



Eutrophication exacerbates the impact of climate warming on lake methane emission

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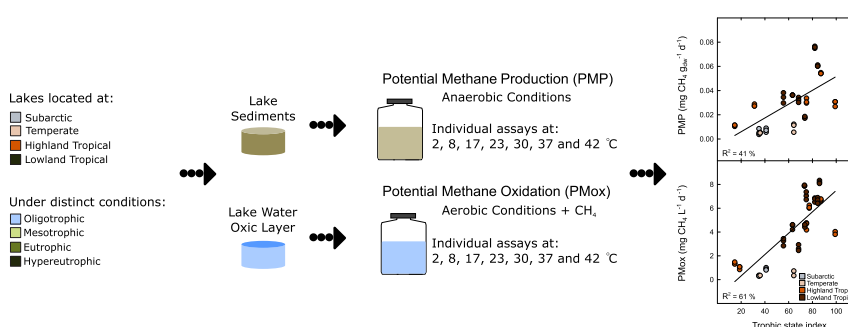
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HIGHLIGHTS

- Net methane emission from lakes depends on methanogenesis and methanotrophy
- Methanogenesis is more sensitive to temperature increments than methanotrophy
- Rates of methanogenesis and methanotrophy are positively influenced by eutrophication
- A temperature buildup of 2 °C would lead to an increase in CH₄ emissions by 47–183%

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 23 February 2018

Received in revised form 19 April 2018

Accepted 20 April 2018

Available online 27 April 2018

Editor: Jay Gan

Keywords:

Climate warming
Greenhouse gases
Lakes
Carbon cycle
Trophic status
Latitudinal gradient

ABSTRACT

Net methane (CH₄) emission from lakes depends on two antagonistic processes: CH₄ production (methanogenesis) and CH₄ oxidation (methanotrophy). It is unclear how climate warming will affect the balance between these processes, particularly among lakes of different trophic status. Here we show that methanogenesis is more sensitive to temperature than methanotrophy, and that eutrophication magnifies this temperature sensitivity. Using laboratory incubations of water and sediment from ten tropical, temperate and subarctic lakes with contrasting trophic states, ranging from oligotrophic to hypereutrophic, we explored the temperature sensitivity of methanogenesis and methanotrophy. We found that both processes presented a higher temperature sensitivity in tropical lakes, followed by temperate, and subarctic lakes; but more importantly, we found that eutrophication triggered a higher temperature sensitivity. A model fed by our empirical data revealed that increasing lake water temperature by 2 °C leads to a net increase in CH₄ emissions by 101–183% in hypereutrophic lakes and 47–56% in oligotrophic lakes. We conclude that climate warming will tilt the CH₄ balance towards higher lake emission and that this impact will be exacerbated by the eutrophication of the lakes.

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1. Introduction

Among the natural sources of CH₄ to the atmosphere, lakes represent a major source of uncertainty to the overall budget (Dean et al., 2018; Holgerson and Raymond, 2016; Saunois et al., 2016; Wik et al., 2016), with annual emissions ranging from 37 to 112 Tg CH₄ to the atmosphere (Saunois et al., 2016). Most lakes are found in northern latitudes, and are relatively small (Verpoorter et al., 2014). However, lakes located in tropical latitudes are highly dynamic, and despite their minor contribution to the overall lake area (~27%), they represent an important source of CH₄ to the atmosphere, contributing ~49% of the global lake and reservoir CH₄ emissions (Holgerson and Raymond, 2016; Bastviken et al., 2011).

Microbial methanogenesis in lakes mainly occurs in anoxic zones, with CH₄ being produced mostly in anoxic sediments, whereas methanotrophy in lakes primarily takes place in oxic zones, consuming CH₄ in the presence of oxygen (O₂). Methanogenesis in lakes is strongly dependent on the availability of labile substrates and is consequently affected by autochthonous (e.g. decaying aquatic biomass such as phytoplankton) and allochthonous (e.g. runoff from adjacent ecosystems) carbon inputs (Conrad, 1999; Grasset et al., 2018; Peura et al., 2014; Schulz et al., 1997; West et al., 2016). Methanotrophy depends on the availability of CH₄ and O₂, and thus primarily occurs in the oxic zones within the water column (Bastviken et al., 2008; Kankaala et al., 2007; Martinez-Cruz et al., 2015). Oxygenated water can be found throughout the entire water column in well mixed or shallow lakes or can be restricted to the epilimnion in some stratified lakes; but it is estimated that a large fraction of the CH₄ migrating through the water column in lakes is suitable for oxidation by methanotrophs (Bastviken et al., 2002; Bastviken et al., 2008; Kankaala et al., 2007). In addition, the availability of dissolved O₂ in lakes varies according to season, trophic state, stratification, and geomorphology; being the depth of particular relevance (Jenny et al., 2016; Lewis, 1996; Nürnberg, 1996), making methanotrophy spatio-temporally variable.

Since methanogenesis and methanotrophy are strongly dependent on temperature (Duc et al., 2010; Dunfield et al., 1993; Lofton et al., 2014; Zeikus and Winfrey, 1976), temperature changes throughout the year can influence the seasonal patterns of CH₄ net emission from lakes (Aben et al., 2017; Davidson et al., 2018; Marotta et al., 2014; Yvon-Durocher et al., 2014; Yvon-Durocher et al., 2017). Methanogenesis increases exponentially with temperature until reaching an optimal value and then declines rapidly due to inactivation and cellular decay (Schulz et al., 1997; Svensson, 1984). Moreover, methanogenesis is carried out by two coexisting biochemical pathways; i.e., autotrophy and heterotrophy, which have different thermal dependencies and distinct temperature optima (Conrad, 1999; Schulz et al., 1997; Svensson, 1984). The thermal dependency of methanotrophy may be less complex. Lofton et al. (2014) reported a linear response of methanotrophy to temperature increase under substrate-saturated conditions, and Duc et al. (2010) and Dunfield et al. (1993) have observed that substrate availability (i.e., CH₄ and O₂) positively affects methanotrophy rates in a stronger way than temperature. The response of methanogenesis and methanotrophy to temperature comprises a complicated matrix of factors, and its understanding is fundamental to predict how net CH₄ emission from aquatic ecosystems will respond to climate warming (Aben et al., 2017; Audet et al., 2017; Davidson et al., 2018; Marotta et al., 2014; Negandhi et al., 2016; Yvon-Durocher et al., 2014; Yvon-Durocher et al., 2017).

In this context, eutrophication, which has become a major problem affecting biodiversity and biogeochemical cycles in aquatic ecosystems (Moss et al., 2011; Schilder et al., 2017; Schindler, 2012), may play an important role in CH₄ cycling, because it modifies O₂ and substrate availability in lakes, regulating methanogenesis and methanotrophy. Furthermore, climate warming exacerbates some symptoms of eutrophication, such as deoxygenation of the water column and increased

phytoplankton biomass (Moss et al., 2011). Currently, eutrophication in lakes, associated to climate warming and anthropogenic activities, has increased, with ecosystems showing scarce or null resilience (Jenny et al., 2016). Therefore, it is plausible that severe eutrophication could alter the magnitude and net balance of CH₄ production and oxidation in lakes (Aben et al., 2017; Adrian et al., 2016; Davidson et al., 2018).

Until now, it has been difficult to determine whether climate warming in conjunction with eutrophication will tilt the CH₄ balance in lakes towards higher or lower CH₄ emissions to the atmosphere. To address this question, we conducted anaerobic potential methanogenic (PMP) and aerobic potential methanotrophic (PMox) assays, using sediments and water samples, respectively. Samples were collected from ten lakes located in tropical, temperate and subarctic latitudes. These lakes varied in their trophic state, ranging from oligotrophic to hypereutrophic. The samples were incubated at temperatures ranging from 2 to 42 °C, and from these assays, the temperature dependence of both methanogenic and methanotrophic processes was determined and used to model the impact of a potential climate warming scenario on net CH₄ emission from lakes.

2. Materials and methods

2.1. Study sites and sampling

Incubations were conducted using sediment and water samples from 10 lakes located across different latitudes (Table 1). Two were located at high-latitude (Alaska), two at temperate latitude (Germany) and six at tropical latitude (Mexico). Among the six lakes located in Mexico, three were in highlands (altitudes between 2255 and 2840 m asl) and three in lowlands (0–20 m asl). The ecosystems were selected according to four main criteria: (i) lake characteristics that are typical from the region, (ii) availability of limnological and biogeochemical information, (iii) accessibility to the ecosystem, and (iv) priority was given to the selection of ecosystems with distinct nutrient regimes. For this purpose, the trophic state index (TSI) and physicochemical parameters, determined in previous studies, were considered for the selection of the study sites (Table 1). The physicochemical parameters discussed in this study included: Secchi disk depth (m), determined with a 0.2 m Secchi disk; ammonium-NH₄⁺ (mg L⁻¹), nitrate-NO₃⁻ (mg L⁻¹), and soluble reactive phosphorous-SRP (mg L⁻¹), determined according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005); chlorophyll *a* (mg L⁻¹), determined in Mexico and Germany according to standard methods, while a calibrated multiparametric probe Hydrolab Data Sonde (Hach, Loveland, Colorado, USA) was used for the chlorophyll *a* analyses of the water samples from Alaskan lakes. Whereas total organic carbon-TOC (mg L⁻¹) and total nitrogen-TN (mg L⁻¹) were measured using carbon elemental analyzers (TOC Shimadzu-Vcsn + TN1 module, in Mexico and Germany; Aurora TOC 1030 W, O-I Analytical, in Alaska).

Surficial sediment samples (for incubation tests) were collected in the central region of each lake using a mud sampler (Ekman dredge for Alaskan and Mexican lakes, and a gravity core for German lakes), with the objective of obtaining samples of the most active anaerobic sediment layer (Marotta et al., 2014). Simultaneously, water samples were collected from the oxycline using a water sampler (2.2 L horizontal Van Dorn Bottle for Alaskan and Mexican lakes and a 2 L Limnos water sampler for German lakes). When the oxycline was absent, samples were taken from the epilimnion at 1 m depth (e.g., Otto and Llano lakes samples), since it has been observed that methanotrophy rates are similar throughout the water column (Martinez-Cruz et al., 2015; Utsumi et al., 1998). Sediment and water samples were stored for about a day at 2 °C, until incubation tests where initiated. The estimation of aerobic and anaerobic methanotrophy occurring on surficial sediments was out of the scope of the present study.

Table 1
Location and characteristics of study sites.

Name	Latitude (°N)	Longitude (°W)	Biome	TSI Classification	TSI ^a	Area (km ²)	MD ^b (m)	References
Goldstream ^c	64.916	147.908	Subarctic yedoma-type thermokarst lake	Mesotrophic	41	0.01	3.3	Sepulveda-Jauregui et al. (2015)
Otto	63.842	149.037	Subarctic non-yedoma-type lake	Oligotrophic	35	0.52	3.1	Sepulveda-Jauregui et al. (2015)
Dagow	53.150	13.051	Temperate lake	Eutrophic	64	0.24	9.5	Casper (1992); Casper et al. (2005)
Stechlin	53.152	13.026	Temperate lake	Oligotrophic	34	4.24	68	Casper (1992); Casper et al. (2005)
Guadalupe	19.627	99.269	Tropical man-made highland lake	Hypereutrophic	85	4.50	17	Sepulveda-Jauregui et al. (2013)
Llano	19.658	99.507	Tropical man-made highland lake	Oligotrophic	22	0.06	14.2	Sepulveda-Jauregui et al. (2013)
Tezozomoc	19.499	99.211	Tropical man-made highland lake	Hypereutrophic	99	0.02	1.2	Martinez-Cruz et al. (2017)
San Julian	19.254	96.262	Tropical lowland lake	Eutrophic	66	0.83	1.5	This study
Olmecca	19.149	96.15	Tropical man-made lowland lake	Hypereutrophic	90	0.62	1.5	Gonzalez-Valencia et al. (2014)
Encanto	19.168	96.156	Tropical man-made lowland lake	Hypereutrophic	80	0.02	1.7	This study

^a TSI = Trophic state index based on reported data.

^b MD = Maximum known depth.

^c Colloquial lake name.

2.2. Experimental setup

The aim of our study was to improve our understanding of the factors influencing methanogenesis and methanotrophy in lakes, including the underlying processes. With that purpose, both processes were quantified through incubations. However, it is of the utmost importance to note that both the CH₄ production and oxidation rates obtained from these incubations do not reflect *in situ* rates and should only be considered as potential. This is mainly due to the disturbance exerted upon the sediment and water samples during the sampling process and the experimental set up (Hoyos-Santillan et al., 2016a; Moore and Dalva, 1997).

The PMP was estimated using surficial sediments from the top 20 cm layer where higher methanogenic activity is located, whereas PMox was estimated using water samples. Sediment used for PMP assays were diluted (1:1 v/v) with CH₄-free anaerobic water that was previously boiled and degassed with Ultra-High Purity N₂ (UHP, 99.999%). From the resulting slurry, 30 mL were transferred to serological bottles (60 mL) under continuous flushing with N₂ UHP. Nitrogen flushing was maintained for an additional five minutes. Subsequently, serological vials were air-tight sealed with rubber stoppers and aluminium crimp caps. Incubations were run in triplicate at 2, 8, 17, 23, 30, 37 and 42 °C for 30 to 45 days. PMox was determined using 30 mL water samples, which were transferred to 60 mL serological bottles under

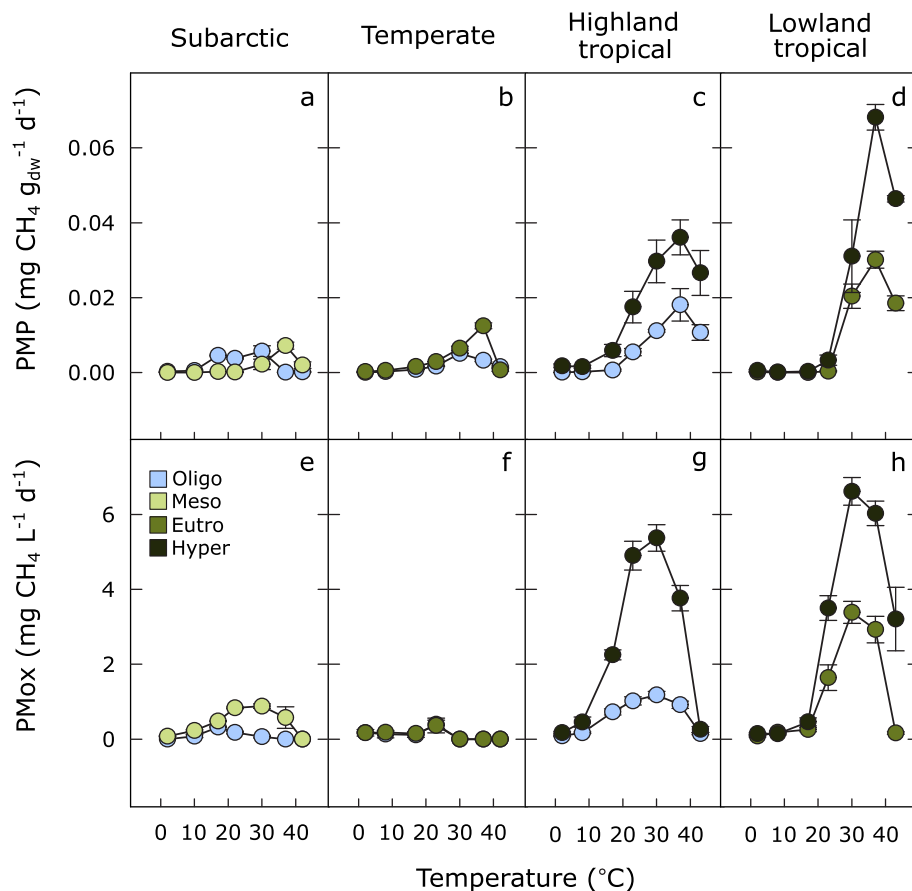


Fig. 1. Temperature sensitivity of the potential CH₄ production-PMP in sediments (a-d) and the potential CH₄ oxidation- PMox in water column (e-h). Colour circles represent the trophic state of the lake; oligotrophic (blue), mesotrophic (light green), eutrophic (medium green) and hypereutrophic (dark green). Errors bars indicate standard error of incubation set of triplicate incubation; two subarctic, two temperate, three highland tropical and three lowland tropical lakes analyzed.

continuous flushing with synthetic air (21% O₂, balance N₂). Flushing was maintained for additional five minutes; afterwards, vials were air-tight sealed with butyl-rubber stoppers and aluminium crimp caps. Each PMox incubation vial was spiked with 1 mL of CH₄ (99.0% CH₄), giving a final gas phase CH₄ concentration of 3.33%. PMox incubations were run by triplicate at 2, 8, 17, 23, 30, 37 and 42 °C for 15 days. PMP and PMox rates were calculated from the linear accumulation or consumption of CH₄ in the headspace over the incubation time, respectively. Concentration of CH₄ was determined by gas chromatography; the chromatograph was equipped with a flame ionization detector and FID detector (Shimadzu 2014 in Alaska and Germany, and Perkin Elmer Clarus 500 in Mexico). Potential rates of methanogenesis and methanotrophy were accepted when the linear regression models presented a $P < 0.001$ and a $R^2 > 0.7$ (data from at least 4 measurements in each triplicate temperature). PMP and PMox rates are reported as mass of CH₄ produced per mass of dry weight of sediment per day (mg CH₄ g_{dw}⁻¹ d⁻¹) and mass of CH₄ oxidized per volume of water per day (mg CH₄ L⁻¹ d⁻¹), respectively.

We used temperature dependence to explore the effect of trophic state and latitude on the potential rates of PMP and PMox. The observed temperature maxima (T_{opt}) is the temperature at which the highest PMP or PMox rates were observed for each lake (Svensson, 1984). PMox assays were conducted without substrate limitation (i.e., O₂ and CH₄), and PMP assays were conducted without substrate addition, but

under well mixed conditions, which reduce potential local limitations of mass transfer, although mixing may disturb microbial consortia present in the natural environment. These conditions were selected to restrain additional factors from influencing T_{opt} .

2.3. Q_{10} and activation energy

The sensitivity of PMP and PMox to temperature was assessed through the Q_{10} coefficient, which corresponds to the magnitude of PMP or PMox rate change as the result of a 10 °C temperature increase (Eq. (1)); where r_a and r_b are the PMP (mg CH₄ g_{dw}⁻¹ d⁻¹) or PMox (mg CH₄ L⁻¹ d⁻¹) rates at the incubation temperatures T_a and T_b (°C), respectively:

$$Q_{10} = \left(\frac{r_b}{r_a} \right)^{\left(\frac{10}{T_b - T_a} \right)} \quad (1)$$

Additionally, the sensitivity of PMP and PMox to temperature was also assessed through the apparent activation energy (E'_a ; kJ mol⁻¹) described by the Arrhenius equation (Duc et al., 2010). The apparent activation energy was calculated from the positive exponential section of the PMP and PMox temperature dependence curves. For this purpose, the Arrhenius equation was linearized by applying natural logarithm

Table 2
 E'_a and Q_{10} values (mean ± SE) for: potential methanotrophic production (PMP) and potential aerobic oxidation of CH₄ (PMox). The summary of the statistical analysis outputs (REML) is also presented. A is the Arrhenius constant, which is a pre-exponential factor that expresses the dependence of the rate coefficient to temperature.

Assay	Lake	Trophic state	E'_a (kJ mol ⁻¹)	Q_{10}	A
PMP	Otto	Oligotrophic	92.6 ± 6.6	3.5 ± 0.6	6.12 × 10 ¹⁶
	Stechlin	Oligotrophic	105.5 ± 1.1	4.9 ± 0.0	1.33 × 10 ¹⁹
	L. Llano	Oligotrophic	127.9 ± 16.1	6.2 ± 0.7	1.53 × 10 ²³
	Goldstream	Mesotrophic	142.8 ± 10.6	8.5 ± 0.1	7.70 × 10 ²⁵
	Dagow	Eutrophic	77.3 ± 1.7	2.9 ± 0.0	1.03 × 10 ¹⁴
	L. Guadalupe	Hypereutrophic	72.4 ± 2.6	2.5 ± 0.0	1.33 × 10 ¹³
	Encanto	Hypereutrophic	194.4 ± 14.3	5.2 ± 1.4	1.74 × 10 ²⁵
	Tezozomoc	Hypereutrophic	230.1 ± 2.6	18.9 ± 0.6	5.17 × 10 ⁴¹
	Olmecca	Hypereutrophic	243.2 ± 30.2	4.3 ± 0.5	1.22 × 10 ⁴⁴
	San Julian	Hypereutrophic	274.1 ± 57.7	7.1 ± 1.1	4.89 × 10 ⁴⁹
PMox	Otto	Oligotrophic	103.7 ± 8.3	4.6 ± 0.4	3.78 × 10 ¹⁸
	Stechlin	Oligotrophic	77.8 ± 13.4	3.4 ± 0.6	7.62 × 10 ¹³
	L. Llano	Oligotrophic	64.9 ± 6.3	2.4 ± 0.1	3.50 × 10 ¹¹
	Goldstream	Mesotrophic	74.6 ± 3.9	2.7 ± 0.3	2.00 × 10 ¹³
	Dagow	Eutrophic	71.4 ± na	2.9 ± na	5.27 × 10 ¹²
	L. Guadalupe	Hypereutrophic	144.3 ± 17.5	3.1 ± 0.2	8.65 × 10 ²⁵
	Encanto	Hypereutrophic	101.5 ± 7.8	3.4 ± 0.2	1.51 × 10 ¹⁸
	Tezozomoc	Hypereutrophic	70.6 ± 2.4	3.0 ± 0.1	3.77 × 10 ¹²
	Olmecca	Hypereutrophic	149.8 ± 27.3	3.8 ± 0.4	8.60 × 10 ²⁶
	San Julian	Hypereutrophic	110.1 ± 13.2	7.4 ± 2.2	5.46 × 10 ¹⁹

Summary of statistical analyses for E'_a and Q_{10} in PMP and PMox^{a,b}

		F	df	P
E'_a	PMP-PMox	22.4	1,74	<0.001
	PMP			
	Trophic state	7.1	3,32	<0.001
	Latitude	6.7	3,32	<0.001
	PMox			
	Trophic state	4.1	3,37	<0.013
	Latitude	0.9	3,37	n.s.
Q_{10}	PMP-PMox	12.8	1,75	<0.001
	PMP			
	Trophic state	1.28	3,32	n.s.
	Latitude	0.59	3,32	n.s.
	PMox			
	Trophic state	1.75	3,37	n.s.
	Latitude	2.97	3,37	0.04

^a E'_a and Q_{10} values were log₁₀ transformed to fulfil the homogeneity of variance requirements of the REML.

^b n.s. = not significant (>0.05).

(Eq. (2)); where r_T is the PMP or PMox rates at T; A is the Arrhenius constant, which is a pre-exponential factor that expresses the dependence of the rate coefficient on temperature; R is the universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}$); T is the absolute incubation temperature expressed in Kelvin (K), and E'_a/R is determined from the slope of the $\ln(r_T)$ vs $1/T$ graph:

$$\ln(r_T) = \ln(A) - \left(\frac{E'_a}{R} \frac{1}{T} \right) \quad (2)$$

2.4. Impact of a temperature increase scenario on lake CH_4 emissions

To evaluate the effect of a potential temperature increase caused by climate warming in CH_4 production and oxidation in lakes, we considered a model lake in which CH_4 production and oxidation comply with the following assumptions: (i) the lake has a homogeneous reference temperature (T) assumed to be 15°C (present temperature assumption) and is subjected to a temperature increase that affects equally the water column and sediments (ΔT); (ii) at T, the total CH_4 production rate in the lake ($R_{\text{MP},T}$; $\text{kg CH}_4 \text{ d}^{-1}$) is a fixed and arbitrary rate, while the total CH_4 oxidation rate in the lake ($R_{\text{MOX},T}$; $\text{kg CH}_4 \text{ d}^{-1}$) is considered to be 60% of $R_{\text{MP},T}$, which is a conservative estimation (Bastviken et al., 2008; Kankaala et al., 2007; Thauer et al., 2008); and (iii) the net CH_4 emission from the lake is exclusively the result of the balance between $R_{\text{MP},T}$ and $R_{\text{MOX},T}$. From these assumptions, we

estimated the impact of ΔT on the balance between CH_4 production and oxidation (Eq. (3)); where ΔCH_4 (%) is the modeled increase in net methane emission and $R_{\text{MP},T}$ and $R_{\text{MOX},T}$ were determined with the Arrhenius equation (Eq. (2)).

$$\Delta \text{CH}_4 (\%) = \left(\frac{R_{\text{MP},T+\Delta T} - R_{\text{MOX},T+\Delta T}}{R_{\text{MP},T} - R_{\text{MOX},T}} - 1 \right) \times 100 \quad (3)$$

2.5. Statistical analysis

We used linear mixed models to assess the effect of the trophic state and latitude on the temperature dependence of PMP and PMox. The models were fitted using residual maximum likelihood (REML) due to the unbalanced nature of the data, a consequence of the experimental program with different number of lakes at each latitude and under distinct trophic states. To analyse the effect of trophic state and latitude on Q_{10} and E'_a from PMP and PMox, trophic state and latitude were used as fixed factors and the individual lakes as random factor. PMP and PMox rates were $\log_{10}$ transformed to fulfil the normality condition of the REML. Level of significance of the differences between the fixed effects was estimated by Wald tests using an F distribution; significance was attributed at $P < 0.05$. To explore the relationship between PMP and PMox rates at T_{opt} against trophic state and physicochemical parameters we used linear regression analyses. Statistical analyses were performed

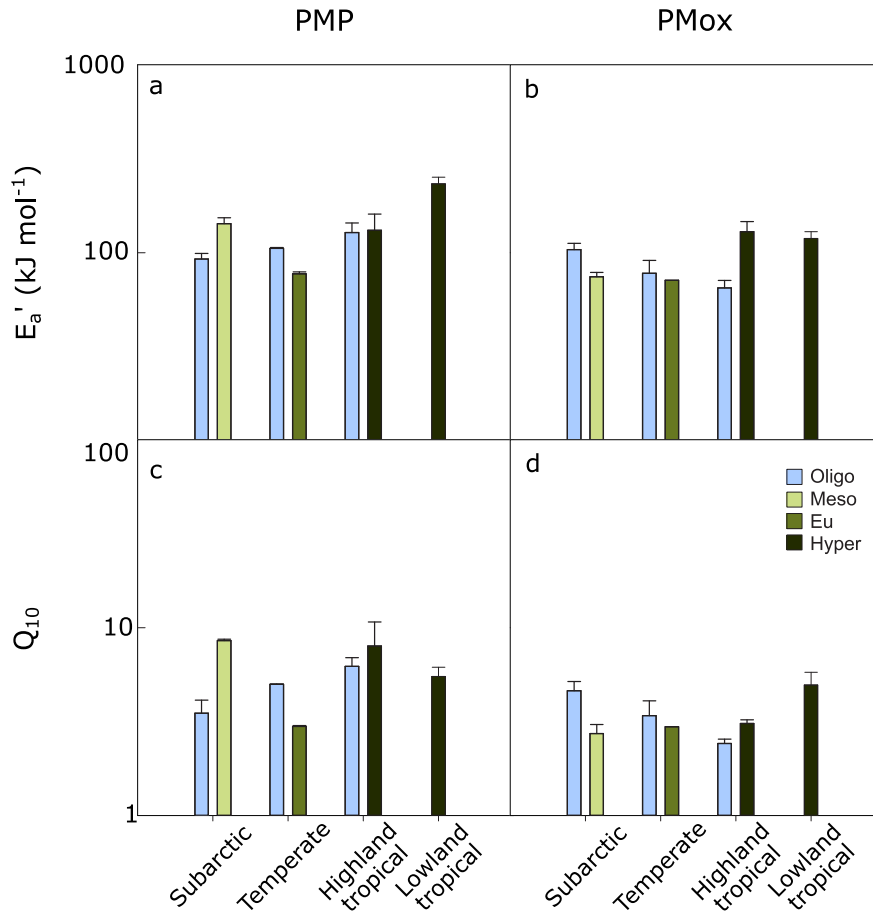


Fig. 2. Temperature sensitivity of the potential CH_4 production-PMP in sediments (a and c) and the potential CH_4 oxidation- PMox in water column (b and d) expressed as the apparent activation energy (E'_a) and Q_{10} coefficient. Colour bars represent trophic state of the lake; oligotrophic (blue), mesotrophic (light green), eutrophic (medium dark green) and hyper-eutrophic (dark green). The y axis is represented in a $\log_{10}$ scale. Errors bars indicate standard error of incubation set of triplicate incubation; two subarctic, two temperate, three highland tropical and three lowland tropical lakes analyzed.

with NCSS 11 Statistical Analysis System software (NCSS LLC, Kaysville UT, USA).

3. Results and discussion

3.1. Influence of eutrophication and latitude on optimum of temperature response

The PMP and the PMox rates showed a clear dependence with temperature (Fig. 1). Minimum PMP and PMox rates of $0.53 \pm 0.14 \mu\text{g g}_{\text{dw}}^{-1} \text{d}^{-1}$ and $0.13 \pm 0.01 \text{ mg CH}_4 \text{ L}^{-1} \text{d}^{-1}$, respectively were observed at the lowest tested temperatures (2°C) (PMP-Temperature of assay: $F_{7,210} = 210.1$, $P < 0.001$; PMox-Temperature of assay: $F_{7,181} = 93.9$, $P < 0.001$). These rates gradually increased until reaching an optimum rate, at different temperatures (T_{opt}), according to the latitude and trophic state (Fig. 1). The PMP presented higher mean T_{opt} values ($34.98 \pm 0.47^\circ\text{C}$) in comparison with PMox ($29.09 \pm 0.70^\circ\text{C}$; T_{opt} : $F_{1,88} = 85$, $P < 0.001$). The T_{opt} for PMP was independent of the trophic state and latitude of the lakes (T_{max} -trophic state: $F_{3,2,3} = 1.4$, $P > 0.05$; T_{opt} -latitude: $F_{3,2,3} = 2.8$, $P > 0.05$) (Fig. 1 a-d). This might be caused by the relatively low sensitivity of the methanogenic community composition to temperature variations, as previously reported (Cui et al., 2015). The mean T_{opt} value for methanogenesis ($34.98 \pm 0.47^\circ\text{C}$) is consistent with mesophilic methanogenesis activity, with optimal temperatures ranging between 30 and 42°C (Blake et al., 2015; Schulz et al., 1997; Zeikus and Winfrey, 1976). This range of temperature suggests that, in most of our assays, methanogenesis was conducted by heterotrophic and hydrogenotrophic (H_2/CO_2) pathways, since these temperatures favor the production of the necessary precursors for syntrophic H_2 production (Schulz et al., 1997). Despite the fact that T_{opt} for PMP was independent of the latitude of the lakes, a CH_4 maximum production rate was observed at 15°C in the sediments from subarctic lakes under oligotrophic conditions (Fig. 1a), but not in lakes located in lower latitudes, suggesting the presence of an active psychrotolerant/psychrophilic methanogenic community in some northern lakes (Nozhevnikova et al., 2003). By contrast, the T_{opt} for PMox rates varied with trophic state and latitude (T_{opt} -Trophic state: $F_{3,7,4} = 6.2$, $P < 0.05$; T_{opt} -latitude: $F_{3,5} = 9$, $P < 0.05$), increasing parallel to the trophic state index and decreasing as latitude increased (Fig. 1 e-h). Under oligotrophic conditions the mean T_{opt} for PMox rates was $25.40 \pm 1.95^\circ\text{C}$, whereas under hypereutrophic conditions, the mean T_{opt} for PMox rates was $31.17 \pm 0.83^\circ\text{C}$. The mean T_{opt} for PMox rates in subarctic lakes was $22.17 \pm 2.61^\circ\text{C}$, which is lower in comparison with the $31.26 \pm 0.81^\circ\text{C}$ observed in lowland tropical lakes. This can be explained by distinct methanotrophic consortia, whose composition is dependent on the average annual temperature in the lake, as an adaptive response to environmental conditions (Martineau et al., 2010; Trotsenko and Murrell, 2008; Wartiainen et al., 2006). Thus, in subarctic lakes, and temperate lakes the T_{opt} corresponded to a methanotroph community rich in psychrotolerant and psychrophilic microorganisms, compared to the mesophilic methanotrophs found in tropical lakes. Indeed, psychrotolerant and psychrophilic methanotrophs exist in the environment, and many ecosystems produce large quantities of CH_4 in cold regions (Ricao Canelhas et al., 2016; Martinez-Cruz et al., 2015; reviewed in Semrau et al., 2010).

Comparing all PMP and PMox rates observed at T_{opt} ($r_{\text{opt,PMP}}$ and $r_{\text{opt,PMox}}$, respectively), we found that both were influenced by the latitude ($r_{\text{opt,PMP}}$ -latitude: $F_{3,2,4} = 13.2$, $P < 0.05$; $r_{\text{opt,PMox}}$ -latitude: $F_{3,4} = 45.5$, $P < 0.01$), with highest values observed in lakes located at lowland tropical regions, whereas subarctic lakes presented the lowest $r_{\text{opt,PMP}}$ and $r_{\text{opt,PMox}}$ (Fig. 1). The $r_{\text{opt,PMP}}$ were also influenced by the trophic state ($r_{\text{opt,PMP}}$ -Trophic state: $F_{3,2,4} = 28.9$, $P < 0.05$), and were 14-fold lower in oligotrophic lakes ($0.005 \pm 0.001 \text{ mg CH}_4 \text{ g}_{\text{dw}}^{-1} \text{d}^{-1}$), compared to hypereutrophic lakes ($0.068 \pm 0.003 \text{ mg CH}_4 \text{ g}_{\text{dw}}^{-1} \text{d}^{-1}$) (Fig. 1 a-d). Similarly, the $r_{\text{opt,PMox}}$ were influenced by the trophic state ($r_{\text{opt,PMox}}$ -Trophic state: $F_{3,6} = 145.8$, $P < 0.001$), and were 20-fold lower under

oligotrophic conditions ($0.344 \pm 0.019 \text{ mg CH}_4 \text{ L}^{-1} \text{d}^{-1}$), than those observed in hypereutrophic lakes ($6.843 \pm 0.287 \text{ mg CH}_4 \text{ L}^{-1} \text{d}^{-1}$) (Fig. 1 e-h).

The PMP presented higher activation energy (E_a) values than PMox, with mean values of $165.34 \pm 13.59 \text{ kJ mol}^{-1}$ and $110.92 \pm 7.30 \text{ kJ mol}^{-1}$, respectively (Table 2; Fig. 2a-b). The highest E_a values for PMP and PMox were observed in hypereutrophic lakes (Fig. 2) (PMP, E_a - trophic state: $F_{3,32} = 7.1$, $P < 0.001$; PMox, E_a - trophic state: $F_{3,37} = 4.1$, $P < 0.001$), suggesting that PMP and PMox sensitivity to temperature is increased in highly eutrophic ecosystems. However, E_a values were similar in ecosystems ranging from oligotrophic to eutrophic, discarding a linear response of temperature sensitivity to eutrophication. The Q_{10} coefficient, which corresponds to the magnitude of rate change as result of a 10°C temperature increase, revealed the same tendency, with values of 5.92 ± 0.68 for PMP and 4.01 ± 0.44 for PMox (Table 2; Fig. 2c-d). The differences between Q_{10} values for methanogenesis and methanotrophy, as well as their magnitude, are consistent with previous reports; with values of up to 28 for methane

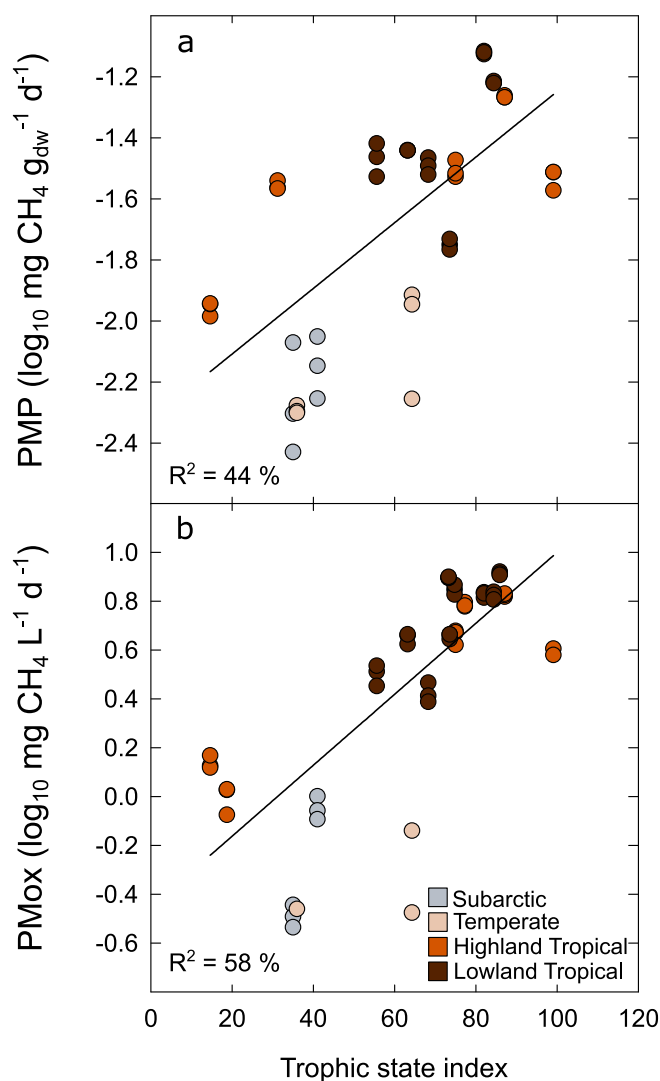


Fig. 3. The potential CH_4 production-PMP at T_{opt} in sediments (a) and the potential CH_4 oxidation-PMox T_{opt} in water column (b) in relation to trophic state index. Circles colour represents latitude location of the lake; subarctic (gray), temperate (pale orange), highland tropical (orange) and lowland tropical (brown). The fitted solid lines correspond to the linear regression estimates for a semi logarithmic data transformation.

production, and 4.1 for methane oxidation (Duc et al., 2010; Dunfield et al., 1993; Segers, 1998).

Our findings further support previous evidence that methanotrophy is less sensitive to temperature increments than methanogenesis, with a systematic approach encompassing, for the first time, methanogenic and methanotrophic processes at four different latitudes and in lakes with contrasting trophic states. As it will be developed hereafter, the main corollary of the lower thermal sensitivity of methanotrophy is that with climate warming a larger net CH₄ emission from lakes is expected. However, it is important to highlight that, in the present work, the results obtained, and their derived conclusions were based on a single time point, without considering seasonal variations. Whereas the results obtained from PMP incubations may be considered poorly sensitive to seasonal variations, as suggested by Yvon-Durocher et al. (2014), for instance Martinez-Cruz et al. (2015) observed strong variations of PMox in subarctic lakes among seasons. Therefore, due to the limited amount of lakes included in our experimental approach, the results presented in this study should be considered with caveats. Additional research including a larger number of lakes and a multi-seasonal approach is required to fully understand the effect of trophic state on the sensitivity of PMP and PMox to temperature changes.

3.2. Potential methanogenesis and methanotrophy in relation to eutrophication

When exploring the relationship between r_{opt} for both methanogenesis and methanotrophy and trophic state, lowland tropical lakes were clearly located in the higher cluster of the data regression, while the rates for subarctic and temperate lakes were located in the lower cluster (Fig. 3). Physicochemical parameters such as TOC, TN, NH₄⁺ and SRP were also positively correlated to $r_{opt,PMP}$ and $r_{opt,PMox}$; these physicochemical parameters explained 13 to 34% and 15 to 46% of the variance in $r_{opt,PMP}$ and $r_{opt,PMox}$, respectively (Table 3). Moreover, $r_{opt,PMP}$ and $r_{opt,PMox}$ were inversely correlated with the Secchi disk depth (Table 3). The relationship observed between the rates of PMP and PMox at T_{opt} and the degree of eutrophication or the physicochemical parameters (apart from chlorophyll-a), is consistent with literature, where the dominant role of nutrients and substrates availability on methanogenesis and methanotrophy rates has been reported (Martinez-Cruz et al., 2015; Schulz et al., 1997; Yvon-Durocher et al., 2017). For example, lakes rich in nutrients produce substantial amounts of labile organic matter from primary production, that can be readily transformed into precursors of CH₄, triggering large CH₄ emission to

the atmosphere (Davidson et al., 2018; Vonk et al., 2015; West et al., 2016; Yvon-Durocher et al., 2017).

3.3. Potential effect of climate warming on PMP and PMox rates in lakes

Given that present-day lake temperatures are lower than the T_{opt} determined for methanotrophy and methanogenesis in this study, a temperature increase caused by climate warming should stimulate both processes. Furthermore, being that methanogenesis is more sensitive to temperature than methanotrophy, CH₄ production should increase in a larger proportion than CH₄ consumption, affecting CH₄ balance in lakes, ultimately increasing their CH₄ emission to the atmosphere. To illustrate such effect, we considered a model lake in which CH₄ production and oxidation are affected by a potential increase in temperature, using a reference temperature of 15 °C (present temperature assumption); Fig. 4 shows the results of ΔCH_4 estimation with respect to ΔT , calculated from the mean A and E_a of two trophic state classes; hypereutrophic ($n = 6$) and oligotrophic lakes ($n = 3$; Table 2). It is relevant to mention that this model does not pretend to simulate what the impact of global warming would be at the global lake ecosystem scale but aims to illustrate the trend of net CH₄ emission in a model lake subject to temperature increase, using an average A and E_a, experimentally determined. In our model, ΔCH_4 does not depend on the fixed $R_{MP,T}$ selected, but is marginally related to the reference T and the percentage of CH₄ oxidized assumed at that T. For instance, with a reference T of 10 °C or 15 °C, the differences between the estimated ΔCH_4 would not exceed 4%. Similarly, if the percentage of CH₄ oxidized is 70% instead of 60%, the difference in ΔCH_4 would range between 16 and 24%.

According to our model (Fig. 4), a temperature increase would have a positive impact on the net CH₄ emission from the lake, which will be intensified by the trophic state. Considering the temperature dependence of CH₄ production and oxidation determined in the present work, a 2 °C temperature increase in hypereutrophic lakes would result in a 101–183% increase of the net CH₄ emission; whereas in oligotrophic lakes, the increase would be limited to 47–56%; both ranges estimated with a confidence interval of 95%.

We have several reasons to consider that our ΔCH_4 estimations are conservative. First, as previously mentioned, an increase in CH₄ production would fuel methanotrophy by increasing CH₄ availability, but would also reduce O₂ availability throughout the water column. This would be beneficial to anaerobic methanogenesis and detrimental to methanotrophy, but has not been considered in our model. Such sequence of reciprocal cause and effect has been observed in subarctic

Table 3
Linear regression models for PMP and PMox^a: physicochemical characteristics of water column.

		Intercept	s.e.	P	Slope	s.e.	P	R ²
PMP								
Secchi disk	(m)	−1.495	0.052	<0.001	−0.123	0.021	<0.001	0.43
Chlorophyll a	(mg L ^{−1})			n.s.			n.s.	–
Trophic state index		−2.322	0.117	<0.001	0.0107	0.001	<0.001	0.44
TOC	(mg L ^{−1})	−1.872	0.080	<0.001	0.006	0.001	<0.001	0.23
TN	(mg L ^{−1})	−1.920	0.069	<0.001	0.055	0.011	<0.001	0.34
NH ₄ ⁺	(mg L ^{−1})	−1.688	0.062	<0.001	0.071	0.027	<0.05	0.13
NO ₃ [−]	(mg L ^{−1})			n.s.			n.s.	–
SRP	(mg L ^{−1})	−1.801	0.060	<0.001	0.431	0.113	<0.001	0.23
PMox								
Secchi disk	(m)	−0.701	0.049	<0.001	−0.228	0.028	<0.001	0.55
Chlorophyll a	(mg L ^{−1})			n.s.			n.s.	–
Trophic state index		−0.453	0.116	<0.001	0.014	0.001	<0.001	0.58
TOC	(mg L ^{−1})	−0.218	0.084	<0.05	0.007	0.001	<0.001	0.26
TN	(mg L ^{−1})	0.087	0.073	>0.05	0.071	0.010	<0.001	0.46
NH ₄ ⁺	(mg L ^{−1})	0.418	0.063	<0.001	0.074	0.021	<0.001	0.20
NO ₃ [−]	(mg L ^{−1})	0.325	0.094	<0.001	0.387	0.188	<0.05	0.05
SRP	(mg L ^{−1})	0.347	0.069	<0.001	0.441	0.139	<0.01	0.15

n.s. = Not significant ($P > 0.05$).

^a PMP and PMox values at T_{opt} were transformed ($\log_{10}$) to fulfil the homogeneity of variance requirement.

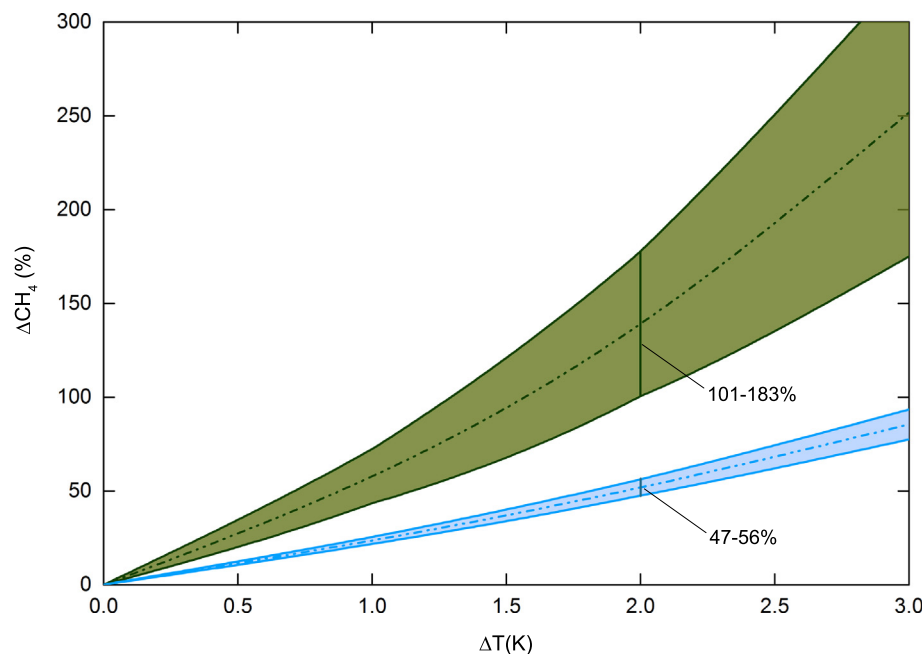


Fig. 4. Impact of a temperature increase scenario on hypereutrophic (green dotted lines) and oligotrophic lakes (blue dotted lines) over the net CH_4 emission balance (ΔCH_4). Blue and green areas show the confidence intervals ($P = 0.05$). The model considers a reference temperature of 15°C and a percentage of CH_4 oxidized at that temperature of 60%.

lakes, where higher CH_4 concentrations were well correlated with lower dissolved O_2 concentrations (Martinez-Cruz et al., 2015). Second, our model did not consider the particular conditions of high-latitude lakes, which are covered each year by a thick layer of ice for several months. This ice layer impedes gas transfer between water-atmosphere; e.g. oxygen supply to the water and affects photosynthesis by reducing light penetration (Clilverd et al., 2009; Vonk et al., 2015), accentuating anoxic conditions and thus further enhancing methanogenesis. Third, our ΔCH_4 estimation did not consider the mixing regime of lakes. Climate warming may increase epilimnion temperature, favoring thermal stratification for longer periods and reducing O_2 availability for methanotrophy in the hypolimnion (DelSontro et al., 2016; Kirillin, 2010; West et al., 2016). Finally, our model does not consider ebullitive emission, which is expected to increase by 6 to 20% per each 1°C temperature increase (Aben et al., 2017; Davidson et al., 2018; DelSontro et al., 2016) and reduced the accessibility of CH_4 for methanotrophs in the water column.

4. Conclusions

The empirical and theoretical results of this study suggest that the severe eutrophication of lakes may exacerbate the net CH_4 emission from lakes in response to climate warming. Further approaches should consider the complex impact of climate warming on oxygen availability for methanotrophy, which depends on many parameters, such as: mixing regime, primary productivity, seasonality, morphology and bathymetry of the lakes. Moreover, additional factors like the mechanisms of CH_4 transport within the water column (e.g., diffusion vs bubbling), quality and quantity of the organic matter available for the syntrophic processes associated to methanogenesis, and the availability of nutrients (e.g., N, S and P) could further improve the current estimates. Nevertheless, even with the partial picture depicted in the present work, most of the parameters that may be affected by climate warming suggest a negative impact on methanotrophy together with a positive impact on methanogenesis, pointing out to a net increase in global CH_4 emissions from lakes.

Acknowledgments

We thank A. Strom, G. Idoate Santaolalla, M. Rodriguez, for field and lab assistance. We gratefully acknowledge the financial support to J.H.-S. (grant #211962), A.S.-J. (grant #232083 and 206621/203709), K.M.-C. (grant #330197/233369), Y.B.-I. (grant #243319) by the Consejo Nacional de Ciencia y Tecnología (CONACyT). This study was funded by SEMARNAT-CONACyT (23661) and by the Leibniz Association within the projects “Climate driven changes in biodiversity of microbiota – TemBi” (SAW-2011-IGB-2), “Aquatic Boundaries and Linkages – Aqualink” (SAW 2012-IGB 4167). Additional support for A.S.-J. and K.M.-C. was provided by Fondo Nacional de Desarrollo Científico y Tecnológico (Fondecyt-Conicyt; grant #11170134 and grant #11160391, respectively). Support for K. W. A. was provided by NASA NNX15AU49A and NSF-1500931.

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