux from our mesocosms. This result agrees with other studies from a range of natural ecosystems, from soils to wetlands, which also highlight the strong temperature dependence of CH_4 efflux (Schutz *et al*., 1990; Whiting & Chanton, 2001; Christensen *et al*., 2003a; Gedney *et al*., 2004). Because of the overriding influence of temperature, the overall rates of CH_4 efflux were consistently elevated in the warmed mesocosms relative to ambient over the course of our annual study, presumably reflecting the strong physiological response to the temperature stimulation of methanogenesis.

To ascertain whether the strong temperature dependence of CH_4 efflux we observed experimentally was due to the physiological stimulation of methanogenesis, we determined the activation energy of methanogenesis in pure cultures at a range of temperatures, and under non-limiting conditions, using previously published data (see S5 in Supporting Information for details of analysis and data collection). Our reanalysis of these data does indeed show that the production of CH_4 in pure cultures has an equally strong temperature dependence (Fig. 6). Interestingly, the temperature dependence of CH_4 efflux at the ecosystem level (E_a = 0.85 eV, 95% confidence interval: 0.64–1.02 eV) was indistinguishable from that for methanogenesis in pure culture (mean $E_a = 0.88$ eV 95% confidence interval: 0.80–0.96 eV). This coherence between organizational scales suggests that much of the potential complexity associated with ecosystem level efflux of CH_4 might be reduced to the first principals of individual/cellular kinetics. This result suggests that whole ecosystem metabolic fluxes can be scaled from the individual to the ecosystem level, in line with predictions derived from the 'metabolic theory of ecology' (Enquist *et al*., 2003; Allen *et al*., 2005; Lopez-Urrutia *et al*., 2006). Our study, therefore, contributes to the growing body of evidence which suggests that metabolism is a fundamental driver of the dynamics of ecological processes across multiple levels of organization, by demonstrating that CH_4 efflux at the ecosystem level appears to be constrained by the activation energy of methanogenesis. Models derived from the metabolic theory of ecology might therefore provide additional insight into the dynamics and temperature response of whole ecosystem CH_4 efflux in aquatic ecosystems.

As well as temperature, primary production has been shown to regulate the efflux of CH_4 to the atmosphere. Such regulation stems from the whole autotrophic assemblage providing structural, labile carbon compounds in the form of dead biomass (Whiting & Chanton, 1993), and vascular plants producing root exudates in the form of organic acids (Chanton *et al*., 1995; Joabssonm & Christensen, 2001; Christensen *et al*., 2003b). Furthermore, rooted aquatic vascular plants can act as conduits for the transport of CH_4 from the anaerobic zone of the sediment to the atmosphere, bypassing the zones of potential CH_4 oxidation in the sediment and water column (Joabsson *et al*., 1999; Joabssonm & Christensen, 2001). In our experiment, however, the efflux of CH_4 did not appear to be limited by substrates from GPP, as suggested by three lines of evidence. Firstly, the weak correlation and gentle slope of the relationship between the efflux of CH_4 and GPP indicated that the flux of CH_4 was relatively independent of the simultaneous rate of photosynthesis and carbon fixation. Secondly, warming had no effect on the intercept of the relationship between ln(CH_4 efflux) vs. $1/kT$ between treatments (Table 2). If organic substrates were limiting for CH_4 production and efflux, we would expect to see a lower intercept in the warmed treatments because elevated physiological rates would be expected to reduce organic substrates more rapidly, resulting in faster substrate limitation and thus a reduced intrinsic capacity for methanogenesis. Thirdly, on average, the efflux of CH_4 represented a very small fraction of GPP (mean annual value = 0.01%). If oxidation of CH_4 production is assumed to be 95% (King *et al*., 1990), from our average annual CH_4 efflux measures (3 μmol m^{-2} h^{-1}), we estimate mean annual CH_4 production to be ~ 290 μmol m^{-2} h^{-1} which would

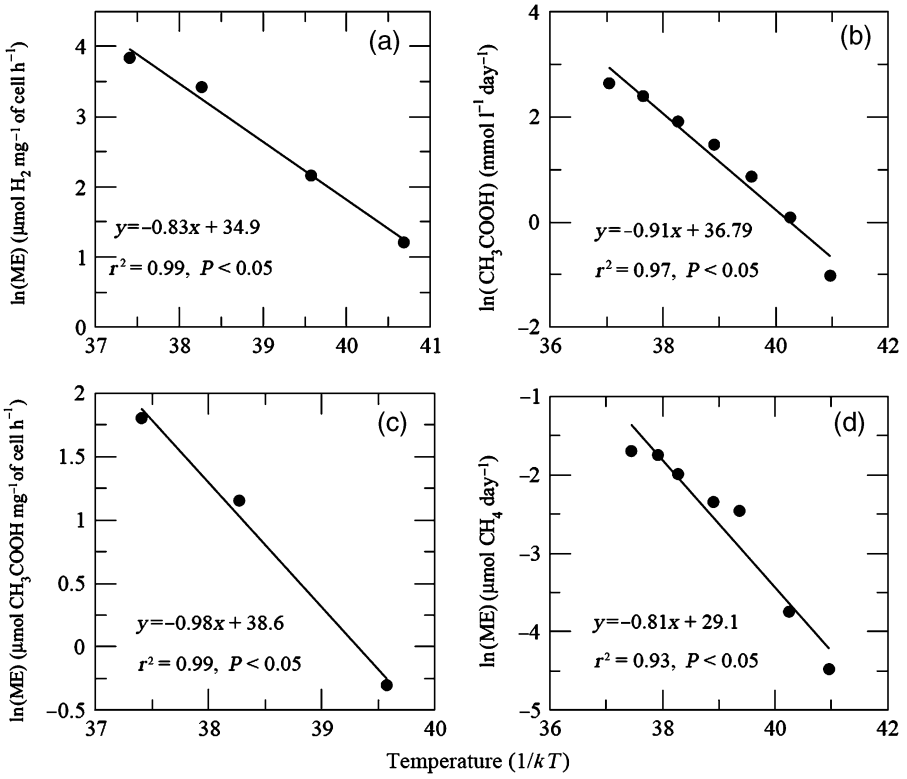

Fig. 6 Temperature dependence of methanogenesis in pure cultures of methanogens. (a) Methanogenesis of *Methanosarcina barkeri* using H_2 as a substrate; (b) methanogenesis of an enrichment culture using CH_3COOH as a substrate; (c) methanogenesis of *M. barkeri* using CH_3COOH as a substrate; and (d) methanogenesis of *Methanothrix soehngenii* using CH_3COOH as a substrate. Data in (a) and (c) were reanalysed from V_{max} values and temperatures reported in Westermann *et al*. (1989). Data for (b) were reanalysed from data on rates of CH_3COOH conversion to CH_4 in Van den Berg *et al*. (1976), and data for (c) were reanalysed from data on rates of CH_4 production in Huser *et al*. (1982). In each case, the slope of the temperature response equates to the activation energy of methanogenesis and agree very well with that derived in Fig. 3.

represent only 10% of the mean annual rate of GPP ($2862\,\mu mol\,m^{-2}\,h^{-1}$). Therefore, carbon sequestration and fixation by photosynthesis is likely to exceed the demand of methanogenesis throughout the annual cycle.

The response of the greenhouse carbon gas balance of freshwater ecosystems to warming could affect the strength of biotic feedbacks on a potentially global scale (Woodwell *et al*., 1998). In our experiment, the fraction of carbon absorbed by GPP and subsequently remineralized via the methanogenic pathway to efflux as CH_4 increased by 20% in response to the simulated global warming scenarios projected for the end of the century. In addition, the efflux of CH_4 as a proportion of ER was 9% greater in the warmed mesocosms. If, as aquatic ecosystems warm, carbon remineralization becomes increasingly dominated by methanogenesis this could result in more CH_4 being emitted to the atmosphere relative to CO_2 emission and carbon draw-down. These patterns can be explained by the differential activation energies of the three metabolic processes involved in the greenhouse carbon balance of ecosystems.

Here, and in previous research (Yvon-Durocher *et al*., 2010), it has been demonstrated that the three key ecosystem level carbon fluxes have progressively higher activation energies (i.e., GPP = 0.45 eV; ER = 0.62 eV; CH_4 efflux = 0.85 eV). The relative response of the greenhouse carbon gas balance to warming observed here might be predictable from these respective activation energies. To test this hypothesis, equations were derived from the metabolic theory of ecology (Brown *et al*., 2004) to predict the response of the mean ratio of CH_4 efflux to GPP over the year between heated and unheated treatments ($R_{\text{fixed}}H:U$) which was given by

$$R_{fixed}H:U = \frac{a_H/p_H}{a_U/p_U} = e^{\dfrac{[(E_a - E_p)(T_H - T_U)]}{kT_HT_U}}, \qquad (4)$$

where a_H and a_U are the allometric equations for the efflux of CH_4 in the heated and unheated treatments respectively, while p_H and p_U are the allometric equations for GPP in heated and unheated treatments.

These allometric equations which describe the temperature and mass dependence of ecosystem-level fluxes were derived assuming that whole ecosystem metabolic fluxes can be approximated from the sum of the individual fluxes of the organisms in the ecosystem (after, Enquist *et al*., 2003; Brown *et al*., 2004; Allen *et al*., 2005; Lopez-Urrutia *et al*., 2006). E_a and E_p are the activation energies for the efflux of CH_4 and GPP in our experiment (0.85 and 0.45 eV, respectively), T_H and T_U are the mean annual temperatures (K) for the heated and unheated treatments, respectively, and k is Boltzmann's constant (see S3 in Supporting Information for the full derivation of Eqn (4)). Eqn (4) predicts a 1.30-fold increase in $R_{\text{fixed}}H{:}U$ (range 1.18–1.38; based on the 95% confidence intervals of the respective empirically measured activation energies),which is very close to our empirically measured value of 1.20. Similarly, the response of the mean ratio for the efflux of CH_4 to ER over the year between heated and unheated treatments ($R_{\text{emitted}}H{:}U$) should be predicted by

$$\mathrm{R}_{emitted}H:U = \frac{a_H/r_H}{a_U/r_U} = e^{\dfrac{[(E_a - E_r)(T_H - T_U)]}{kT_HT_U}}, \qquad (5)$$

where E_a and E_r are the empirically determined activation energies for the efflux of CH_4 and ER (0.85 and 0.62 Ev, respectively), respectively (see S4 in Supporting Information for the full derivation of Eqn (4)). Using Eqn (5), we would expect a 1.16-fold increase in $R_{\text{emitted}}H:U$ (range 0.17–1.24; based on the 95% confidence intervals of the respective empirically measured activation energies). Our empirically measured value of $R_{\text{fixed}}H:U$ was 1.09, again very close to our prediction.

Interestingly, Eqns (4 and 5) suggest that the relative offset of the carbon balance between the ambient and warmed mesocosms can be predicted by the differences in activation energies of metabolism and the degree of expected warming. This result is important because it highlights the potential for a positive feedback between warming and the carbon cycle of freshwater ecosystems, especially given the greater radiative forcing potential of CH_4 (Rodhe, 1990; Lelieveld *et al*., 1991; Whiting & Chanton, 2001). Finally, the close coherence between the activation energy of methanogenesis in pure culture and that of whole system CH_4 efflux, suggests that much of the complexity of ecosystem level fluxes can be reduced to produce simpler predictive models.

Acknowledgements

We thank Brian Godfrey, Dan Perkins, and the Freshwater Biological Association for their help with the experiment. Andrew P Allen and Ricard Solé discussed ideas and provided comments on early drafts. G. Yvon-Durocher was supported by a Natural Environment Research Council studentship (NER/S/A2006/14029). J. Montoya was funded by the NERC Fellowship Scheme (NE/C002105/1), and a Ramon y Cajal Fellowship (RYC-2008-03664).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Diagram of the gas trap sampler used to measure whole ecosystem methane emission (ME).

Figure S2. Example of field measurements of methane efflux (ME) from mesocosm 1 (June 2007) to the atmosphere. After approximately 7 h ME reaches an asymptote (dashed line). This might be an artefact of the gas trap enclosure and artificial changes in partial pressure and/or temperature, but may also include a decline in photosynthesis and gas transfer through the aerenchyma in early afternoon as photosynthesis declines. As a result the shortest possible time (typically 1 h) to generate a significant ($P < 0.05$) flux (insert) was used for determination of flux rates.

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