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Temperature fluctuation promotes the thermal adaptation of soil microbial respiration

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The magnitude of the feedback between soil microbial respiration and increased mean temperature may decrease (a process called thermal adaptation) or increase over time, and accurately representing this feedback in models improves predictions of soil carbon loss rates. However, climate change entails changes not only in mean temperature but also in temperature fluctuation, and how this fluctuation regulates the thermal response of microbial respiration has never been systematically evaluated. By analysing subtropical forest soils from a 2,000 km transect across China, we showed that although a positive relationship between soil microbial biomass-specific respiration and temperature was observed under increased constant incubation temperature, an increasing temperature fluctuation had a stronger negative effect. Our results further indicated that changes in bacterial community composition and reduced activities of carbon degradation enzymes promoted the effect of temperature fluctuation. This adaptive response of soil microbial respiration suggests that climate warming may have a lesser exacerbating effect on atmospheric CO_2 concentrations than predicted.

Soil respiration, the release of carbon dioxide from the soil surface to the atmosphere, is one of the most important terrestrial carbon fluxes[1,2]. Short-term (that is, days to months) experiments have shown that soil microbial respiration increases exponentially with mean temperature[3,4], and the incorporation of this response into soil carbon and Earth system models has resulted in predictions that the recent increase in global temperature resulting from climate change has caused soil microbial respiration rates to rise[5–7]. However, the thermal responses of microbial respiration to changes in mean temperature might not be invariable. Much research on the thermal responses of microbial respiration to variation in mean temperature has shown that this thermal response can either decrease ("thermal adaptation")[8] or increase ("enhancing response")[9,10] in different ecosystems, indicating that the strength of the soil-carbon–climate feedback may decrease or increase. Understanding the thermal responses is thus an essential research priority to help accurately predict how climate warming will affect soil carbon flux.

However, climate warming entails changes not only in mean thermal conditions but also in the patterns of temperature fluctuation[11].

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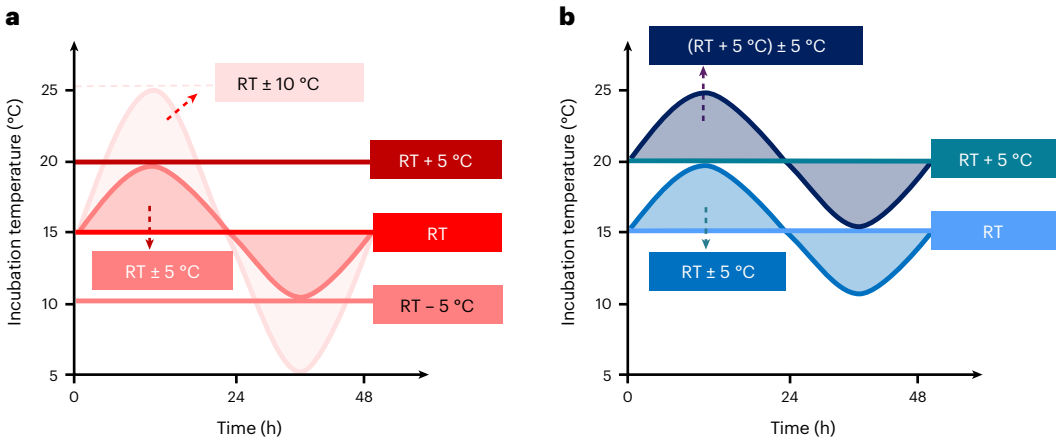

Fig. 1 | The thermal regimes used in the two incubation experiments. a,**b**, Soil incubations were performed under six thermal regimes representing a constant mean temperature or fluctuation around the mean across the two experiments, with temperatures in the fluctuation treatments varying between 5 and 25 °C over a period of 48 h. The treatment temperatures are expressed relative to an RT of 15 °C, which represents the approximate mean annual temperature at the soil collection sites; the thermal regimes at these sites show comparable temperature fluctuations. Later, when conducting the respiration assays, we used three temperatures covering the same range (5, 15 and 25 °C). Panel **a** shows the experiment to determine the independent effects of temperature fluctuation and mean temperature using the following two groups of three temperature regimes: (1) RT ± 0 °C (15 °C), RT ± 5 °C (15 ± 5 °C) and RT ± 10 °C (15 ± 10 °C) and (2) (RT − 5 °C) ± 0 °C (10 °C), RT ± 0 °C (15 °C) and (RT + 5 °C) ± 0 °C (20 °C). Panel **b** shows the experiment to determine the joint effect of temperature fluctuation and mean temperature by including an additional group of incubation temperature regimes: RT ± 0 °C (15 °C), RT ± 5 °C (increased temperature fluctuation, 15 ± 5 °C), (RT + 5 °C) ± 0 °C (increased mean temperature, 20 °C) and (RT + 5 °C) ± 5 °C (simultaneously increased mean temperature and temperature fluctuation, 20 ± 5 °C). Both experiments lasted 200 d.

Indeed, meteorological data have indicated that temperature fluctuations have increased substantially since the mid-twentieth century; specifically, the interannual temperature range has increased at approximately the same pace as the annual mean temperature over the past 30 years[12,13]. In addition, the intensity and frequency of climatic extremes are expected to further increase, according to the Intergovernmental Panel on Climate Change[14,15]. To comprehensively understand how climate warming will affect the thermal response of soil microbial respiration, it is critical to determine the impacts of warming under both constant and fluctuating conditions. Given the paucity of empirical data on temperature fluctuations included in current Earth system models[13], there is an urgent need for experimental studies to examine the response patterns of soil microbial respiration to increases in both constant thermal conditions and temperature fluctuations.

Previous research on thermal adaptation has typically focused on the effects of changes in mean temperature, but few biological or ecological systems experience constant ambient temperature. Indeed, the climatic variability hypothesis suggests that the physiological adaptability and stability of organisms are strengthened by high annual temperature fluctuations[16]. A previous study showed that microbial activities were inhibited by fluctuations in temperature, suggesting that an increase in temperature fluctuation could induce the adaptive response of the soil microbial community[17]. Such thermal adaptation is driven by changes in the structure, composition and/or physiology of the microbial communities[18,19], and these modifications can have an important influence on soil enzymatic reactions[20–23].

Several research groups have revealed that shifts in the relative abundance of microbial taxa with different functional capabilities induce changes in soil enzyme activities[24,25]. For example, a decreased proportion of Proteobacteria in the community may cause a reduction in enzyme activities (for example, *N*-acetyl-β-glucosaminidase and β-xylosidase) involved in decomposing soil carbon under warming[26]. We thus hypothesized that temperature fluctuation can decrease the thermal response of soil microbial respiration and that these effects of temperature fluctuation can be mediated by shifts in microbial community composition and reduced enzyme activities; this information could provide a general basis to forecast the feedback between soil carbon flux and global climate warming[27].

To determine whether temperature fluctuation can influence the thermal response of soil microbial respiration and whether this process is driven by changes in the composition and physiology of the microbial community, we collected soil samples from six evergreen broad-leaved forests at similar subtropical latitudes along an approximately 2,000-km-long west–east transect in China (Supplementary Table 1), with comparable approximate mean annual temperatures of 15 °C and interannual temperature fluctuations of approximately 10 °C at the soil surface. These soil samples were used in two 200 d laboratory incubation experiments incorporating six thermal regimes to explore the independent and combined effects of changes in mean temperature and the magnitude of temperature fluctuation on the thermal response of soil microbial respiration (Fig. 1).

Results

Thermal response of soil microbial respiration

To evaluate the effects of temperature fluctuation and mean temperature on the thermal response of soil microbial respiration, we incubated soil samples under a total of six temperature regimes in two separate experiments, using the mean annual temperature at the collection sites (15 °C) as a reference point (Fig. 1). The first experiment explored the independent effects of these variables. Differences in the thermal regime were generated by altering the magnitude of temperature fluctuation while maintaining a fixed mean temperature (that is, 15 ± 0 °C, 15 ± 5 °C and 15 ± 10 °C) for some of the samples, while other samples were incubated at different constant mean temperatures (that is, 10, 15 and 20 °C). The second experiment aimed to disentangle their joint effects using the same soils incubated under four thermal regimes in which both the magnitude of temperature fluctuation and the mean temperature were altered—that is, increased mean temperature (20 °C), increased temperature fluctuation (15 ± 5 °C), simultaneously increased mean temperature and temperature fluctuation (20 ± 5 °C) and a control treatment (15 °C). We characterized the thermal adaptation of soil microbial respiration as the attenuation of biomass-specific respiration (R_{mass}) following prolonged warming or recovery to the initial level

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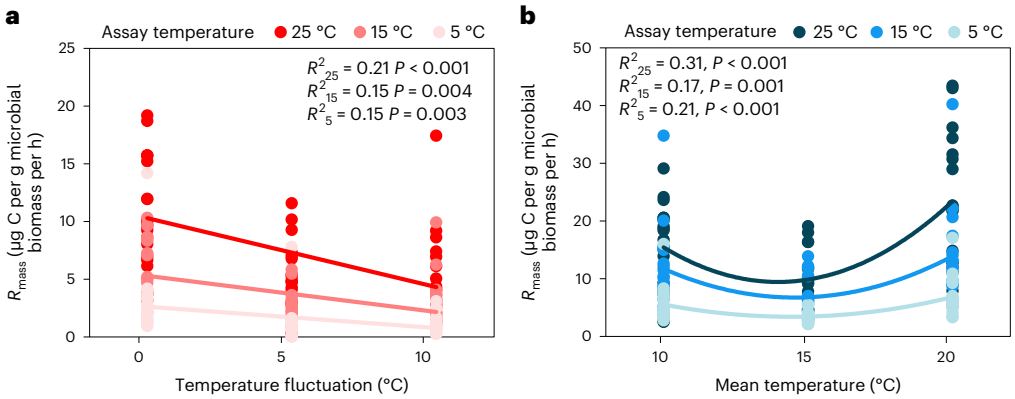

Fig. 2 | Thermal treatment response of R_{mass} under three assay temperatures (5, 15 and 25 °C). a, The adaptive response of respiration to temperature fluctuations of varying amplitude relative to the RT of 15 °C during incubation. R_{mass} decreased under greater temperature fluctuation. **b**, R_{mass} was quadratically related to the mean incubation temperature under all three assay temperatures; R_{mass} was the lowest at the intermediate mean temperature. The relationship between R_{mass} and mean temperature or temperature fluctuation was tested with mixed-effects models, with soil collection site as a random factor. The best models were fitted as the solid lines, and P values were estimated using a two-tailed t-test. Marginal R^2 was calculated, and R^2 represents the explained variation in fixed factors; n = 18 independent soil samples.

before warming, as described previously[19,28]. We thus assayed respiration rates at three temperatures (5, 15 and 25 °C) after incubation under the various experimental temperature regimes for 200 d, with the timescale chosen to induce adaptive responses under these regimes[29].

The relationships of temperature treatments and microbial respiration were quantified using mixed-effects regression models with site as a random factor to avoid the spatial autocorrelation of our soil collection sites, and the best fits of the candidate models were verified by the Akaike information criterion corrected for small sample size (AIC_c) (Methods). Our results demonstrated that temperature fluctuation had a negative effect on R_{mass} at a constant assay temperature according to linear mixed-effects modelling analysis (Fig. 2a). This finding is consistent with the concept of a thermal adaptive response. However, R_{mass} was quadratically related to the mean incubation temperature under all three assay temperatures (Fig. 2b), suggesting that R_{mass} was enhanced in response to both increased and decreased reference temperatures (RTs). We further found that the thermal adaptive response could also be induced by the interactive effect of simultaneous increases in temperature fluctuation and mean temperature (Fig. 3). Our results suggest that temperature fluctuation promotes microbial thermal adaptation even within the scenario of increased mean temperature. In addition, to determine whether the thermal adaptation due to temperature fluctuation changed the temperature sensitivity of R_{mass} (Q_{10}), we calculated the Q_{10} of microbial respiration and found that it was not affected by the different temperature regimes (Supplementary Table 2).

Factors driving the thermal response

To identify the effects of the various experimental thermal regimes on the microbial community, we used high-throughput sequencing to characterize the microbial community composition, and employed the Mantel test to evaluate the dissimilarity in the microbial community composition and temperature treatments. The results showed that increased mean temperature and temperature fluctuation both affected the composition of the microbial community (Table 1 and Supplementary Figs. 1 and 2). Specifically, increased temperature fluctuation predominantly affected bacterial community composition, while increased mean temperature affected fungal and bacterial community composition similarly (Table 1). These effects varied among the dominant taxa within each community (Supplementary Fig. 3). For example, temperature fluctuation had significant impacts on the relative abundances of the major phyla in both the bacterial and fungal communities, while increased mean temperature had no significant effect on the dominant phyla in the fungal community.

Furthermore, we constructed structural equation models (SEMs) to identify how these shifts in microbial community composition mediate enzyme-catalysed carbon degradation reactions under the different experimental temperature regimes and explore the factors driving the observed changes in soil microbial respiration. The model results suggest that two contrasting pathways predominantly mediated R_{mass} via temperature fluctuation (Fig. 4a). On the one hand, temperature fluctuation might attenuate R_{mass} by promoting shifts in bacterial community composition and by enhancing the negative relationship between changes in bacterial community composition and enzyme activities. On the other hand, temperature fluctuation had a weak positive impact on R_{mass} by facilitating enzyme activities and enhancing the positive relationship between changes in fungal community composition and enzyme activities. Our results demonstrate that microbial physiological adjustment was a dominant factor modulating R_{mass} under the various thermal regimes and that changes in bacterial composition accounted for these adaptive responses (Supplementary Fig. 4a).

We also found two opposite pathways by which mean temperature mediated R_{mass} (Fig. 4b and Supplementary Fig. 4b). First, mean temperature facilitated a positive relationship between fungal community composition and enzyme activities, resulting in an increase in R_{mass}. Second, an increase in the mean temperature also promoted shifts in fungal composition and thereby decreased R_{mass}.

Discussion

In this study, we provided experimental evidence that an increase in temperature fluctuation induced the thermal adaptation of soil microbial respiration. We also observed enhanced R_{mass} under both increased and decreased mean temperature from the RT (Fig. 2b and Fig. 3). However (and more importantly), although shifts in mean temperature and temperature fluctuation had opposite independent effects on R_{mass}, we showed that the thermal adaptation of microbial respiration also occurred when temperature fluctuation and an increase in mean temperature occurred simultaneously (Fig. 3). These findings suggest that previous studies reporting the response of microbial respiration on the basis of mean temperature alone are inaccurate since they ignored temperature fluctuation[30,31].

In this study, we considered the mean annual temperature of the soil surface as the RT. Although we observed an enhancing response of R_{mass} under increased mean temperature, the enhanced R_{mass} under decreased mean temperature suggests that soil microbial respiration can thermally adapt to changed mean constant temperature, as the

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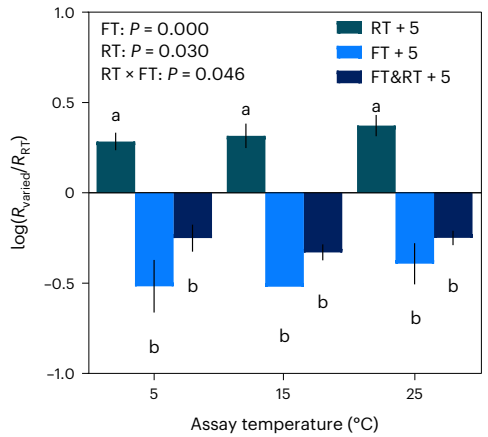

Fig. 3 | Log response ratios of R_{mass} under three assay temperatures (5, 15 and 25 °C) for three incubation scenarios. The incubation scenarios are increased mean temperature, temperature fluctuation, and both increased mean temperature and temperature fluctuation. The RT is 15 °C; RT + 5 represents an increase in the mean temperature of 5 °C; FT + 5 represents the pattern in which the temperature fluctuated by 5 °C and FT&RT + 5 represents the treatment in which both the temperature fluctuated by 5 °C and the mean temperature was increased by 5 °C. A linear mixed-effect model was used to examine the significance of the effects of mean temperature, temperature fluctuation and their interaction on the R_{ratio} values (Supplementary Table 3). Means ± s.e. are presented. One-way repeated-measures analysis of variance (ANOVA) was used to explore the differences in the log response ratios of R_{mass} under three temperature regimes within each assay temperature, and post hoc analyses were performed with Tukey's multiple comparisons test (Supplementary Table 4). Significant differences are indicated by lowercase letters; $n = 18$ independent soil samples.

Table 1 | Relationships between dissimilarity in bacterial and fungal community composition, temperature fluctuation, and mean temperature

Taxa	Temperature fluctuation		Mean temperature	
	Mantel *r*	*P*	Mantel *r*	*P*
Bacteria	0.717	<0.001	0.736	<0.001
Fungi	0.499	<0.001	0.767	<0.001

The Mantel test is widely used to evaluate the correlations between corresponding positions in two dissimilarity or distance matrices, the results of which can represent the actual effects of the selected variates (Mantel *r*). In our study, Bray–Curtis distances were used to test for dissimilarities in the bacterial and fungal community compositions. n=54 independent soil samples.

current understanding of thermal adaptation indicates that microbial communities would upregulate respiration following a sustained decrease in mean temperature[19,29]. In many previous incubation studies to evaluate the effect of mean temperature change, the thermal responses of microbial respiration decreased[28,32], increased[18,29] or remained stable[23,33] under a single heating or cooling (for reducing substrate loss) treatment. Since a lower R_{mass} was observed at intermediate mean temperatures (Fig. 2b), our results suggest that any change in mean temperature (a decrease or an increase) can influence microbial community composition as a result of increased R_{mass}. Considering the background of global warming, our results suggest that the increase in mean temperature may lead to an enhancement of the thermal response of soil respiration in our incubation experiment.

In addition to the finding that mean temperature has strong effects on microbial community structure, as shown previously[34,35], we found that temperature fluctuation had a non-negligible influence (Supplementary Fig. 1a,c and Supplementary Table 5). We further demonstrated that temperature fluctuation had a stronger effect on the dominant microbial taxa than the mean temperature (Supplementary Fig. 3). As global temperature changes entail alterations not only to mean temperature but also to temperature fluctuation[36], these results underline the limitations of only considering changes in mean temperature when examining the effects of temperature on microbial community structure and function.

Changes in community composition can affect the physiological capacity of the bacterial community, because the enzymatic capacity for the initial steps of degradation occurs within a comparatively limited number of bacterial species[37,38]. However, our findings suggest that the shifts in bacterial community composition are not associated with enzyme activities or R_{mass} under increased mean temperature (Fig. 4b), which is consistent with previous studies of weak linkages between shifts in microbial community composition and the thermal response of microbial respiration with changing mean temperature. However, our results showed that shifts in bacterial composition played a key role in decreasing R_{mass} (Supplementary Fig. 4a), and temperature fluctuation had a greater effect on bacterial community composition than on fungal community composition (Table 1). Accordingly, we also found that temperature fluctuation had a direct impact on the relative abundance of major phyla in the bacterial community (Supplementary Fig. 1a). Specifically, the relative abundance of Proteobacteria decreased with increased temperature fluctuation, while the relative abundance of Planctomycetes increased (Supplementary Fig. 3a); both of these bacterial taxa are commonly found in soil and are considered key microorganisms in regulating the temperature response of soil respiration[24,39]. Bacteria exert a stronger influence than fungi on the thermal response to temperature changes[40], which may result in the Q_{10} of the temperature fluctuation treatment being greater than that of the mean temperature treatment (Supplementary Table 2). Our results provide strong empirical evidence of linkages between bacterial community composition and changes in temperature fluctuation, and highlight the importance of incorporating the thermal responses of key soil microorganisms into predictions of soil-carbon–climate feedbacks.

Although increased mean temperature and temperature fluctuation had dissimilar effects on specific taxa in the fungal community (Supplementary Fig. 3c,d), the results indicated that both of these variables could have a positive effect on R_{mass} by promoting shifts in fungal composition and synchronously increasing enzyme activities (Fig. 4). Fungi are considered the major decomposers in forest ecosystems, and substrate preferences vary among fungal taxa, meaning that changes in structure–function relationships in fungal communities are probably prerequisites for the optimization of soil substrate utilization[41]. We demonstrated that shifts in the fungal community induced positive feedback in various patterns of temperature-related changes in enzyme activities, thus resulting in continuous soil carbon loss.

Three types of thermal adaptation of microbial respiration are recognized[28]: type I involves a decrease in the Q_{10} of R_{mass}, with the lowest R_{mass} at intermediate temperatures; type II involves no changes in Q_{10} and a lower R_{mass} regardless of the assay temperature; and type III takes the form of a higher optimum temperature for R_{mass}. Our observations indicated that the thermal adaptation induced by temperature fluctuation was predominantly type II (Supplementary Table 2), which is considered to represent the strongest ability for microorganisms to adapt to changing temperatures[19,23]. This finding suggests that future soil microbial respiration may not be as high as currently predicted but that the temperature sensitivity would be consistent at both longer and shorter timescales.

Overall, our study reveals that temperature fluctuation critically affects the thermal adaptation of soil microbial respiration by causing shifts in bacterial composition and decreasing soil enzyme activities. We thus offer a framework that can be used to describe the interplay between temperature and soil microbial physiology relevant to soil carbon dynamics and provide a basis for future research focusing

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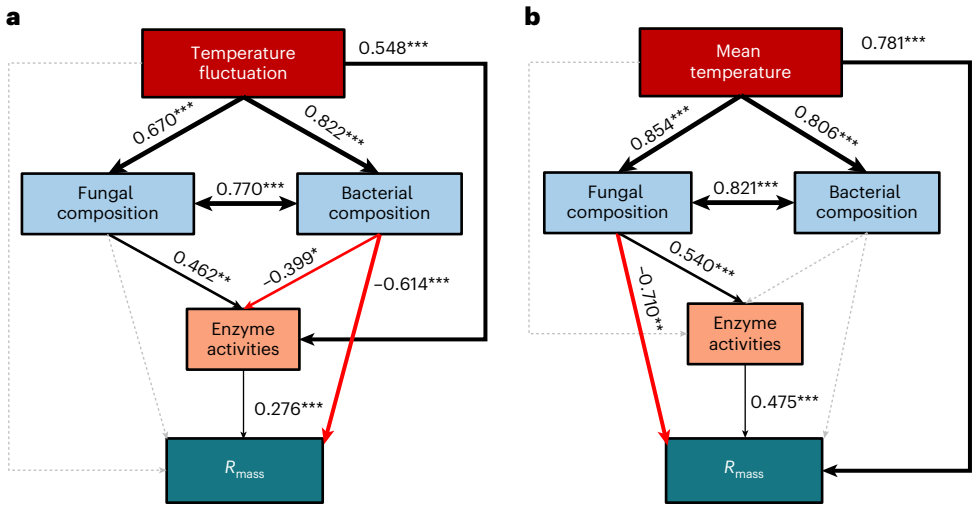

χ^2 = 2.943, P = 0.230, R^2_m = 0.24, d.f. = 2, n = 162

χ^2 = 0.287, P = 0.867, R^2_m = 0.24, d.f. = 2, n = 162

Fig. 4 | Structural equation modelling of the effects of selected drivers on R_{mass}. **a**, Model for the temperature fluctuation treatments. **b**, Model for the mean temperature treatments. The SEMs describe connections among R_{mass}, enzyme activities, fungal community composition, bacterial community composition and the effects of the different temperature treatments on these variables. The values used to represent enzyme activities were calculated as the sum of four soil hydrolytic enzymes involved in carbon degradation. The numbers adjacent to the arrows represent the standardized path coefficients. The solid black and red arrows represent positive and negative pathways, respectively; the grey arrows indicate non-significant pathways ($P > 0.05$). The bidirectional arrows connecting bacterial composition and fungal composition account for their shared fluctuation and indicate a bidirectional relationship between these variables. The widths of the arrows indicate the approximate strength. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; two-sided t-tests. A total of 162 observations (6 soil samples × 3 soil replicates × 3 temperature regimes × 3 assay temperatures) were conducted in the SEM analyses for the two group experiments (that is, changed temperature fluctuation and changed mean temperature). R^2_m represents the explained variation in fixed factors. Goodness-of-fit statistics are shown below each model.

on how to better constrain feedback from soils to climate change. Glucose was chosen as a representative labile substrate commonly available to soil microorganisms and is readily assimilated without exoenzyme breakdown by soil microorganisms[42,43]. Our study focused on the thermal response of soil microbial respiration under excess labile carbon, and most of the microbial communities were active during this duration. However, substrate availability is an important factor affecting the thermal response of soil microbial respiration, and our single substrate additions may not be representative of the natural carbon inputs in terrestrial ecosystems[44], either in bulk soils when the substrate availability is limited or in rhizosphere soils[45], and how differences in the substrate influence the thermal response of soil microbial respiration need to be clarified in future studies.

We expect that our results contribute to improving the characterization of the thermal adaptation of microbial respiration and its incorporation in land surface models by providing robust laboratory evidence of its occurrence. Our laboratory experiments have thus far focused on stable temperature fluctuations. However, given that strong asymmetries in temperature fluctuation occur during different periods (for example, night-time temperatures increase more rapidly than daytime temperatures[46]), future studies are still needed to focus on the ecological consequences of temperature fluctuation under more complex scenarios to gain an even better understanding of the thermal response of soil microbial respiration.

Methods

Field sampling and soil analysis

Soils were collected from six sites along an approximately 2,000-km-long west–east transect extending across subtropical evergreen broad-leaved forests in China. To avoid extremely hot or cold sites and thereby allow the generalization of the soil microbial respiration response across diverse common thermal regimes, we selected these sites on the basis that they had a mean annual land surface temperature of 15 °C and an annual temperature range of approximately 10 °C according to the China Meteorological Data Service Center (http://data.cma.cn/) and spanned a longitudinal range of 103.56° E to 118.42° E. At each site, we established three 1 × 1 m^2 sampling plots at random locations more than 20 m apart from one another. Surface soils (the top 10 cm) were used because this is where microbial activity is generally the highest[47]. The coordinates and elevation of each site were recorded in situ (Supplementary Table 1). The soil samples were transported to the laboratory, where they were sieved to 2 mm; screened to remove the remaining roots, visible stones and litter fragments; and homogenized on the basis of sites. One part of the sample was stored at 4 °C for incubation and community composition analyses, and the other part was air dried for chemical determination.

Soil pH was determined in a 1:2.5 soil–water slurry using a glass pH meter (SevenExcellence, Mettler Toledo). The initial soil total carbon and total nitrogen contents of the sieved composite samples were measured in triplicate using an elemental analyser (Flash*Smart* Organic Elemental Analyser, Elementar) after grinding with a ball grinder (RM200, Retsch), and the soil organic carbon content was determined after the removal of carbonates. Soil dissolved organic carbon concentrations in non-fumigated soils and soils fumigated with chloroform were measured using a TOC analyser (Vario TOC cube, Elementar) after extraction with 0.5 M K_2SO_4 (ratio: 1:2.5 w/v). The soil moisture content was determined by drying 'fresh' soil samples at 105 °C until a constant mass was reached, and the water-holding capacity was determined after the samples had been wetted beyond field capacity and permitted to drip drain for 2 h[28].

Thermal adaptation assays

We tested for soil microbial thermal adaptation by conducting two 200 d incubation experiments; this mid-long-term timescale is sufficient to induce microbial thermal adaptation[29]. Considering the mean temperature and fluctuation around the mean observed at the

sites where the soil samples were collected, the assays involved first incubating the soils at 60% water-holding capacity[48] simultaneously for 200 d under six temperature regimes varying between 5 and 25 °C every 48 h on the basis of an RT of 15 °C. These temperature treatments were used across two separate experiments. The first experiment explored the independent effects of mean temperature and temperature fluctuation on soil microbial respiration. The experimental thermal regimes were generated as follows: (1) alteration of the magnitude of temperature fluctuation while maintaining a fixed mean temperature (that is, RT ± 0 °C (15 °C), RT ± 5 °C (15 ± 5 °C) and RT ± 10 °C (15 ± 10 °C)) and (2) incubation at different constant mean temperatures (that is, (RT − 5 °C) ± 0 °C (10 °C), RT ± 0 °C (15 °C) and (RT + 5 °C) ± 0 °C (20 °C)). The second experiment aimed to determine the joint effects of mean temperature and temperature fluctuation. We incubated the same soils under four thermal regimes in which both the magnitude of temperature fluctuation and the mean temperature were changed—that is, RT ± 0 °C (15 °C), RT ± 5 °C (increased temperature fluctuation, 15 ± 5 °C), (RT + 5 °C) ± 0 °C (increased mean temperature, 20 °C) and (RT + 5 °C) ± 5 °C (simultaneously increased mean temperature and temperature fluctuation, 20 ± 5 °C).

During the incubation periods, a glucose solution corresponding to 3 mg C per g dry soil was added to the jars to keep the excessive substrate before our measurements (Supplementary Fig. 5), and water loss was corrected by weighing the jars and adding the corresponding amount of water at intervals of two weeks. We measured microbial respiration in the middle of each of these intervals (the eighth day after the last addition) under the three assay temperatures, with the time point avoiding the stimulating effect of glucose addition. We used glucose as a substrate because it is a common constituent of fast-cycling soil carbon pools, the turnover of which fuels much of the total soil microbial respiration[49,50]. The substrate concentrations and incubation times used in this experiment were adapted from previous studies[32,51]; dose–response experiments confirmed that the substrate concentrations used were in excess of demand (Supplementary Fig. 5), which prevents the confounding effect of substrate limitation when assessing the response of enzyme-catalysed reactions to temperature fluctuation[51,52]. We used 600 ml containers with caps modified for gas analysis and placed approximately 120 g dry-weight-equivalent aliquots of soil in each container. The incubation jars were placed in a cryogenic thermostatic bath (DC3050, Dawei Instrument Corp.). The incubator automatically fluctuated the incubation temperature over a 48 h cycle at a constant rate for the fluctuation treatments, with the incubation period lasting 100 cycles. Data loggers (HOBO TidbiT Temp Data Logger, UTBI-001) were placed in the water bath to confirm that the temperatures in the incubators followed the designated experimental temperature regime. We used 5, 15 and 25 °C for the assay temperatures to derive the response curves of soil microbial respiration, covering all experimental temperatures used during the incubation phase, to test whether the thermal regimes could be considered the cause of the observational patterns. We did not include a preincubation phase in the experiment because our objective was to test for thermal adaptation to the experimental temperature regimes regardless of where the soil samples were collected.

The microbial respiration measurements followed the methods used by Li et al.[53]. The jars were allowed to remain at the three assay temperatures (5, 15 and 25 °C) for 2 h to allow respiration to stabilize. Fresh air via a gas distribution system was continuously passed through the headspace of each incubation jar at a rate of 0.75 l min^{-1}. Microbial respiration was measured by sealing the incubation jars and immediately removing a 5 ml gas sample from the headspace. The same volume of CO_2-free air was injected to balance the air pressure. After two hours, a second 5 ml gas sample was obtained, and the incubation jar was opened to allow fresh air circulation. The CO_2 concentration in the gas sample was measured using a gas chromatograph (Agilent 6890, Agilent Corp.) equipped with a flame ionization detector. The microbial respiration rate was calculated as the difference in concentrations between the first and second sampling times.

To assess the thermal adaptation of soil microbial respiration at the different assay temperatures, it was necessary to control for soil microbial biomass, given that biomass itself is a factor that regulates soil respiration rates[33]. Thermal adaptation is often tested on the calculation of biomass-specific respiration (R_{mass})[28,54] or the consideration of microbial biomass as a covariate control for microbial biomass[55]. A variable that can be considered a covariate should not be influenced by intervention (that is, the temperature treatments in our study) and can explain the variability in the outcome (that is, microbial respiration in our study)[56]. However, the changes in temperature fluctuation and mean temperature could significantly and marginally significantly influence microbial biomass, respectively (Supplementary Table 6). In addition, the biomass could not explain the variability in microbial respiration under changed temperature fluctuations (Supplementary Table 7). It therefore may not be appropriate to regard biomass as a covariate in our study. R_{mass} is a measure of the ecophysiological status of soil microorganisms, describing the microbial activities at the synecological level by regarding microbial biomass as a single ecological entity[57]. Our analyses showed that R_{mass} was better fitted for describing the relationship between temperature and microbial respiration for each assay temperature, as all these relationships were statistically significant within each assay temperature (Supplementary Tables 8 and 9). The thermal adaptation of soil microbial respiration was therefore tested on R_{mass}. Soil microbial biomass was estimated using the chloroform-fumigation extraction method[58], and soil microbial respiration was expressed per unit microbial biomass-C (μg C per g microbial biomass C per h). The soil microbial biomass was estimated for each lab replicate (that is, each jar), and the analysis for this characteristic was consistent with the corresponding dataset of microbial respiration. In total, we ran 972 assays (6 soil samples × 3 soil replicates × 6 temperature regimes × 3 assay temperatures × 3 laboratory replicates).

Soil microbial species composition analyses

Soil DNA extraction was conducted with a PowerSoil DNA Isolation Kit (Qiagen) according to the manufacturer's instructions. The template DNAs isolated from the laboratory replicates from each site were pooled so that each replicate was equally represented. In total, we evaluated 108 DNA samples (6 soil samples × 3 soil replicates × 6 temperature regimes). The quality of the DNA samples was evaluated using an Invitrogen Qubit 4.0 Fluorometer (Thermo Fisher Scientific) with a Qubit dsDNA HS Assay Kit after extraction, and the DNA was stored at −80 °C for high-throughput sequencing analysis. The composition of the bacterial and fungal communities was assessed by high-throughput sequencing analysis of the 16S rDNA gene with primers 338F/806R (338F: 5′-ACT CCT ACG GGA GGC AGC A-3′; 806R: 5′-GGA CTA CHV GGG TWT CTA AT-3′) and the internal transcribed spacer 1 (ITS1) gene with primers ITS1f/ITS2 (ITS1: 5′-CTT GGT CAT TTA GAG GAA GTA A-3′; ITS2: 5′-GCT GCG TTC TTC ATC GAT GC-3′). Sequencing was conducted using an Illumina NovaSeq platform according to the standard protocols.

The raw FASTQ files were filtered using Trimmomatic (version 0.33)[59]. The primer region was then trimmed from all reads using Cutadapt (version 1.9.1)[60]. Read 1 and read 2 were joined into full-length sequences using USEARCH version 10 (UCHIME 4, version 8.1)[61,62]. In the Quantitative Insights into Microbial Ecology 2 pipeline (QIIME 2, version 2020.6), the sequences were processed with DADA2 to denoise and remove chimaeric sequences, generate non-chimaeric reads and classify amplicon sequence variants[63,64]. All singletons were removed from the analysis. Taxonomic annotation of the amplicon sequence variant sequences was performed with a Bayesian classifier using SILVA (release 132, http://www.arb-silva.de) for bacteria and UNITE (release 8.0, https://unite.ut.ee/) for fungi as reference databases[65,66]. On average, 23,308 quality-controlled bacterial 16S sequences and 20,760 quality-controlled fungal ITS sequences were obtained per sample.

Fluorometric enzyme assays

We measured the activities of four hydrolytic enzymes covering a range of soil microbial carbon processes—namely, α-glucosidase, β-glucosidase, β-D-cellubiosidase and β-xylosidase—according to the fluorometric protocol of German et al.[67]. Briefly, after the 200 d incubation period, assays were conducted by homogenizing 2.75 g of soil (dry equivalent) from each incubation jar in 90 ml of sodium acetate buffer using a hand blender. The slurries were then added to wells in 96-well microplates. Fluorescent substrate proxies specific to each enzyme were added to the assay wells at a substrate concentration of 200 μM. The assays were run with two standard columns containing soil homogenate and methylumbelliferone, the fluorescent tag attached to each substrate proxy. Each enzyme was assayed at 25 °C for 3 h. Following the termination of each reaction, we used a fluorimeter (Synergy 2; BioTek) set at 365 nm excitation and 450 nm emission to measure fluorescence. On the basis of these fluorescence values, we calculated enzyme activity as the rate of substrate conversion in nmol per g dry soil per h. The final enzyme activity values are summed across all four hydrolytic enzymes considered.

Statistical analysis

To evaluate the response of soil microbial respiration to temperature fluctuation and mean temperature, we chose the sites where we collected the soil samples to represent the various soil conditions in forests under similar thermal regimes; thus, the data are presented as the mean values from the six sites. To avoid the spatial autocorrelation of our soil collection sites, we constructed mixed-effect regression models to test for the effects of the different experimental temperature regimes on microbial respiration with site as a random factor. We evaluated several candidate models that are used to test the effect of temperature treatments on soil microbial respiration and chose the best ones according to the lowest ΔAIC_c values (Supplementary Table 10). Given that ΔAIC_c values lower than 2 were considered equally good[68], we chose the formulas R_{mass} ~ FT × AT + (1|site) (model d) and R_{mass} ~ FT × AT + FT^2 × AT + (1|site) (model e) for the temperature fluctuation treatments and the formula R_{mass} ~ MT × AT + MT^2 × AT + (1|site) (model e) for the mean temperature treatments. A better fit of the linear mixed-effect model was verified by the simpler model (Occam's razor—for example, model d) for the temperature fluctuation treatments. We considered coefficients with $P < 0.05$ to be significant. The mixed-effect model was also used to examine the significance of the effects of mean temperature, temperature fluctuation and their interaction on R_{mass}. One-way repeated-measures ANOVA was used to test for differences in the log response ratios of R_{mass} under the four temperature regimes at the three assay temperatures, and post hoc analyses were performed with Tukey's multiple comparisons test.

We fit the exponential model ($R_{mass} = R_{10} \times e^{E_0}\left(\frac{1}{56.02} - \frac{1}{AT - -227.1}\right)$) to the microbial respiration rates calculated for each temperature treatment[69,70]. In the model, R_{mass} represents the microbial respiration at the assay temperature (that is, 5, 15 or 25 °C), AT is expressed in K, R_{10} is a basal respiration rate at 10 °C and E_0 is related to the relative temperature sensitivity of respiration, which is proportional to Q_{10}, where $Q_{10} = e^{10 \times E_0/(T-227.1)^2}$. We calculated Q_{10} at the RT of 15 °C. One-way ANOVA was used to examine the differences in the Q_{10} values under the various temperature regimes.

The differences in microbial community composition associated with the experimental thermal regimes were assessed by non-metric multidimensional scaling and permutational multivariate ANOVA constructed independently for bacteria and fungi. The function mantel from the R package vegan was used to test for differences in community composition in response to temperature changes[71]. One-way ANOVA was used to test for effects of different temperature treatments on the dominant phyla. We further built an SEM to evaluate the factors that directly and indirectly regulate R_{mass} and to evaluate how these factors contribute to the standardized total effect (direct plus indirect effects, Supplementary Fig. 4). We considered the collection site as a random factor, and the data were fitted to the SEM using the maximum likelihood estimation method. Because the quadratic curves were found to be more appropriate to describe the relationship between increased mean temperature and microbial respiration for each assay temperature, we used the squared values of mean constant temperature in the SEM analysis. The best model was based on the overall adequacy of χ^2, the goodness-of-fit index and the root mean squared error of approximation index. All analyses were conducted with R statistical software (version 4.0.4).

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 the present study have been deposited in the NCBI GenBank Short Read Archive under accession number PRJNA809849. All data supporting the findings of the statistical analyses are publicly available at https://doi.org/10.5281/zenodo.6153431.

Code availability

All the R code for our statistical analyses is publicly available at https://doi.org/10.5281/zenodo.6153431.

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Author contributions

M.N. developed the original ideas presented in the manuscript. Y.Z. completed the experiments with assistance from M.N., J.-Q.L., J.-T.L., C.L., C.-M.F. and X.X. Y.Z. performed the overall analysis with assistance from X.-N.X., H.-Y.C., T.Z., J.-J.X., M.N. and B.L. Y.Z. and M.N. 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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Software and code

Policy information about availability of computer code

Data collection	All data were obtained by our laboratory experiments. No software was used for data collection.
Data analysis	All the data analyses were conducted in the R (version 4.0.4) statistical environment. The mixed–effect models used the “nlme” package, Mantel test used the "vegan"package, and structural equation model used the "piecewiseSEM" package, respectively.

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Sequence data generated in the present study were deposited in NABI GenBank Short Read Archive (SRA) under accession number PRJNA809849. All data in the support of these findings and the R code for the statistical analyses will be publicly available at: https://doi.org/10.5281/zenodo.6153431.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Our study does not involve this respect.
Population characteristics	None
Recruitment	None
Ethics oversight	None

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
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Ecological, evolutionary & environmental sciences study design

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Study description	We collected soil samples from 6 sites along a 2,000-km-long west-east transect extending across subtropical forests in China and used them in a 200-d laboratory experiment involving various temperature regimes (See Fig. 1) to explore how temperature fluctuation influences the thermal response of soil microbial respiration and the underlying mechanisms of this process. We evaluated the consequences of changes in temperature fluctuation on the thermal adaptation of soil microbial respiration by explicitly manipulating the temperature fluctuation and holding the mean temperature constant while also exploring the independent effect of mean temperature and the interplay of mean temperature and temperature fluctuation on this process. In total, we ran 972 assays (6 soil samples ×3 soil replicates × 6 temperature regimes × 3 assay temperatures ×3 laboratory replicates). The means of replicates were used as the attribute values for each site, and the means of six sites were randomly assigned to different analyses in our study.
Research sample	Soil samples from six forests at similar latitudes in China (Table S1). These sites were selected spanned a longitudinal range of 103.56° to 118.42°, having a mean annual land surface temperature of 15 °C (reference temperature during our incubation experiments) and a similar interannual temperature variation of approximately 10 °C.
Sampling strategy	Soil samples from six sites in September, 2019. At each site, we established three 1 × 1 m sampling plots at random locations more than 20 m apart from one another. Surface soils (the top 10 cm) were used because this is where microbial activity is generally highest. The soil samples were transported to the laboratory, where they were sieved to 2 mm, screened to remove the remaining roots, visible stones and litter fragments, and homogenized on the basis of sites. One part of the sample was stored at 4 °C for incubation and microbial measurement, and the other part was air-dried for chemical determination.
Data collection	The original data of this study was collected mainly by Yan Zhang and Jintao Li with assisance of Siyuan Xu and Xinyue Qi. Soil respiration measurements were obtained using a gas chromatograph (Agilent 6890; Agilent Corp., USA) equipped with a flame ionization detector, conducted by Yan Zhang and Hongyang Chen. Soil enzyme assays by a fluorimeter (Synergy 2; BioTek, Winooski, Vermont, USA), conducted mainly by Yan Zhang and help from Jintao Li and Hongyang Chen. Soil microbial species composition analyses were determined using an Illumina NovaSeq platform according to standard protocols, conducted by Yan Zhang, Ting Zhu and Jianjun Xu.
Timing and spatial scale	Timing scale: Soil samples from six sites in September, 2019, which represents the mean thermal conditions of the selected soils. Soils were incubated and analyzed from Oct 2019 to Oct 2020.Spatial scale: Soil samples from 6 sites along a 2,000-km-long westeast transect extending across subtropical forests in China, spanned a longitudinal range of 103.56° to 118.42°.
Data exclusions	No data were excluded from this study.
Reproducibility	In the whole experiment, all the findings can be replicated as all the incubation and measurement techniques are widely used and can be reproduced. For example, the treatments for various thermal conditions could be realized in a cryogenic thermostatic bath (DC3050; Dawei Instrument Corp. Ltd., Hangzhou). The incubator automatically regulated the incubation temperature, which gradually fluctuated around the experimental incubation temperature regimes at any speed as designed. Soil respiration measurements were obtained using a gas chromatograph (Agilent 6890; Agilent Corp., USA) and soil enzymes measurements conducted by a fluorimeter (Synergy 2; BioTek, Winooski, Vermont, USA), following the previous studies . Information about the methods used in this paper are included in our material and methods.
Randomization	The means of replicates were used as the attribute values for each site, and the means of six sites were randomly assigned to different analyses in our study. A total of 972 assays (6 soil samples ×3 soil replicates × 6 temperature regimes × 3 assay temperatures ×3 laboratory replicates) were conducted.
Blinding	Our graduate research assistants had no idea what the bottle labels meant. Microbial community composition was measured at a different institution where the investigators had only a sample number and hence no knowledge of where the sample came from.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	These sites we selected having a mean annual land surface temperature of 15 °C (reference temperature during our incubation experiments) and an annual temperature range of approximately 10 °C. More details see Table S1.

Location	Latitude: 25°25'28'' N to 30°54'47'' N, Longitude: 103°33'47'' E to 118°25'27'' E, Elevation: 379 to 1143 m.
Access & import/export	No permit was required, all field work was performed around research stations. Soil samples were sealed in sterile polypropylene bags and transported to laboratory using iceboxes by high-speed railway.
Disturbance	To minimize the effects of our sampling, we backfill the soil profiles.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	*Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Validation	*Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.*

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	*State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.*
Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*
Mycoplasma contamination	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

Specimen provenance	*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.*
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	*For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.*
Wild animals	*Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Reporting on sex	*Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference
(See Eklund et al. 2016)

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity	*Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*
Graph analysis	*Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*
Multivariate modeling and predictive analysis	*Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*