LETTER

doi:10.1038/nature13164

Methane fluxes show consistent temperature dependence across microbial to ecosystem scales

Gabriel Yvon-Durocher[1], Andrew P. Allen[2], David Bastviken[3], Ralf Conrad[4], Cristian Gudasz[5,6]†, Annick St-Pierre[7], Nguyen Thanh-Duc[8] & Paul A. del Giorgio[7]

Methane (CH_4) is an important greenhouse gas because it has 25 times the global warming potential of carbon dioxide (CO_2) by mass over a century[1]. Recent calculations suggest that atmospheric CH_4 emissions have been responsible for approximately 20% of Earth's warming since pre-industrial times[2]. Understanding how CH_4 emissions from ecosystems will respond to expected increases in global temperature is therefore fundamental to predicting whether the carbon cycle will mitigate or accelerate climate change. Methanogenesis is the terminal step in the remineralization of organic matter and is carried out by strictly anaerobic Archaea[3]. Like most other forms of metabolism, methanogenesis is temperature-dependent[4,5]. However, it is not yet known how this physiological response combines with other biotic processes (for example, methanotrophy[6], substrate supply[3,7], microbial community composition[8]) and abiotic processes (for example, water-table depth[9,10]) to determine the temperature dependence of ecosystem-level CH_4 emissions. It is also not known whether CH_4 emissions at the ecosystem level have a fundamentally different temperature dependence than other key fluxes in the carbon cycle, such as photosynthesis and respiration. Here we use meta-analyses to show that seasonal variations in CH_4 emissions from a wide range of ecosystems exhibit an average temperature dependence similar to that of CH_4 production derived from pure cultures of methanogens and anaerobic microbial communities. This average temperature dependence (0.96 electron volts (eV)), which corresponds to a 57-fold increase between 0 and 30 °C, is considerably higher than previously observed for respiration (approximately 0.65 eV)[11] and photosynthesis (approximately 0.3 eV)[12]. As a result, we show that both the emission of CH_4 and the ratio of CH_4 to CO_2 emissions increase markedly with seasonal increases in temperature. Our findings suggest that global warming may have a large impact on the relative contributions of CO_2 and CH_4 to total greenhouse gas emissions from aquatic ecosystems, terrestrial wetlands and rice paddies.

Biogenic CH_4 fluxes are a major component of global CH_4 emissions, yet they are poorly constrained[2,13,14]. There are large uncertainties not only in the current magnitude of these fluxes but also in the factors that regulate them[2,13]. In particular, there is substantial uncertainty in the parameterization of the temperature dependence of natural CH_4 emissions in process-based biogeochemistry models[15–18], which greatly hinders our ability to predict the response of this key component of the carbon cycle to global warming. For example, temperature dependencies for ecosystem-level CH_4 emissions have reported apparent activation energies that vary from 0.2 to 2.5 eV (refs 6, 19–21) (1 eV = 96 kJ mol^{-1}).

In a bid to reduce this uncertainty, which is fundamental to improving projections of feedbacks between the carbon cycle and future climate change[15–18], we quantified variation in the temperature dependence of CH_4 fluxes in meta-analyses of three different types of experiments (pure cultures of methanogens, laboratory incubations of anaerobic sediments, and seasonal field surveys of CH_4 emissions) that correspond to three distinct levels of biological organization (population, community and ecosystem). In particular, we assess whether ecosystem-level CH_4 emissions exhibit temperature dependencies similar to those of the underlying methanogenic process. To do this, we first established the magnitude and variability of the temperature dependencies of key rate processes for populations of methanogens in culture (methanogenesis, growth) and laboratory incubations of anaerobic microbial communities from natural sediment samples (CH_4 production). We then assessed whether these temperature dependencies differ from those observed in an ecosystem-level analysis of the seasonal temperature dependence of CH_4 emissions from aquatic, wetland and rice-paddy ecosystems.

To characterize the temperature dependencies of physiological rate processes for methanogens, we fit the Boltzmann–Arrhenius function (which describes the exponential relationship between metabolic rate and temperature, assuming a single enzyme-catalysed reaction is rate-limiting[22]), separately, to the data compiled from the population and community-level experiments using linear mixed-effects models (see Methods).

The population-level analysis reveals that the average temperature dependencies for the rates of methanogenesis and growth are similar. Specifically, the improvement in model fit going from a null model, which assumes a common average activation ($\overline{E_M}$ in equation (1), see Methods) energy for both rate processes to an alternative model, which assumes a distinct average activation energy for each rate process, is not statistically significant (likelihood ratio test: $\chi^2 = 0.39$, d.f. = 1, $P = 0.53$). Thus, the average temperature dependencies for both rate processes (methanogenesis and growth) can be characterized using the same average apparent activation energy ($\overline{E_M} = 1.10$ eV, 95% confidence interval of 0.93–1.27 eV; Fig. 1a).

The community-level analysis of CH_4 production rates from anaerobic sediment incubations produces a similar value for the average activation energy ($\overline{E_M} = 0.93$ eV, 95% confidence interval of 0.82 – 1.03 eV), suggesting that the temperature dependence of CH_4 production at the community level largely reflects the kinetics of the physiological processes generating this flux. More detailed analyses of these community-level data indicate that the average activation energies for the sediments from three broadly defined ecosystem types (that is, aquatic ecosystems, wetlands and rice paddies) are statistically indistinguishable (likelihood ratio test comparing a null model with a single activation energy to an alternative model with separate activation energies for each ecosystem type: $\chi^2 = 1.62$, d.f. = 2, $P = 0.44$). However, for both the population- and community-level analyses, temperature dependencies do vary among experimental units, *i*, as indicated by the statistical significance of random effects on the activation energies (characterized by $\varepsilon_{E_M,i}$ in equation (1), see Methods and Table 1), owing, for example, to inherent physiological

[1]Environment and Sustainability Institute, University of Exeter, Penryn, Cornwall, TR10 9EZ. UK. [2]Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia. [3]Department of Thematic Studies – Water and Environmental Studies, Linköping University, SE-581 83 Linköping, Sweden. [4]Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany. [5]Department of Ecology and Environmental Sciences, Umeå University, Linnaeus väg 6, SE-901 87 Umeå, Sweden. [6]Department of Ecology and Genetics, Limnology, Uppsala University, Norbyvägen 18D, SE-752 36, Uppsala Sweden. [7]Département des sciences biologiques, Université du Québec à Montréal, Montréal, Province of Québec, H2X 3X8, Canada. [8]Earth Systems Research Center, Institute for the Study of Earth, Oceans, and Space, University of New Hampshire, Durham, New Hampshire 03824, USA. †Present address: Department of Ecology and Evolutionary Biology, Princeton University, Princeton, 106A Guyot Hall, New Jersey 08544, USA.

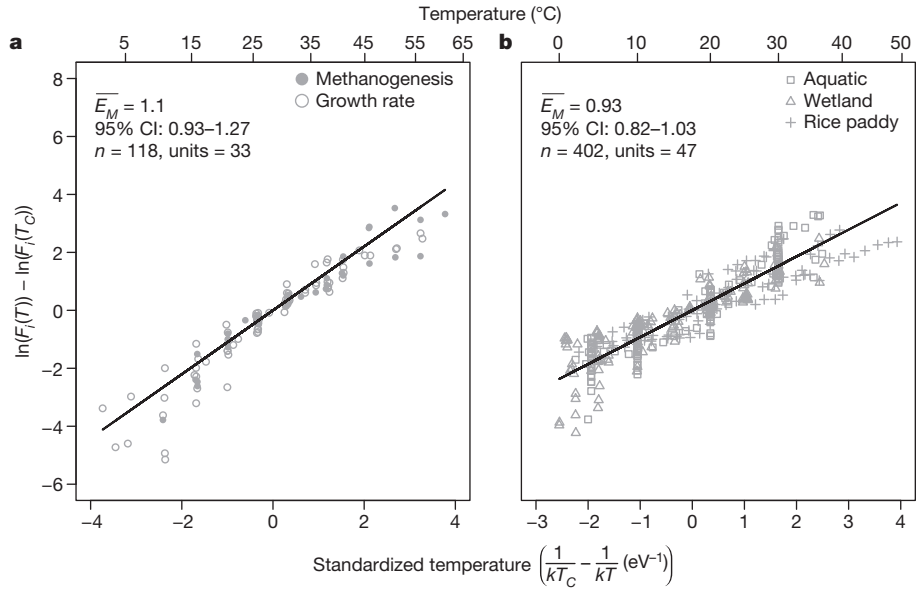


Figure 1 | Temperature dependence of CH_4 production and related processes at population and community levels. Temperature dependencies for methanogen populations in culture (**a**) and anaerobic microbial communities from natural sediment samples (**b**) are separately characterized using mixed-effects models by fitting Boltzmann-Arrhenius functions with experimental-unit-level random effects on the apparent activation energy and rate at fixed temperature (see equation (1) in the Methods). Analyses reveal that average temperature dependencies are statistically indistinguishable for rates of growth and methanogenesis (**a**, open circles and filled circles, respectively) by populations of methanogens ($P = 0.53$), and rates of CH_4 production by laboratory-incubated anaerobic microbial communities from aquatic, wetland and rice-paddy sediments (**b**, squares, triangles and crosses, respectively) ($P = 0.44$). The fitted solid lines correspond to the average apparent activation energies estimated from the mixed-effects models ($\overline{E_M} = 1.10$ and 0.93 eV for the population (**a**) and community (**b**) analyses, respectively). Data have been standardized by subtracting from each measurement the estimated experiment-specific intercept; that is, the estimated rate at fixed temperature, $\ln F(T_C)$, where T_C is the average of the temperature measurements for the population-level data (27.2 °C in **a**) and the community-level data (17.5 °C in **b**). These standardizations are used for visualization of the data only.

differences between strains of methanogens in culture and variation between sediment samples in microbial community composition.

To assess how the average temperature dependence of CH_4 production for methanogenic populations and communities compares to that of CH_4 emissions from entire ecosystems, we fit the Boltzmann–Arrhenius equation to a database of 1,553 measurements of CH_4 emission and temperature, measured seasonally for 127 field sites that span the globe and encompass wetlands, rice paddies and aquatic ecosystems. Analysis revealed that the estimated average activation energies are statistically indistinguishable for these three ecosystem types (likelihood ratio test comparing a null model with a single activation energy to an alternative model with separate activation energies for each ecosystem type: $\chi^2 = 4.97$, d.f. = 2, $P = 0.10$), and can therefore be characterized by a common parameter ($\overline{E_M} = 0.96$ eV; 95% confidence interval of 0.86–1.07; Fig. 2). This average temperature dependence is statistically indistinguishable to those observed for CH_4 production from populations in culture and incubations of microbial communities from sediments—that is, all three estimates for $\overline{E_M}$ have 95% confidence intervals that overlap (Table 1)—which is remarkable given the multitude of processes that may confound the temperature dependence of CH_4 emissions over a seasonal cycle at the ecosystem level. Thus, our analysis is consistent with the hypothesis that the seasonal temperature dependence of CH_4 emissions at the ecosystem level largely reflects the kinetics of the methanogenic processes generating this flux (see Supplementary Information section 2 for further discussion of the potential mechanisms governing the scaling of the temperature dependence of CH_4 fluxes from populations to ecosystems).

Importantly, the average apparent activation energy we report here for the seasonal temperature dependence of ecosystem-level CH_4 emissions ($\overline{E_M} = 0.96$ eV) is considerably higher than that reported previously for CO_2 fluxes attributable to respiration (~0.65 eV)[11] and photosynthesis (~0.3 eV)[12], which could have important implications for the effect of global warming on the balance of CH_4 and CO_2 emissions from ecosystems[23]. Given these differential temperature dependencies, we expected the ratio of CH_4 to CO_2 emissions to increase, on average, with seasonal increases in temperature for a collection of sites (see Supplementary Information section 1).

To test this prediction, we analysed ecosystem-level data for the subset of sites in our compilation where simultaneous measurements of CH_4 and CO_2 fluxes have been made, enabling us to calculate the efflux ratio of these greenhouse gases in response to seasonal variation in temperature. This data set comprises 177 estimates from 38 field sites. In exactly the same way as for the analyses of CH_4 emissions, we fit a linear mixed-effects model using the Boltzmann–Arrhenius function

Table 1 | Estimates of the parameters used to characterize the temperature dependencies of CH_4 production, emission and related variables

Rate or ratio	Average activation energy (eV) (95% CI)	Standard deviation of random effect on activation energy	Standard deviation of random effect on rate or ratio at fixed temperature
Population-level rate of methanogenesis or growth	1.10 (0.93–1.27)	0.42	2.28
Community-level rate of CH_4 production	0.93 (0.82–1.03)	0.32	2.45
Ecosystem-level CH_4 emission	0.96 (0.86–1.07)	0.42	2.17
Ecosystem-level CH_4:CO_2 emission ratio	0.71 (0.46–0.97)	0.59	1.03

Parameters were determined by fitting Boltzmann–Arrhenius functions to rates (equation (1)) or ratios of rates (equation (2)) using mixed-effects models. The standard deviations of the random effects used to characterize differences among sites or experimental units in temperature dependencies ($\varepsilon_{E_M,j}$ and $\varepsilon_{E_{M,C},j}$ in equations (1) and (2) of the Methods, respectively) are significantly greater than zero in all analyses ($P < 0.05$), as are the standard deviations of the random effects used to characterize differences in rates (or rate ratios) among sites or experimental units at fixed temperature ($\varepsilon_{F,j}$ and $\varepsilon_{Q,j}$, respectively) ($P < 0.001$).

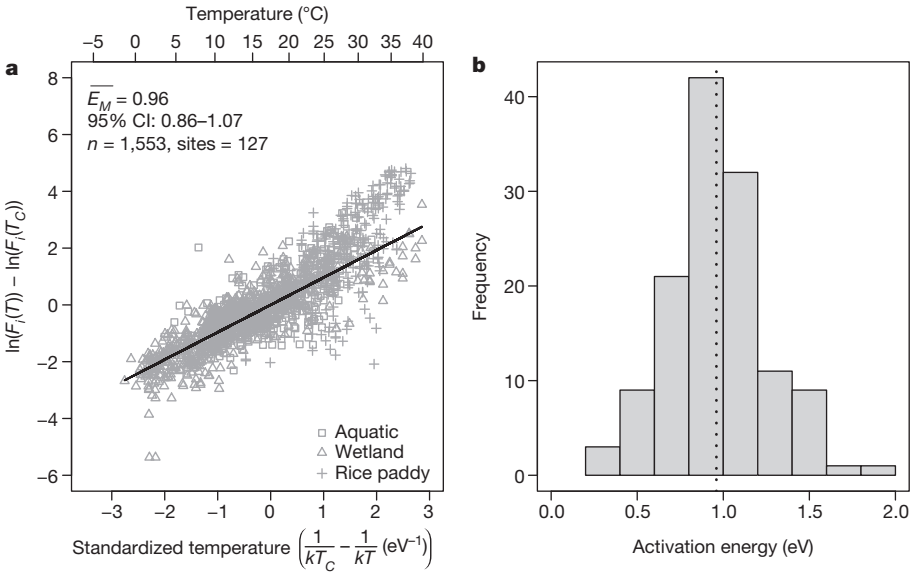


Figure 2 | Temperature dependence of CH_4 emissions at the ecosystem level. Temperature dependencies in **a** are characterized using a mixed-effects model by fitting a Boltzmann–Arrhenius function to the emission data with site-level random effects on the apparent activation energy and rate at fixed temperature (see equation (1) in the Methods). Analysis reveals that CH_4 emissions exhibit average temperature dependencies that are statistically indistinguishable for aquatic (squares), wetland (triangles) and rice-paddy (crosses) ecosystems ($P = 0.10$). The fitted solid line corresponds to the average apparent activation energy estimated from the mixed-effects model ($\overline{E_M} = 0.96$ eV). The distribution of apparent activation energies among sites (**b**) yields an average ($\overline{E_M} = 0.96$ eV, represented by a dashed line) that is statistically indistinguishable ($P > 0.05$) from the averages derived from the population- and community-level analyses (Fig. 1 and Table 1). In **a**, data have been standardized by subtracting from each measurement the estimated site-specific intercept; that is, the estimated rate of CH_4 emission at fixed temperature, $\ln F(T_C)$, where T_C is the average of the temperature measurements in the field emissions data set (15.6 °C). This standardization is used for visualization of the data only.

to the natural logarithm of the CH_4:CO_2 flux data. As predicted, this ratio increases with increasing temperature for the majority of the field sites (35 of 38), yielding average temperature dependence across sites (characterized by $\overline{E_{M:C}}$ in equation (2), see Methods) of 0.71 eV (95% confidence interval of 0.46–0.97 eV; Fig. 3). This finding suggests that, on average, the relative contribution of CH_4 to total greenhouse gas emissions increases with seasonal increases in temperature due to the differences in the biochemical kinetics of methanogenesis, respiration and photosynthesis.

Our analyses demonstrate that, on average, CH_4 emissions for a wide range of ecosystems show a temperature dependence similar to that of methanogenesis by methanogen populations in culture and CH_4 production from incubations of microbial communities from sediments. Moreover, this average temperature dependence is much higher than that of both respiration[11] and photosynthesis[12], resulting in a greater relative contribution of CH_4 to total carbon emissions at higher temperatures. Although this consistent temperature dependence of CH_4 fluxes from microbes to ecosystems is remarkable, our results also emphasize that temperature is not the only variable that controls CH_4 emissions from ecosystems. The substantial site-to-site variation we report for the temperature dependence of ecosystem-level CH_4 emissions (see Fig. 2b and Table 1) highlights the importance of other variables in driving deviations from the underlying physiological response. Moreover, the average apparent activation energy we report here ($\overline{E_M} = 0.96$ eV), based on the seasonal temperature dependence of CH_4 emissions within sites, does not apply across sites along geographic temperature gradients. Indeed, the response of CH_4 emissions to geographic variation in temperature (Extended Data Figs 1 and 2) is consistently weaker than the

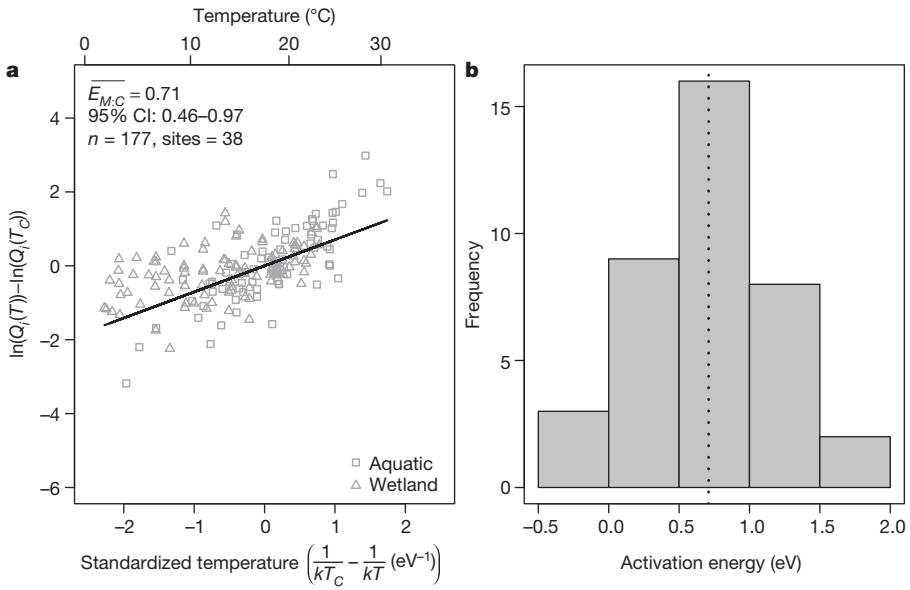


Figure 3 | Temperature dependence of the CH_4:CO_2 emission ratio. Temperature dependencies are characterized in **a** using a mixed-effects model by fitting a Boltzmann–Arrhenius function with site-level random effects on the temperature dependence and ratio at fixed temperature (see equation (2) in the Methods). The fitted solid line in **a** corresponds to the average temperature dependence estimated from the mixed-effects model ($\overline{E_{M:C}} = 0.71$ eV). The distribution of site-level temperature dependencies (presented as a histogram of slope estimates in **b**) yields an average ($\overline{E_{M:C}} = 0.71$ eV, represented by a dashed line in **b**) that is positive and significant ($P > 0.05$). Data in **a** have been standardized by subtracting from each measurement the estimated site-specific intercept; that is, the estimated ratio of CH_4:CO_2 emissions at fixed temperature, $\ln F(T_C)$, where T_C is the average temperature for the ratio data (15.3 °C). This standardization is used for visualization of the data only.

response to seasonal variation within sites (see Fig. 2). Furthermore, for natural wetlands, which span the broadest gradient in average temperature, we observe a significant negative correlation between the site-level intercept—that is, CH_4 emissions at fixed temperature, $\ln F(T_C)$ (see equation (1) in Methods)—and average site temperature (Extended Data Fig. 2e). These results suggest that temperature-dependent variation in other biotic (for example, methanotrophy[6], substrate supply[3,7], microbial community structure and/or composition[8], physiological acclimation and/or adaptation[24]) and abiotic variables (for example, water-table depth[9,10]) may have an important role in constraining the response of CH_4 emissions to warming in the long-term, as has been suggested for rates of ecosystem respiration[11].

Overall, our findings provide an empirical basis for refining representations of the temperature dependence of CH_4 fluxes in coupled climate–carbon cycle models[15–18,25]. In particular, they provide evidence that the temperature dependencies of methanogenesis, CH_4 production and CH_4 emissions are substantially higher than two other key rate processes in the carbon cycle, heterotrophic respiration and photosynthesis. The observation of a general increase in the CH_4:CO_2 ratio with increasing temperature, driven by the relatively high temperature dependence of CH_4 production, may have important implications for the magnitude of future positive feedbacks between global warming and the carbon cycle given the greater potency of CH_4 compared to CO_2 as a greenhouse gas[2,13].

METHODS SUMMARY

We used linear mixed-effects modelling[26] to quantify the temperature dependencies of metabolic rate processes for pure cultures of methanogens, anaerobic microbial communities and seasonal field surveys of CH_4 emissions by fitting Boltzmann–Arrhenius functions of the form:

$$\ln F_i(T) = \left(\overline{E_M} + \varepsilon_{E_M,i}\right)\left(\frac{1}{kT_C} - \frac{1}{kT}\right) + \left(\overline{\ln F(T_C)} + \varepsilon_{F,i}\right) \quad (1)$$

where $\ln F_i(T)$ is the natural logarithm of the rate of CH_4 flux at absolute temperature, T (K), for some arbitrary experimental unit, i. In this expression, each experimental unit, i, corresponds to a distinct strain of methanogen (population-level analysis), sediment sample (community-level analysis) or site (ecosystem-level analysis). The parameter $\overline{E_M}$ (in eV) corresponds to an average among experimental units for the apparent activation energy, which characterizes the temperature dependence of $F_i(T)$, and k is the Boltzmann constant (8.62×10^{-5} eV K^{-1}). We centred the temperature data separately in each analysis using the mean temperature for the data set, T_C, so that $\ln F(T_C)$ corresponds to an average among experimental units for the rate at T_C. For each analysis, we expect estimates of the apparent activation energy, E_M, and the rate of CH_4 production at a fixed temperature, $\ln F(T_C)$, to vary between experimental units. We account for this variation by treating the slopes and intercepts as random variables with averages of $\overline{E_M}$ and $\overline{\ln F(T_C)}$, respectively, and deviations from these averages of $\varepsilon_{E_M,i}$ and $\varepsilon_{F,i}$ for each experimental unit, i.

We used linear mixed-effects modeling[26] to quantify the temperature dependence of the CH_4:CO_2 emission ratio using a Boltzmann–Arrhenius-type approximation (see Supplementary Information section 1) of the form:

$$\ln Q_i(T) = \left(\overline{E_{M:C}} + \varepsilon_{E_{M:C},i}\right)\left(\frac{1}{kT_C} - \frac{1}{kT}\right) + \left(\overline{\ln Q(T_C)} + \varepsilon_{Q,i}\right) \quad (2)$$

where $Q_i(T)$ is the ratio of CH_4 to CO_2 emissions for site i at temperature T, $\overline{E_{M:C}}$ and $\overline{\ln Q(T_C)}$ are the respective averages across sites for the temperature dependence of this ratio and the magnitude of the ratio at a fixed temperature T_C; $\varepsilon_{E_{M:C},i}$ and $\varepsilon_{Q,i}$ are random-effects terms used to represent site-level deviations from these averages.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 2 October 2013; accepted 21 February 2014.
Published online 19 March 2014.

1. Forster, P. *et al.* in *Climate Change 2007: the Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Solomon, S. *et al.*) (Cambridge Univ. Press, 2007).
2. Kirschke, S. *et al.* Three decades of global methane sources and sinks. *Nature Geosci.* **6,** 813–823 (2013).
3. Thauer, R. K., Kaster, A.-K., Seedorf, H., Buckel, W. & Hedderich, R. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Rev. Microbiol.* **6,** 579–591 (2008).
4. Westermann, P., Ahring, B. K. & Mah, R. A. Temperature compensation in *Methanosarcina barkeri* by modulation of hydrogen and acetate affinity. *Appl. Environ. Microbiol.* **55,** 1262–1266 (1989).
5. Zinder, S. H., Anguish, T. & Cardwell, S. C. Effects of temperature on methanogenesis in a thermophilic (58°C) anaerobic digester. *Appl. Environ. Microbiol.* **47,** 808–813 (1984).
6. Segers, R. Methane production and methane consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry* **41,** 23–51 (1998).
7. Whiting, G. J. & Chanton, J. P. Primary production control of methane emission from wetlands. *Nature* **364,** 794–795 (1993).
8. Fey, A. & Conrad, R. Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. *Appl. Environ. Microbiol.* **66,** 4790–4797 (2000).
9. Moore, T. R., Roulet, N. T. & Waddington, J. M. Uncertainty in predicting the effect of climatic change on the carbon cycling of Canadian peatlands. *Clim. Change* **40,** 229–245 (1998).
10. Moore, T. R. & Roulet, N. T. Methane flux: water table relations in northern wetlands. *Geophys. Res. Lett.* **20,** 587–590 (1993).
11. Yvon-Durocher, G. *et al.* Reconciling the temperature dependence of respiration across time scales and ecosystem types. *Nature* **487,** 472–476 (2012).
12. Allen, A. P., Gillooly, J. F. & Brown, J. H. Linking the global carbon cycle to individual metabolism. *Funct. Ecol.* **19,** 202–213 (2005).
13. Bridgham, S. D., Cadillo-Quiroz, H., Keller, J. K. & Zhuang, Q. Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales. *Glob. Change Biol.* **19,** 1325–1346 (2013).
14. Bastviken, D., Tranvik, L. J., Downing, J. A., Crill, P. M. & Enrich-Prast, A. Freshwater methane emissions offset the continental carbon sink. *Science* **331,** 50 (2011).
15. Gedney, N., Cox, P. M. & Huntingford, C. Climate feedback from wetland methane emissions. *Geophys. Res. Lett.* **31,** L20503 (2004).
16. Riley, W. J. *et al.* Barriers to predicting changes in global terrestrial methane fluxes: analyses using CLM4Me, a methane biogeochemistry model integrated in CESM. *Biogeosciences* **8,** 1925–1953 (2011).
17. Spahni, R. *et al.* Constraining global methane emissions and uptake by ecosystems. *Biogeosciences* **8,** 1643–1665 (2011).
18. Wania, R., Ross, I. & Prentice, I. C. Implementation and evaluation of a new methane model within a dynamic global vegetation model: LPJ-WHyMe v1.3.1. *Geosci. Mod. Dev.* **3,** 565–584 (2010).
19. Christensen, T. R. *et al.* Factors controlling large scale variations in methane emissions from wetlands. *Geophys. Res. Lett.* **30,** 1414 (2003).
20. Crill, P. M. *et al.* Methane flux from Minnesota peatlands. *Glob. Biogeochem. Cycles* **2,** 371–384 (1988).
21. Walter, B. P. & Heimann, M. A process-based, climate-sensitive model to derive methane emissions from natural wetlands: application to five wetland sites, sensitivity to model parameters, and climate. *Glob. Biogeochem. Cycles* **14,** 745–765 (2000).
22. Eyring, H. The activated complex and the absolute rate of chemical reactions. *Chem. Rev.* **17,** 65–77 (1935).
23. Yvon-Durocher, G., Montoya, J. M., Woodward, G., Jones, J. I. & Trimmer, M. Warming increases the proportion of primary production emitted as methane from freshwater mesocosms. *Glob. Change Biol.* **17,** 1225–1234 (2011).
24. Bradford, M. A. *et al.* Thermal adaptation of soil microbial respiration to elevated temperature. *Ecol. Lett.* **11,** 1316–1327 (2008).
25. Melton, J. R. *et al.* Present state of global wetland extent and wetland methane modelling: conclusions from a model inter-comparison project (WETCHIMP). *Biogeosciences* **10,** 753–788 (2013).
26. Zuur, A., Ieno, E., Walker, N. & Saveliev, A. *Mixed Effects Models and Extensions in Ecology with R* (Springer Verlag, 2009).

Supplementary Information is available in the online version of the paper.

Acknowledgements We thank M. Trimmer for early discussions that inspired much of this work, as well as P. Cox and T. Lenton for comments on earlier drafts of the manuscript.

Author Contributions G.Y.-D., D.B. and C.G. had initial discussions. G.Y.-D. conceived the study, analysed the data and wrote the first draft of the manuscript. D.B., P.A.d.G., C.G., N.T.-D., R.C. and A.S. contributed original data. A.P.A. wrote the theory for the CH_4:CO_2 temperature dependence. All authors contributed to revisions of the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to G. Y.-D. (g.yvon-durocher@exeter.ac.uk).

METHODS

Statistical analyses. We used linear mixed-effects modelling[26] to quantify the temperature dependencies of metabolic rate processes for pure cultures of methanogens, anaerobic microbial communities, and seasonal field surveys of CH_4 emissions, by fitting Boltzmann–Arrhenius functions of the form:

$$\ln F_i(T) = \left(\overline{E_M} + \varepsilon_{E_M,i}\right)\left(\frac{1}{kT_C} - \frac{1}{kT}\right) + \left(\overline{\ln F(T_C)} + \varepsilon_{F,i}\right) \quad (1)$$

where $\ln F_i(T)$ is the natural logarithm of the rate of CH_4 flux at absolute temperature, T (K), for some arbitrary experimental unit, i. In this expression, each experimental unit, i, corresponds to a distinct strain of methanogen (population-level analysis), sediment sample (community-level analysis) or site (ecosystem-level analysis). The parameter $\overline{E_M}$ (in eV) corresponds to an average among experimental units for the apparent activation energy, which characterizes the temperature dependence of $F_i(T)$, and k is the Boltzmann constant (8.62×10^{-5} eV K^{-1}). We centred the temperature data separately in each analysis using the mean temperature for the data set, T_C, so that $\ln F(T_C)$ corresponds to an average among experimental units for the rate at T_C. For each analysis, we expect estimates of the apparent activation energy, E_M, and the rate of CH_4 production at a fixed temperature, $\ln F(T_C)$, to vary between experimental units, which we account for by treating the slopes and intercepts as random variables with averages of $\overline{E_M}$ and $\overline{\ln F(T_C)}$, respectively, and deviations from these averages of $\varepsilon_{E_M,i}$ and $\varepsilon_{F,i}$ for each experimental unit, i.

We used linear mixed-effects modeling[26] to quantify the temperature dependence of the CH_4:CO_2 emission ratio using a Boltzmann–Arrhenius-type approximation (see Supplementary Information section 1) of the form:

$$\ln Q_i(T) = \left(\overline{E_{M:C}} + \varepsilon_{E_{M:C},i}\right)\left(\frac{1}{kT_C} - \frac{1}{kT}\right) + \left(\overline{\ln Q(T_C)} + \varepsilon_{Q,i}\right) \quad (2)$$

where $Q_i(T)$ is the ratio of CH_4 to CO_2 emissions for site i at temperature T, $\overline{E_{M:C}}$ and $\overline{\ln Q\ (T_C)}$ are the respective averages across sites for the temperature dependence of this ratio and the magnitude of the ratio at a fixed temperature T_C; $\varepsilon_{E_{M:C},i}$ and $\varepsilon_{Q,i}$ are random-effects terms used to represent site-level deviations from these averages.

Mixed-effects models are the most appropriate tool for meta-analyses of the kinds of data compiled for this study because they allow for nested covariance structures, where site- or experimental-unit-level relationships are nested within overall relationships, and can accommodate unbalanced designs, where the number of measurements vary among experimental units[26,27]. This approach enabled us to determine the overall average temperature dependence of flux ($\overline{E_M}$, represented by a 'fixed' effect) for a collection of experiments or sites, i, while accounting for the fact that multiple flux–temperature relationships (corresponding to 'random' effects on the slope ($\varepsilon_{E_M,i}$) and intercept ($\varepsilon_{F,i}$) for each i) are nested within this overall relationship. It also allowed us to explicitly quantify the variation in temperature dependencies among sites (or experimental units).

The appropriateness of this technique is perhaps most easily explained for the analysis of field data in this study. Given that there are many other factors, besides temperature, that influence CH_4 emissions, we expect the estimated apparent activation energy to exhibit variation among sites. In particular, we expect the apparent activation energy to differ from that of the physiological flux whenever other variables that affect the rate process exhibit covariation with temperature (see ref. 11 for a theoretical derivation of this result). In this analysis, we are interested in two types of quantities: first, the fixed effects representing the average temperature response of CH_4 emissions across all 127 sites, $\overline{E_M}$, and the average emission rate at fixed temperature, $\ln F(T_C)$; and second, the random effects ($\varepsilon_{E_M,i}$,$\varepsilon_{F,i}$) representing the magnitudes of the deviations from these fixed effects for each of the 127 sites. The deviation from the fixed effects for any given site will be driven by other variables, besides temperature, that affect CH_4 emission and seasonally covary with temperature in the field; for example, methanotrophy[6], water-table depth[9,10], substrate supply[7], community composition[8]. Similar reasoning can be used to justify applying the mixed-effects modelling approach to the population- and community-level data. Although this statistical approach does not allow us to identify the particular variables that contribute to differences in flux–temperature relationships among sites or experimental units, it does allow us to quantify the overall magnitude of their effects as standard deviations of the random-effects terms. Given that the magnitudes of these deviations are assumed to represent a sample from a multivariate normal distribution, the resulting model can be generalized to a larger population of sites.

Parameters were estimated by fitting the Boltzmann–Arrhenius equation to the data using mixed-effects models via the 'lmer' function in the 'lme4' package of R statistical software (v 3.0.2)[28]. For these analyses, we adopted a top-down approach, starting with the most complex model, to determine the significance of the fixed and random effects in a two-stage analysis[26]. In stage one, we determined whether it was necessary to include random effects corresponding to variation in both the slope and intercept among units, i, by using a likelihood ratio test[26,29] to assess the improvement in model fit going from a null model, which includes all potential fixed effects and only one random effect (corresponding to intercept variation), to an alternative model, which includes all potential fixed effects and both random effects (that is, on the slope and intercept). Analysis revealed that the random-effects structure that best described each data set included random variation in both the slope and the intercept (see Table 1 for details of the statistics). In the second stage, we applied the random-effects structure determined in stage one to assess the significance of the fixed effects (averages across sites for the apparent activation energy and intercept), and other potential covariates, using likelihood ratio tests. The results of these likelihood ratio tests are given below and in the main text.

Data compilations. For population-level experiments, measurements of rates of methanogenesis and temperature from cultures of methanogenic Archaea were compiled based on an exhaustive search of the literature. Experiments were only included in the analysis if they met the following criteria: rates were measured at three or more distinct temperatures at or below the optimum; rates were measured during the logarithmic (that is, exponential) phase of population growth, either in batch or continuous culture; rates could be normalized by population biomass. These criteria were met for 12 separate strains. Only data up to the optimum temperature were analysed. Two experiments of enrichment cultures for acetic acid conversion to CH_4 were also included because these culturing techniques result in numerical dominance by methanogenic populations[30]. In our analysis, these enrichment cultures had estimates of the apparent activation energy within the range exhibited by the pure cultures.

To explore further the temperature dependence of metabolic rate processes in methanogens, we compiled additional data on methanogen growth rates, which have been reported more frequently in the literature than rates of methanogenesis. Given the rapid growth rates of these populations (that is, doubling times of less than 1 day), and that the energy transformations required to fuel this growth result from methanogenesis, growth rates of methanogens are likely to be closely linked to rates of methanogenesis and hence ecosystem-level CH_4 emissions. Growth rate data meeting the same three criteria listed above for methanogenesis were compiled for 21 strains of methanogens, and encompass both the acetoclastic and hydrogentrophic groups.

Mixed-effects model analysis indicates that the average activation energies are not significantly different for rates of methanogenesis and growth (likelihood ratio test comparing a null model with a single activation energy to an alternative model with separate activation energies for methanogenesis and growth rate; $\chi^2 = 0.39$, d.f. = 1, $P = 0.53$). These findings suggest that methanogen growth efficiency is approximately independent of temperature for populations that are growing exponentially and are not therefore substrate limited. We also found no significant difference between the acetoclastic or hydrogenotrophic methanogens in the temperature dependencies of either growth rate or methanogenesis (likelihood ratio test comparing a null model with a single activation energy to an alternative model with separate activation energies for acetoclastic or hydrogenotrophic methanogens; $\chi^2 = 1.30$, d.f. = 1, $P = 0.25$). We therefore combined the data on rates of growth and methanogenesis for both groups to characterize an overall average temperature dependence for these metabolic rate processes in methanogen populations (Fig. 1a).

For community-level experiments, rates of CH_4 production were compiled from the literature from experiments in which sediment samples from aquatic, wetland and rice-paddy ecosystems, where incubated anaerobically under laboratory conditions at multiple temperatures. Our data compilation consists of 402 estimates of CH_4 production from 47 separate anaerobic communities sampled from 16 aquatic ecosystems, 26 wetlands and 5 rice-paddy ecosystems. Unlike the culture data, these data characterize the overall effect of temperature on the production of CH_4 by the entire sediment microbial community[31,32].

CH_4 is produced as the terminal product of organic matter decomposition, and is known to be dependent on a series of syntrophic associations with anaerobic bacteria, protozoa and fungi, which supply the methanogens with the precursors for methanogenesis[3], H_2, CO_2 and acetate. It is accomplished by the more or less sequential reduction of NO_3^-, Mn(IV), Fe(III), and SO_4^{2-}, before CH_4 production becomes the sole process of organic matter degradation[31,32]. The length of this reduction period, during which organic matter is oxidised to CO_2, and which precedes the production of CH_4, is determined by the relative amounts of organic matter and inorganic electron acceptors in the sediment[31]. These factors are likely to vary between experiments carried out on sediments from different sites. Thus, between-experiment variation in the length of the incubation period over which the estimate of CH_4 production is determined may influence the measured rate, and may also interact with temperature (for example, if the sequential reduction of electron acceptors proceeds faster at higher temperatures[32]) to influence the estimated apparent activation energy.

The experimental techniques applied in these studies were heterogeneous with respect to the duration over which the flux of CH_4 was determined—ranging from 2 to 30 days—and with respect to whether or not there was a pre-incubation period. We found no evidence that length of the incubation duration affected estimates of the apparent activation energy when this variable was included as a fixed factor in our linear mixed-effects model (likelihood ratio test comparing an alternative model including an interaction between temperature and the length of the incubation duration to a null model with no interaction term: $\chi^2 = 0.95$, d.f. = 1, $P = 0.33$). These findings suggest that the estimated apparent activation energy is largely unaffected by potential effects of temperature on the speed of alternative electron acceptor reduction. Among the 47 studies, 15 entailed pre-incubation periods ranging from 1 to 60 days in the dark at 4 °C. We found no evidence that the presence or absence of an incubation period affected the estimated temperature dependence when this variable was included as a binary fixed factor in our mixed-effects model (likelihood ratio test comparing a null model with a single activation energy to an alternative model with separate activation energies for experiments with and without pre-incubation periods; $\chi^2 = 1.92$, d.f. = 1, $P = 0.38$). In summary, estimates of the apparent activation energy appear to be largely unaffected by differences in experimental protocols among studies.

Our database of ecosystem-level CH_4 emission data comprises both published data compiled from the literature and unpublished data analysed here for the first time (see below for data-collection methods for the unpublished data set). In total, this database comprises 1,553 estimates of CH_4 emission and temperature taken from 127 sites across the globe, including 57 aquatic ecosystems, 51 wetlands and 19 rice paddies (Extended Data Fig. 1). CH_4 emissions were estimated using one of three methods: the floating-chamber technique; the eddy-covariance method; or occasionally (<5% of the estimates) the modelled-diffusion method, which yields estimates based on the concentration of CH_4 just below the air–water interface. The estimated apparent activation energies of seasonal CH_4 emissions did not vary significantly among the three methods when this variable was included as a fixed factor in the mixed-effects model (likelihood ratio test comparing a null model with a single activation energy to an alternative model with separate activation energies for each measurement method; $\chi^2 = 0.52$, d.f. = 2, $P = 0.77$), which justifies combining data obtained using all three methods for the analysis.

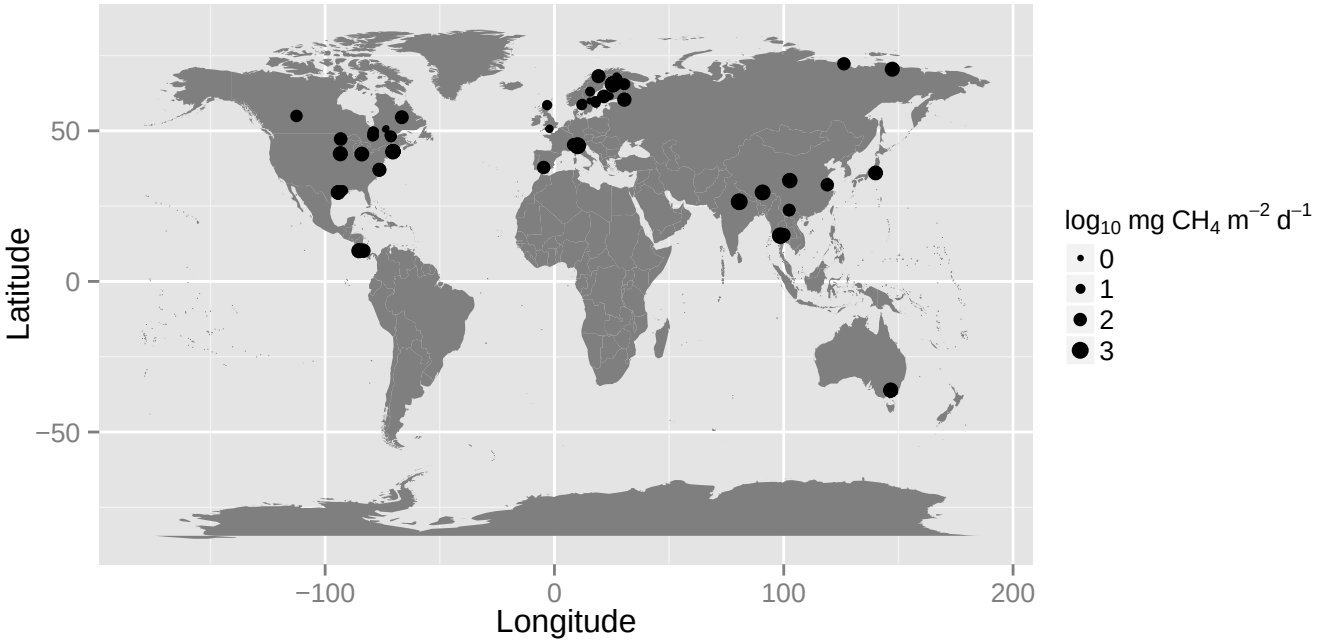
We assessed potential differences in the temperature dependence of CH_4 emissions among three 'ecosystem types'—aquatic, wetland and rice-paddy ecosystems—owing to differences in the physical and chemical characteristics of these ecosystems. 'Aquatic' ecosystems were defined as those that have standing water throughout the year, and included lakes and rivers. Wetlands were defined as natural systems with either permanently or seasonally water-saturated soils, and include peatlands, bogs and swamps. Finally, rice paddies were defined as man-made water-saturated sites established for rice cultivation. We separated these systems for the analysis, because the differences in their physical and chemical characteristics have the potential to modify the seasonal temperature dependence of CH_4 emission in a systematic way. For example, methanotrophy in aerobic zones can re-oxidize up to 90% of the CH_4 produced in anaerobic sediments[6]. Among these three ecosystem types, aquatic systems (lakes and rivers) are expected to have the largest aerobic zones beneath the air–water interface, and may therefore be expected to exhibit temperature dependencies that deviate furthest from the intrinsic temperature dependence of methanogenesis. However, as noted in the main text, although the temperature dependencies clearly varied between sites (Fig. 2b), we found no evidence of systematic differences in temperature dependence between the three ecosystem types (likelihood ratio test comparing a null model with a single activation energy to an alternative model with separate activation energies for each ecosystem type; $\chi^2 = 4.97$, d.f. = 2, $P = 0.10$). This finding is broadly consistent with recent studies[33–35], which show that methanotrophy is primarily limited by substrate availability under *in situ* CH_4 concentrations in aquatic ecosystems, and exhibits no temperature dependence when CH_4 is limiting. Therefore, our results, and those of refs 33–35, suggest that methanotrophy generally does not interact with CH_4 production and temperature under *in situ* CH_4 concentrations. Rather, methanotrophy seems to track the seasonal dynamics of CH_4 production, resulting in little modulation of the observed temperature dependence.

Another factor that may systematically affect the observed temperature dependence of CH_4 emissions, at least for the fluxes estimated through the floating-chamber technique, is the relative contributions of fluxes attributable to diffusion versus ebullition. This is because ebullition has the potential to bypass the methanotrophic microbial populations in the oxic zones of the sediment and water column. We believe that the estimates of CH_4 emissions in our database are largely attributable to diffusive fluxes, due to the extensive sampling effort (in both space and time) required to accurately quantify ebullition fluxes. Although none of our flux estimates could be partitioned into diffusive and ebullition fluxes, we attempted to assess indirectly whether ebullition influenced the estimated temperature dependence by including the duration over which the flux chamber was deployed as a covariate in our mixed-effects model. In carrying out this analysis, we reasoned that ebullition fluxes were more likely to be assayed for chambers deployed for longer periods of time. However, we found no evidence of a significant interaction between 'flux duration' and 'temperature' in the mixed-effects model (likelihood ratio test comparing an alternative model including an interaction between temperature and flux duration to a null model with no interaction term: $\chi^2 = 0.85$, d.f. = 1, $P = 0.36$) when analysing fluxes estimated using the floating-chamber technique ($n = 1{,}007$, 118 sites).

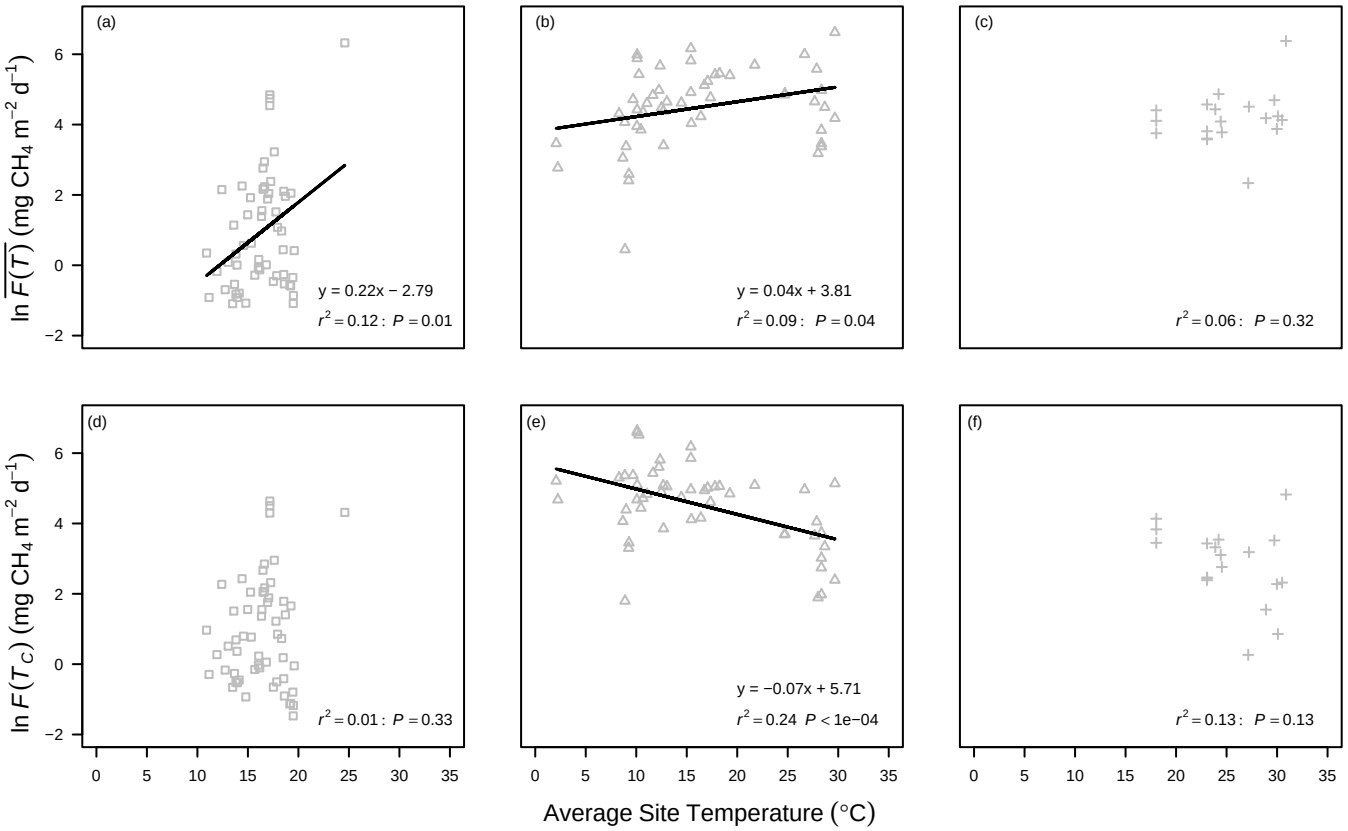
The unpublished emission data included in our analysis come from lakes and rivers located in four distinct boreal regions of Québec, eastern Canada: Abitibi south (48.5° N, 79° W), Abitibi Borth (51° N, 79° W), Chibougamau (49.5° N, 74° W) and Chicoutimi (48° N, 71° W), and in boreal Sweden (58–59° N, 12–18° E). Geographic and climatic details of these regions can be found in ref. 36 and at http://www.smhi.se, respectively. The selected lakes and rivers were visited from three to 12 times during the open water season (May to November 2012). Surface-water partial pressures of CO_2 (pCO_2; Canadian sites only) and of CH_4 (pCH_4) were measured using the headspace method. Water was sampled at 0.1–0.5 m from the surface from the deepest measured point of lakes, and near the shores of streams, rivers and wetlands. Samples were taken using a 60-ml polypropylene syringe by extracting 30 ml of water, followed by 30 ml of ambient air, to create a headspace in a 1:1 ratio of ambient air to water. The syringe was then vigorously shaken for 1 min in order to equilibrate the gases in water and air. Ambient pCO_2 was measured *in situ* using an EGM-4 infrared gas analyser (PP-systems). pCH_4 samples (in duplicate) were placed in 30-ml glass vials equipped with crimped rubber stoppers, filled with saturated saline solution, and then stored inverted until subsequent analysis in laboratory with Shimadzu GC-8A Gas chromatograph with FID (flame ionization detector). In Canada, the CO_2 and CH_4 fluxes were measured on the basis of changes in pCO_2 and pCH_4 with time in floating chambers. The chamber was connected in closed loop to an EGM-4 infrared gas analyser (PP-systems), with pCO_2 in the chamber measured every minute for 10 min. The accumulation rate was estimated from these data using linear regression. Samples for pCH_4 were taken from a sampling port every 10 min, and analysed as described above. Flux calculations were carried out following ref. 37. The measurements in Sweden focused on CH_4 only allowing 24 h floating flux chamber deployments. The details of field approaches and flux calculations for these measurements are found in ref. 38.

For ecosystem-level studies including CH_4:CO_2 ratio, the seasonal temperature dependence of the CH_4:CO_2 efflux ratio was estimated for the subset of field sites in our database (38 of 127) with simultaneous measurements of CO_2 and CH_4 emissions.

27. Bolker, B. M. *et al.* Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* **24,** 127–135 (2009).
28. R core development team R: a language and environment for statistical computing. http://www.R-project.org (R Foundation for Statistical Computing, 2014).
29. Pinheiro, J. & Bates, D. M. *Mixed-effects models in S and S-PLUS* (Springer Verlag, 2000).
30. Van den Berg, L., Patel, G. B., Clark, D. S. & Lentz, C. P. Factors affecting the rate of methane formation from acetic-acid by enriched methanogeneic cultures. *Can. J. Microbiol.* **22,** 1312–1319 (1976).
31. Yao, H. & Conrad, R. Effect of temperature on reduction of iron and production of carbon dioxide and methane in anoxic wetland rice soils. *Biol. Fertil. Soils* **32,** 135–141 (2000).
32. van Bodegom, P. M. & Stams, A. Effects of alternative electron acceptors and temperature on methanogenesis in rice paddy soils. *Chemosphere* **39,** 167–182 (1999).
33. Lofton, D., Whalen, S. & Hershey, A. Effect of temperature on methane dynamics and evaluation of methane oxidation kinetics in shallow Arctic Alaskan lakes. *Hydrobiologia* **721,** 209–222 (2014).
34. Duc, N. T., Crill, P. & Bastviken, D. Implications of temperature and sediment characteristics on methane formation and oxidation in lake sediments. *Biogeochemistry* **100,** 185–196 (2010).
35. Megonigal, J. P. & Schlesinger, W. H. Methane-limited methanotrophy in tidal freshwater swamps. *Glob. Biogeochem. Cycles* **16,** 1088–1095 (2002).
36. Lapierre, J.-F. & del Giorgio, P. A. Geographical and environmental drivers of regional differences in the lake pCO_2 versus DOC relationship across northern landscapes. *J. Geophys. Res.-Biogeosci.* **117,** (2012).
37. Vachon, D., Prairie, Y. T. & Cole, J. J. The relationship between near-surface turbulence and gas transfer velocity in freshwater systems and its implications for floating chamber measurements of gas exchange. *Limnol. Oceanogr.* **55,** 1723–1732 (2010).
38. Bastviken, D. *et al.* Methane emissions from Pantanal, South America, during the low water season: toward more comprehensive sampling. *Environ. Sci. Technol.* **44,** 5450–5455 (2010).



Extended Data Figure 1 | Global distribution of the field sites included in our analysis of ecosystem-level CH_4 emissions. The size of each point relates to the logarithm (base 10) of the mean rate of CH_4 emission (mg CH_4 per m^2 per day) over the duration of the experiment.



Extended Data Figure 2 | Correlations of average site temperatures with average CH_4 emissions and CH_4 emissions at fixed temperature for globally distributed ecosystems. Average CH_4 emissions are shown in **a**–**c**, and CH_4 emissions at fixed temperature are shown in **d**–**f**. Average site temperature is positively correlated with average CH_4 emissions, $\ln \overline{F(T)}$, for aquatic ecosystems (**a**) and natural wetlands (**b**), although temperature explains only 12% and 9% of the variance, respectively, for CH_4 emissions from these two ecosystem types. In contrast, it is not significantly correlated with average CH_4 emissions for rice paddies (**c**). Average site temperature is also not correlated with CH_4 emissions at fixed temperature, $\ln F(T_C)$ (where T_C is the average temperature across the field emissions data set (15.6 °C)), for aquatic ecosystems (**d**) and rice paddies (**f**), but is significantly negatively correlated for natural wetlands (**e**). The latter finding suggests that temperature-dependent biotic (for example, methanotrophy, substrate supply, microbial community structure, physiological acclimation and/or adaptation) and abiotic factors (for example, water-table depth) may play an important role in constraining variation in total CH_4 emissions among wetlands along geographic temperature gradients.