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Warming Increases the Proportion of Primary Production Emitted as Methane from Freshwater Mesocosms



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Keywords:	Global Warming, Methane, Carbon Cycle, Metabolic Theory, Primary Production, Ecosystem Respiration
Abstract:	Methane and carbon dioxide are the dominant gaseous end products of the remineralisation of organic carbon and also the two largest contributors to the anthropogenic greenhouse effect. We investigated whether warming altered the balance of methane efflux relative to primary production and ecosystem respiration in a freshwater mesocosm experiment. Whole ecosystem CH ₄ efflux was strongly related to temperature with an apparent activation energy of 0.85eV. Furthermore, CH ₄ efflux increased faster than ecosystem respiration or primary production with temperature, with all three processes having sequentially lower activation energies. Warming of 4°C increased the fraction of primary production effluxing as methane by 20% and the fraction of ecosystem respiration as methane by 9%, inline with the offset in their respective activation energies. Because methane 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.

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For Review Only

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4 1 **Warming Increases the Proportion of Primary Production Emitted as**
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11 4 **Running title:** Warming Alters Methane Emissions in Freshwater Mesocosms
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17 7 **Trimmer¹**
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47 23 **Keywords:** Global Warming, Methane, Carbon Cycle, Metabolic Theory, Primary Production,
48 24 Ecosystem Respiration.
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3 28 **Abstract**
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5 29 Methane and carbon dioxide are the dominant gaseous end products of the remineralisation of
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8 30 organic carbon and also the two largest contributors to the anthropogenic greenhouse effect. We
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10 31 investigated whether warming altered the balance of methane efflux relative to primary
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12 32 production and ecosystem respiration in a freshwater mesocosm experiment. Whole ecosystem
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14 33 CH₄ efflux was strongly related to temperature with an apparent activation energy of 0.85eV.
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17 34 Furthermore, CH₄ efflux increased faster than ecosystem respiration or primary production with
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20 35 temperature, with all three processes having sequentially lower activation energies. Warming of
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22 36 4°C increased the fraction of primary production effluxing as methane by 20% and the fraction
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24 37 of ecosystem respiration as methane by 9%, inline with the offset in their respective activation
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26 38 energies. Because methane is 21 times more potent as a greenhouse gas, relative to CO₂, these
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29 39 results suggest freshwater ecosystems could drive a previously unknown positive feedback
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31 40 between warming and the carbon cycle.
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51 Introduction

52 The two most important gaseous end products of the remineralisation of organic carbon, carbon
53 dioxide (CO₂) and methane (CH₄), are also the two largest contributors to the anthropogenic
54 greenhouse effect (IPCC 2007). The net emission of greenhouse carbon gases from an ecosystem
55 is the balance between the CO₂ absorbed by the ecosystem by gross primary production (GPP)
56 and the carbon that is respired and released as CO₂ and/or CH₄ (Whiting & Chanton 2001).
57 Further, the fraction of fixed carbon that is respired and released as either CO₂ or CH₄ may be
58 decisive for future global warming, as shifts in this balance will affect the greenhouse gas efflux
59 potential of ecosystems, because CH₄ has 21 times the radiative forcing potential of CO₂ over
60 periods of up to 20 years (Rodhe 1990, Lelieveld *et al.* 1991, Whiting & Chanton 2001).

61 Methanogenesis in freshwater ecosystems is the result of complex and often interrelated
62 biotic and abiotic processes (Christensen *et al.* 2003a). Methane is produced under strictly
63 anaerobic conditions during organic matter mineralisation but the net efflux of CH₄ from
64 ecosystems can be considerably reduced through oxidation by methanotrophs, which can
65 consume significant quantities of the CH₄ produced in the sediments of lakes (Kuivila *et al.*
66 1988) and wetlands (Bartlett & Harriss 1991, Segers 1998). Primary production by plants also
67 influences CH₄ production (Joabsson *et al.* 1998, 1999, Christensen *et al.* 2003b), and this has
68 been attributed to the co-variability of organic carbon through root exudation (Chanton *et al.*
69 1995), the turnover of labile carbon, and/or litter production (Joabsson *et al.* 1999, Christensen *et al.*
70 2003b). These lines of evidence are supported by isotopic data which have shown that a large
71 fraction of the organic material that fuels methanogenesis in wetlands is derived from recently
72 synthesised carbon (Chanton *et al.* 1995, Joabsson *et al.* 1999). Vascular plants can also enhance

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3 73 emissions of CH₄ to the atmosphere via root aerenchyma that act as conduits across zones of
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5 74 potential CH₄ oxidation in soils and sediments (Kelker & Chanton 1997, King *et al.* 1998).
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8 75 Clearly the mechanisms that influence CH₄ efflux are diverse, however, when all other
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10 76 limiting factors (e.g. substrate limitation, water-table depth) are equal, temperature, via the
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12 77 physiological stimulation of microbial metabolism, has been shown to exert strong control on
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14 78 CH₄ efflux (Schutz *et al.* 1990, Christensen *et al.* 2003a, Gedney *et al.* 2004). Recently,
15
16 79 significant focus has been given to the temperature dependence of the biotic components of the
17
18 80 carbon cycle, and how the “metabolic balance” of ecosystems, that is the balance between the
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20 81 gross sequestration and release of CO₂, may respond to future global warming (Allen *et al.* 2005,
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22 82 Lopez-Urrutia *et al.* 2006, Yvon-Durocher *et al. in press*). Recent evidence suggests that
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24 83 autotrophic and heterotrophic metabolisms (e.g. photosynthesis and respiration) have different
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26 84 temperature dependencies (or activation energies when depicted in an Arrhenius plot) at the
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28 85 ecosystem level, such that respiration increases more rapidly with temperature than does
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30 86 photosynthesis (Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006, Yvon-Durocher *et al. in press*). We
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32 87 have recently shown in an aquatic mesocosm experiment, that the differential temperature
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34 88 dependence of these two processes reduced the ability of the warmed systems to sequester CO₂
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36 89 because more of the carbon fixed by primary production was respired (Yvon-Durocher *et al. in*
37
38 90 *press*). The response of the greenhouse gas efflux potential of aquatic ecosystems to warming is
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40 91 further complicated by considering the balance of CH₄ efflux in relation to carbon sequestration
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42 92 and CO₂ emission.
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50 93 A substantial body of work over the last two decades has established the strong
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52 94 temperature dependence of methanogenesis in a wide range of ecosystems (i.e. from landfill sites
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54 95 to high latitude wetlands) and from pure cultures of methanogens to whole ecosystem-level
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3 96 production (Schutz *et al.* 1989, Westermann *et al.* 1989, Conrad & Wetter 1990, Schutz *et al.*
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5 97 1990, Walter & Heimann 2000, Christensen *et al.* 2003a, Gedney *et al.* 2004). Studies of the
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7
8 98 temperature dependence of methanogenesis in pure cultures (under optimal conditions) have
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10 99 revealed that its activation energy is typically higher than for other forms of metabolism, due to
11
12 100 the relatively large entropy change of the reaction (Westermann *et al.* 1989, Conrad & Wetter
13
14 101 1990, Segers 1998). Methanogenesis and potentially CH₄ efflux may, therefore, be especially
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16 102 sensitive to increases in temperature which raises a number of important unanswered questions.
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18 103 First, does the temperature dependence of CH₄ efflux at the ecosystem scale differ from that of
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20 104 primary production and ecosystem respiration? Second, how will the balance between carbon
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22 105 sequestration, ecosystem respiration and CH₄ efflux respond to warming?
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27 106 Although our knowledge of CH₄ efflux and its regulation by temperature is extensive, it
28
29 107 is largely based on seasonal field surveys (e.g. in wetlands, soils, lakes) and laboratory
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31 108 experiments (e.g. with peat monoliths and rice paddy-soil incubations) which cannot fully
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33 109 address these unanswered questions. We sort to extend this knowledge using a controlled
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35 110 freshwater mesocosm experiment where we compared the efflux of CH₄ with rates of gross
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37 111 primary production (GPP) and whole ecosystem respiration (ER) in replicated mesocosms
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39 112 maintained at either ambient temperature or ~ 4°C above ambient, in line with warming scenarios
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41 113 predicted for temperate latitudes by the end of the 21st century (IPCC 2007).
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114 **Materials and Methods**

115 **Experimental Design**

116 The experiment was carried out between December 2005 and April 2008 at the Freshwater
117 Biological Association River Laboratory (2°10`W, 50°13`N) East Stoke, Dorset, UK. The
118 experiment consisted of twenty freshwater mesocosms (~1 m³, 0.5 m water depth): ten replicates
119 remained at ambient temperature, whilst the other ten were maintained at 3-5°C (mean 4°C)
120 above ambient. Warming was achieved by an electronic heating element at the base of the
121 mesocosm connected to a thermocouple which monitored the temperature in a given heated and
122 unheated treatment pair. Treatments were arranged in a randomised block design (5 blocks of 4
123 mesocosms) such that each block contained two replicates of each treatment. The mesocosms
124 were seeded in December 2005 with organic substrates and a suite of organisms [species list in
125 (Yvon-Durocher *et al. in press*)] to mimic the organismal composition and physical structure of
126 shallow lake ecosystems [e.g., after (McKee *et al. 2003*)]. The submerged macrophytes *Elodea*
127 *canadensis* Michaux, *Myriophyllum spicatum* L. and *Ceratophyllum spicatum* L. were added to
128 each pond in equal quantities (250g wet weight) and *Chara contraria* A. Braun ex Kutz
129 colonised all 20 ponds during the experiment. The biota was left to establish for ten months prior
130 to experimental warming, which commenced in September 2006.

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132 **Dissolved Methane**

133 The concentration of dissolved CH₄ was measured by removing a water sample (30 mL in a gas-
134 tight syringe) and gently transferring it to a gas tight vial (12.5 mL Exetainers, Labco, High
135 Wycombe, UK), allowing it to overflow, fixing it with a bactericide (100 µL 50 % w/v ZnCl₂)
136 and sealing it. Samples were collected at hourly time intervals (in total 6 to 10 hours depending

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3 137 on the time of year) over a day for each replicate on alternate months for one year (April 2007 to
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5 138 April 2008, $n = 1416$ individual measurements). Upon return to the laboratory, a headspace (2
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7 139 mL analytical grade helium) was introduced to the gas tight vial and the sample was shaken
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9 140 vigorously for 0.5 minutes and then allowed to stand for a further 30 minutes to allow for
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11 141 headspace equilibration, before analysis of the headspace concentration of CH_4 using a gas
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13 142 chromatograph after Sanders *et al.* (2007). Samples (50 μL) were withdrawn from the headspace
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15 143 of the sample vials and injected into a gas-chromatograph fitted with a flame ionising detector
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17 144 (GC/FID; Agilent Technologies, UK). Headspace concentrations of CH_4 were calculated from
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19 145 peak areas calibrated against known standards (Scientific and Technical gases, Staffs, UK) and
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21 146 the total amount of CH_4 in the gas tight vial (water plus headspace) was calculated using the
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23 147 appropriate solubility coefficients (Yamamoto *et al.* 1976). Finally, the 1416 individual
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25 148 measurements were reduced in each case to give an average daily pool of dissolved CH_4 for each
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27 149 pond (140 measures over the year for 70 heated and 70 ambient).
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36 151 **Methane Efflux**

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38 152 Measurements of the efflux of CH_4 were made simultaneously to those of dissolved CH_4 . A
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40 153 single gas chamber was positioned at the water surface of each mesocosm on each sampling
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42 154 occasion. The chambers were made of polycarbonate and enclosed a headspace (300 mL) of
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44 155 ambient air at the air-water interface of the mesocosm (see figure S1 in supporting online
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46 156 material). The lid of the gas chamber was equipped with a Teflon septum port, through which
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48 157 samples of gas (1 mL) were removed using a gas-tight syringe (2 mL VICI gas tight syringe)
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50 158 every 15 minutes for the first hour of the incubation, then hourly for up to 10 hours thereafter.
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52 159 The samples were then transferred to water filled gas tight vials (3 mL, Exetainers; Labco, High
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3 160 Wycombe, UK) through a two way valve with venting through a narrow bore needle. The gas-
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6 161 tight vials were then stored upside down prior to analysis.

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8 162 The concentration of CH₄ in the headspace of the sample was determined by gas
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10 163 chromatography as described above. The efflux of CH₄ across the water-air interface was
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12 164 calculated by regression analysis of the change in concentration of CH₄ in the chamber
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15 165 headspace over time. Subsequently, one hour was used as an appropriate duration for accurately
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17 166 estimating the flux of CH₄ (Lambert & Frechette 2005) (see figure S2 in supporting online
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20 167 material). As such, only data from the 1st hour of the incubation were used to estimate the efflux
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22 168 of CH₄ and we made a total of 140 measurements (70 heated and 70 ambient) over the annual
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25 169 cycle. Regression slopes with a significance of $P > 0.05$ and/or an R -squared of below 0.9 were
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27 170 considered non-significant and were excluded from further analyses (9 from the 140 individual
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29 171 flux measurements).

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33 34 173 **Determination of GPP and ER**

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36 174 GPP and ER were estimated simultaneously with the measurements of dissolved CH₄ and CH₄
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38 175 efflux, by applying the well established single station dissolved oxygen (DO) change technique
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40 176 (Odum 1956, Marzolf *et al.* 1994, Mulholland *et al.* 2001). This technique assumes that changes
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43 177 in DO concentration over a diel cycle represent the metabolic activity (photosynthetic and
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46 178 respiratory) of an aquatic ecosystem. YSI 600XLM multiparameter sondes equipped with 6562
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48 179 rapid pulse™ dissolved oxygen sensors were deployed for 24 hours in each heated and unheated
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50 180 treatment pair on each of the seven sampling occasions over the year. Measurements of DO and
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53 181 temperature were taken every 15 minutes for 24 hours at the mid depth (0.25m) in the water
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56 182 column of each pond. The daylight and night-time analysis periods were delimited as follows:

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3 183 the total analysis period was defined from the minimum O₂ concentration on the 1st night and
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5 184 extended for 24 hours to include the minimum O₂ concentration on the 2nd night. Photosynthetic
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8 185 dawn was identified as the minimum O₂ concentration after which all subsequent values were
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10 186 greater than it. Photosynthetic dusk was defined as the maximum O₂ concentration after which
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12 187 all subsequent values were lower (Mulholland *et al.* 2001, Bales 2007). The change in dissolved
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14 188 oxygen (ΔDO) over each 15 minute time interval was calculated as the difference in O₂
15
16 189 concentration between t_1 and t_2 (i.e., $t_2 - t_1$). Each ΔDO value was then assigned to a day or night-
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18 190 time category. The metabolic parameters GPP and ER were calculated, in turn, by numerical
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20 191 integration according to:

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

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27 193 where R_{day} is day-time respiration. Since it is impossible to measure R_{day} directly it was
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29 194 estimated, in keeping with the literature, by extrapolating the mean night time respiration value
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31 195 across the hours of daylight (Cole *et al.* 2000). Current biogeochemical techniques cannot
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33 196 discriminate between autotrophic and heterotrophic respiration at the ecosystem level
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35 197 (Mulholland *et al.* 2001) and preclude the estimation of photorespiration (Marzolf *et al.* 1994).
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37 198 Our measures of GPP using the DO change technique may, therefore, be slightly overestimated
38
39 199 given the inclusion of heterotrophic respiration in calculation of R_{day} . ER was calculated as:

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

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202 **Estimated Gas Transfer Velocity**

50 203 Any systematic variability in the rate of gas transfer between the surface water and the
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52 204 atmosphere due to physical forcing (e.g. advection) induced by the experimental treatment (e.g.
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54 205 warming) may have biased the interpretation of our results.
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3 206 The rate of exchange of a gas between the surface water and the atmosphere is dependent
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6 207 on two principal parameters: the concentration gradient of the gas between the water and the
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8 208 atmosphere, and the gas transfer velocity, k . The gas transfer velocity is frequently modelled as a
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11 209 function of wind speed, which controls the rate of gas transfer by determining turbulence in the
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13 210 surface water (Cole & Caraco 1998). When wind speed drops below about 3 m s^{-1} , however, as
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15 211 was the case in > 97 % of our measurements (*see Yvon-Durocher et al. in press*), gas transfer
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17 212 becomes independent of wind speed, though it can remain substantial by being governed by
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20 213 other physical (advective) processes besides wind (Cole & Caraco 1998). To determine whether
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22 214 our experimental warming systematically altered the gas transfer velocity we estimated k from
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24 215 our measurements of the efflux of CH_4 and dissolved CH_4 from:

$$27 \quad 216 \quad k = f / (C_{\text{water}} - C_{\text{eq}}) \quad (3)$$

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30 217 where f is the measured efflux of CH_4 across the air-water interface, $C_{\text{water}} - C_{\text{eq}}$ is the
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32 218 concentration gradient of the gas in the water and the concentration in the water at equilibrium
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34 219 with the atmosphere (C_{eq}). C_{eq} was calculated using the equations of Yamamoto *et al.* (1976) and
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36 220 the measured mixing ratio for CH_4 in the air and temperature of the water on each occasion.
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41 222 **Statistical Analyses**

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44 223 All data were checked for normality using the Shapiro Wilks test for normality and were natural
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46 224 log transformed prior to statistical analysis where necessary. The activation energy of any
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48 225 metabolism is given by the slope of the relationship of an Arrhenius plot between $\ln(x \text{ flux})$ and
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50 226 $1/kT$, where k is Boltzmann's constant and T is absolute temperature (K). The temperature
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52 227 dependence of $\ln(\text{CH}_4 \text{ efflux})$, $\ln(\text{GPP})$ and $\ln(\text{ER})$ (i.e. the activation energy) was determined by
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54 228 ANCOVA. Furthermore, we used ANCOVA to test for statistical differences in the slopes and
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3 229 intercepts of these relationships between treatments and months (i.e. $n = 7$ sampling occasions),
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6 230 to identify the most parsimonious model for determining the activation energy (i.e. the
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8 231 temperature dependence). Model comparison was carried out using the Akaike Information
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10 232 Criterion (AIC). In the ANCOVA, temperature was delimited as a continuous variable and
11
12 233 defined as $1/kT$. To account for temporal pseudo-replication in the statistical model pond identity
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15 234 ($n = 131$) was nested within sampling occasion. ANCOVA computations were carried out in R
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17 235 statistical software (R. Development. Core. 2006).

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20 236 Categorical analyses (treating temperature as a fixed factor i.e. heated or unheated) of
21
22 237 CH_4 efflux, dissolved CH_4 pool, k gas transfer, CH_4 efflux /GPP and CH_4 efflux /ER was
23
24 238 conducted with restricted maximum likelihood methods using the *lme* (linear mixed-effects
25
26
27 239 model) function in R (R. Development. Core. 2006). In the model treatment (heated or unheated)
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29 240 was the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms
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31 241 over the year was accounted for by including mesocosm identity nested with sampling occasion
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34 242 as random effects. The repeated measures model was used to test for overall statistical
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36 243 differences between treatments in mean annual values of the above parameters.
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244 **Results**

245 The concentration of CH₄ exhibited clear and near identical seasonal trends in the water of both
246 the heated and ambient mesocosms and, on average, over the year, was not significantly different
247 between treatments (Fig. 1a and Table 1). The concentration of CH₄ ranged from 0.04 μmol L⁻¹
248 to 6.8 μmol L⁻¹, though the distribution of CH₄ concentration exhibited strong positive skew
249 (Shapiro Wilks test; $W = 0.63$; $P < 0.005$), such that 75% of all measurements were less than
250 0.66 μmol L⁻¹ over the seasonal cycle. Using the 75th percentile for dissolved CH₄ as a
251 conservative estimate, and the average concentration of CH₄ that would be at equilibrium with
252 the atmosphere ($\sim 3.55 \times 10^{-3}$ μmol L⁻¹), we estimated that the mesocosms were about 128 times
253 supersaturated with respect to CH₄ and the atmosphere.

254 Similarly, the rate of CH₄ efflux showed strong seasonal trends with peaks in early
255 summer and lowest rates in winter (Fig. 1b) and was strongly positively correlated ($r = 0.94$;
256 $P < 0.005$) with the concentration of CH₄ in the water column. The rate of CH₄ efflux ranged from
257 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
258 warmed mesocosms. The distribution of CH₄ efflux between heated and ambient mesocosms was
259 also strongly positively skewed (Shapiro Wilks test; $W = 0.74$; $P < 0.005$), with 75% of
260 measurements falling below 3.6 μmol m⁻² h⁻¹. Furthermore, the rate of CH₄ efflux was elevated
261 in the warmed mesocosms over parts of the seasonal cycle (April, August and February), and, on
262 average, the mean annual rate of CH₄ efflux was significantly greater in the warmed mesocosms
263 (Table 1). The gas transfer velocity, k , exhibited no clear seasonal variability (Fig. 2) and was not
264 significantly different between treatments (Fig. 2 and Table 1).

265 The rate of CH₄ efflux was strongly related to temperature ($1/kT$) (Table 2 and Fig. 3),
266 with an apparent activation energy in the order of 0.85 eV (95% confidence interval: 0.64 to 1.02

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3 267 eV). In addition, CH₄ efflux was also related to GPP, though much more weakly (Table 2 and
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5 268 Fig. 4), with temperature explaining 43% of the variance in rate of CH₄ efflux, while GPP
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8 269 explained only 23% (Fig. 2 and Fig. 4).
9

10 GPP and ER were also strongly related to temperature, as has been described previously
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12 270 (Yvon-Durocher *et al. in press*), with apparent activation energies in the order of 0.45 eV (95%
13 271 confidence interval 0.38 to 0.53 eV) and 0.62 eV (95% confidence interval 0.55 to 0.69 eV) for
14 272 each, respectively. Importantly, here, the temperature dependence of CH₄ efflux was
15 273 significantly higher than that for either GPP or ER (Table 2) and, correspondingly, CH₄ efflux
16 274 increased more rapidly in response to warming than did either GPP or ER.
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25 276 The balance between carbon absorption and CH₄ emission is given by the ratio of CH₄
26 277 efflux to GPP, which was found to be significantly elevated in the heated mesocosms, on
27 278 average, over the year (Table 1 and Fig 5a). The mean annual ratio of CH₄ efflux to GPP was
28 279 elevated by 20 % in response to the ~4°C experimental warming. Similarly, the ratio of CH₄
29 280 efflux to ER was significantly elevated in the heated mesocosms, on average, over the annual
30 281 cycle (Table 1 and Fig 5b), with warming elevating the mean annual ratio of CH₄ efflux to ER by
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3 283 **Discussion**
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5 284 Mesocosm experiments represent a compromise between the control and replication of
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8 285 laboratory studies and the realism of descriptive field surveys but, despite their limitations, can
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10 286 provide a fundamental tool for predicting how global change scenarios might affect ecosystem
11
12 287 level processes (Benton *et al.* 2007). Our measured rates of mesocosm CH₄ efflux (0.35 – 8.14
13
14 288 $\mu\text{mol CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) were comparable to those measured in natural shallow lakes (Rudd &
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16 289 Hamilton 1978, Bastviken *et al.* 2004), suggesting that our experimental scale was sufficient
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18 290 enough to reproduce the complex biogeochemical cycling of carbon observed in natural
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20 291 ecosystems.
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24 292 Experimental warming might have artificially stimulated advective processes resulting in
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26 293 elevated gas transfer at the air-water interface and a detailed consideration of this potential
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28 294 artefact is fundamental before interpretation of our results. Our estimates of the gas transfer
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30 295 velocity, based on detailed measurement of dissolved CH₄ and rates of CH₄ efflux, however,
31
32 296 suggest that this was not the case. If the gas transfer velocity was systematically enhanced by
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34 297 artificial warming, we would have expected to observe considerable differences in the
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36 298 concentration of dissolved CH₄ between treatments, but, again, this was not the case. At first
37
38 299 glance, the consistency in both the pool size of dissolved CH₄ and the estimated gas transfer
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40 300 velocity between treatments appears at odds with the elevated efflux of CH₄ measured in the
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42 301 warmed mesocosms. According to equation 3, the gas transfer velocity and the concentration
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44 302 gradient (i.e. between the water and the atmosphere) drive the efflux of gas across the air-water
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46 303 interface (Cole & Caraco 1998). This discrepancy can be explained when the relative magnitudes
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48 304 of the respective processes and pool sizes are taken into consideration. Using the 75th percentiles
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50 305 for both CH₄ efflux and dissolved CH₄ (scaled to a whole mesocosm) of 11 $\mu\text{mol CH}_4$
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3 306 mesocosm⁻¹ h⁻¹ and 662 μmol CH₄ mesocosm⁻¹, respectively, 75% of our measurements of the
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5 307 efflux of CH₄ represented <1.7% of the total pool of dissolved CH₄. The subtle differences
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8 308 detected in the efflux of CH₄ between treatments would have been masked when analysing for
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10 309 treatment effects at the level of the pool, because the overall magnitude of the pool size of
11
12 310 dissolved CH₄ was considerable relative to its minor diurnal variability, which drives the efflux.
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15 311 Therefore, any error associated with the measurement of the CH₄ pool would likely overwhelm
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17 312 the detection of any subtle statistical differences between treatments. This evidence suggests that
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20 313 the physical influence of heating the mesocosms by ~4°C had little discernable effect on
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22 314 advective processes. We can therefore be confident that the biogeochemical patterns revealed by
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24
25 315 our experiment are due to the biological consequences of warming on aquatic communities.

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27 316 Our experimental results have implications for understanding the mechanisms controlling
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29 317 CH₄ efflux from freshwater ecosystems, and how CH₄ dynamics in relation to carbon
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31 318 sequestration rates might be affected by future global warming. Our experiment revealed that
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34 319 temperature was the dominant driver of CH₄ efflux from our mesocosms. This result agrees with
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36 320 other studies from a range of natural ecosystems, from soils to wetlands, which also highlight the
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38 321 strong temperature dependence of CH₄ efflux (Schutz *et al.* 1990, Whiting & Chanton 2001,
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40 322 Christensen *et al.* 2003a, Gedney *et al.* 2004). Because of the overriding influence of
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43 323 temperature, the overall rates of CH₄ efflux were consistently elevated in the warmed mesocosms
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46 324 relative to ambient over the course of our annual study, presumably reflecting the strong
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48 325 physiological response to the temperature stimulation of methanogenesis.

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50 326 To ascertain whether the strong temperature dependence of CH₄ efflux we observed
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52 327 experimentally was due to the physiological stimulation of methanogenesis, we determined the
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55 328 activation energy of methanogenesis in pure cultures at a range of temperatures, and under non-

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3 329 limiting conditions, using previously published data (see S5 in supporting online information for
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5 330 details of analysis and data collection). Our re-analysis of these data does indeed show that the
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8 331 production of CH₄ in pure cultures has an equally strong temperature dependence (Fig. 5).
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10 332 Interestingly, the temperature dependence of CH₄ efflux at the ecosystem level ($E_a = 0.85$ eV,
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12 333 95% confidence interval: 0.64 to 1.02 eV) was indistinguishable from that for methanogenesis in
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14 334 pure culture (mean $E_a = 0.88$ eV 95% confidence interval: 0.80 to 0.96 eV). This coherence
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16 335 between organisational scales suggests that much of the potential complexity associated with
17
18 336 ecosystem level efflux of CH₄ might be reduced to the first principals of individual/cellular
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20 337 kinetics. This result suggests that whole ecosystem metabolic fluxes can be scaled from the
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22 338 individual to the ecosystem level, in line with predictions derived from the “metabolic theory of
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24 339 ecology” (Enquist *et al.* 2003, Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006). Our study, therefore,
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26 340 contributes to the growing body of evidence which suggests that metabolism is a fundamental
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28 341 driver of the dynamics of ecological processes across multiple levels of organisation, by
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30 342 demonstrating that CH₄ efflux at the ecosystem level appears to be constrained by the activation
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32 343 energy of methanogenesis. Models derived from the metabolic theory of ecology might therefore
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34 344 provide additional insight into the dynamics and temperature response of whole ecosystem CH₄
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36 345 efflux in aquatic and other ecosystems.
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43 346 As well as temperature, primary production has been shown to regulate the efflux of CH₄
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45 347 to the atmosphere. Such regulation stems from the whole autotrophic assemblage providing
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47 348 structural, labile carbon compounds in the form of dead biomass (Whiting & Chanton 1993), and
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49 349 vascular plants producing root exudates in the form of organic acids (Chanton *et al.* 1995,
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51 350 Joabsson & Christensen 2001, Christensen *et al.* 2003b). Furthermore, rooted aquatic vascular
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53 351 plants can act as conduits for the transport of CH₄ from the anaerobic zone of the sediment to the
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3 352 atmosphere, bypassing the zones of potential CH₄ oxidation in the sediment and water column
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5 353 (Joabsson *et al.* 1999, Joabsson & Christensen 2001). In our experiment, however, the efflux of
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7 354 CH₄ did not appear to be limited by substrates from GPP, as suggested by three lines of
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9 355 evidence. Firstly, the weak correlation and gentle slope of the relationship between the efflux of
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11 356 CH₄ and GPP indicated that the flux of CH₄ was relatively independent of the simultaneous rate
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13 357 of photosynthesis and carbon fixation. Secondly, warming had no effect on the intercept of the
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15 358 relationship between ln(CH₄ efflux) vs 1/kT between treatments (Table 2). If organic substrates
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17 359 were limiting for CH₄ production and efflux, we would expect to see a lower intercept in the
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19 360 warmed treatments because elevated physiological rates would be expected to reduce organic
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21 361 substrates more rapidly, resulting in faster substrate limitation and thus a reduced intrinsic
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23 362 capacity for methanogenesis. Thirdly, on average, the efflux of CH₄ represented a very small
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25 363 fraction of GPP (mean annual value = 0.01%). If oxidation of methane production is assumed to
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27 364 be 95% (King *et al.* 1990), from our average annual CH₄ efflux measures (3 μmol m⁻² h⁻¹), we
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29 365 estimate mean annual CH₄ production to be ~290 μmol m⁻² h⁻¹ which would represent only 10%
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31 366 of the mean annual rate of GPP (2862 μmol m⁻² h⁻¹). Therefore, carbon sequestration and fixation
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33 367 by photosynthesis is likely to exceed the demand of methanogenesis throughout the annual cycle.

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41 368 The response of the greenhouse carbon gas balance of freshwater ecosystems to warming
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43 369 could affect the strength of biotic feedbacks on a potentially global scale (Woodwell *et al.* 1998).
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45 370 In our experiment, the fraction of carbon absorbed by GPP and subsequently remineralised via
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47 371 the methanogenic pathway to efflux as CH₄ increased by 20% in response to the simulated global
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49 372 warming scenarios projected for the end of the century. In addition, the efflux of CH₄ as a
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51 373 proportion of ER was 9% greater in the warmed mesocosms. If, as aquatic ecosystems warm,
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53 374 carbon remineralisation becomes increasingly dominated by methanogenesis this could result in
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375 more CH₄ being emitted to the atmosphere relative to CO₂ emission and carbon draw-down.
 376 These patterns can be explained by the differential activation energies of the three metabolic
 377 processes involved in the greenhouse carbon balance of ecosystems.

378 Here, and in previous research (Yvon-Durocher *et al. in press*), it has been demonstrated
 379 that the three key ecosystem level carbon fluxes have progressively higher activation energies
 380 (i.e., GPP = 0.45eV; ER = 0.62eV; CH₄ efflux = 0.85eV). The relative response of the
 381 greenhouse carbon gas balance to warming observed here might be predictable from these
 382 respective activation energies. To test this hypothesis, equations were derived from the metabolic
 383 theory of ecology (Brown *et al.* 2004) to predict the response of the mean ratio of CH₄ efflux to
 384 GPP over the year between heated and unheated treatments ($R_{\text{fixed}}\text{H:U}$) which was given by:

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

386 where a_H and a_U are the allometric equations for the efflux of CH₄ in the heated and unheated
 387 treatments respectively, while p_H and p_U are the allometric equations for GPP in heated and
 388 unheated treatments. E_a and E_p are the activation energies for the efflux of CH₄ and GPP in our
 389 experiment (0.85 and 0.45eV respectively), T_H and T_U are the mean annual temperatures (K) for
 390 the heated and unheated treatments, respectively, and k is Boltzmann's constant (see S3 in
 391 supporting online information for the full derivation of equation 4). Equation 4 predicts a 1.30
 392 fold increase in $R_{\text{fixed}}\text{H:U}$ (range 1.18 to 1.38; based on the 95% confidence intervals of the
 393 respective empirically measured activation energies), which is very close to our empirically
 394 measured value of 1.20. Similarly, the response of the mean ratio for the efflux of CH₄ to ER
 395 over the year between heated and unheated treatments ($R_{\text{emitted}}\text{H:U}$) should be predicted by:

$$R_{\text{emitted}}\text{H:U} = \frac{a_H/r_H}{a_U/r_U} = e^{\frac{[(E_a - E_r)(T_H - T_U)]}{kT_H T_U}} \quad (5)$$

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3 397 where E_a and E_r are the empirically determined activation energies for the efflux of CH_4 and ER
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5 398 (0.85 and 0.62eV respectively), respectively (see S4 in supporting online information for the full
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8 399 derivation of equation 4). Using equation 5, we would expect a 1.16 fold increase in $R_{\text{emitted}}\text{H:U}$
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10 400 (range 0.17 to 1.24; based on the 95% confidence intervals of the respective empirically
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12 401 measured activation energies). Our empirically measured value of $R_{\text{fixed}}\text{H:U}$ was 1.09, again very
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14 402 close to our prediction.

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17 403 Interestingly, equations 4 and 5 suggest that the relative offset of the carbon balance
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19 404 between the ambient and warmed mesocosms can be predicted by the differences in activation
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21 405 energies of metabolism and the degree of expected warming. This result is important because it
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23 406 highlights the potential for a positive feedback between warming and the carbon cycle of
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25 407 freshwater ecosystems, especially given the greater radiative forcing potential of CH_4 (Rodhe
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27 408 1990, Lelieveld *et al.* 1991, Whiting & Chanton 2001). Finally, accepting the caveats associated
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29 409 with mesocosms, the close coherence between the activation energy of methanogenesis in pure
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31 410 culture and that of whole system CH_4 efflux, suggests that much of the complexity of ecosystem
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33 411 level fluxes can be reduced to produce simpler predictive models.
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42
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44
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46
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Variable	d.f.	F-ratio	P – Value
ln(CH ₄ Pool)	1,123	0.0001	0.992(NS)
ln(CH ₄ efflux)	1,123	6.22	0.014
ln (<i>k</i>)	1,123	3.46	0.068(NS)
ln(CH ₄ efflux) / ln(GPP)	1,123	6.33	0.013
ln(CH ₄ efflux) / ln(ER)	1,123	6.23	0.0139

530

531 **Table 1.** Linear mixed effects model analysis. Analysing differences between heated and
 532 unheated treatments in the annual means of methane efflux [ln(CH₄ efflux)], the dissolve
 533 methane pool [ln(CH₄ Pool)], the ratio of methane efflux to GPP [ln(CH₄ efflux)/ln(GPP)], and
 534 the ratio of methane efflux to ER [ln(CH₄ efflux)/ln(ER)]. Significant *P*-values are given in bold.

535

Relationship	d.f.	F-ratio	P – Value
ln(CH ₄ efflux) vs 1/kT	1,127	97.98	<0.0001
Difference in slope of ln(CH ₄ efflux) vs 1/kT between treatments	1,127	1.23	0.23
Difference in intercept of ln(CH ₄ efflux) vs 1/kT between treatments	1,127	2.29	0.132
ln(CH ₄ efflux) vs ln(GPP)	1,127	38.61	<0.0001
Difference in intercept of ln(CH ₄ efflux) vs ln(GPP) between treatments	1,127	1.28	0.26
Difference in slope of ln(CH ₄ efflux) vs ln(GPP) between treatments	1,127	0.38	0.54
Difference in slope between ln(CH ₄ efflux) vs 1/kT and ln(GPP) vs 1/kT	1,258	21.61	<0.0001
Difference in slope between ln(CH ₄ efflux) vs 1/kT and ln(ER) vs 1/kT	1,258	8.36	0.0042

536

537 **Table 2.** Analysis of Co-Variance table for the relationships between ln(CH₄ efflux), ln(GPP)
 538 and ln(ER) vs 1/kT. Significant *P*-values are given in bold.

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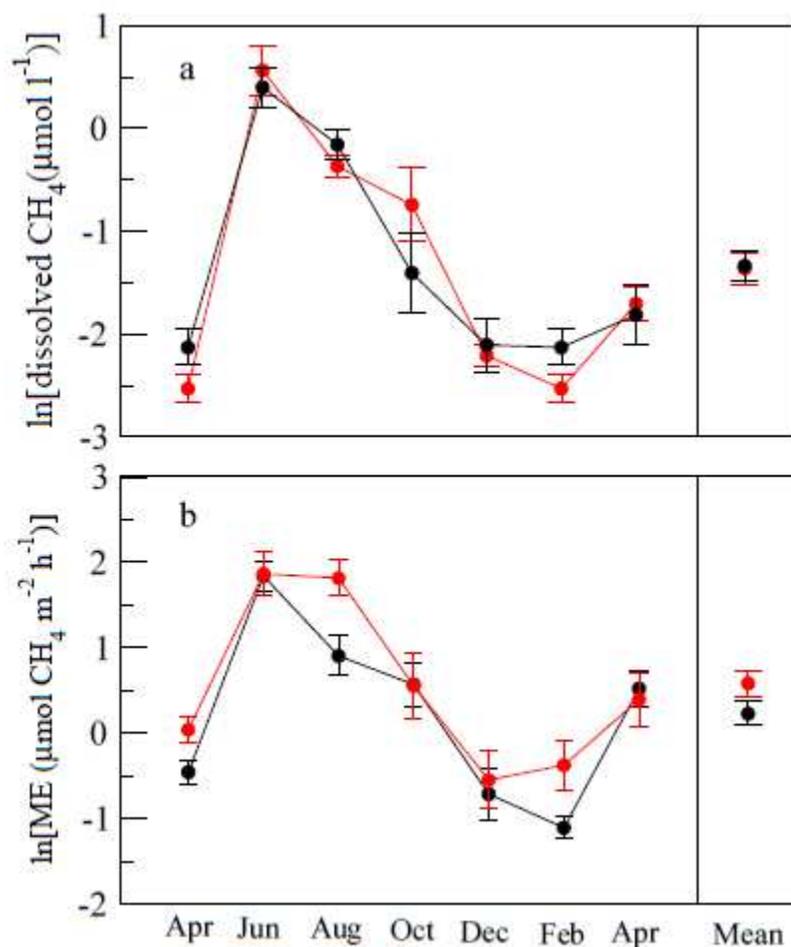


Fig. 1. (a) Differences in the pool of dissolved methane [$\ln(\text{CH}_4)$] (\pm SE) between heated (red lines) and unheated treatments (black lines). The pool of dissolved methane exhibited strong seasonal trends and which were identical between treatments. Furthermore, the average annual pool of dissolved methane was identical between treatments (Table 1). (b) Differences in methane efflux, $\ln(\text{ME})$ (\pm SE) between heated (red lines) and unheated (black lines) 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).

149x179mm (72 x 72 DPI)

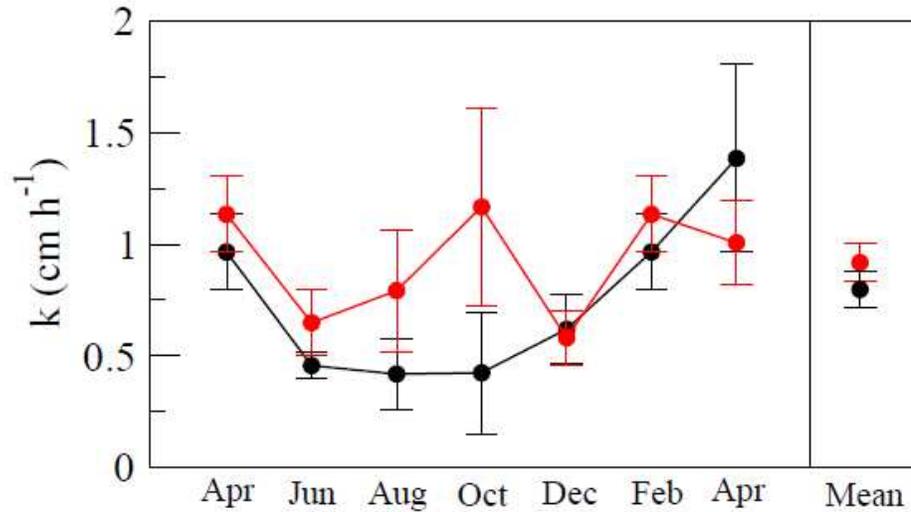


Fig. 2. Seasonal trends in the gas transfer velocity (k), between heated (red lines) and unheated (black lines) treatments. The gas transfer exhibited little seasonal variability and was not systematically affected by the heating of the mesocosms (Table 1). Data were natural log transformed for statistical analysis but presented here untransformed for ease of interpretation.
213x126mm (72 x 72 DPI)

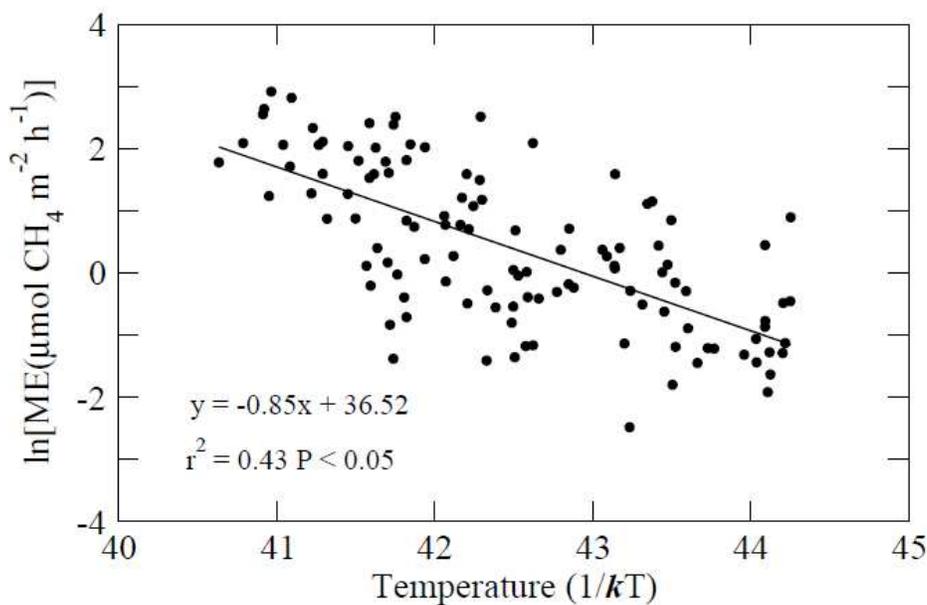


Fig. 3. Temperature dependence of whole ecosystem methane efflux. The slope of the temperature response of methane efflux in our experiment was equivalent to the activation energy of methanogenesis. Each data point corresponds to the CH_4 efflux from a single mesocosm on each of the seven sampling occasions ($n = 131$). 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 to characterise the activation energy.

254x160mm (72 x 72 DPI)

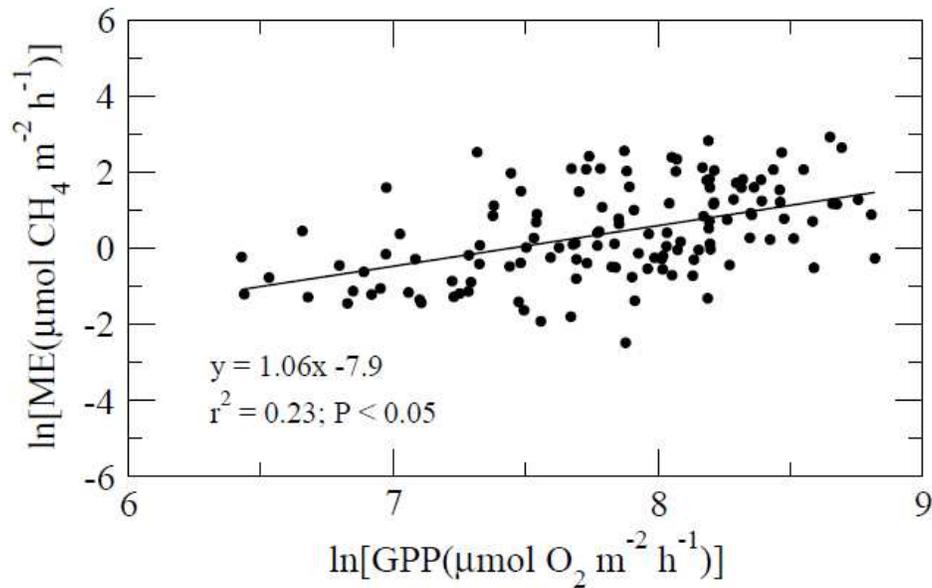


Fig. 4. Positive correlation between methane efflux [$\ln(\text{ME})$] and gross primary production [$\ln(\text{GPP})$]. Each data point corresponds to the CH_4 efflux and GPP of a single mesocosm on each of the seven sampling occasions ($n = 131$). There were no significant differences in the slope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

239x149mm (72 x 72 DPI)

Only

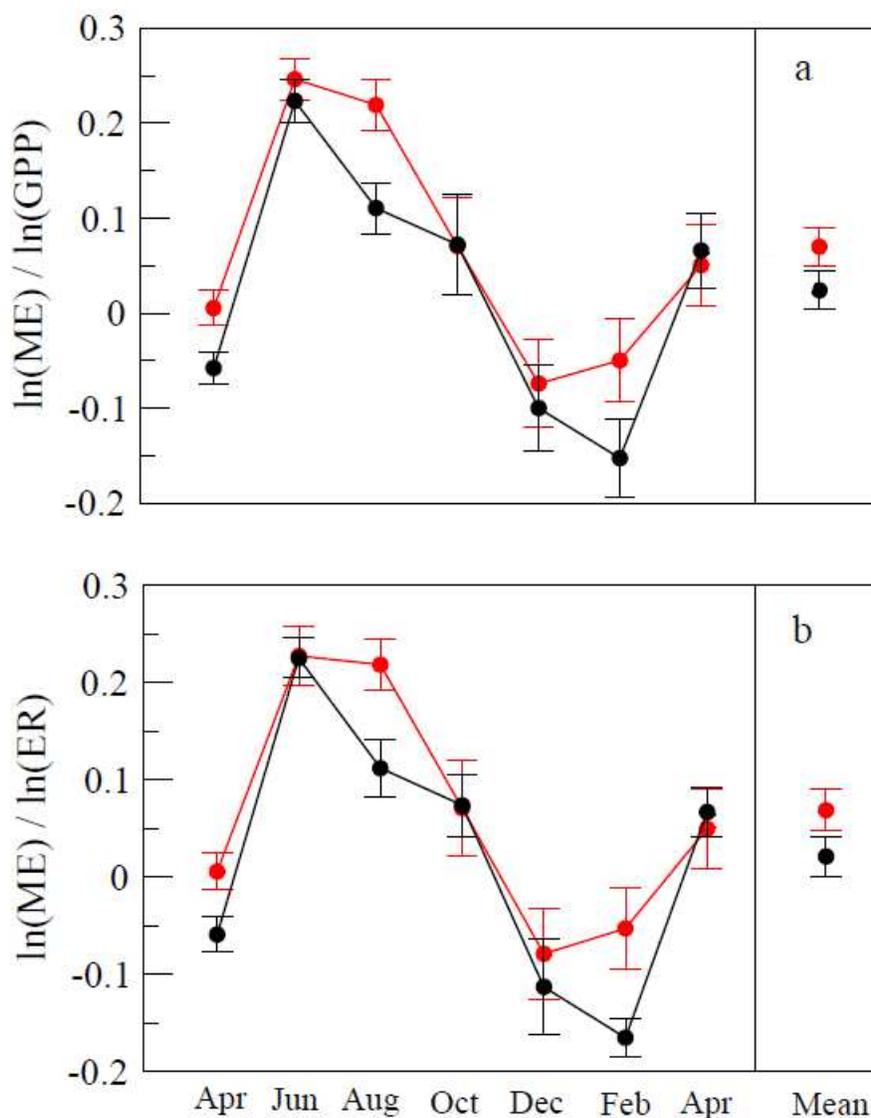


Fig. 5. (a) Differences in the ratio of methane efflux to GPP [$\ln(\text{ME})/\ln(\text{GPP})$] ($\pm\text{SE}$) and (b) methane efflux to ER $\ln(\text{ME})/\ln(\text{ER})$ ($\pm\text{SE}$) between heated (red lines) and unheated (black lines) experimental treatments. Both $\ln(\text{ME})/\ln(\text{GPP})$, and $\ln(\text{ME})/\ln(\text{ER})$, were elevated, on average over the annual cycle in the warmed treatments. The magnitude of the increase in $\ln(\text{ME})/\ln(\text{GPP})$ and $\ln(\text{ME})/\ln(\text{ER})$ 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.

220x264mm (72 x 72 DPI)

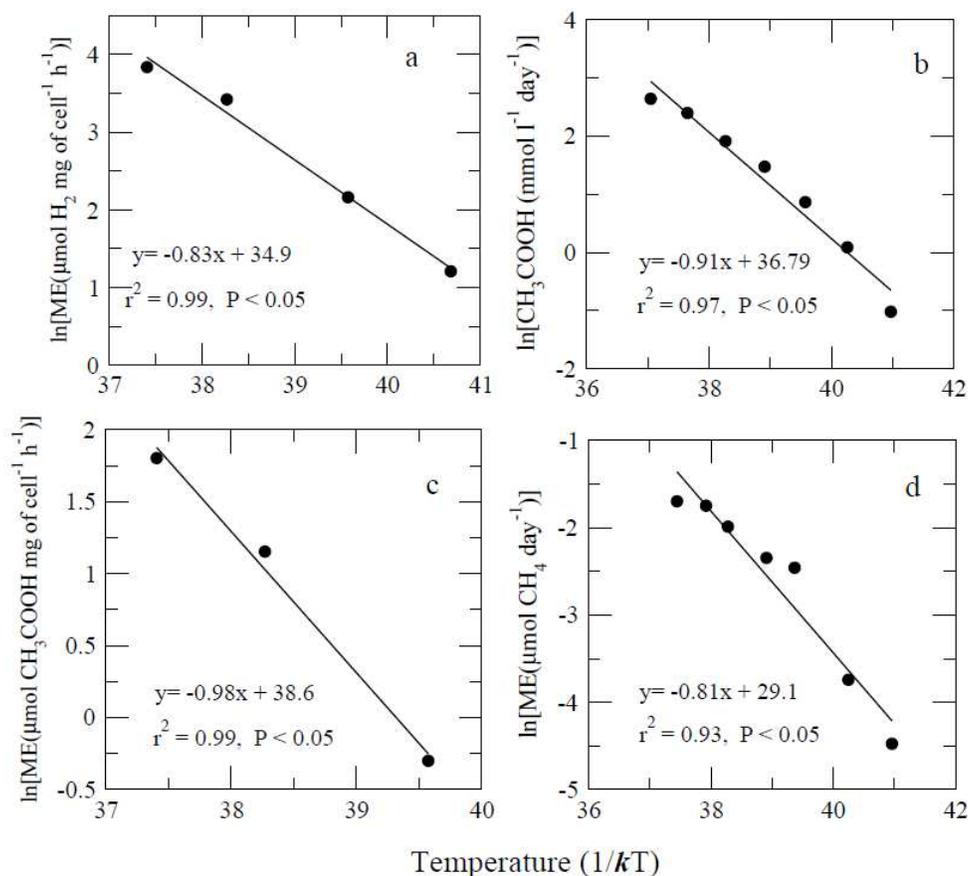


Fig. 6. Temperature dependence of methanogenesis in pure cultures of the methanogens. (a) Methanogenesis of *Methanosarcina barkeri* using H₂ as a substrate; (b) methanogenesis of an enrichment culture using CH₃COOH as a substrate; (c) methanogenesis of *M. barkeri* using CH₃COOH as a substrate; and (d) methanogenesis of *Methanothrix soehngenii* using CH₃COOH as a substrate. Data in a and c were reanalysed from Vmax values and temperatures reported in Westermann et al. (1989). Data for b was reanalysed from data on rates of CH₃COOH conversion to CH₄ in Van den Berg et al., (1976), and data for c was reanalysed from data on rates of CH₄ 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.

294x267mm (72 x 72 DPI)