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Higher temperature sensitivity of forest soil methane oxidation in colder climates

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Forest soils, serving as an important sink for atmospheric methane (CH_4), modulate the global CH_4 budget. However, the direction and magnitude of the forest soil CH_4 sink under warming remain uncertain, partly because the temperature response of microbial CH_4 oxidation varies substantially across geographical scales. Here, we reveal the spatial variation in the response of forest soil microbial CH_4 oxidation to warming, along with the driving factors, across 84 sites spanning a broad latitudinal gradient in eastern China. Our results show that the temperature sensitivity of soil microbial CH_4 oxidation significantly declines with increasing site mean annual temperature, with a range of 0.03 to 0.77 μg CH_4 g^{-1} soil d^{-1} $°C^{-1}$. Moreover, soil resources and type II methanotrophs play crucial roles in shaping the temperature sensitivity of soil microbial CH_4 oxidation. Our findings highlight the importance of incorporating climate, soil resources, and methanotroph groups into biogeochemical models to more realistically predict forest soil CH_4 sink under warming.

Methane (CH_4) is a highly potent greenhouse gas that contributes disproportionately to Earth's ongoing warming[1,2], and has increased rapidly from 0.7 ppm to over 1.9 ppm since the industrial revolution[3]. This increase poses a significant challenge to the world's goal of restricting global temperature rise to 1.5–2 °C over pre-industrial temperatures[4]. However, forest soils can provide an important terrestrial CH_4 sink, owing primarily to the activity of methanotrophs[5], and this sink is expected to strengthen in the face of climate warming[6–8]. As a result, the warming-induced CH_4 sink changes may mitigate the growth in atmospheric CH_4 concentration caused by human activities and climate change, triggering a negative feedback that may slow down the rate of global warming[9]. However, the magnitude of this forest soil CH_4 sink in response to warming remains uncertain[10], in part because it is not well understood how the temperature sensitivity of forest soil microbial CH_4 oxidation (i.e., CH_4 uptake by soil methanotrophs) varies over a broad geographical scale[11–16].

Soil methanotrophs show very different responses in CH_4 oxidation capacities with increasing temperature depending on the climates and soil properties in which they are embedded[17,18]. It is clear that soil CH_4 oxidation is temperature-sensitive and variable across forest ecosystems[11,12,18], as a result of variation in climate[19,20], soil abiotic factors[21–23], and the microbial community properties[24,25]. For instance, previous studies have shown that increased mean annual temperature reduces the abundance of methanotrophs[26] and inhibits the activity of enzymes involved in CH_4 oxidation[27] that would lead to reduced CH_4 uptake[20]. Moreover, sandy soils have been demonstrated to be generally more efficient at oxidizing CH_4 than silty soils, likely due to their better ability to diffuse gases[28,29]. Along with soil texture, soil nitrogen is a crucial factor in controlling CH_4 oxidation since it is an essential nutrient element for the growth of methanotrophs[30,31]. How these biotic and abiotic factors interact to impact the temperature sensitivity of forest soil CH_4 oxidation, however, remains largely unidentified and thus is not explicitly represented in process-based models[10,32]. In addition, the current modeling efforts generally use a fixed temperature sensitivity parameter and do not incorporate spatial variation in temperature sensitivity of CH_4 oxidation, which will lead to large uncertainty in global forest soil CH_4 sink estimation[9,33,34]. By evaluating the spatial variation and ecological drivers of the temperature sensitivity of forest soil CH_4

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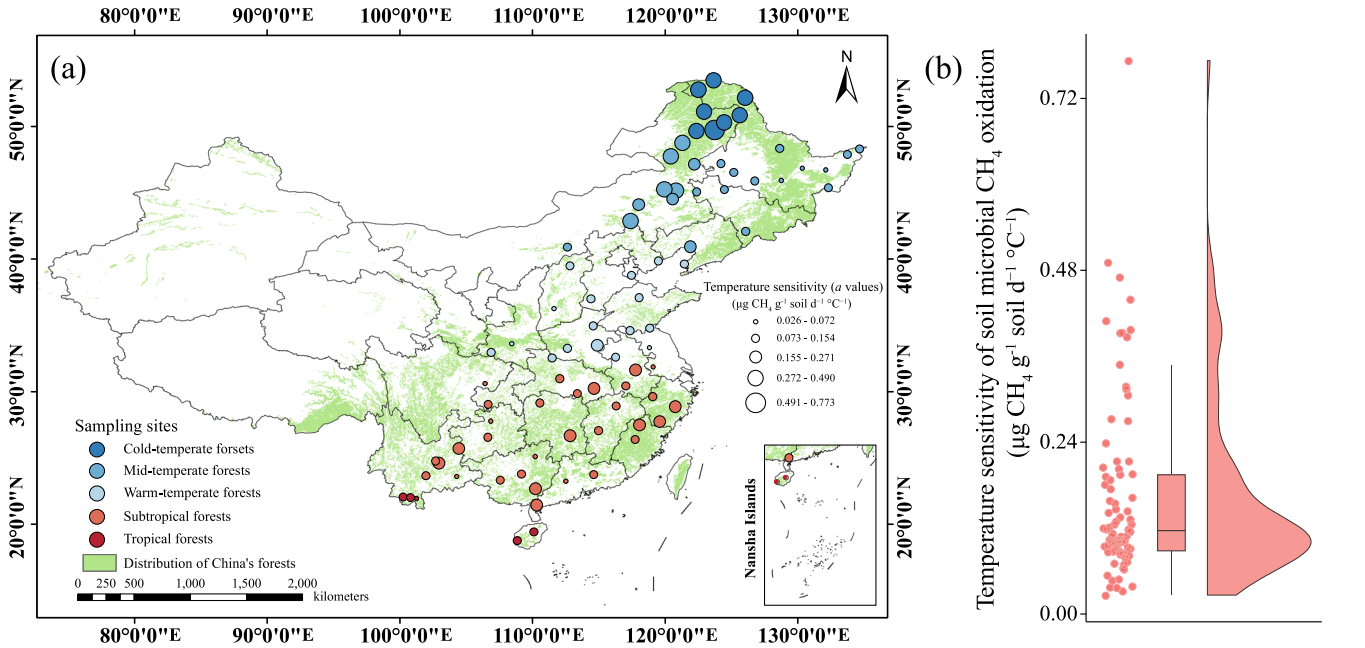

Fig. 1 | The temperature sensitivity of forest soil microbial CH_4 oxidation across the 84 sites in eastern China. a Geographic variation in the temperature sensitivity of soil microbial CH_4 oxidation. **b** Distribution of the temperature sensitivity of soil microbial CH_4 oxidation (n = 84). Horizontal lines inside the box represent the median. Box limits are the upper and lower quartiles. Whiskers are 1.5 times interquartile range. The temperature sensitivity of forest soil microbial CH_4 oxidation was quantified using the partial derivative of the soil microbial CH_4 oxidation rate with respect to temperature (parameter *a* values, see "Methods").

oxidation, we can begin to gain more accurate predictions of the magnitude and direction of the response of soil CH_4 sink to warming. This would allow us to improve processed-based models to quantify the relative importance of soil in the global CH_4 cycle and its potential ability to reduce atmospheric CH_4 under future global change scenarios.

To better understand the temperature sensitivity of forest soil microbial CH_4 oxidation at a large scale, we evaluated the temperature response curves of soil microbial CH_4 oxidation using soils sampled from 84 sites along a ~4000 km north-south transect in eastern China. This transect included high levels of variation in both mean annual temperature and mean annual precipitation (Supplementary Data 1) across many climate zones. Our main objectives are to answer the following questions: (i) How does the temperature sensitivity of forest soil microbial CH_4 oxidation change at large geographic scales? (ii) What are the important factors of temperature sensitivity in forest soil microbial CH_4 oxidation?

Results

Across the latitudinal gradient, we observed a high degree of variation in temperature sensitivity values, reflecting the increase in soil microbial CH_4 oxidation rate per degree of warming (Fig. 1a). These sensitivity values ranged from 0.03 to 0.77 $\mu g\, CH_4\, g^{-1}$ soil $d^{-1}\, °C^{-1}$ (Fig. 1b and Supplementary Data 1), and increased along a latitudinal gradient from tropical to cold-temperate forests (Supplementary Table 1). Similar patterns were observed in the temperature sensitivity of soil microbial CH_4 oxidation across different temperature ranges (Supplementary Fig. 1).

We found that the temperature sensitivity of soil microbial CH_4 oxidation was significantly influenced by variations in climate factors, soil resources (e.g., total carbon, total nitrogen, and available nitrogen content), bacterial community composition, and methanotroph traits, while soil properties (i.e., pH and clay content) had no notable effect (Fig. 2). Variance decomposition analysis revealed that climate factors accounted for 44.45% of the variation in the temperature sensitivity, surpassing the individual contributions of soil resources, soil properties, bacterial community composition, and methanotroph traits (Fig. 3a; $R^2 = 0.52$). The key role of climate factors in driving the temperature sensitivity of soil microbial CH_4 oxidation was further confirmed by independent random forest analyzes (Fig. 3b), which identified mean annual temperature as the primary factor determining the observed spatial patterns of temperature sensitivity. Specifically, the temperature sensitivity of soil microbial CH_4 oxidation exhibited a significant negative correlation with mean annual temperature (Fig. 4).

Structural equation model (SEM) was used to further assess the direct and indirect effects of climate factors, soil resources (including total carbon, total nitrogen, and ammonium nitrogen content), soil properties (i.e., pH and clay content), bacterial community composition, and methanotroph traits on the temperature sensitivity of soil microbial CH_4 oxidation (Fig. 5). The SEM revealed that climate factors and the relative abundance of type II methanotrophs had direct effects on the temperature sensitivity of soil microbial CH_4 oxidation (Fig. 5). Additionally, soil resources and the relative abundance of *Verrucomicrobiota* had indirect effects on temperature sensitivity of soil microbial CH_4 oxidation, mediated through changes in the relative abundance of type II methanotrophs (Fig. 5). Furthermore, climate factors indirectly influenced the shifts in the relative abundances of both type II methanotrophs and *Verrucomicrobiota* by altering soil resources (Fig. 5).

Discussion

Although forest soils play an important role in the global CH_4 sink[5], the magnitude of this effect can be strongly but variably influenced by climate warming. Our findings show that colder regions had the highest CH_4 oxidation potential, as well as the greatest increase in forest soil CH_4 oxidation capacity in response to warming (Fig. 4 and Supplementary Table 1). As a consequence, we expect forest soils in colder regions to become more effective at oxidizing CH_4 as the global climate warms, especially since these regions account for more than half of the world's forested area[35]. Given that global warming tends to be most pronounced at high latitudes[36], these colder regions will play a vital role in mitigating the rise in atmospheric CH_4 concentrations by enhancing microbial-driven CH_4 oxidation capacity in a warming

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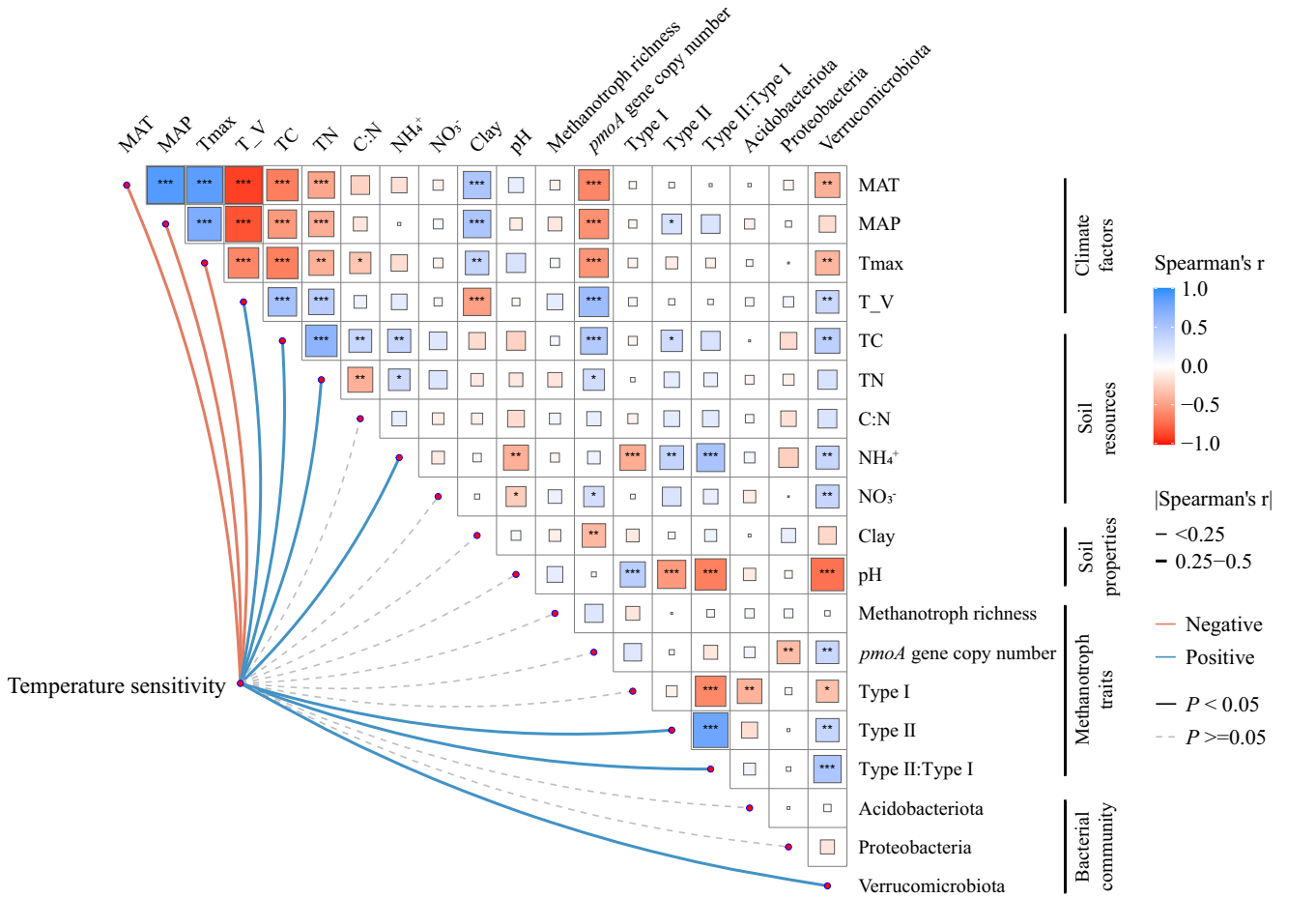

Fig. 2 | The relationship between the temperature sensitivity of soil microbial CH_4 oxidation and climate factors, soil properties, soil resources, bacterial community composition, and methanotroph traits. The climate factors include mean annual temperature (MAT), mean annual precipitation (MAP), annual maximum temperature (Tmax), annual temperature variation (T_V). The soil resources include total carbon content (TC), total nitrogen content (TN), ammonium nitrogen content (NH_4^+), nitrate nitrogen content (NO_3^-), soil C:N ratio (C:N). The soil properties include soil pH (pH) and clay content (Clay). The bacterial community composition includes relative abundances of *Proteobacteria*, *Acidobacteriota*, and *Verrucomicrobia*. The methanotroph traits include methanotroph richness, *pmoA* gene copy number, relative abundance of type I methanotrophs (Type I), relative abundance of type II methanotrophs (Type II), the ratio of type II to type I methanotrophs (Type II: Type I). The temperature sensitivity of forest soil microbial CH_4 oxidation was quantified using the partial derivative of the soil microbial CH_4 oxidation rate with respect to temperature (parameter *a* values, see "Methods"). Statistical significance was tested using a two-sided test. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

climate. Furthermore, it is important to note that the warming-induced enhancement of soil CH_4 oxidation capacity may lead to a potential increase in CO_2 emissions from the soil, with significant implications for the global greenhouse gas budget[37,38].

Unlike other soil carbon cycling processes, CH_4 uptake in forest soils is driven by methanotrophs, which primarily rely on CH_4 as their carbon and energy source[39–42]. Previous studies have shown a strong relationship between soil CH_4 oxidation rates and the abundance of key methanotroph functional groups[25,40,43]. Similarly, our finding indicates that the temperature sensitivity of soil microbial CH_4 oxidation is significantly influenced by the predominant methanotroph functional group, with sensitivity increasing in relation to the relative abundance of type II methanotrophs (Supplementary Figs. 2 and 3). These results suggest that variations in type II methanotrophs abundance may significantly impact methanotroph-mediated CH_4 oxidation under warming, thus affecting the capacity of soil CH_4 sinks. However, current terrestrial ecosystem models do not account for the role of dominant methanotroph functional groups in CH_4 oxidation and its temperature sensitivity[9,21,32]. Our findings emphasize the importance of incorporating variations in type II methanotrophs abundance into biogeochemical models to improve estimates of global forest CH_4 sinks under climate change.

The abundance and functional characteristics of soil microbial groups are influenced by soil resource availability and interactions with other microbial groups[44]. As expected, our results showed that soil resources directly promote the relative abundance of type II methanotrophs (Fig. 5). This is likely due to the nitrogen requirements of methanotrophs, which typically need a 1:4 nitrogen-to-carbon ratio[25,45–47]. Additionally, soil resources indirectly affect the type II methanotrophs by influencing the abundance of the predominant bacterial phylum, *Verrucomicrobiota* (Fig. 5). Given the critical role of type II methanotrophs in regulating the temperature sensitivity of soil microbial CH_4 oxidation, soil resources are key in shaping this sensitivity. Moreover, climate has been proven to influence soil weathering, leading to significant variability in soil resources on a large-scale[48,49]. These findings highlight the significance of the interactive effects of climate, soil resources, and key microbial groups in regulating the temperature sensitivity of soil microbial CH_4 oxidation, suggesting that considering these interactions is essential for robust prediction of future global forest soil CH_4 uptake under warming.

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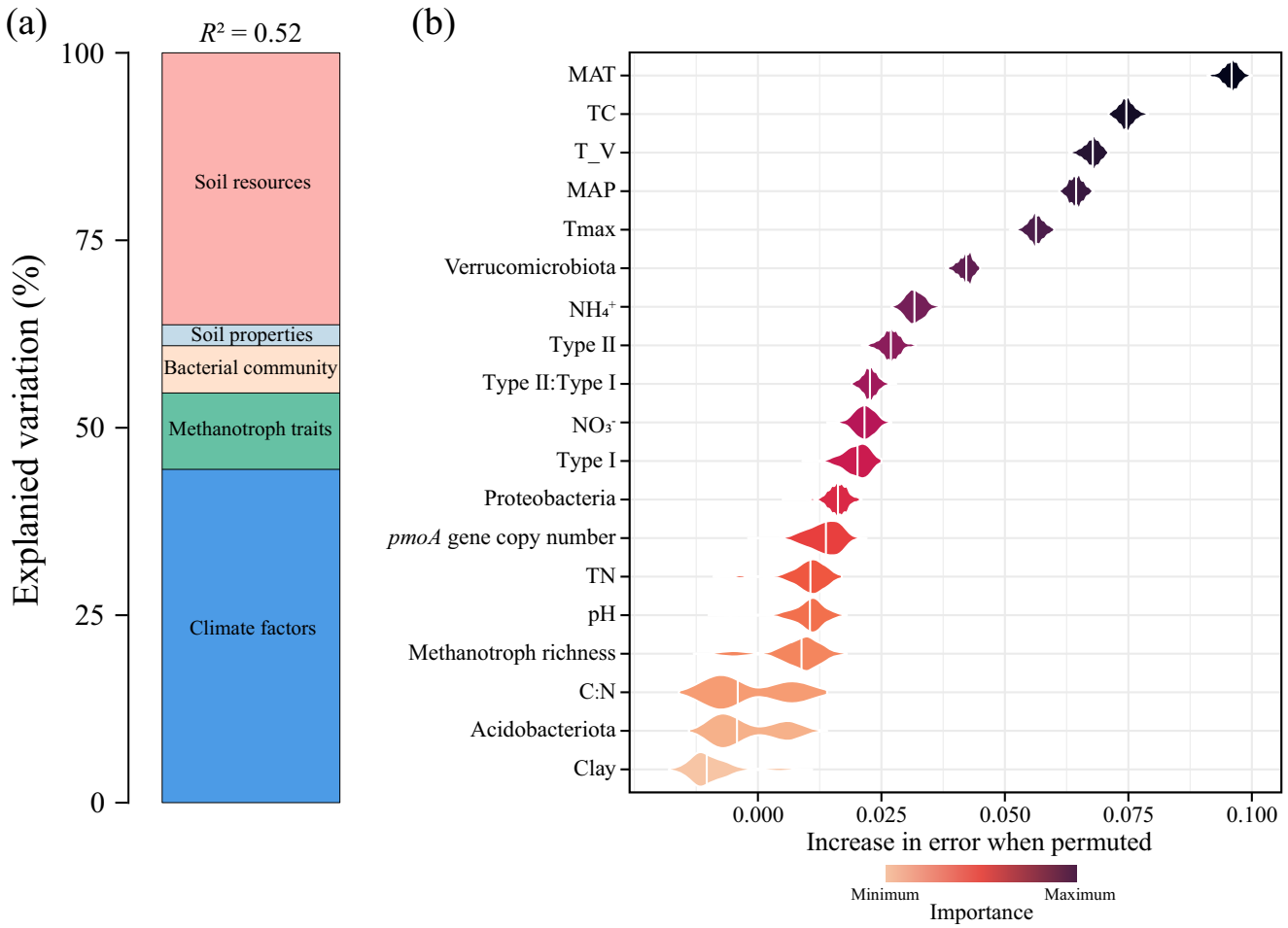

Fig. 3 | Relative importance of biotic and abiotic variables in determining the temperature sensitivity of forest soil microbial CH_4 oxidation. a Variance decomposition analysis evaluating the proportion of variation in the temperature sensitivity of forest soil microbial CH_4 oxidation explained by all variables. **b** Results from random forest analysis displaying the relative importance of the individual variable in predicting the temperature sensitivity of forest soil microbial CH_4 oxidation. The climate factors include mean annual temperature (MAT), mean annual precipitation (MAP), annual maximum temperature (Tmax), annual temperature variation (T_V). The soil resources include total carbon content (TC), total nitrogen content (TN), ammonium nitrogen content (NH_4^+), nitrate nitrogen content (NO_3^-), soil C:N ratio (C:N). The soil properties include soil pH (pH) and clay content (Clay). The bacterial community composition includes relative abundances of *Proteobacteria*, *Acidobacteriota*, and *Verrucomicrobia*. The methanotroph traits include methanotroph richness, *pmoA* gene copy number, relative abundance of type I methanotrophs (Type I), relative abundance of type II methanotrophs (Type II), the ratio of type II to type I methanotrophs (Type II: Type I). The temperature sensitivity of forest soil microbial CH_4 oxidation was quantified using the partial derivative of the soil microbial CH_4 oxidation rate with respect to temperature (parameter *a* values, see "Methods").

While our study provides a consistent way to examine the temperature sensitivity of forest soil microbial CH_4 oxidation across a broad geographic scale, some uncertainties remain. The temperature sensitivity values we reported (Supplementary Data 1) are higher than those found in previous studies[12,16,18]. One possibility is that CH_4 oxidation rates in our study were measured under conditions of ample CH_4 substrate and optimal moisture, which may have mitigated the limiting effects of environmental factors at elevated temperatures, thus increasing temperature sensitivity[22,50]. Additionally, the temperature sensitivity of soil microbial CH_4 oxidation was assessed within a range of 15–25 °C, reflecting the average growing season temperatures at the sites, which ranged from 10.04 to 24.19 °C (Supplementary Data 1). However, this range may not fully capture in situ responses of forest soil CH_4 oxidation to ambient temperature changes[51], especially in regions where temperatures are low during the non-growing season. To better understand how microbial CH_4 oxidation responds to climate warming, future research should focus on capturing seasonal variations in the temperature sensitivity through in situ observations.

Overall, our research reveals the broad-scale spatial variation and key drivers of forest soil CH_4 oxidation in response to warming. We found that the temperature sensitivity of soil microbial CH_4 oxidation was highest in colder regions, suggesting a greater potential for the CH_4 sink in these areas under global warming. Additionally, our results emphasize the critical roles of soil resources and type II methanotrophs in shaping the spatial patterns of temperature sensitivity in soil microbial CH_4 oxidation. Our findings provide a previously unrecognized perspective that improves our understanding of forest soil CH_4 oxidation and its response to warming in the face of variable climate and edaphic factors, thereby helping to refine and validate biogeochemical models that predict the global CH_4 sink under warming.

Methods

Study area and soil sampling

Between June and August 2023, we collected 336 topsoil (0–10 cm) samples from 84 forest sites across eastern China along a ~4000 km south–north transect. This transect spanned a wide range of latitudes (18.7°–53.4° N) and longitudes (100.3°–134.7° E) and covered five climatic zones (cold-temperate, mid-temperate, warm-temperate, subtropical and tropical zones). The mean annual temperature of these sites ranges from −4.6 to 24.19 °C, and the mean annual precipitation

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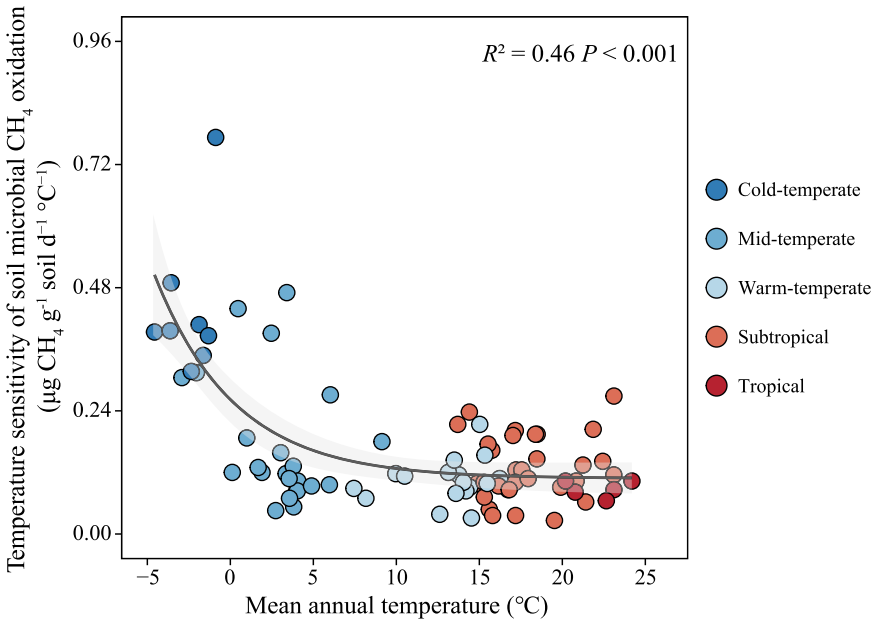

Fig. 4 | The relationship between the temperature sensitivity of forest soil microbial CH_4 oxidation and mean annual temperature across 84 forest sites. The fitted solid line was estimated from a mixed-effects model, with the shaded region corresponding to the 95% confidence interval. The temperature sensitivity of forest soil microbial CH_4 oxidation was quantified using the partial derivative of the soil microbial CH_4 oxidation rate with respect to temperature (parameter *a* values, see "Methods").

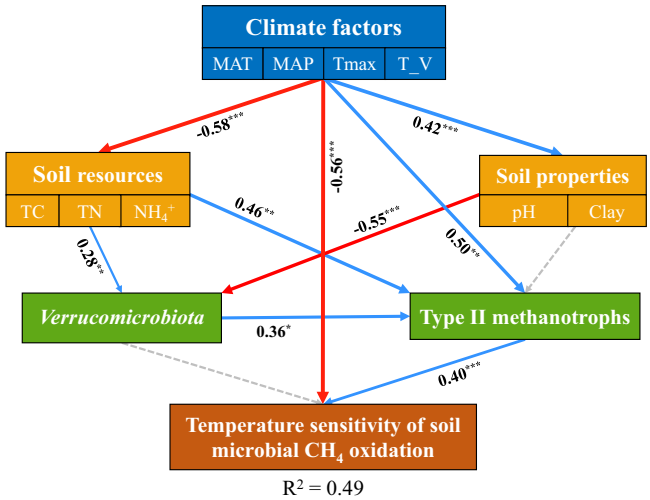

Fig. 5 | Structural equation model (SEM) examining the multivariate effects on the temperature sensitivity of forest soil microbial CH_4 oxidation. Results of the final model fitting: $R^2 = 0.49$, $P = 0.221$, Fisher's $C = 10.667$. The blue and red lines indicate positive and negative relationships, respectively; gray lines indicate insignificant relationships ($P > 0.05$). The thickness of the lines represents the magnitude of the path coefficient, with thicker lines indicating larger coefficients. The numbers adjacent to the arrows are standardized path coefficients. The model includes the following variables: mean annual temperature (MAT), mean annual precipitation (MAP), annual maximum temperature (Tmax), annual temperature variation (T_V), total carbon content (TC), total nitrogen content (TN), ammonium nitrogen content (NH_4^+), soil pH (pH), clay content (Clay), relative abundance of *Verrucomicrobia* (*Verrucomicrobia*), relative abundance of type II methanotrophs (Type II methanotrophs). The temperature sensitivity of forest soil microbial CH_4 oxidation was quantified using the partial derivative of the soil microbial CH_4 oxidation rate with respect to temperature (parameter *a* values, see Methods).

varies from 337 to 1884 mm. Detailed information about the sites is provided in Supplementary Data 1.

At each forest sampling site, we randomly established four 10 × 10 m sampling plots separated by more than 100 m from each other. We took six soil core samples (0–10 cm) following a zigzag path through each plot and then mixed them to form a composite sample. We then sieved the samples through a 2 mm mesh and divided them into three subsamples. One subsample was stored at 4 °C for soil incubation, one was stored at −20 °C for microbial DNA extraction, and the third was air-dried to constant weight and processed for measurements of soil properties. The soil samples were kept at 4 °C for no longer than 4 weeks before the incubation experiment began. Previous studies have shown that storing soil samples at 4 °C for up to 7 weeks does not significantly affect microbial activity or its temperature sensitivity[52,53]. Therefore, the storage conditions used in this study are unlikely to have a major impact on the subsequent analyzes of microbial CH_4 oxidation rates[52,54].

Incubation experiments

The incubation experiment aimed to assess the intrinsic temperature sensitivity of soil microbial CH_4 oxidation, which is defined as the change in CH_4 oxidation rate with temperature under otherwise constant conditions[55]. For each site, 10 g of dry-weight fresh soil, with four replicates, was weighed into 140 mL incubation bottles. To control for potential variations in soil moisture across different sites, we adjusted the soil moisture to 60% of the water-holding capacity using sterile deionized water, which is optimal for microbial activity and gas diffusion[56]. To activate microorganisms and minimize possible disturbances, we performed a 3-day pre-incubation at 20 °C, a period sufficient to restore soil microbial CH_4 oxidation activity (Supplementary Fig. 4). Since the growing season is typically the most critical period for soil CH_4 oxidation[51], with average growing season temperatures at the sampling sites ranging from 10.04 °C to 24.19 °C (Supplementary Data 1), the subsequent incubation experiments were conducted at three temperatures—15 °C, 20 °C, and 25 °C—to capture the temperature response of microbial CH_4 oxidation during the active period of methanotrophs in forest soils.

During the incubation, each bottle was sealed with a butyl rubber stopper, and pure CH_4 was injected to approach a concentration of ~200 ppm. This concentration was chosen because it is high enough to enable the maximum potential CH_4 oxidation rate across different soils, as lower, atmospheric-like CH_4 concentrations might limit the

activity of soil methanotrophs (Supplementary Fig. 5). Next, a 5 mL gas sample was collected using a syringe, and 5 mL CH_4-free air was immediately injected into the bottle to avoid the air pressure changes in the incubation bottles. The sealing times for the bottles at 15 °C, 20 °C, and 25 °C were ~3, 2, and 2 h, respectively. After the sealing period, another 5 mL gas sample was collected from the headspace. The CH_4 concentrations in the gas samples were measured using gas chromatography (Agilent 7890 A, Agilent Technologies Inc., Santa Clara, California, USA). The potential soil CH_4 oxidation rate was then calculated as follows:

$$P = \frac{dc}{dt} \times \frac{Vh}{Ws} \times \frac{MW}{MV} \times \frac{Ts}{T} \quad (1)$$

where P indicates the potential soil CH_4 oxidation rate (μg CH_4 g^{-1} soil d^{-1}), dc/dt is the rate of CH_4 concentration change. Vh indicates incubation bottle headspace volume (m^3), and Ws indicates soil dry-weight (g). MW and MV indicate molar mass of CH_4 (16 g mol^{-1}) and gas molar volume under standard air pressure (22.4 L mol^{-1}), respectively. Ts and T represent standard temperature (273.15 K) and incubation temperature (K), respectively.

Model choice for soil methane oxidation rate and its temperature sensitivity

The response of biological processes to temperature change can often be fitted using different functional forms[57]. In this study, we evaluated the performance of three widely used models—the linear, exponential, and Arrhenius equations—to describe the relationship between soil microbial CH_4 oxidation rate and temperature. Our analysis revealed that the linear equation provided the best fit for the majority of sampling sites, outperforming both the exponential and Arrhenius models (Supplementary Table 2). Therefore, we determined the temperature sensitivity of microbial CH_4 oxidation as the slope of the linear relationship between soil CH_4 oxidation rate and temperature change (Supplementary Fig. 6), as follows:

$$P = a * T + b \quad (2)$$

where P indicates the potential CH_4 oxidation rate (μg CH_4 g^{-1} soil d^{-1}), T indicates incubation temperature (°C), and a and b are fitting parameters.

Given that climate warming exhibits marked non-uniformity across the globe, the intrinsic temperature sensitivity is thus defined here as the change rate of P with respect to temperature while all other variables are held constant[58]. Mathematically, this corresponds to the partial derivative of P with respect to temperature, $\partial P/\partial T$, meaning the absolute change of P for a given unit change in temperature[58,59]. In this study, the parameter a value represents the temperature sensitivity of soil microbial CH_4 oxidation and allows direct comparisons of the absolute change in soil CH_4 oxidation rate for a 1 °C temperature increase over a large geographic scale. Indeed, the temperature sensitivity of biological processes expressed as partial derivatives has been widely used[60,61].

Furthermore, we calculated the Q_{10} value, which reflects the relative increase in microbial metabolic rate with a 10 °C rise in temperature[62], for soil microbial CH_4 oxidation rate at both lower (between 15 and 20 °C) and higher (between 20 and 25 °C) temperature ranges. The Q_{10} value was calculated using the following equation:

$$Q_{10} = \left(\frac{R(T_2)}{R(T_1)}\right)^{\frac{10}{(T_2 - T_1)}} \quad (3)$$

where $R(T_1)$ and $R(T_2)$ are soil CH_4 oxidation rates in temperatures T_1 and T_2, respectively.

Climate data and soil analysis

Climate data, including mean annual temperature, mean annual precipitation, annual maximum temperature, and annual temperature variation (defined as the difference between the annual maximum and minimum temperatures), were extracted from the Worldclim dataset for the period 1970–2000[63].

Soil water content was determined by drying samples at 105 °C for 24 h. The maximum water-holding capacity of the soil was measured using the funnel-filter paper-drainage method[64]. In this process, deionized water was used to pre-saturate the filter paper in the funnel. Then, 10 g of air-dried soil was placed into the wet filter paper funnel, and the soil was saturated by wetting the outer rim of the exposed filter paper. Once the soil appeared glistening and a small amount of water was visible on the surface, the funnel was covered with cling film that had small holes to minimize evaporation. After 6 h, the drained soil was weighed and then oven-dried to determine its dry weight.

Total carbon (TC) was measured using a TOC analyzer (Multi N/C 3100, Germany), while total nitrogen (TN) was quantified using the Kjeldahl method[65]. The contents of ammonium (NH_4^+) and nitrate (NO_3^-) in the soil were determined in 2 M KCl extracts (with a soil-to-extract ratio of 1:4 by weight) using a flow injection analyzer (AutoAnalyzer 3 SEAL, Bran and Luebbe). Soil pH was measured using a 1:2.5 soil-to-water suspension and a pH meter. Soil texture was analyzed using a particle size analyzer (BT-9300ST, China).

DNA extraction, PCR amplification, and high-throughput sequencing

Soil total DNA was extracted from 300 mg of composite frozen soil sample from each site using the ALFA-SEQ Magnetic Soil DNA Kit (Findrop Biosafety echtnology (Guangzhou) Co. Ltd) according to the manufacturer's instructions and was stored at −80 °C for later use. The *pmoA* gene, which encodes the critical enzyme methane monooxygenase (particulate MMO), is commonly employed to identify methanotroph communities. We determined the copy numbers of the *pmoA* gene using quantitative PCR (qPCR) with the primer pairs pmof1/pmor (Supplementary Table 3). We determined the bacterial community and methanotroph community using high-throughput sequencing analysis of the 16S rRNA gene with primers 515F/806R and the *pmoA* gene with primers pmof1/pmor (Supplementary Table 3). This *pmoA* primer has proven useful for amplifying both cultured and uncultured methanotrophs (Supplementary Table 4)[66,67]. Samples with clear, bright main bands were selected for further experiments. The sequencing library was prepared and sequenced on an Illumina NovaSeq 6000 platform at Guangdong Magigene Biotechnology Co., Ltd, Guangzhou, China. This allowed for a comprehensive analysis of the microbial communities present in the soil samples.

The QIIME2 was used to process the raw sequences[68]. DADA2, integrated within QIIME2, was employed to cluster the sequences into amplicon sequence variants (ASVs) after filtering out adaptor sequences, low-quality reads, ambiguous nucleotides, and barcodes. Taxonomic assignment of the 16S rRNA and *pmoA* sequences was performed using the Ribosomal Database Project (RDP) classifier, referencing the SILVA database (Version 138) for 16S rRNA and the FunGene database (Version 1.0) for *pmoA* sequences. To ensure adequate representation of each community's structure, we removed bacterial samples with fewer than 70,320 sequences per sample and methanotroph samples with fewer than 32,140 sequences per sample, leaving 60 sites for further analysis. Rarefaction curves confirmed that the sequencing depth was sufficient to assess the diversity and community composition of the microbial populations in the soil (Supplementary Fig. 7). Richness, which describes the microbial alpha diversity, was used as a key metric to quantify the diversity within each community[69].

Statistical analyzes

We divided five types of explanatory factors related to the temperature sensitivity of soil microbial CH_4 oxidation: climate factors (mean annual temperature, mean annual precipitation, annual maximum temperature and annual temperature variation), soil resources (total carbon content, total nitrogen content, soil C:N ratio, ammonium and nitrate content), soil properties (soil pH and clay content), bacterial community composition (relative abundances of *Proteobacteria*, *Acidobacteriota*, and *Verrucomicrobia*), and methanotroph traits (*pmoA* gene copy number, methanotroph richness, relative abundance of type I methanotrophs, relative abundance of type II methanotrophs, and the ratio of type II to type I methanotrophs). Spearman correlation analysis was conducted to identify the positive or negative correlations among the explanatory factors as well as between each factor and the temperature sensitivity of soil microbial CH_4 oxidation. Variance decomposition analysis was performed to quantify the relative importance of climate factors, soil resources, soil properties, bacterial community composition, and methanotroph traits in explaining the temperature sensitivity of soil microbial CH_4 oxidation, using the package of "glmm.hp"[70,71].

Additionally, independent random forest analysis was used to identify the key drivers of temperature sensitivity in soil microbial CH_4 oxidation[72]. The importance of each predictor was determined using the R package "spatialRF"[73], which reduces multicollinearity, identifies relevant variable interactions, and assesses model transferability via spatial cross-validation[74]. To evaluate the relationships between the temperature sensitivity of soil microbial CH_4 oxidation and each variable, a mixed-effects model with the sampling site as a random factor was performed with "nlme" package[75]. One-way ANOVA was conducted to examine differences in soil CH_4 oxidation rates and their temperature sensitivity across different climatic zones.

Structural equation model

To explore the complex and potentially simultaneous interactions between climate factors, soil resources, soil properties, bacterial community composition, and methanotroph traits influencing the temperature sensitivity of soil microbial CH_4 oxidation, we applied a structural equation model (SEM). Given the correlations among these factors (Fig. 2), we first performed principal components analysis (PCA) to create three new composite indices for climate factors, soil resources, soil properties. The first component (PC1), which explained 48.9–82.9% of the total variance across these groups, was introduced as a new variable in the SEM. We fit and evaluated the model using restricted maximum likelihood with the "nlme"[75] and "piecewiseSEM"[76] packages. After determining the best model, we conducted a goodness-of-fit evaluation for piecewise SEM based on Fisher's *C* and chi-squared tests ($P > 0.05$). All statistical analyzes were performed using R statistical software (v.4.2.0).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The sequence data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) under project accession number PRJNA1212223. All data generated and analyzed in this study have been deposited in the Figshare database (https://doi.org/10.6084/m9.figshare.28234274.v2).

Code availability

Codes for processing the data in this study have been deposited in the Figshare database (https://doi.org/10.6084/m9.figshare.28234274.v2).

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H.C. developed the original ideas presented in the manuscript; B.J. designed the research with the assistance from H.C.; B.J., Z.W., M.G. and J.Z. conducted the overall experiment and measurements with the assistance from T.Y.; B.J. analyzed the data with the assistance from H.C. and X.Z.; B.J. and H.C. wrote the first draft, and all authors jointly revised the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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