Temperature sensitivity of anaerobic methane oxidation versus methanogenesis in paddy soil: Implications for the CH$_4$ balance under global warming

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Abstract
The global methane (CH$_4$) budget is based on a sensitive balance between methanogenesis and CH$_4$ oxidation (aerobic and anaerobic). The response of these processes to climate warming, however, is not quantified. This largely reflects our lack of knowledge about the temperature sensitivity ($Q_{10}$) of the anaerobic oxidation of CH$_4$ (AOM)—a ubiquitous process in soils. Based on a $^{13}$CH$_4$ labeling experiment, we determined the rate, $Q_{10}$ and activation energy of AOM and of methanogenesis in a paddy soil at three temperatures (5, 20, 35°C). The rates of AOM and of methanogenesis increased exponentially with temperature, whereby the AOM rate was significantly lower than methanogenesis. Both the activation energy and $Q_{10}$ of AOM dropped significantly from 5–20 to 20–35°C, indicating that AOM is a highly temperature-dependent microbial process. Nonetheless, the $Q_{10}$ of AOM and of methanogenesis were similar at 5–35°C, implying a comparable temperature dependence of AOM and methanogenesis in paddy soil. The continuous increase of AOM $Q_{10}$ over the 28-day experiment reflects the successive utilization of electron acceptors according to their thermodynamic efficiency. The basic constant for $Q_{10}$ of AOM was calculated to be 0.1 units for each 3.2 kJ mol$^{-1}$ increase of activation energy. We estimate the AOM in paddy soils to consume 2.2–5.5 Tg CH$_4$ per year on a global scale. Considering these results in conjunction with literature data, the terrestrial AOM in total consumes ~30% of overall CH$_4$ production. Our data corroborate a similar $Q_{10}$ of AOM and methanogenesis. As the rate of AOM in paddy soils is lower than methanogenesis, however, it will not fully compensate for an increased methane production under climate warming.

Keywords
$^{13}$C labeling, anaerobic oxidation of methane, global warming, methane emission, methanogenesis, paddy soil, temperature sensitivity

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1 | INTRODUCTION

Methane (CH$_4$) is an effective greenhouse gas with a global warming potential (GWP) 25 times higher than that of carbon dioxide (CO$_2$) on a time scale of 100 years (Forster et al., 2007). The modern global net emission to the atmosphere is $\sim$580 Tg CH$_4$ year$^{-1}$ (He et al., 2020) and increasing by $\sim$5 Tg CH$_4$ year$^{-1}$ (Saunois et al., 2016). CH$_4$ is produced in the last step of anaerobic organic carbon reduction (methanogenesis; Megonigal et al., 2004) by various methanogenic archaea (including hydrogenotrophs, formotrophs, acetotrophs, methyloptrophs, and alcoholotrophs; Bridgham et al., 2013; Chen et al., 2013; Jongejans et al., 2021; McNicol et al., 2020; Serrano-Silva et al., 2014). In turn, CH$_4$ itself can serve as a carbon and energy source for specialized aerobic and anaerobic microorganisms (methanotrophs) in sediments and soils (see Knittel & Boetius, 2009; Serrano-Silva et al., 2014 for reviews). Similar to methanogens, aerobic CH$_4$ oxidizing-bacteria have been known for over a century (Troitsenko & Murrell, 2008). The anaerobic oxidation of methane (AOM) by microorganisms, in contrast, has long been thought to be thermodynamically and mechanistically impossible (Thauer & Shima, 2008). The paradigm changed in the 1970s, when AOM was first identified in marine sediments (Barnes & Goldberg, 1976; Reeburgh, 1976). Over the last four decades, AOM has been widely explored in modern and ancient marine settings (Bhattacharjee et al., 2019; Reeburgh, 2007; Thiel, 2020), but only recently received increasing attention in terrestrial ecosystems (Gauthier et al., 2015; Smemo & Yavitt, 2011). AOM was estimated to consume $\sim$300 Tg CH$_4$ year$^{-1}$ in global marine environments (Hinrichs & Boetius, 2002), and $\sim$200 Tg CH$_4$ year$^{-1}$ in global freshwater wetlands (Segarra et al., 2015). This makes AOM a significant component of the global carbon cycle and represents a potential constraint on climate warming. Despite its clear significance, AOM in terrestrial ecosystems is not yet included in most modern process-based biogeochemical models due to the lack of information about the underlying mechanisms and controls.

Mechanistically, AOM is linked to electron acceptors other than oxygen (O$_2$), namely (in the order of decreasing electron acceptor affinity): nitrate (Haroon et al., 2013), nitrite (Ettwig et al., 2009), humic substances (Bai et al., 2019), metal oxides (e.g., Mn$^{4+}$, Fe$^{3+}$; Beal et al., 2009), and sulfate (Valentine, 2002). These alternative electron acceptors (AEAs) are used by anaerobic methanotrophic archaean (ANMEs) to oxidize CH$_4$ to CO$_2$ via the "reverse methanogenesis" pathway, which is performed independently or in association with syntrophic bacteria. Another AOM pathway is driven by anaerobic methanotrophic bacteria (NC10) via the "intracellular-aerobic" pathway (Ettwig et al., 2010).

The potential of AOM in marine and terrestrial habitats has been studied under controlled conditions in relation to CH$_4$ concentrations, electron acceptor availability, and environmental parameters such as temperature, salinity, alkalinity, and pressure (Bhattacharjee et al., 2019; Fan et al., 2019). Among these factors, temperature appears to be a decisive selection parameter for the distribution of ANMEs (Bhattacharjee et al., 2019) and NC10 (He et al., 2015). Moreover, AOM metabolic processes are, as all chemical and biochemical reactions, temperature-dependent (He et al., 2015), but the temperature sensitivity of AOM in soils has not yet been described.

The temperature sensitivity of metabolic reactions is commonly expressed as the Q$_{10}$ value, that is, the factor by which the reaction rate increases with a 10°C rise in temperature. The Q$_{10}$ value can be used to predict the potential feedback of metabolic reactions to climate change (Davidson & Janssens, 2006) and thus plays a pivotal role in global biogeochemical models. In the global carbon cycle, the CH$_4$ flux to the atmosphere is a result of three processes: methanogenesis, aerobic CH$_4$ oxidation, and AOM (Figure 1). The rates of these processes, therefore, determine the amount of CH$_4$ emitted to the atmosphere, whereas the Q$_{10}$ describes the proportional increase of these rates in response to global warming. In nature, Q$_{10}$ commonly decreases with increasing temperature (Davidson & Janssens, 2006; Karhu et al., 2014; Mahecha et al., 2010). For example, Q$_{10}$ values of methanogenesis decrease with temperature and range between 1.4–15.4 (Huang et al., 2016; Wei et al., 2021). In comparison, the Q$_{10}$ of aerobic CH$_4$ oxidation in paddy soil ranges from 2.2 to 9.8 (Cai & Yan, 1999). The Q$_{10}$ is directly related to the activation energy of a given biochemical reaction (Davidson & Janssens, 2006). The activation energy is described by the Arrhenius equation, which links the reaction rate with temperature. Higher activation energy should correspond to a higher Q$_{10}$ (Arrhenius, 1889). Perkins et al. (2012) demonstrated that the temperature dependence (Q$_{10}$) of respiration across ecosystems was equivalent to the activation energy. Nonetheless, the real-world values will additionally depend on the biochemical and biophysical conditions in the soil, for example, methanogenesis requires a certain low redox potential ($<$–150 mV; Wang et al., 1993), which is below that for the reduction of NO$_3$-NO$_2$, Mn$^{4+}$, Fe$^{3+}$, and SO$_4^{2–}$ (Patrick Jr & DeLaune, 1977). Sound predictions of the future global CH$_4$ budget under climate warming must include the feedback mechanisms between methanogenesis and AOM. Unfortunately, no experimental confirmations are yet available.

Rice paddies are hotspots of methanogenesis and emit $\sim$31 Tg CH$_4$ per year, thus representing 9% of the anthropogenic input into the atmosphere (Keppeler et al., 2006). Submerged paddy soils provide an ideal habitat for AOM-related microorganisms (Hu et al., 2014; Vaksmaa et al., 2017) due to the abundance of AEAs from organic and mineral fertilization (NO$_3$/NO$_2$, SO$_4^{2–}$ and dissolved organic matter) and from (sub)tropical soil development (Mn$^{4+}$, Fe$^{3+}$). Several metabolic pathways of AOM are active in paddy soils (Fan et al., 2021). The available data indicate that AOM could offset the CH$_4$ efflux from paddy soils by 10%–20% (Fan et al., 2019, 2020). Here, we experimentally tested the response of methanogenesis and AOM to warming within a range of temperatures common in paddy soils from temperate to tropical climates (5–35°C). Based on the obtained temperature sensitivity of these processes, and using available literature data, we compared the rates and Q$_{10}$ values of AOM and methanogenesis, and predicted the significance of AOM as a CH$_4$ sink in response to climate change.
2 MATERIALS AND METHODS

2.1 Soil collection and samples

The sampling site is located near Jining town of Hunan province in China (28°33′04″N, 113°19′52″E). Soils were sampled from an ongoing long-term field experiment with different fertilization treatments for rice cultivation (Shen et al., 2014). The typical paddy field has a tillage history of more than 1000 years of rice production. In a previous study, we found that soils from 20–30 cm depth under pig manure fertilization showed the highest AOM potential (Fan et al., 2020). We therefore chose soils from this depth and fertilization treatment for the experiment. The fertilization treatment comprised 60 Mg pig manure ha⁻¹ year⁻¹ (containing 250 g C kg⁻¹, 16.8 g N kg⁻¹, 5.3 g P kg⁻¹, 2.5 g K kg⁻¹; pH 8.0), plus conventional background fertilization (60 kg N ha⁻¹ year⁻¹ as urea, 18 kg P ha⁻¹ as Ca(H₂PO₄)₂, and 83 kg K ha⁻¹). Samples from four soil cores were mixed to form one composite sample per plot. The samples were not sieved to minimize exposure to air because both methanogenesis and AOM are highly sensitive to oxygen contamination.

The soil is classified as Hydragric Anthrosol developed from red granite parental material. The soil organic matter content was 15.7 g C kg⁻¹; total nitrogen content, 1.85 g kg⁻¹; soil microbial biomass carbon, 1.13 g kg⁻¹; extractable dissolved organic carbon, 63.4 mg kg⁻¹; NO₃⁻, 10.0 mg kg⁻¹; NH₄⁺, 2.13 mg kg⁻¹; total S, 408 mg kg⁻¹; total Fe, 21.3 g kg⁻¹; bulk density, 1.26 g cm⁻³; soil pH, 5.2.

2.2 Experimental design

Twenty grams of field-moist soil (30% soil weight-based water content) and 15 ml deionized water were loaded into 100-ml Kimble KIMAX borosilicate laboratory glass jars to prepare the soil slurry. Before use, all jars and septa for sealing were autoclaved twice at 121°C for 20 min. Anoxic conditions were created by cycles of head-space evacuation and N₂ refilling (Fan et al., 2019). The slurry was pre-incubated in the dark at either 5 ± 0.5, 20 ± 0.5, or 35 ± 0.5°C for 2 weeks to establish a new equilibrium and consume any O₂ remaining in the microcosms. To raise the representativeness of soil samples, two jars were set up for every plot. To exclude contamination with atmospheric O₂, all manipulations were conducted in an anaerobic glovebox (N₂/H₂, 97/3%).

Subsequently, 5 ml of CH₄ containing 10 atom% ¹³CH₄ were injected to quantify the net anaerobic oxidation of ¹³CH₄ to ¹³CO₂.
This yielded an initial average headspace CH$_4$ concentration of 3.1%. A similar volume of N$_2$ instead of CH$_4$ was used to maintain the same gas pressure in the natural abundance control. Gas samples were collected at 3 and 7 days during the pre-incubation period, and at 3, 7, 14, 21, 28 days after $^{13}$CH$_4$ injection. Gas sampling and all measurements, including stable C isotope analysis of CO$_2$ using isotope ratio mass spectrometry, were conducted according to (Fan et al., 2020). Isotope data are reported as $\delta^{13}$C-values relative to the Vienna Pee Dee Belemnite standard.

2.3 Calculations

The rate of methanogenesis was calculated using the difference of headspace CH$_4$ concentration between two sampling time points during the pre-incubation period (i.e., before $^{13}$CH$_4$ injection). AOM, as expressed by the amount of $^{13}$CO$_2$ (i.e., the end-product of AOM) generated, was calculated using the isotope mixing-model:

$$C_{OX} = \frac{(\delta^{13}C_{Total} - \delta^{13}C_{Control})}{(\delta^{13}C_{OX} - \delta^{13}C_{Control})} \times C_{Total}, \tag{1}$$

where $C_{OX}$ (µg) is the amount of $^{13}$CH$_4$ oxidized based on the released $^{13}$CO$_2$, $C_{Total}$ is the total amount of C in the corresponding pool (i.e., CO$_2$), $\delta^{13}C_{Total}$ is the delta value of $^{13}$CO$_2$ in the samples treated with $^{13}$CH$_4$, $\delta^{13}C_{Control}$ is the delta value of $^{13}$CO$_2$ in the reference (no $^{13}$CH$_4$ addition), and $\delta^{13}C_{OX}$ is the delta value of the added tracer $^{13}$CH$_4$ with 10 atom% enrichment.

The $Q_{10}$ of methanogenesis and AOM were calculated by the transformed Arrhenius equation (Arrhenius, 1889):

$$R = a \exp(b \cdot T), \tag{2}$$

$$Q_{10} = \exp^{(10-b)}, \tag{3}$$

where $R$ is the methanogenesis or AOM rate (ng C g$^{-1}$ DW (dry weight) h$^{-1}$), $T$ is the incubation temperature (°C), $a$ and $b$ are fitted coefficients.

The activation energy was calculated by the Arrhenius equation (Arrhenius, 1889):

$$k = A \exp\left(-\frac{E_a}{RT}\right), \tag{4}$$

where $k$ is the rate (ng C g$^{-1}$ soil h$^{-1}$), $A$ is the frequency of molecular collisions, $R$ is the universal gas constant (8.314 J K$^{-1}$.mol$^{-1}$), and $T$ is the temperature in Kelvin (273.15 K). The activation energy ($E_a$, J mol$^{-1}$) was calculated as the slope of ln($k$) versus (-1/RT).

2.4 Data collection from the literature

We extracted data on methanogenesis and AOM rates from peer-reviewed articles published until April 2021 using Web of Science (http://apps.webofknowledge.com/) and Google Scholar (http://scholar.google.com/) databases. The following key words were used for the search of (a) methanogenesis rate: “methane production” or “CH$_4$ production” or “methanogenesis”; (b) AOM rate: “anaerobic oxidation of methane” or “anaerobic oxidation of CH$_4$” or “anaerobic methane oxidation” or “anaerobic CH$_4$ oxidation” or “AOM”. Both parameters were further refined by the key words “soil” or “wetland” or “peatland” or “river sediment” or “lake sediment” or “freshwater sediment.”

Experiments to be included in the dataset had to meet the following criteria: (a) laboratory incubation; (b) declared incubation temperature; (c) the unit can be transferred to ng C g$^{-1}$ h$^{-1}$; (d) data were collected only from natural, untreated samples. The latter are often reported as control/reference for various treatments. For example, amendments with electron acceptors for AOM, or added substrates for methanogenesis, were not considered. If a paper reported multiple temperature treatments, then each treatment was included separately in the dataset. If a paper reported rates at different depths of soils/sediments, then their average values were included. Likewise, if a paper reported rates at different incubation times, average values were included. The data obtained in our current study were incorporated into the dataset. In total, we considered 83 studies with 375 values of methanogenesis rates and 30 studies with 94 values of AOM rates reported in terrestrial ecosystems (Tables S1 and S2). To extract data from figures, the WebPlotDigitizer 4.2 (https://automeris.io/WebPlotDigitizer) was used.

2.5 Statistical analyses

To exclude outliers in the prepared literature datasets (Tables S1 and S2), the rates of methanogenesis and AOM in each dataset were normalized by temperature (i.e., rate/temperature); the normalized rates were thus transferred to z-scores. A given rate was considered as an outlier when its z-score was >3 or <-3. The correlations between rates (methanogenesis and AOM) and temperature were fitted by six observations in the Equation (2).

Two-way ANOVA of variance was used to determine differences in CH$_4$ and CO$_2$ production, $\delta^{13}$C-CO$_2$, and cumulative AOM against incubation time and temperatures. The normality and homogeneity of the residuals of the variances were tested before applying ANOVA. $t$-tests were used to characterize the differences between methanogenesis and AOM. We used an exponential model ($y = y_o + a(1 - e^{-bt})$) to fit the values of $Q_{10}$ change with incubation duration (time). SPSS 19.0 software (SPSS) and Sigmaplot software 14.0 (Systat Software, Inc.) were used to perform the statistical analyses and draw figures.
3 | RESULTS

3.1 | CH₄ and CO₂ production and δ¹³C values of CO₂

CH₄ and CO₂ production increased with temperature during pre-incubation (Figure 2a,b). After the ¹³CH₄ injection, both incubation duration and temperature affected the CO₂ concentration [CO₂]. The δ¹³C values of CO₂ clearly increased (became less negative) over time and with temperature (−21‰, −16‰, and −12‰ at 5, 20, and 35°C, respectively; *p* < 0.001; Figure 3a; Table S3). In the control soil, the average δ¹³C of CO₂ did not exceed −23‰; a slight depletion was recorded during the 28-day experiment, but this trend was insignificant (Figure 3a).

3.2 | Rates of methanogenesis and AOM

The cumulative AOM increased with incubation temperature (*p* < 0.01; Table S4) and peaked at 370 ng C per gram dry soil after 28 days at 35°C. This was 17 and 2.8 times more than at 5 and 20°C, respectively (Figure 3b). The methanogenesis rate increased exponentially with temperature (Figure 3c), as did the AOM rate (Figure 3d), the latter being 0.07, 0.34, and 0.85 ng C g⁻¹ soil h⁻¹ at 5, 20, and 35°C, respectively. The methanogenesis rates over the 5–35°C temperature range were significantly higher than the AOM rates in the studied paddy soil.

3.3 | Temperature sensitivity and activation energy of methanogenesis and AOM

The temperature sensitivity (Q₁₀) of methanogenesis was similar at 5–20°C and at 20–35°C (Figure 4a). In comparison, the Q₁₀ of AOM was significantly higher for the 5–20°C (2.7) than the 20–35°C (1.8) interval (Figure 4b); moreover, it increased non-linearly for both the 5–20 and 20–35°C intervals over the 28-day period (Figure 4d). Note, however, that the Q₁₀ of AOM and of methanogenesis were similar at both 5–20°C and at 20–35°C, or for the overall 5–35°C range in paddy soil (Figure 4a–c).

The Q₁₀ and Eₐ of AOM were higher at 5–20 than at 20–35°C (1.8) (Figure 5b). The overall (5–35°C) Eₐ of methanogenesis was higher than that of AOM (Figure 5c). The Eₐ of AOM increased linearly at a rate of 3.2 kJ mol⁻¹ by every 0.1 unit of Q₁₀ (Figure 5d).

3.4 | Literature data on methanogenesis and AOM rates

To elucidate the temperature dependency of AOM versus methanogenesis, we collected literature data reporting the rates of these processes in natural samples at various temperatures (after outliers scanning: 364 methanogenesis rates from 82 studies; 91 AOM rates from 28 studies including our own data; see chapter 2.4., Tables S1 and S2). The literature dataset corroborated the exponential increase of both rates with temperature (Figure 6a,b). The mean methanogenesis rate across terrestrial ecosystems was about 3.6 times higher than that of AOM (Figure 6c), whereas the mean Q₁₀ and Eₐ of both processes were similar (Figure 6d,e).

4 | DISCUSSION

The increase in δ¹³C values of CO₂ after injecting ¹³C-labeled CH₄, as compared to the unlabeled control, is clear evidence of AOM in the anoxic paddy soil (Figure 3a). The ¹³C enrichment of CO₂ was higher at 20 and 35°C than at 5°C, reflecting an increase of the AOM rate with temperature, as expected. Based on our results and literature data, the AOM and methanogenesis rates exponentially increase with soil temperature (Figures 3c,d and 6a,b), and thus follow the general pattern of the temperature effect on microbial respiration (Karhu et al., 2014).
**FIGURE 3** Methanogenesis and anaerobic oxidation of methane (AOM) in paddy soil. (a) Dynamics of $\delta^{13}C$ value of $\text{CO}_2$ over 28 days of incubation with $^{13}\text{CH}_4$ versus soil without $^{13}\text{CH}_4$ injection (=natural abundance unlabeled control; means ± SEs, $n = 6$). (b) Cumulative AOM at three temperature levels (5, 20, 35°C) during the 28-day incubation (means ± SEs, $n = 6$). (c) Average methanogenesis rate for each temperature, and the exponential relationship between the rate and temperature. (d) Average AOM rate for each temperature level, and the exponential relationship between AOM rate and temperature. Blue dashed lines: 95% confidence interval of regression lines. Box plots: upper and lower bars—maximum and minimum observations, respectively; top and bottom of boxes—third and first quartiles; thin horizontal solid lines in boxes—median values; dashed horizontal lines—mean values

**FIGURE 4** Temperature sensitivity ($Q_{10}$; a–c) of methanogenesis and anaerobic oxidation of methane (AOM) and $Q_{10}$ changes of AOM with incubation duration (d) in paddy soil. (a) and (b): 5–20 and 20–35°C temperature intervals, (c) total 5–35°C range. *Significant difference ($p < 0.05$) by t test. Solid lines: regressions of $Q_{10}$ and incubation duration at 5–20°C ($y = 2.01 + 1.28^*(1 - e^{-0.13x}), R^2 = 0.95$) and at 20–35°C ($y = 1.73 + 134^*(1 - e^{-0.0001x}), R^2 = 0.72$). Blue dashed lines: 95% confidence interval of regression analysis
FIGURE 5 Activation energy ($E_a$; a–c) of methanogenesis and anaerobic oxidation of methane (AOM) and linear relationship between $E_a$ and $Q_{10}$ of AOM in paddy soil. (a) and (b) represent the 5–20 and 20–35°C temperature intervals, (c) total 5–35°C range. *Significant difference ($p < 0.05$) by t-test. Blue dashed lines: 95% confidence interval of regression analysis. Horizontal dashed purple line in (b) and (c): activation energy level of AOM corresponding to $Q_{10} = 2.0$.

FIGURE 6 Literature data on rates (a–c), activation energy ($E_a$; d) and temperature sensitivity ($Q_{10}$; e) of methanogenesis ($n = 364$) and anaerobic oxidation of methane (AOM; $n = 91$). Yellow points in (a) and (b): rates observed in the current study (see Figure 3). Standard errors of means in (d) and (e) were estimated by the exponential regression. Blue dashed lines: 95% confidence interval of regression analyses. Horizontal dashed purple line in (d): activation energy level of AOM corresponding to $Q_{10} = 2.0$. *Significant difference ($p < 0.05$) by t-test. For details of data selection and sources, see text and Supporting Information tables.
The $Q_{10}$ of AOM decreased from 2.7 (5–20°C) to 1.8 (20–35°C) in paddy soil (Figure 4b, $p = 0.024$), consistent with theoretical predictions (Davidson & Janssens, 2006). Therefore, AOM is a strongly temperature-dependent microbial process, and the AOM-driven CH$_4$ sink is more effective in a low- versus the high-temperature environment expected as a consequence of global warming. The physiological temperature optimum of rice growth, however, is typically above the low-temperature range (Peng et al., 2004), and most CH$_4$ is produced during the rice growth season (Huang et al., 1997; Tokida et al., 2011). Notably, while the $Q_{10}$ of methanogenesis and AOM were similar, the methanogenesis rate was higher in both low- and high-temperature ranges (Figures 3c,d, 4c, and 6). Thus, the counterplay of CH$_4$ production versus AOM still yields net CH$_4$ emission from anoxic soils.

Compared to methanogens, anaerobic methanotrophs respond faster to warming at low than at high temperatures (Figure 4b). Consequently, AOM activity is more temperature-dependent than methanogenesis. The activation energy ($E_a$) of AOM decreased from 5–20 to 20–35°C, but the $E_a$ of methanogenesis remained stable (Figure 5a,b). This additionally supports the conclusion that methanogenesis is less temperature-dependent but relied more on the redox potential and/or substrate availability. The higher $E_a$ of methanogenesis than that of AOM in paddy soil (Figure 5c) demonstrated that methanogens needed more energy to overcome the reaction threshold of anaerobic organic matter fermentation (acetoclastic pathway) and/or CO$_2$ reduction with hydrogen (hydrogenotrophic pathway) to form CH$_4$ (Parkin, 1993; Whiticar et al., 1986). In contrast, anaerobic methanotrophs more easily reduce NO$_3^-$/NO$_2^-$, humic acids, Mn$^{4+}$, Fe$^{3+}$, and SO$_4^{2-}$ to oxidize CH$_4$ anaerobically (Conrad, 2009; Fan et al., 2021; Smemo & Yavitt, 2011). The $E_a$ of AOM shows a linear relationship with $Q_{10}$ (Figure 5d). This agrees with previous findings that the temperature dependence of respiration across ecosystems is consistent with the activation energy (Perkins et al., 2012). Based on this linear relationship, we calculated an increase of $E_a$ by 3.2 kJ mol$^{-1}$ for each 0.1 unit of $Q_{10}$. This fundamental constant for the $E_a$ and $Q_{10}$ changes of AOM with temperature is crucial for process-based modeling of CH$_4$ oxidation in paddy soils under climate warming.

The $Q_{10}$ of AOM falls quite well into the $Q_{10}$ range of CO$_2$ efflux from soils (1.3–3.6; Lenton & Huntingford, 2003), which is commonly used in the global-scale models (Friedlingstein et al., 2006; Mahecha et al., 2010). Considering environmental conditions, the $Q_{10}$ of AOM could be affected by abiotic (e.g., electron acceptors availability, substrate diffusion coefficient) and biotic factors (e.g., AOM-related microbiota, involved enzymes). The $Q_{10}$ values increase with the incubation period (Fuchs et al., 2016; Wei et al., 2021), that was also observed in our 28-day experiment (Figure 4d). We explain this $Q_{10}$ increase by the initial preferential consumption of more energetically efficient AEAs (e.g., NO$_3^-$/NO$_2^-$), leaving AEAs with a lower redox potential (e.g., SO$_4^{2-}$) for use in the later stages of the incubation. First, decreasing the availability of suitable AEAs with incubation duration should increase $Q_{10}$ values of AOM because the microorganisms coping with the AEAs also have different affinities to substrates. For instance, the microbial affinity constant of NO$_3^-$/NO$_2^-$-dependent AOM ($<$0.6 μM) is four orders of magnitude higher than that of SO$_4^{2-}$-dependent AOM ($>$16 mM; Raghoearsingh et al., 2006). Second, the $Q_{10}$ of AOM should increase in the following order depending on the thermodynamic energy yield of electron acceptors: NO$_3^-/NO_2^-$ < humic substances < Mn$^{4+}$ < Fe$^{3+}$ < SO$_4^{2-}$ (Figure 1; see detailed results of the effects of AEAs on AOM potential for the same paddy soil in Fan et al., 2020; Smemo & Yavitt, 2011). Third, the restricted diffusion of CH$_4$ and electron acceptors in soils is a limiting factor for AOM; accordingly, the $Q_{10}$ of AOM should be higher in soils than in aquatic environments. Nevertheless, substrate diffusion/supply co-varies with temperature, and this confounding effect could induce a relatively higher $Q_{10}$ value, that overestimates each individual process (e.g., AOM, methanogenesis; Davidson et al., 2006). A consistent parameterization of the temperature sensitivities beyond $Q_{10}$ (e.g., $E_a$) is therefore urgently needed (Davidson et al., 2006; Lane & Martin, 2010; Perkins et al., 2012).

Anaerobic oxidation of methane is ubiquitous in soils but is still an underappreciated CH$_4$ sink (Gauthier et al., 2015). Based on the global rice paddy area (167 million ha; FAO [Food & Agriculture Organization of the United Nations], 2018) and the mean bulk soil density of the plow layer (1.3 g cm$^{-3}$; Pan et al., 2004), our conservative estimation is that AOM in global paddy soils annually consumes between $-2.2$ Tg (moderate temperature, 20°C) and $-5.5$ Tg (high temperature, 35°C) of CH$_4$. For the current conditions, which fit on average to the moderate temperature range, AOM could reduce approximately $-60$ Tg of CO$_2$ equivalents year$^{-1}$ (100-year GWP) of the global greenhouse gas potential. Considering these results in conjunction with the literature data, AOM consumes about 30% of the total CH$_4$ production in terrestrial ecosystems. Our data provide the first experimentally based concept on the temperature sensitivity of AOM. They should be further verified and integrated into the process-based biogeochemical models balancing the CH$_4$ efflux with AOM in the terrestrial carbon cycle.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information of this article.
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