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Cross-biome patterns in soil microbial respiration predictable from evolutionary theory on thermal adaptation

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Climate warming may stimulate microbial metabolism of soil carbon, causing a carbon-cycle–climate feedback whereby carbon is redistributed from the soil to atmospheric CO_2. The magnitude of this feedback is uncertain, in part because warming-induced shifts in microbial physiology and/or community composition could retard or accelerate soil carbon losses. Here, we measure microbial respiration rates for soils collected from 22 sites in each of 3 years, at locations spanning boreal to tropical climates. Respiration was measured in the laboratory with standard temperatures, moisture and excess carbon substrate, to allow physiological and community effects to be detected independent of the influence of these abiotic controls. Patterns in respiration for soils collected across the climate gradient are consistent with evolutionary theory on physiological responses that compensate for positive effects of temperature on metabolism. Respiration rates per unit microbial biomass were as much as 2.6 times higher for soils sampled from sites with a mean annual temperature of −2.0 versus 21.7 °C. Subsequent 100-d incubations suggested differences in the plasticity of the thermal response among microbial communities, with communities sampled from sites with higher mean annual temperature having a more plastic response. Our findings are consistent with adaptive metabolic responses to contrasting thermal regimes that are also observed in plants and animals. These results may help build confidence in soil-carbon-climate feedback projections by improving understanding of microbial processes represented in biogeochemical models.

Temperature is a dominant control on the rate of carbon cycle processes[1]. Warmer temperatures accelerate the rate at which carbon fixed by primary producers is broken down and respired by microorganisms into products such as CO_2. This temperature dependence of microbial decomposition is a key parameter in biogeochemical models, including those embedded in the Earth system models used to project feedbacks between the carbon cycle and climate system[2]. However, the ability of these models to predict the magnitude of these feedbacks is constrained by uncertainty in the extent to which climate warming will accelerate losses from soil carbon to atmospheric CO_2[3–5]. The uncertainty arises, at least in part, because the form of the temperature–decomposition relationship is under intense debate as new data and ideas emerge regarding the physical, chemical and biological processes that collectively regulate the vulnerability of soil carbon to respiratory loss[6–8].

One approach to building confidence in the projected range of soil carbon–climate feedbacks is to compare models that represent both established and emerging conceptions of how the temperature-decomposition response is regulated[2,9,10]. This approach acknowledges process-level uncertainties in soil carbon dynamics. Many of these uncertainties are reflected in the recent proliferation of soil biogeochemical models that variously represent microbial physiology, population dynamics and community composition as controls on the size of soil carbon stocks[11–14]. Comparisons among these models suggest that the magnitude of soil carbon stock responses to warming depends strongly on the specific representations of microbial processes, such as growth, respiration and community turnover[9,10,15]. A key challenge now is to collect data that facilitate evaluation and refinement of the contrasting microbial-process representations[2,9,10].

Here, we test two alternate hypotheses about how soil microbial processes will respond to climate warming. Both hypotheses are based on the knowledge that temperature is a strong selective force that acts on the structure and function of cells and populations, resulting in both individual and community responses. At the cellular level, temperature directly selects for enzyme and membrane structures that help to maintain physiological process rates as temperatures cool, and constrain them as temperatures warm[16]. This selection operates via evolutionary trade-offs in the structure and function of enzymes and membranes that, for example, counteract the positive effects of temperature on reaction rates[16]. As such, the expectation is that these trade-offs will serve to mute soil microbial respiration responses to warming, at least on a per-biomass level (the 'compensation' hypothesis; Fig. 1). Alternatively, the 'enhancement' hypothesis posits that warming will enhance soil respiration responses as microbial communities shift in terms of their dominant functional traits[17] (Fig. 1). For example, higher respiratory costs might be associated with indirect temperature selection for taxa that specialize on more recalcitrant soil carbon compounds[17–19]. The indirect mechanisms proposed include depletion of labile substrates, and the fact that warming should help overcome the activation energies required to grow on carbon substrates that are less energetically efficient[20,21].

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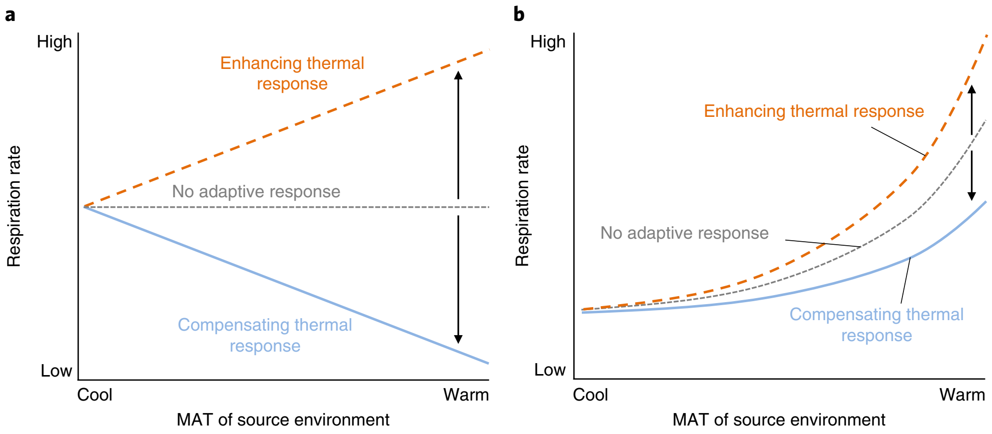

Fig. 1 | Competing assumptions for adaptive responses of soil microbial respiration to changes in thermal climate. a, The compensation hypothesis (solid blue line in both panels) holds that metabolic activity per unit biomass—when measured at a common temperature with substrate availability in excess—decreases with adaptation to warmer conditions. **b**, When metabolic activity is then measured at the temperature regime to which organisms adapted, the expectation is that respiration rates—also at a common microbial biomass and with substrate in excess—respond positively to temperature but to a lesser extent than would be observed if there was no thermal adaptation (grey dotted lines). In contrast with this compensating response, the enhancement hypothesis (orange dashed lines) suggests that there will be an enhancing response caused by a shift in the functional traits of organisms in communities. Note that the curvilinear plots in **b** represent—regardless of whether adaptive responses are assumed or not—a short-term, positive respiration response to temperature, as is commonly observed for soil respiration, although the exact form of the respiration–temperature relationship varies, and at higher temperatures can peak and then decline.

We tested the competing expectations of the compensation and enhancement hypotheses (Fig. 1) on heterotrophic soil respiration rates using two approaches. For the first approach, differences in thermal regime were generated by sampling soils from across a gradient in mean annual temperature (MAT) spanning artic to tropical biomes (see Methods and Supplementary Table 1). Soils from this MAT gradient were then assayed at three temperatures (12, 20 and 28 °C), with favourable moisture and excess carbon substrate. We also measured other variables (for example, texture, pH and soil carbon) that affect soil respiration rates, and then built multiple-regression models to estimate respiration rates at the mean microbial biomass across samples (see Methods). This approach permitted us to test for patterns in soil respiration that were consistent with one of the competing hypotheses: either decreasing respiration per unit biomass with increasing MAT (per the compensation hypothesis) or increasing respiration per unit biomass with increasing MAT (per the enhancement hypothesis) (Fig. 1). For the second approach, we then incubated the same soils for 100 d at one of 3 thermal regimes, ranging from cool (12 °C) to warm (28 °C), before again assaying them and building regression models to estimate respiration rates per unit biomass (see Methods). This incubation-gradient approach included weekly additions of carbon substrate, permitting us to test for effects of thermal regime independent of changes in other potentially causal variables—such as substrate availability—that co-vary with MAT across spatial gradients[22]. Overall, we then addressed the hypotheses using two different approaches: an observational MAT gradient and a 100-d experimental incubation, in line with recent calls to use multiple approaches to test a single question[23].

Results and discussion

MAT gradient. Soil respiration rates respond strongly to the availability of labile carbon substrates and contemporary temperature[24,25]. Not surprisingly, we therefore saw strong positive responses of respiration to substrate addition (mean rates ~4.4 times greater; mean ± s.d. μg C-CO_2 g dry-weight soil^{-1} h^{-1}: water only = 0.62 ± 1.45 ($n = 198$) versus with substrate = 2.73 ± 10.86 ($n = 594$)) and assay temperature, with potential respiration rates as much as tripling as the assay temperature was increased from 12 to 28 °C (Fig. 2). Also in line with expectations[26,27], we found that soils with higher microbial biomass respired more, with the effect of microbial biomass measured as almost twice that of assay temperature for a one s.d. increase in these controls (compare the standardized coefficients in Table 1).

Previous work has shown that seasonal temperature variation affects soil microbial respiration rates per unit biomass[28]. However, temperature data collected at each of the 11 field locations in the 1, 2 and 3 months before the soil samplings in 2010, 2011 and 2012 correlated strongly with MAT, which most strongly affected the respiration patterns. As such, MAT was used in the final statistical models to represent location-level temperature conditions. There was a clear decrease in potential respiration rates (at the mean microbial biomass across samples and with the substrate in excess) as MAT increased (Fig. 2a and Table 1). This negative relationship was robust to model specification (Supplementary Table 3) and is consistent with the compensation hypothesis (Fig. 1). Specifically, in warmer environments, the lower conformational flexibility of enzymes and decreasing cell-membrane permeability translate to lower respiration rates per unit biomass than for similar individuals conditioned to cooler environments, when assayed under common conditions and intermediate temperatures[16].

Admittedly, we assessed respiration rates at the community level and not the individual level, making it hard to pinpoint the specific mechanisms that might underlie a compensation response. Yet, the patterns we observed did not appear to arise through differences in the physiology of the community related to such phenomena as its carbon use efficiency. For example, the amount of substrate assimilated that is allocated to respiration is expected to increase with temperature and to vary markedly with different substrates[15,29]. However, the MAT–respiration relationship was consistently negative regardless of assay temperature and whether glucose, glycine or oxalic acid was used as the assay substrate (Table 1, Fig. 2 and Supplementary Fig. 1). These findings support model expectations that compensatory responses arising through shifts in enzyme and membrane properties have effects on respiration rates that are independent of changes in carbon use efficiency[15]. Furthermore, the lack of a strong interaction between MAT and assay temperature suggested

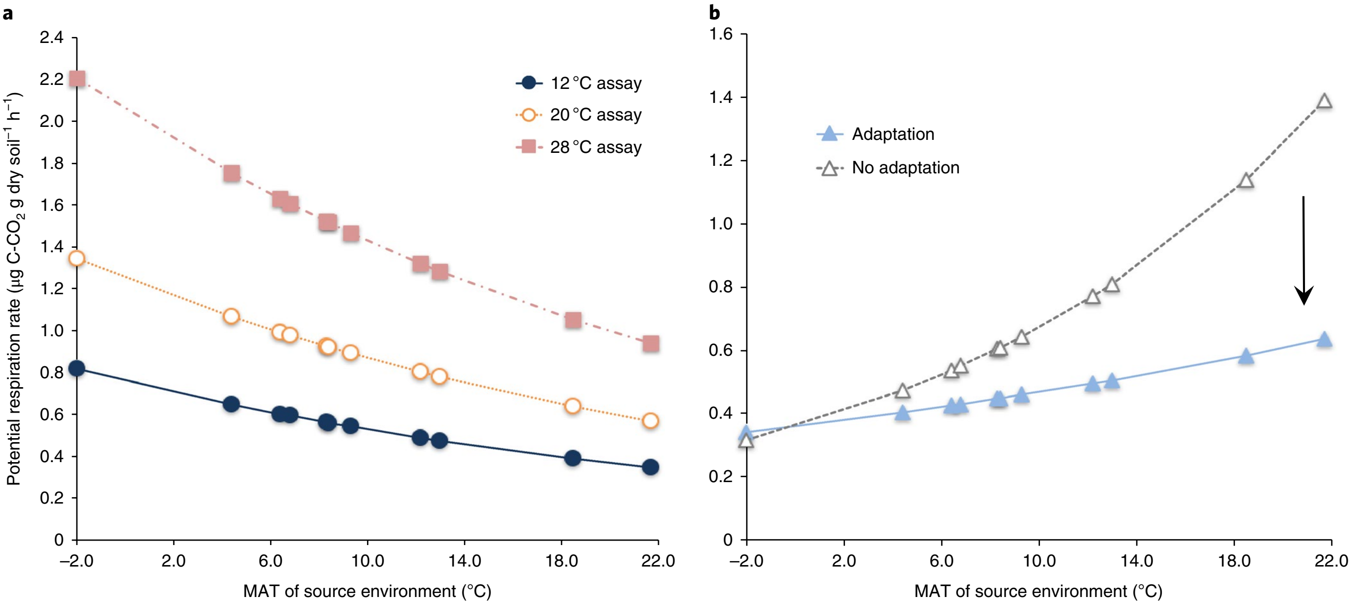

Fig. 2 | Estimated effects of spatial variation in thermal climate (observational gradient) on soil microbial respiration rates. Soils were collected across a MAT gradient to generate differences in the thermal regime to which the soil microbial communities had been exposed. Respiration rates were measured with carbon substrate in excess and are shown for the mean microbial biomass value across all samples. **a**, Effect of differences in assay temperature. Symbols are point estimates and lines are only shown as guides to help discern the patterns for each assay condition. Point estimates were made using the unstandardized coefficients (see Methods) from the MAT-gradient statistical model presented in Table 1 (which also shows the s.d. for the slope coefficients), with MAT values in the regression equation systematically increased from the lowest to highest value for the 11 locations where soils were sampled in 2010, 2011 and 2012 (Supplementary Table 1). The rates shown are for glucose assays, but the qualitative patterns are identical to those for glycine and oxalic acid assays (Supplementary Fig. 1). The negative slope for each line in **a** is consistent with the compensation hypothesis. **b**, Estimates of potential respiration rates for each soil at an assay temperature that matched its source location MAT value, where thermal adaptation dampens (depicted by the downward arrow) the positive response of soil respiration to increasing assay temperature compared with a no-compensatory-adaptation scenario. To generate the point estimates in **b**, we used the unstandardized negative coefficient for the MAT term from the MAT-gradient statistical model presented in Table 1 for the adaptation scenario, then set this coefficient to zero for the no-adaptation scenario.

that there was little evidence of different temperature optima for microbial activity across the soils (Supplementary Table 3). Measurement of microbial growth rates, and how they are related to respiration rates, would be necessary to evaluate these possibilities[30]. Nevertheless, the negative response of respiration rates at a common biomass to warmer environments is consistent with a compensatory response, and has also recently been observed in global drylands[31].

Notably, respiration responses are rarely perfectly compensatory[16]. That is, at higher in situ temperatures, organisms should have lower respiration rates per unit biomass than would be expected in the absence of adaptation, but there should still be a positive, albeit muted, response of respiration to increasing temperature[16]. We tested for this possibility by estimating the potential respiration rates of our soils for the MAT value of the location from which they were sampled. We did this using the negative coefficient for MAT (Table 1) and—to generate a second scenario with no compensation—by setting this coefficient value to zero (see Methods). In both instances, there was a positive response of respiration to increasing temperature, as is expected for reaction rates measured within the lower-to-middle temperature range typical for biological activity[32,33]. However, the adaptation scenario had a more muted temperature response (Fig. 2b), supporting the idea of a partial compensatory response, as is typically observed in animals and plants[16,34].

Incubator gradient. Given the inherent challenges in identifying causation with observational data, we further evaluated our hypotheses using an experimental design where we could manipulate the thermal regime independent of other factors. Specifically, soils collected in 2012 were maintained at optimal moisture conditions, with continual or no substrate supply (glucose versus water addition), at 3 temperature regimes for 100 d, before again being assayed to assess the potential respiration rates at a common biomass (see Methods). The cool and warm regimes (12 versus 28 °C) involved maintenance at a constant temperature, whereas a third regime varied between 12 and 28 °C every 3.5 d to give a mean temperature condition of 20 °C (analogous, then, to MAT). This constant versus varying thermal regime had little influence on the respiration response to the thermal incubation regime (Supplementary Table 4), so we used the mean temperatures (12, 20 and 28 °C) in the full statistical models. We note that thermal regimes for in situ soils are rarely constant but vary diurnally and seasonally; we did not intend to simulate realistic regimes, but rather created experimental regimes to test whether the thermal regime (that is, MAT) was plausibly the cause of the observational patterns (that is, Fig 2).

As for the MAT-gradient dataset, biomass had a strong positive effect on respiration rates, as did the addition of assay substrate (that is, glucose versus water; Table 1). Furthermore, the soils maintained at a higher temperature across the incubation had lower potential respiration rates under the 20 °C assay conditions, when estimated at the mean biomass across samples, compared with soils maintained at lower temperatures (Fig. 3). That is, potential respiration rates at a common biomass were higher (when assayed with the substrate in excess and at a common intermediate temperature) for soils previously maintained at cooler thermal regimes. These patterns were not the result of substrate limitation, which has been invoked as an explanation in other studies[35], because the pattern was evident

Table 1 | Coefficients (mean ± s.d.), significance and r^2 values for the linear mixed models used to evaluate the influence of thermal regime on soil microbial respiration rates

	Model			
	MAT(unstandardized)	**MAT(standardized)**	**Incubator(unstandardized)**	**Incubator(standardized)**
Intercept	−2.18 ± 0.157	−0.27 ± 0.062	−2.75 ± 0.316	−1.01 ± 0.086
Assay temperature	**0.062 ± 0.0024**[a]	**0.81 ± 0.031**[a]	NA	NA
Glucose	0.075 ± 0.0381[b]	0.075 ± 0.0381[b]	NA	NA
Oxalic acid	**−0.283 ± 0.0381**[a]	**−0.283 ± 0.0381**[a]	NA	NA
MAT	**−0.036 ± 0.0117**[a]	**−0.45 ± 0.146**[a]	0.041 ± 0.0234[b]	−0.048 ± 0.215
Microbial biomass	**0.0055 ± 0.00098**[a]	**1.49 ± 0.264**[a]	**0.0021 ± 0.00074**[a]	**0.38 ± 0.137**[a]
Clay	0.0072 ± 0.00544	0.18 ± 0.138	0.0011 ± 0.00755	0.029 ± 0.1973
Carbon	0.020 ± 0.0249	0.21 ± 0.260	**0.057 ± 0.0241**[a]	**0.48 ± 0.203**[a]
pH	−0.00028 ± 0.0027	−0.015 ± 0.1459	−0.0023 ± 0.0039	−0.11 ± 0.188
Incubation temperature	NA	NA	0.016 ± 0.0118	**−0.23 ± 0.066**[a]
Incubation substrate	NA	NA	**1.20 ± 0.218**[a]	**0.79 ± 0.0773**[a]
Assay substrate	NA	NA	**0.84 ± 0.066**[a]	**0.84 ± 0.0655**[a]
Incubation temperature × incubation substrate	NA	NA	**−0.021 ± 0.0101**[a]	**−0.27 ± 0.132**[a]
Incubation temperature × MAT	NA	NA	**−0.0022 ± 0.0008**[a]	**−0.38 ± 0.143**[a]
d.f.	540, 477 or 54	540, 477 or 54	222, 196 to 10	222, 196 to 10
Fixed r^2	66.6	66.6	62.0	62.0
Full r^2	86.9	86.9	74.9	74.9

Shown are the statistical models for the MAT- and incubator-gradient thermal regimes. Unstandardized coefficients were used to plot Figs. 2, 3 and 4, where predicted rates were back-transformed from natural-log values before plotting. Standardized coefficients are also presented, to facilitate comparisons of the effect size of predictor variables on different unit scales, and to facilitate interpretation of the main effects involved in the two-way interactions (see Methods). Note that the coefficient for the incubation temperature is negative when standardized in the incubator model, consistent with expectations of thermal adaptation. Also consistent with theory on thermal adaptation, the MAT term is negative in the MAT models. Mean coefficients and their s.d. were estimated using a Markov chain Monte Carlo sampling approach. Significance was calculated with the Satterthwaite approximation for restricted maximum likelihood models. Model r^2 values were calculated using a method that retains the random effects structure (see Methods). Random effects for the MAT models assumed a common slope, and for the intercept, year (2010–2012) was nested in cover (forest or grass), nested in location (Supplementary Table 1). Only 2012 soils were used in the incubator model, in which a common slope was also assumed, and for the intercept, cover (forest or grass) was nested within location. Sensitivities of the coefficient estimates to model structure and the observations evaluated are reported in Supplementary Tables 2 and 4. Predictor variables for the MAT model were: assay temperature; the carbon substrate used in the assays (that is, glucose or oxalic acid; when both binary variables were set to 0, glycine was used); microbial biomass; MAT at the site from which the soil was sourced; the soil physicochemical properties from those sourced soils (namely, soil texture as a percentage of the clay content (clay), and as a percentage of the soil carbon concentration (carbon)); and pH. Predictor variables for the incubator model included the majority of these variables, in addition to the incubation temperature regime, incubation substrate (glucose or water) and assay substrate (glucose or water). The incubator model did not use glycine or oxalic acid, and only used a single assay temperature (20 °C; see Methods and Supplementary Tables 2–5). Model r^2 values are given for the fixed and full (that is, fixed + random) effects. Degrees of freedom (d.f.) are first given for the number of observations on which each model was based, and then for the range in d.f. on which significance was based (see Supplementary Tables 2 and 4). There was low correlation among the main predictor variables (see Supplementary Tables 3 and 5). [a]Significant ($P < 0.05$) coefficients (also shown in bold). [b]Marginally significant ($P < 0.1$) coefficients. NA, not applicable.

for the subset of soils that received an excess supply of labile organic carbon (glucose) across the 100-d incubations. As such, potential soil respiration rates (at the mean microbial biomass) responded to the different experimental thermal regimes in a manner consistent with the negative response to MAT in the observational part of our study. These patterns are consistent with compensatory responses observed when individual microbes, animals and plants physiologically acclimatize to the thermal regime[16,34,36,37].

In contrast with the MAT-gradient data, the 100-d incubations altered the effect of MAT (of the source location) on respiration rates. Specifically, there was a significant and strong interaction between MAT and incubation temperature (Table 1), and model sensitivity analysis highlighted the importance of retaining this interaction to generate robust coefficients (Supplementary Tables 4 and 5). The interaction arose because MAT was now positively related to potential respiration rates for soils incubated at the coolest thermal regime, albeit still negatively related for those incubated at the warmest regime (Fig. 3). We cannot ascertain the precise mechanisms underlying this difference in the direction of the MAT effect, but it appears evident that soils sampled from a higher MAT location showed a more plastic response to the incubation thermal regime than those from cooler locations. For example, for soils from the location with a MAT of 21.8 °C, the estimated respiration potentials (at a common intermediate temperature) for soils from the 12 °C thermal regime were more than double those of the soils exposed to 28 °C for 100 d. In contrast, for soils from the location with a MAT of −2.0 °C, there was essentially no difference (Fig. 3).

The apparently greater plasticity of the respiration response for soils from warmer locations is consistent with empirical data and the expectation that microbes in more benign or favourable abiotic environments have more plastic phenotypes[38–41]. In contrast, in harsher (for example, dry, cold and/or substrate-limited) environments, genome size is expected to be smaller, and traits more constitutively expressed. This apparent difference in plasticity was also evident with the MAT-gradient data, where the magnitude of compensation was greater for soils sampled from locations with higher MAT values (Fig. 2b). However, further research is required to tease out how and why the in situ thermal environment might shape the plasticity of respiration rates. For example, our soils differed markedly in soil properties such as total soil carbon and texture (Supplementary Table 1), and would also be expected to differ in other respects such as mineral surface reactivity, which could control the availability of carbon substrates to the microbial community[6]. Furthermore, the weekly glucose additions across the 100-d experimental incubations probably shifted the microbial community composition towards faster-growing taxa[42], meaning that our results might only apply to those environments within soils where low-molecular-weight substrates are in high supply (for example,

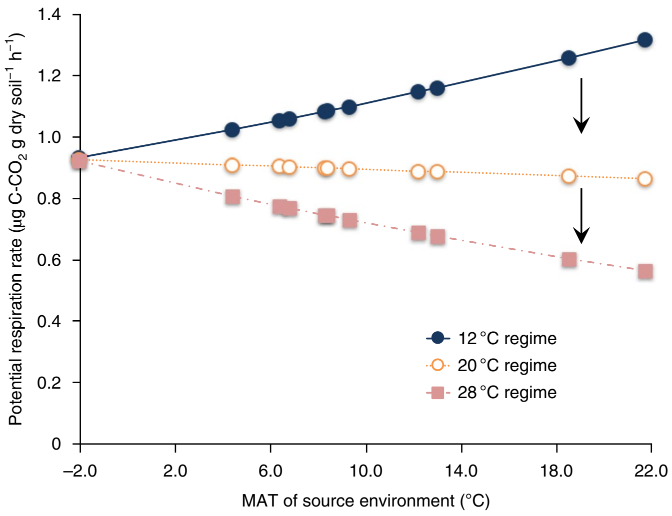

Fig. 3 | Estimated effects of experimental variation in thermal climate (incubator gradient)—with substrate supply—on soil microbial respiration rates. Soils were collected across a MAT gradient (*x* axis), then incubated for 100 d, with carbon substrate supplied, under 1 of 3 different mean thermal regimes (12, 20 and 28 °C). Respiration rates were measured at the end of the 100-d incubations at 20 °C with glucose in excess, and are shown for the mean microbial biomass value across all soils receiving glucose across the 100-d incubations. Symbols are point estimates and lines are only shown as guides to help discern the patterns for each assay condition. Point estimates were made using the unstandardized coefficients (see Methods) from the incubator regime statistical model presented in Table 1 (which also shows the s.d. for the slope coefficients), with MAT values in the regression equation systematically increased from the lowest to highest value for the 11 locations where soils were sampled in 2012 (Supplementary Table 1). The lower rates for the soils incubated for 100 d at warmer thermal regimes are consistent with the compensation hypothesis (depicted by the downward arrows). The magnitude of response to the incubation-gradient thermal regimes was greater for soils sampled from locations with higher MAT values.

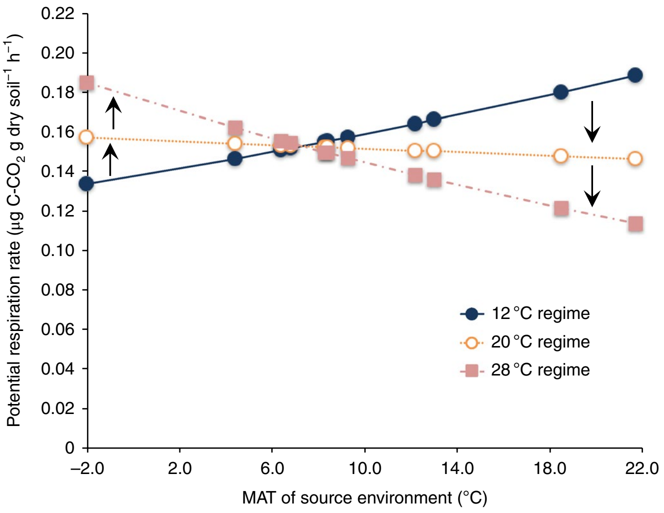

Fig. 4 | Estimated effects of experimental variation in thermal climate (incubator gradient)—without substrate supply—on soil microbial respiration rates. Soils were collected across a MAT gradient (*x* axis), then incubated for 100 d, without carbon substrate supplied, under 1 of 3 different mean thermal regimes (12, 20 and 28 °C). Respiration rates were measured at the end of the 100-d incubations at 20 °C with water only (no glucose), and are shown for the mean microbial biomass value across all soils receiving only water across the 100-d incubations. These conditions (no substrate supply and assayed without substrate addition) approximate those where previous work has detected enhancing thermal responses (Fig. 1). Point estimates were made using the unstandardized coefficients (see Methods) from the incubator regime statistical model presented in Table 1 (which also shows the s.d. for the slope coefficients), with MAT values in the regression equation systematically increased from the lowest to highest value for the 11 locations where soils were sampled in 2012 (Supplementary Table 1). Upward and downward arrows represent enhancing and compensating thermal responses, respectively, as observed in previous studies among soils sampled across climate gradients where incubations and assays were conducted without a substrate supply.

the rhizosphere). However, our results might still be representative, given that soil community composition acts as a control on the temperature sensitivity of soil respiration across latitudinal gradients[43], and because low-molecular-weight substrates are thought to contribute to as much as half of the heterotrophic respiration observed in soils[44]. Yet, even if our experimental manipulations fail to replicate in situ conditions, such experimental data can be used to evaluate competing structural assumptions in microbial-explicit soil biogeochemical models, and hence help identify how microbial processes might be most appropriately represented[10].

Regardless of the underlying mechanisms, the MAT-dependent plasticity in the potential respiration rates that we observed (Figs. 2b and 3) fits with mechanisms proposed to explain the enhancement hypothesis[17,18] (that is, the hypothesis that warming selects for communities with higher respiration rates, when biomass is equal). If warmer temperatures select for communities with higher biomass-specific respiration rates, the negative MAT–respiration relationship we observed with the MAT-gradient data (Fig. 1) reflects a net compensation effect. As such, the negative slope will be smaller than if only compensatory mechanisms were operating, because the slope will have been offset by responses expected under enhancing mechanisms. The balance between the mechanisms operating under these apparently conflicting hypotheses may help to explain the contrasting results observed in previous studies. That is, some soils experience a net enhancing thermal response for respiration, whereas others experience net compensating responses under warming[17,18] (Fig. 1).

Resolving compensatory versus enhancing responses. A further reason why both compensating and enhancing responses may be observed among studies is that researchers generally focus on understanding respiration patterns by resolving different mechanisms. For example, to test for compensatory responses caused by enzyme or membrane structure–function trade-offs, controlled conditions are required to discern the effects independent of multiple other controls on respiration rates[16,35]. Hence, and as we do here, such investigations standardize temperature, provide substrate in excess and express respiration rates for a common biomass[28]. However, where enhancing (or null) responses have been found, substrate has not been provided in excess for assays, nor provided across the course of long-term incubations of soils held at different thermal regimes[17,27]. Such differences in protocols, as well as the inappropriate analysis of respiration-to-biomass ratios (see Methods), may affect conclusions about the temperature sensitivity of soil respiration[33,40,45].

We therefore also established soils that were maintained with water only in our 100-d incubations, and that were assayed at the end of the 100 d with water, to discern whether protocol differences in substrate availability might explain alternative conclusions. Respiration rates under conditions of substrate limitation were lower than when we added substrate (compare the *y* axes in Figs. 3 and 4). Under these conditions of substrate limitation, we saw patterns that fit with some soils exhibiting an enhancing response, some exhibiting no response and some exhibiting a compensatory response (Fig. 4). These results therefore help to resolve apparently contradictory findings among past studies[17,27,28,46], revealing that conclusions about whether warming elicits net compensatory or enhancing responses are method dependent. Carbon substrate availability also varies across space and time[22,24], and so might help explain why there is limited evidence of acclimatization to experimental warming when soil respiration rates are measured in the field[27,32,47,48]. Perhaps most importantly, however, we show that when compensatory responses underlain by expected biochemical trade-offs are tested for following protocols established in the physiological evolution literature, there is consistent evidence for compensation (Figs. 2 and 3). Notably, using the same approaches and our incubation data, we also find evidence that enhancing mechanisms may offset the magnitude of these compensatory responses[17,40].

Conclusions

Our MAT and incubation thermal regimes yield data on respiration rates at a common biomass that are consistent with evolutionary theory on structure–function trade-offs in enzymes and membranes in response to thermal regime. The respiration responses we observe may occur through individual acclimatization, population- and/or community-level shifts[35]. Regardless of the level of organization at which they operate, the underlying biochemical mechanisms are expected to act via common trade-offs between the structural stability of an enzyme or membrane versus the rate at which it facilitates metabolic activity[16]. Notably, similar to animals and plants, we find evidence for partial, as opposed to 'full', compensation. These insights can inform the structural assumptions in the growing number of soil biogeochemical models that explicitly represent microbial physiological, population and community dynamics as controls on soil carbon turnover[10–14]. However, our data are not suitable for model parameterization because they are conducted with idealized conditions (for example, excess substrate) that permit us to test between the competing hypotheses (Fig. 1) but not estimate in situ respiration rates. Nevertheless, there is an important need to test among structural assumptions because model comparisons show that soil carbon dynamics depend strongly on how microbial processes are represented[9,10,14,49,50]. As such, empirical tests of model structural assumptions, and consequent model refinements, are required to build confidence in the projected magnitude of soil-carbon–climate feedbacks[2]. In particular, our findings suggest the need to represent microbial physiological and community shifts, to understand how warming will influence soil carbon turnover.

Methods

Study design and respiration measures. *Soil collection and subsampling.* Soils were collected from 11 locations ranging from Hawaii to Northern Alaska, using a sampling design intended to generate marked variation in abiotic conditions, including temperature and precipitation (Supplementary Table 1)[51]. The locations included 10 US Long Term Ecological Research (LTER) stations—Andrews Experimental Forest, Oregon (44.21° N, −122.26° E), the Boreal Ecology Cooperative Research Unit, Alaska (64.85° N, −147.84° E), Cedar Creek Ecosystem Science Reserve, Minnesota (45.40° N, −93.20° E), Coweeta LTER, North Carolina (35° N, −83.5° E), Hubbard Brook Experimental Forest, New Hampshire (43.94° N, −71.75° E), Harvard Forest, Massachusetts (42.53° N, −72.19° E), Kellogg Biological Station, Michigan (42.4° N, −85.4° E), Konza Prairie Biological Station, Kansas (39.09° N, −96.57° E), Luquillo LTER, Puerto Rico (18.3° N, −65.8° E) and Niwot Ridge LTER, Colorado (39.99° N, −105.37° E)—and the Hawaii Experimental Tropical Forest, Institute of Pacific Islands Forestry, Hawaii (19.81° N, −155.26° E). Each location included paired sites that had natural forest versus grassland cover, where the grasslands were either natural or maintained by low-intensity (annual mowing) management (Supplementary Table 1).

To capture temporal variation in microbial community attributes, three replicate samples were collected from each sampling site over three years (2010, 2011 and 2012), with the sampling date varying between seasons (spring, summer and autumn, respectively). We used surface soils (top 10 cm) because this is where microbial activity, and hence contributions to heterotrophic soil respiration, are generally highest. On receipt at Yale, soils were sieved to 2 mm, screened to remove the remaining root and litter fragments, and then homogenized to provide a total of 66 soil samples (11 locations × 2 cover types × 3 sampling years). In each year, soils were then subsampled for respiration measurements, soil properties and microbial biomass (see below). In 2012, additional soil subsamples were taken for the 100-d experimental incubations (see 'Incubation-gradient approach').

MAT-gradient approach and respiration measures. The MAT gradient provided by sampling from the 11 field locations in 2010, 2011 and 2012 permitted a regression-based experimental design with 66 soils (6 from each location with 2 per sampling year: 1 from forest and 1 from grassland). To compare the respiration potentials of the microbial communities in each of the 66 soils, we used a catabolic profiling assay following the methodology described by Crowther et al.[51] Briefly, three organic carbon substrates that are readily assimilated without exoenzyme breakdown by soil microbes—namely, glucose, glycine and oxalic acid—were added in 8 ml of deionized water solution to 4 g (dry-weight equivalent) of each soil. Soils were shaken for 1 h, capped and flushed with CO_2-free air to obtain headspace concentrations to ~0 ppm. They were then capped and the net CO_2 accumulation was measured on an infrared gas analyser after an incubation period at 20 °C of ~4 h, from which respiration rates were calculated knowing the soil mass, time of incubation, CO_2 concentration change and headspace volume[28]. The substrates were chosen to represent labile substrates commonly available to soil microorganisms (that is, sugars, amino acids and organic acids, respectively)[44]. Substrate concentrations and incubation times were modified from Crowther et al.[51], following preliminary dose–response assays with 2010 soils to determine concentrations (from as low as 0.075 mM to as high as 750 mM) and times (from 2 to 24 h) that were non-limiting (that is, gave a maximum respiratory response) across the assays. Specifically, 4-h incubation times maximized rates across soils, as did substrate concentrations of 75 (glucose), 100 (oxalic acid) and 300 mM (glycine). Assays were conducted in duplicate at 12, 20 and 28 °C, yielding 1,584 assays in total (66 soils × 3 substrates plus a water-only control × 3 incubation temperatures × 2 analytical repeats per substrate per soil). In general, analytical repeats varied minimally from one another (for example, in 2012 with glucose at 20 °C, the mean ± s.d. among duplicate samples for the 22 field soils was 3 ± 5%). Hence, the mean of the two analytical repeats was used in the statistical analyses, giving 792 observations. We chose assay temperatures ranging from 12 to 28 °C because they represent a wide temperature variation but also are within the favourable range for biotic activity for microbes sampled from cold to warm seasonal environments[52].

To estimate the thermal regime at each location, climate data were collected from the US National Climatic Data Center (www.ncdc.noaa.gov/cdo-web/datasets) for weather stations local to or maintained at each location. The MAT for each location was determined from the 1981–2010 climate norms using this 30-year temperature period. When data were available for multiple stations, the mean value across stations was used. Monthly averages were also obtained for 2010, 2011 and 2012 to calculate the mean monthly temperature in the 3-, 2- and 1-month period before soil sampling at each location, to test for seasonal responses of respiration potentials to temperature. However, given strong correlations between MAT and the monthly data (r values of 0.62–0.74; $n = 33$ per 1-, 2- and 3-month period), it was not possible to discern any seasonal patterns independent of the MAT effects.

Incubation-gradient approach. Soils sampled in 2012 were maintained under three thermal regimes, with or without substrate addition. Specifically, 30 g (dry-weight equivalent) of each soil was placed in plastic containers with perforated lids to permit gas exchange (that is, CO_2 efflux and O_2 influx). Soils were adjusted to 65% water-holding capacity, which is optimal for microbial activity[53], and placed in the dark within incubators maintained at 12 or 28 °C. A total of 6 containers for each soil were established, with 2 of each container maintained for 100 d at 12 °C, 2 containers at 28 °C, and 2 containers switched from 12–28 °C and back every 3.5 d (giving an average temperature across the 100 d of 20 °C, which conceptually we consider analogous to MAT for the field). As for the assays of the field soils, the temperatures of 12–28 °C fall within the favourable range for the metabolic activity of both cold- and warm-adapted microbes[52]. One of each container under each of the thermal regimes received a weekly addition of 0.8 mg carbon (glucose) g soil^{-1} (dry-weight equivalent)—an addition rate that maintains (but does not exceed) substrate supply to soil microbes[54]. The second container received only the deionized water that the glucose solution was made up in. Weekly solution volumes were adjusted to maintain the water-holding capacity at 65% across the 100 d, and the water or glucose solution was slowly pipetted across the full surface of the receiving soils for each application. The design should have provided 132 containers (22 soils × 3 thermal regimes × 2 substrates (glucose or water)), but because of limited soil for some locations, there were 120. Specifically, there was not enough Harvard Forest soil from the forest site to establish any containers, nor enough Andrews, Hubbard Brook or Niwot Ridge forest soils to establish water-only containers. As such, soils from all 11 locations were represented, but for the glucose incubations there were 21 soils represented (that is, no Harvard Forest soil from the forest site) and for the water additions there were 18 soils represented (that is 4 locations had grassland soils only).

At the end of 100 d, the container soils were subsampled for the respiration potential assays as described for the observational gradient part of the study. However, we only assayed the soils at 20 °C and with glucose, given that the patterns in the MAT-gradient part of the study were essentially independent of the assay temperature and substrate. In total, this gave 234 observations (39 soils receiving water or glucose over 100 d × 3 thermal regimes × 2 assay substrates (water or glucose)).

Additional measurements. *Phospholipid fatty acid (PLFA) analysis.* Microbial biomass was estimated as the total PLFA. As with other common methods to measure microbial biomass in soils, PLFA measures provide an estimate of the standing biomass. All of the common methods are correlated with one another, at least to a certain extent[55,56]. As such, following previous work[28,57], we verified the robustness of our inferences using a second biomass method. Specifically, we checked the robustness of the MAT coefficient in the model MAT_1 (Supplementary Table 2) when biomass was instead estimated using a modified version of the substrate-induced respiration approach[28,58]. The MAT coefficient remained significantly negative. However, we used total PLFA in our main analyses because the substrate-induced respiration approach is—like our assays—based on respiration measures, which led to a previous critique that its use might then confound interpretation[57].

On subsampling the field soils received at Yale, or at the end of the 100-d experimental incubations, soils were placed in Whirl-Pak bags at −80 °C until PLFA analysis at the University of Kentucky. The methodology followed that of Findlay and Dobbs[59]. Specifically, samples were homogenized, and 5 g moist soil was extracted in a single-phase, phosphate-buffered dichloromethane solution to remove PLFAs. These lipids were further separated by silicic acid chromatography, and phospholipids were derivatized in an alkaline solution to form fatty acid methyl esters (FAMEs). FAME purification was performed with C18 reverse plasma chromatography. These were then separated and quantified by capillary gas chromatography with a flame ionization detector (a Shimadzu GC-2014) equipped with a Restek Rtx-1 column. FAME peaks were identified and concentrations calculated based on a Supelco 37 Component FAME Mix (Sigma–Aldrich) run as a standard every third sample. The bacterial biomass was calculated as the sum of the following fatty acids: i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, 16:1n9, 16:1n7, cy17:0, 18:1n7, 18:1n5, cy19:0, 14:0, 15:0, 17:0, 18:0, 10Me16, 10Me17, 10Me18 and i17:1n7 (nmol g^{-1} soil). The fungal biomass was the sum of 18:2n6 and 18:1n9 (nmol g^{-1} soil). The total microbial biomass was calculated as the sum of bacterial and fungal PLFAs.

Soil physical and chemical properties. On subsampling the field soils received at Yale, soils were placed at 4 °C in sealed plastic bags until pH and moisture analyses, or air-dried before elemental and texture determinations. Soils were analysed for pH by mixing water to soil in a 1:1 volumetric ratio, and the gravimetric moisture was determined by oven drying to a constant mass at 105 °C. These measurements were also performed for each soil at the end of the 100-d experimental incubations. As with texture, total soil carbon and nitrogen contents were determined for 2012 soils only because these variables change little year to year and soils were sampled from the same spot in 2010, 2011 and 2012. For carbon and nitrogen, air-dried soils were milled to a fine powder, then run on an elemental analyser (Flash 2000; Thermo Fisher Scientific). The texture (that is, sand, silt and clay contents) was measured using a simplified version of the hydrometer method following Grandy et al.[60].

Data and inferential analysis. *Overview of approach.* We built linear regression models structured to represent, and test between, assumptions of the compensation versus enhancement hypotheses of soil respiration potentials (Fig. 1). Multivariate linear regression permitted us to compare estimated effect sizes on the respiration of location MAT or incubation temperature regimes when all other predictor variables were held constant at their mean value. Our approach follows that of Bradford et al.[61] and permits the influence of a single controlling variable to be examined when the response variable of interest is controlled by multiple causative variables. Specifically, we estimated the relative effect size for both MAT and incubation temperature to assess how the MAT or incubation gradient influenced patterns in respiration rates. The regression approach enabled us to hold microbial biomass constant (at the mean value for all observations within each dataset), which is important given the expectation that it has a strong effect on respiration rates and so should be controlled for when assessing patterns of thermal adaptation[35,54]. Note that instead of calculating mass-specific respiration rates (that is, respiration/biomass), we include biomass as a predictor variable to avoid the many pitfalls of analysing ratios[62]. The relative effect size of the thermal regime was then estimated using the slope coefficient for the MAT and/or incubation temperature variables, the slope coefficient for any interactions they were involved in, and the range of observed MAT or experimental incubation temperature values. All other predictor variables (for example, assay substrate or assay temperature) were fixed at a common value for these estimations (for example, the mean for microbial biomass). We generated the coefficients for the predictor variables by fitting linear mixed-effect models (LMMs; see 'Statistical model specifics').

The choice of variables to measure and then include in our statistical models (described next) was based on the approach of Hobbs et al.[63], which rejects model selection on philosophical and operational grounds. Philosophically, we investigated only variables for which the biological mechanism of their influence on soil respiration is firmly established. Operationally, there is subjectivity and a lack of agreement in statistical model selection approaches, with different decisions leading to markedly different conclusions about effect sizes. Instead, coefficients, and hence effect sizes, are generally most robust when all terms are retained, assuming that each is included with a well-established biological foundation and in the absence of strong collinearity among predictors. Nevertheless, there was some collinearity among predictors (Supplementary Tables 3 and 4), and we also fit relevant two-way interactions and detected some outliers in our data. To ensure that our inferences were robust to the precise model specification, we checked the sensitivity of our coefficient estimates to exclusion of observations, exclusion of predictors and exclusion of interactions, as well as a reduced dataset (see Supplementary Tables 2 and 4).

Testing among the competing hypotheses. When biomass and assay temperature are held constant, and substrate is provided in excess of respiratory demand, biochemical theory on thermal adaptation predicts that there should be a negative relationship between respiration rates (at a common biomass) and the temperature of the thermal regime from which organisms are sampled (Fig. 1). As such, a negative coefficient for the MAT variable in the observational gradient dataset, and for the incubation temperature variable in the incubator-gradient dataset, would be consistent with the biochemical adaptation hypothesis. In contrast, a positive coefficient for these predictor variables would be consistent with the idea of an enhancing thermal response, whereas a coefficient of approximately zero would suggest no adaptive response was apparent at the level of the microbial community.

In both the MAT- and incubation-gradient models, we included continuous variables known to exert a strong influence on soil respiration rates: percentage soil carbon, texture, pH and microbial biomass. Soil moisture was standardized given the assay design and so was not included in the models. We did not include cover (that is forest or grassland) as a predictor variable because its effects on respiration are probably mediated by its influence on other predictors, such as pH, microbial biomass and soil carbon. Hence, cover was included in the study design primarily to generate variation in these other predictors. However, we verified that our inferences were robust to its inclusion in the full MAT-gradient model (see Supplementary Table 2). In addition, we used cover in the random effects to account for the hierarchical spatial and temporal design of our study (see below). Furthermore, in addition to the thermal regime variables (that is MAT or incubation gradient), we included the predictor variables that we imposed, and that would be expected to strongly affect respiration rates. For the MAT gradient, these variables were the assay temperature and substrate identity (glucose, oxalic acid or glycine). The assay temperature was represented as a continuous variable and the substrate by binary variables (glucose: 1 or 0; oxalic acid: 1 or 0; where both predictors had a value of 0, glycine was added). For the incubation-gradient dataset, these variables were the incubation substrate (glucose or water added: 1 or 0) and assay substrate (glucose or water added: 1 or 0). In the incubation-gradient model, we also included two-way interactions between the incubation temperature and incubation substrate, and the incubation temperature and MAT. The two-way interactions were included to account for the possibility that adaptive responses to the thermal regime would be greater when there was more substrate available to facilitate microbial growth and hence turnover (that is, when there was glucose versus water addition during the incubations), and if the MAT of the thermal regime from where the soils were collected influenced the plasticity of the microbial taxa (see section 'Incubator gradient' in the main text).

Statistical model specifics. The LMMs were fit in the 'lme4' package for the 'R' statistical programme (version 3.1.3) using the 'lmer' function. Potential respiration rates were expressed as μg $\mathrm{C\text{-}CO_2}$ g dry-weight $\mathrm{soil^{-1}\,h^{-1}}$. For the MAT-gradient models, location, cover type and year were fit as random variables to the LMMs, with the finer-scale variables nested within the broader-scale variables to account for potential spatial and temporal autocorrelation[64,65]. That is, the random error structure accounted for the hierarchical design (year nested within cover type, with cover type nested within location), assuming a common slope but spatially dependent intercept. In the incubator-gradient dataset, given that only 2012 soils were used, the random error structure comprised cover type nested within location.

Before we tested the model structures described above, we tested the data distributions. Soil respiration data are commonly highly skewed to the right, and our data were no exception. Natural-log transformation produced a distribution that fit with model assumptions of normality. There were three influential observations (based on Cook's D) in the MAT-gradient dataset, but they were retained because the coefficient estimates, significance, model r^2 values and model assumptions were essentially insensitive to their inclusion (Supplementary Table 2). In the incubation-gradient dataset, a single observation was influential but its inclusion only slightly affected the coefficients (Supplementary Table 4). For both datasets, second-order terms were fit for both MAT and assay temperature, but were uninformative: coefficients and model fits were unchanged. Soil pH (back-transformed to H^+ ion concentration), percentage soil carbon and texture (as percentage clay) were all included in the models for both datasets. Despite the fact that the square-root variance inflation factors in the final models were <2.0 for the main effects (when interactions were dropped), suggesting acceptably low collinearity, soil carbon and texture were, to a certain extent, correlated with MAT and microbial biomass (Supplementary Tables 3 and 5). However, our inferences as to the effects of thermal regimes were essentially insensitive to their inclusion in the models (Supplementary Tables 2 and 4).

Standardized coefficients were calculated by running the same statistical models but with the observed values of each predictor variable transformed by subtracting the mean for the variable derived from all observations, then dividing by two s.d. for the same distribution[66]. This method of standardizing coefficients permits coefficients to be directly compared for variables measured on different unit scales and when both continuous and binary predictor variables are included in a model (as is the case for our analysis). Furthermore, standardization facilitates the interpretation of main effects in the presence of interactions[66].

Models were fit using restricted maximum likelihood and P values derived using the Satterthwaite approximation for degrees of freedom, given that this approach minimizes the type I error rate and so is considered conservative[67]. We considered coefficients with $P<0.05$ to be significant and coefficients with $P<0.10$ to be marginally significant[68]. We calculated the r^2 values for each model following Nakagawa and Schielzeth[69]. Calculation of r^2 values is common practice when modelling ecosystem processes, and a high value associated with a specific explanatory variable is often associated with that variable having a strong effect size. This reasoning makes no sense within the context of our study[61] because some variables were experimentally controlled (for example, assay substrate and temperature) and hence accurately measured for all soils, whereas the other variables relied on observed variation and measurements that represented—but probably did not fully characterize—the conditions that acted on respiratory activity (for example, the impact of MAT and microbial biomass). The imprecision introduced by such measurements make data more 'noisy', lowering r^2 values, but in the absence of systematic bias will not change the coefficient estimates and hence effect sizes[61,70]. We therefore only report the r^2 value for the overall models, to verify that they had the potential to explain a substantive degree of the variance in respiration rates and to show that the coefficient estimates were robust.

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

Data availability

Data in the support of these findings and the R code for the statistical models are available via the Dryad Digital Repository (https://doi.org/10.5061/dryad.s87008d).

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Author contributions

M.A.B., R.L.M. and N.F. co-designed the study and wrote the application for the grant that funded the work. M.A.B., R.L.M., E.E.O. and T.W.C. collected the data and performed the laboratory work. S.A.W. and M.A.B. carried out the statistical analyses. All authors contributed to interpreting the data and writing the paper.

Competing interests

The authors declare no competing interests.

Additional information

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☒	☐	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection - experimental observations are reported.
Data analysis	All analyses were in the statistical freeware R. The full code for the analyses is available open access along with the data via. the Dryad repository. That is: Data package title: Data from: Cross-biome patterns in soil microbial respiration predictable from evolutionary theory on thermal adaptation Journal: Nature Ecology & Evolution Provisional DOI: doi:10.5061/dryad.s87008d Data files: BradfordetalSTARObsData BradfordetalSTARIncData BradfordetalSTARCODE

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Data in the support of these findings and the R code for the statistical models are available via the Dryad Digital Repository (doi:10.5061/dryad.s87008d).

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Eleven locations were chosen given that six points or more along a continuous axis is considered to give much greater power (with regression designs) than 5 points or less. To generate replication around these eleven points, we then sampled two cover types in each location (22 samples) and repeated the sampling of new soils in each of three years (giving 66 samples). We accounted for the spatial and temporal design using linear mixed effects models, and carried out assays with multiple substrates and at multiple temperatures, giving for the spatial design (MAT-gradient) an n of 594 for a model we expected to include between 5 to 9 independent variables. Similarly, for the incubation design we used multiple temperatures and substrates giving an n of 234 for a similarly low variable number regression model. Our intention was to generate sample sizes for the models that exceeded the standard n value of 30 for every independent variable included (helping to ensure that we had sufficient replication to generate robust coefficient estimates).
Research sample	Soils were collected from 11 locations ranging from Hawaii to Northern Alaska, using a sampling design intended to generate marked variation in abiotic conditions including temperature and precipitation.
Sampling strategy	Please see "Study description" section above
Data collection	Data collection on soils was performed in the laboratories at Yale and the University of Kentucky. At Yale, soil processing and data collection was performed by MAB, TWC and EEO. At University of Kentucky, the lab manager Jim Nelson ran and performed all PLFA analyses, with RLM screening and interpreting data before submitting it to MAB for the statistical analyses.
Timing and spatial scale	For the temporal part of the study , soils were collected in project years 2010, 2011 and 2012, immediately shipped to Yale, and analyzed on receipt. For PLFA analyses, soils were stored frozen and then analyzed in a single extraction and analysis, to ensure data consistency. For the lab incubation at Yale, 2012 soils were incubated for 100 days and then analyses were performed at the end of the incubation in 2013. The PLFAs from these soils were then also frozen and run in a single batch.
Data exclusions	There were 3 outliers in the MAT-gradient and 1 main outlier in the incubation-gradient dataset. Using a sensitivity analysis to assess how influenced by model structure and data inclusion the coefficients were, we ascertained that the coefficients were robust to inclusion or exclusion of these outliers.
Reproducibility	We used 11 locations and 22 soils (2 from each location). We sampled soils in each of three years - in different seasons - and performed the same assays. The intent being to establish whether our spatial effects (i.e. MAT-gradient) were reproducible if repeated in different years (they were). Further, we also tested the same hypotheses with a different approach (i.e. we applied triangulation). That is, following the work on the soils collected in each of three years across the spatial climate gradient, we then subjected the soils collected in the third year to laboratory incubations where incubation temperature was directly manipulated. That is, we tested whether the correlative spatial pattern was causative by using a controlled experimental design.
Randomization	We used a stratified regression design, which is more powerful than a simply random design given that we deliberately exploited natural variation to generate a broad distribution in the values of our predictor variables. Specifically, we chose locations along a broad climate gradient on the basis that there was a long-term research site present. As such, we systematically chose locations along a climate gradient, but not in a manner that controlled potential influential variables other than climate. Then at each location we asked a site manager to choose a forest and a grassland site, separating sample (i.e. soil) selection from those working in the lab who processed those field soils on receipt. As such, our sample collection design meant that we expected high variation in controls (e.g. soil texture and carbon content) not under direct investigation for the hypotheses, thereby improving the reliability and generality of the climate coefficients.
Blinding	Investigators were part blinded. Specifically, those processing the field soils on receipt from the field were not, but instead a competing hypothesis framework was employed, where the directionality of effects were opposite for different hypotheses,

separating the investigators from potential bias toward a specific directional effect. In addition, a key variable for assessment of the hypotheses (i.e. PLFA microbial biomass) was measured at a different institution where the investigators had only a sample number and hence no knowledge of where the sample came from. As such, some of the essential variables were measured "blind", ensuring that the eventual dataset contained some variables where the measurements were performed entirely blind.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	To capture temporal variation in microbial community attributes, three replicate samples were collected from each sampling location (see next) over three years (2010, 2011 and 2012), with sampling date varying between seasons (spring, summer and fall, respectively). For high latitude and elevation locations, snow and ice precluded safe sampling of soils in winter, so soils were collected for return to the lab at Yale only when sites were accessible by 4-WD (as opposed to snow mobile).
Location	Locations included 10 U.S. Long Term Ecological Research stations (Andrews Experimental Forest, Oregon 44.21°N, -122.26°E (AND), Boreal Ecology Cooperative Research Unit, Alaska 64.85°N, -147.84°E (BNZ), Cedar Creek Ecosystem Science Reserve, Minnesota 45.40°N, -93.20°E (CDR), Coweeta LTER, North Carolina 35°N, -83.5°E (CWT), Hubbard Brook Experimental Forest, New Hampshire 43.94°N, -71.75°E (HBR), Harvard Forest, Massachusetts 42.53°N, -72.19°E (HFR), Kellogg Biological Station, Michigan, 42.4°N, -85.4°E (KBS), Konza Prairie Biological Station, Kansas 39.09°N, -96.57°E (KNZ), Luquillo LTER, Puerto Rico 18.3°N, -65.8°E (LUQ), Niwot Ridge LTER, Colorado 39.99°N, -105.37°E (NWT)), and the Hawaii Experimental Tropical Forest, Institute of Pacific Islands Forestry, Hawaii 19.81°N, -155.26°E (HAW). Each location included paired sites that had natural forest versus grassland cover, where the grasslands were either natural or maintained by low-intensity (annual mowing) management
Access and import/export	Permission and/or permits were sought from site managers, or broader regulatory bodies where relevant (e.g. the US Forest Service in Hawaii) before soil samples were taken. Then, for all locations (e.g. Alaska, Puerto Rico, Hawaii) that required soil import licenses, a US Department of Agriculture soil import permit was obtained and maintained by M.A. Bradford, including all relevant stipulations for the safe handling and disposal of foreign soils.
Disturbance	Three 0.16 m2 quadrats of soil down to 10 cm depth were taken at each site, in each sampling year. Hence, there was minimal disturbance to the locations where the work was conducted.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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