Community-level respiration of prokaryotic microbes may rise with global warming

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Understanding how the metabolic rates of prokaryotes respond to temperature is fundamental to our understanding of how ecosystem functioning will be altered by climate change, as these micro-organisms are major contributors to global carbon efflux. Ecological metabolic theory suggests that species living at higher temperatures evolve higher growth rates than those in cooler niches due to thermodynamic constraints. Here, using a global prokaryotic dataset, we find that maximal growth rate at thermal optimum increases with temperature for mesophiles (temperature optima \( \leq 45^\circ \text{C} \)), but not thermophiles (\( \geq 45^\circ \text{C} \)). Furthermore, short-term (within-day) thermal responses of prokaryotic metabolic rates are typically more sensitive to warming than those of eukaryotes. Because climatic warming will mostly impact ecosystems in the mesophilic temperature range, we conclude that as microbial communities adapt to higher temperatures, their metabolic rates and therefore, biomass-specific \( \text{CO}_2 \) production, will inevitably rise. Using a mathematical model, we illustrate the potential global impacts of these findings.

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A general understanding of how individual organisms respond to changing environmental temperature is necessary for predicting how populations, communities and ecosystems will respond to a changing climate. Because fundamental physiological rates of ectotherms are directly affected by environmental temperature, climatic warming may be expected to lead to ectotherm communities with higher metabolic rates on average. How environmental temperature drives metabolic rates of prokaryotes (bacteria and archaea) is of particular importance because they are globally ubiquitous, estimated to comprise up to half of the planet’s global biomass, and are the end users of the majority of net primary production. Therefore, climate-driven changes in prokaryotic metabolic rates are expected to significantly alter ecosystem productivity, nutrient cycling and carbon flux. Indeed, increased carbon efflux (CO2 emission) has been observed in experimental measures of soil CO2 loss to warming, as well as the responses of other microbial metabolic processes to increased temperature such as methanogenesis.

However, whether the short-term (timescales of minutes to days) thermal responses of prokaryotes can be compensated by acclimation (physiological phenotypic plasticity) or longer-term (timescales of months, years or longer) evolutionary adaptation is currently unclear. The most recent study to investigate this idea concluded that both short- and long-term responses of ecosystem-level heterotrophic respiration were similar. However, this study quantified short-term responses by aggregating day-level carbon fluxes across sites, and did not have data on the direct respiratory contributions of prokaryotes per se.

The short term, or instantaneous response of metabolic traits of individual organisms to changing temperature (the intra-specific thermal response) is typically unimodal, with the thermal performance curve (TPC) of the trait increasing with temperature up to a peak value (TPk), before decreasing as high temperature becomes detrimental to metabolic or cellular processes. The Tp for maximal population growth rate (rmax, a direct measure of fitness, often used as a proxy for metabolic rate), sometimes termed the thermal optimum, is expected to correspond to the typical thermal environment in which the organism’s population has evolved (the long-term response). The Hotter-is-Better (HiB) hypothesis posits that trait performance at TPk (henceforth denoted by Ppk) is also expected to increase inevitably in a similar manner to the short-term intra-specific response, because of the positive temperature-dependence of rate-limiting enzymes operating at their thermal optimum (a thermodynamic constraint), i.e., Ppk increases with TPk. This hypothesis essentially links the short-term TPC of trait performance to the longer-term performance mediated by evolution. The HiB hypothesis is also implicit in the universal temperature-dependence concept of the Metabolic Theory of Ecology (MTE). However, whether the HiB hypothesis holds across organisms and environments is a question that is still debated. Deviations from a HiB pattern would indicate that either thermodynamic constraints do not exist, or are compensated for by other mechanisms. In particular, an alternative hypothesis is that natural selection acts to override thermodynamic constraints, allowing peak trait performance and fitness to be, on average, equalised across different adaptation temperatures. Intermediate scenarios are also possible, where adaptation of optimal trait performance or fitness is only partially constrained thermodynamically. Moreover, trade-offs between protein rigidity and activity at high temperatures may in fact cause hot-adapted organisms to display depressed maximal fitness (Ppk decreasing with TPk). Indeed, the existence of thermal constraints leading to an upper limit of prokaryotic growth rates has been shown recently. A comparison of short- and long-term (HiB) responses of prokaryotic populations has neither been made nor the potential effects of responses at different timescales on ecosystem fluxes studied.

Under MTE, the global thermodynamic constraint is expected to centre n ~0.65 eV for heterotrophs. This is the long-term, inter-specific (across-species) thermal sensitivity around which species are expected to evolve, which we term E0 (see Fig. 1). Mean intra-specific thermal sensitivities (i.e., acute organism or species-level responses, here termed Ei) have been found to be very similar to this value, although the distribution is right-skewed with a median value of ~0.55 eV. However, these values are derived from data sets which have largely or entirely excluded prokaryotes. Previous work on sub-groups of prokaryotes—cyanobacteria and methanogenic archaea—has indicated comparatively high thermal sensitivities which deviate from the eukaryote-derived MTE expectations. Whether this deviation from MTE is a property of prokaryotes in general has never been thoroughly tested. Given the ubiquity of prokaryotes, this is a matter of particular importance for theories applying MTE to whole ecosystems.

Here, we build and analyse a global data set of TPCs in bacteria and archaea to quantify general patterns in both the short-term

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**Fig. 1** Three alternative hypotheses for short- vs. long-term thermal responses. **a** Hotter-is-Better: organisms adapt around a global, inter-specific, thermal constraint (black line, Boltzmann–Arrhenius fitted to intra-specific curve peaks), such that the average intra-specific (short-term) activation energy (Ei) is statistically indistinguishable from the inter-specific (long-term) activation energy of the group of organisms (E0), and both are greater than zero. See the Methods section for more details on the definition and estimation of E0 and Ei, and the statistical methods used to differentiate between them. Note that each intra-specific TPC represents the short-term thermal response of each organism’s population. Inset panel illustrates how this would look in an Arrhenius plot. **b** Equalisation of fitness: selection overrides thermodynamic constraints, such that trait performance at Tpk is on average the same (Ei = 0). Alternatively, the same effect of Ei = 0 may occur due to or thermodynamic constraints on enzymes in fact restricting metabolic rate (and therefore fitness) at higher temperatures. **c** Weak biochemical adaptation: an intermediate scenario where Ei > 0, but significantly less than E0. Panel **c** also illustrates the the Sharpe–Schoolfield TPC model parameters (Eq. (1), Methods).
Adaptation to culture conditions. First, we compared each strain’s thermal optimum (Peak growth temperature, $T_{pk}$) with the laboratory growth temperature ($T_{lab}$) to determine whether the TPCs reflect adaptation to growth temperature. For both bacteria and archaea, we find a strong and significant ($p < 0.00001$, linear regression) association between $T_{pk}$ and $T_{lab}$ (Fig. 2; bacteria $R^2 = 0.92$, archaea $R^2 = 0.96$), indicating that these strains are generally well-adapted to their culturing temperature. In both archaea and bacteria data subsets, the $T_{pk}$ vs $T_{lab}$ line deviates significantly from a slope of 1 (bacteria slope = 0.88, 95% CI ± 0.04, $n = 165$; archaea slope = 0.92, 95% CI ± 0.05, $n = 58$) because $T_{pk}$ tends to fall below culturing temperature at high temperatures (Fig. 2).

Comparison of short- and long-term thermal responses. Next, we tested the HiB hypothesis by comparing the short-term (intraspecific) and long-term (inter-specific) thermal responses (see Fig. 1; Methods). If there is a universal thermodynamic constraint, peak fitness ($P_{pk}; r_{max}$ at $T_{pk}$) across strains should increase with each strain’s respective $T_{pk}$ (parameter $E_L$; Fig. 1) at the same rate as $r_{max}$ would increase with temperature (parameter $E_S$), on average, within single a strain’s TPC. Our analysis relies on $P_{pk}$-$T_{pk}$ pairs across strains because data within strains are largely lacking, and the HiB pattern is expected to apply across strains within monophyletic taxonomic groups (such as archaea and bacteria). Analysing this relationship across 416 bacterial and 82 archaeal strains, we find that hotter is indeed better (HiB holds) across mesophilic bacteria ($E_S$ and $E_L$ are >0, and there is significant overlap of their 95% CIs; Fig. 3 and Table 1). However, this result does not extend to thermophiles, where instead fitness is on average invariant with respect to temperature. The outcome is less clear for mesophilic archaea as whilst $E_L > 0$, there is <50% overlap of $E_L$ and $E_S$ CIs.

Variation in thermal sensitivity. We find mean thermal sensitivity ($E_S$) for bacteria = 0.88 eV and $E_S$ for archaea = 0.95 eV. These are significantly greater than the 0.65 eV global inter-specific constraint expected under MTE and previously observed mean intra-specific values (calculated primarily from eukaryote data). The data are right-skewed (as observed by Dell et al.), but even after accounting for this skew by taking the median, activation energy still falls significantly >0.65 eV (bacteria median = 0.84 eV, archaea median = 0.80 eV; Supplementary Fig. 1). Furthermore, we see a consistent pattern of median thermal sensitivity >0.65 eV throughout the lower taxonomic groupings (Fig. 4a).
To further understand these findings, we investigated differences between groups of strains sharing functional traits, such as their pathogenicity and their main energy-generating metabolic pathway (Fig. 4b). Again we find mean and median thermal sensitivity \( >0.65 \text{ eV} \) in the majority of functional groups, suggesting that this high \( E \) is a trait generally conserved across prokaryotic organisms.

Here, we have focused on the TPCs (and activation energies) of population growth rate. However, to understand the implications of the short- and long-term thermal responses of prokaryotes for ecosystem functioning, it is necessary to test whether these reflect the activation energies of underlying metabolic flux rates. To investigate this, we assembled another thermal response data set (Methods) for metabolic fluxes recorded in prokaryotes (mainly anaerobic respiratory fluxes e.g., sulfur oxidation) and asked whether, on average, thermal sensitivity is equivalent for growth rate and metabolic fluxes. We find that average intra-specific \( E \) values for growth rate TPCs were similar to, and statistically indistinguishable from the mean activation energy for metabolic fluxes (bacteria flux \( E_S = 0.82 \text{ eV} \); archaea flux \( E_S = 1.01 \text{ eV} \); Fig. 5a, see Supplementary Table 1 for a list of fluxes analysed). Furthermore, we compared both the prokaryotic growth rate and flux \( E_S \) distributions, with a new data set (Methods) on thermal sensitivity of respiration in autotrophic eukaryotes. The results (Fig. 5d) further support a lower thermal sensitivity of short-term responses for eukaryotes than prokaryotes (\( E_S = 0.67 \text{ eV} \) with CI = 0.63–0.72, median = 0.57).

| Kingdom | Thermal niche | \( n \) | \( E_S \) | \( \bar{E}_S \) | \( E_L > 0 \) | \( E_L \approx E_S \) | HiB |
|---------|---------------|-----|--------|--------|----------|----------------|------|
| Bacteria | Mesophile | 264 | 0.98 (0.70–1.25) | 0.87 (0.82–0.93) | TRUE | TRUE | TRUE |
| Bacteria | Thermophile | 114 | −0.07 (−0.26–0.12) | 0.85 (0.78–0.92) | FALSE | FALSE | FALSE |
| Archaea | Mesophile | 21 | 0.97 (0.69–2.26) | 0.60 (0.50–0.70) | TRUE | FALSE | FALSE |
| Archaea | Thermophile | 60 | −0.09 (−0.21–0.17) | 1.11 (0.95–1.28) | FALSE | FALSE | FALSE |

Estimated mean intra-specific (short-term, \( \bar{E}_c \)) and inter-specific (long-term, \( \bar{E}_S \)) thermal sensitivities (95% CI ranges in parentheses) for bacteria and archaea split by thermal niche (also see Fig. 3). As the same data are used to calculate \( \bar{E}_S \) and \( \bar{E}_L \), the number of data points (\( n \)) applies to both. The last column indicates whether or not the HiB hypothesis is supported. The HiB result for archaea may be ambiguous, as described in the Results and Discussion section.
Fig. 4 Variation in thermal sensitivity amongst prokaryotic groups. Comparison of intra-specific population growth rate activation energies ($E_S$) across taxonomic levels (a) and functional trait groupings (b) for archaea (orange) and bacteria (blue). Points and error bars represent weighted mean and 95% CIs of $E_S$ for each group. Groups shown are those with at least five data points, the number in brackets indicates the number of data points from which $E_S$ was calculated for each grouping. The dotted line marks 0.65 eV, the mean $E_S$ previously reported within the MTE framework. Grey triangles mark the median $E_S$ for each group. Source data are provided as a source data file.
Potential ecosystem-level impacts. Our results suggest that higher sensitivity of both short- (higher intra-specific activation energies) and long-term (higher inter-specific activation energies — a HiB constraint) thermal responses in mesophilic prokaryotes may have profound implications for responses of ecosystem fluxes to climatic warming. To illustrate this, we built a simple mathematical model to calculate the potential change in the current ecosystem carbon fluxes without evolution or acclimation in response to, and each sit above 0.65 eV (dotted line) for both archaea and bacteria (bacteria growth $E_a = 0.88$ eV, $n = 416$; bacteria flux $E_{fl} = 0.82$ eV, $n = 28$; archaea growth $E_a = 0.95$ eV, $n = 82$; archaea flux $E_{fl} = 1.01$ eV, $n = 20$). b Density plot of flux $E_{fl}$ values for archaea and bacteria. c Density plot of growth rate $E_a$ values for archaea and bacteria. d Density plot of $E_c$ values for respiration rate TPCs in autotrophic eukaryotes, showing comparatively lower mean thermal sensitivity than those of the distributions for prokaryotes ($E_c = 0.67$ eV, $n = 381$). Source data are provided as a source data file.

**Fig. 5** Differences in short-term thermal sensitivity across taxonomic groups. a Comparison of the intra-specific thermal sensitivity ($E_a$) for growth (green triangles) and metabolic fluxes (purple circles). Error bars represent 95% CIs. CIs for growth rate thermal sensitivity fall within those for metabolic fluxes, relative to ecosystems with all components having the same (0.65 eV) average activation energy. The scale of emergent activation energies and each sit above 0.65 eV (dotted line) for both archaea and bacteria (bacteria growth $E_a = 0.88$ eV, $n = 416$; bacteria flux $E_{fl} = 0.82$ eV, $n = 28$; archaea growth $E_a = 0.95$ eV, $n = 82$; archaea flux $E_{fl} = 1.01$ eV, $n = 20$). b Density plot of flux $E_{fl}$ values for archaea and bacteria. c Density plot of growth rate $E_a$ values for archaea and bacteria. d Density plot of $E_c$ values for respiration rate TPCs in autotrophic eukaryotes, showing comparatively lower mean thermal sensitivity than those of the distributions for prokaryotes ($E_c = 0.67$ eV, $n = 381$). Source data are provided as a source data file.

**Fig. 6** Potential changes in ecosystem carbon flux due to differences in $E$ between taxa. a Heatmap of % short-term increase in flux with 10°C temperature increase (as may occur during daily fluctuations) of model ecosystems with bacteria having a different activation energy on average than eukaryotes, relative to ecosystems with all components having the same (0.65 eV) average activation energy. The flux change is shown over a range of ecosystem biomass compositions in terms of heterotrophs vs. autotrophs and bacterial proportion of the heterotrophs. The scale of emergent activation energies and $Q_{10}$ values for the ecosystems with amplified flux are also shown. b Similar to a, but for long-term flux increase under a 4°C warming scenario due to climate change. Values for the short- and long-term thermal sensitivity of bacterial thermal responses used in these calculations are our estimated $E_a$ and $E_c$, respectively, for mesophilic bacteria (Table 1). The mathematical model is described in the Methods section.

Potential ecosystem-level impacts. Our results suggest that higher sensitivity of both short- (higher intra-specific activation energies) and long-term (higher inter-specific activation energies — a HiB constraint) thermal responses in mesophilic prokaryotes may have profound implications for responses of ecosystem fluxes to climatic warming. To illustrate this, we built a simple mathematical model to calculate the potential change in the relative contribution of heterotrophs to ecosystem carbon ef

$\text{short-term relative flux increase} = \frac{\text{flux due to warming on current ecosystem carbon fluxes}}{\text{flux without warming}}$
fluctuations that organisms may typically experience\textsuperscript{39}). When we consider the effects of longer-term warming (such as through gradual global climate change) on the prokaryotic subcommunity using the inter-specific (evolutionary) thermal sensitivity, \( E_k \) (0.98 eV), we find that modelled ecosystem flux increases by \(~\sim\%) 5\% with 4 °C warming (again with 50% heterotrophs of which 50% are bacteria) compared with a baseline where the long-term thermal sensitivity is 0.65 eV for all components of the ecosystem. The actual increase in flux may indeed be higher, but is dependent upon the ratio of prokaryotic biomass to eukaryotic biomass within the ecosystem, a quantity for which estimates vary widely\textsuperscript{8,13,40}. In our model, each percentage point increase in prokaryotic biomass within the heterotrophic component causes a flux increase of 0.05—0.15%, depending on the quantity of prokaryotic biomass already in the system.

**Discussion**

Our results demonstrate that mean thermal sensitivities for both bacteria and archaea fall significantly >0.65 eV (Fig. 5, bacteria \( E_b = 0.88 \text{ eV}; \) archaea \( E_a = 0.95 \text{ eV}), suggesting that prokaryotes operate under different thermal constraints to more complex eukaryotes. Indeed, we also present a new data set of autotrophic eukaryotes which show thermal sensitivity consistent with the 0.65 eV MTE generalisation. These findings of high (relative to eukaryotes) intra-specific thermal sensitivities in prokaryotes are consistent with previous work on methanogenic archaea\textsuperscript{17} and cyanobacteria\textsuperscript{32}, but have never been demonstrated across all major lineages of prokaryotes. In particular, Yvon-Durocher et al.\textsuperscript{17} have argued that the high \( E_S \) of methanogens are expected to translate into an increased ecosystem-level methane production at longer temporal and spatial scales. Our results suggest how these two different scales of response may be related—the short-term responses may be coupled with a HiB constraint which results in the flux at thermal optimum also increasing with (longer-term) adaptation. Moreover, this coupling across timescales is expected not just in methanogens but across most major mesophilic prokaryotes, including those involved in aerobic respiration.

The data do not allow us to determine the timescale of the adaptation resulting in the HiB pattern, but numerous previous studies have shown rapid adaptation of prokaryotes to experimental warming conditions\textsuperscript{13—15}. Due to this adaptive capacity, as global temperatures rise prokaryotes would be expected to respond to new environmental temperatures rapidly, in effect pushing them further along the global (inter-specific) HiB curve (Fig. 1a). Alternatively, species sorting may occur such that prokaryotes inherently better-adapted to higher temperatures take advantage of temperature increases. This would have the same overall effect, because these prokaryotes would also effectively be further up the inter-specific temperature response curve (Fig. 3). In either case, under HiB, we can expect global warming to result in prokaryotic communities with higher metabolic rates on average. Whilst temperature is an important constraint, metabolic rates are also mediated by resource availability\textsuperscript{48}. This can be seen in studies which show that carbon availability and use efficiency, community composition, and changes in microbial abundance all play roles in soil carbon loss under warming\textsuperscript{46}. Furthermore, moisture is expected to play a significant role in microbial CO\(_2\) eflux from soils\textsuperscript{48}, a factor which is itself likely to change with global warming. Thus overall, our results suggest that further production of greenhouse gases from the prokaryotic component of ecosystems is likely to increase at a greater rate than by component eukaryotic organisms (Fig. 6), albeit mediated by other biotic and abiotic factors. Our data comprising TPCs of exponential growth rates under weak nutrient limitation may not be specific to all natural systems, however, recent work shows that repeated assembly dynamics following perturbations are key to understanding ecosystem functioning\textsuperscript{37—39}. Therefore, our empirical results and our model may be interpreted as being especially relevant to ecosystem respiration under intermittent perturbations, as would be expected in natural, open ecosystems. This also means that future work should focus on quantifying the TPCs of prokaryotic populations under different levels of nutrient limitation.

While in general, we see a tendency towards high thermal sensitivity (\( E_S \)) in prokaryotes, there are taxonomic sub-groups within our data set for which this is not the case (Fig. 4). For example, \( E_S \) for mesophilic archaea as a whole does not deviate significantly from the MTE 0.65 eV average (Table 1). This is largely because this subgroup is primarily comprised strains from *Halobacteria*, which have thermal sensitivities significantly <0.65 eV (Halobacteria \( E_S = 0.46; CI = 0.38–0.58; \) Fig. 4a). This is likely a result of their ecologically extreme niche imposing unusual constraints on their physiology (these archaea have been isolated only from high salinity lakes). In addition, despite mesophilic archaea displaying a clear long-term increase in rate with temperature, we found only a very small overlap in CIs between \( E_S \) and \( E_k \) for these strains which may not be enough to infer equivalence\textsuperscript{30}, although mapping \( p \)-values onto % overlap of bootstrapped CIs is not a trivial task\textsuperscript{51}. One possibility is that mesophilic archaea follow an adaptive long-term response which is not coupled to their short-term thermal sensitivity, however, this result may also be due to shortcomings of our data set for these prokaryotes. In general, it may be harder to make generalisations about short- and long-term thermal responses across taxa for archaea as a whole, because these prokaryotes are partly typified by their propensity to adapt to different types of extreme environments\textsuperscript{52}. That is not to say that archaea do not contribute significantly to ecosystem functioning in benign environments, however (as demonstrated through our exhaustive data collection), the thermal performance of these organisms has been generally less well-characterised. More work is necessary in order to fully understand the coupling of short- and long-term thermal responses in archaea.

We also note that while the majority of heterotrophic bacteria in our data set respire aerobically, there are a number of anaerobic strains, the majority of which were grown under various fermentation conditions. However, when we consider these groups of bacteria separately, we see no significant difference between their mean intra-specific thermal sensitivities (aerobic \( E_b = 0.86; CI = 0.81–0.91, n = 221; \) fermentation \( E_b = 0.86, CI = 0.77–0.96, n = 62 \)). Ultimately, despite all this variation, we find that both, the short-term (intra-specific) and long-term (HiB hypothesis) amplification of metabolic rate holds true for the mesophiles (\( \leq 45^\circ C \)), temperatures which encompass most of the biomass on the planet.

Following previous approaches applying MTE to ecosystem functioning, such as Enquist et al.\textsuperscript{35} and Schramski et al.\textsuperscript{35}, our model assumes that biomass remains constant with temperature, and therefore does not vary at the timescale of the calculation. That is, it assumes that only biomass-specific CO\(_2\) efflux changes with temperature. However, realised changes to net ecosystem flux will also depend on changes in the biomass of different ecosystem components with temperature. How warming is likely to alter the overall abundances of ecosystem constituents, from microbes to plants and animals, is currently not well-understood. Future work to establish the effects of warming on population dynamics is therefore needed in order to fully understand the implications of our findings. Also, for simplicity, when parameterising our ecosystem model we used \( E_S \) and \( E_G \) calculated from all of the mesophilic bacteria in the data set, as we expect a huge amount of variation in the taxa present at the ecosystem scale. Future work
can build on this by considering more specific situations where certain prokaryotes dominate in certain environments based on global biogeographic studies. However, the majority of microbial taxa are known only from sequencing data, for example Acidobacteria are thought to make up in the region of 50% of soil biodiversity, yet very few strains have actually been cultured and therefore have TPCs available. Thus in practice, it may not be feasible to accurately parameterise this sort of model based on patterns of microbial biogeography and therefore, using a global average is appropriate. Climate warming may also be accompanied by more extreme fluctuations which may be large enough to push organisms beyond their operational temperature range (OTR), within which the Boltzmann-Arrhenius model is appropriate. If these fluctuations were frequent enough to have strong effects on the community, using the Sharpe–Schofield model as an alternative to Boltzmann–Arrhenius may be an interesting approach to predicting the effects of extreme warming events at the ecosystem level. However, parameterising this would again require the data on the specific species present in a given ecosystem and their individual TPCs, which is outside of the scope of our current study.

We have focused on the ecosystem consequences in the face of global change, but our results also have implications for understanding prokaryotic physiology. We are not aware of any previous work showing that prokaryotes differ systematically in their thermal sensitivity from eukaryotes. Therefore, further studies are needed to explore the mechanistic basis of this difference, and may reveal a major physiological transition mediated by an increase in cellular complexity as well as multi-cellularity in eukaryotes. Also, our comparisons for growth rate and metabolic flux $E$ are simply averages across strains. Direct within-strain comparisons of growth rate (a slower thermal response) and the more instantaneous metabolic flux TPCs will be needed in order to fully understand the coupling of positive intra-specific and inter-specific thermal responses we have found here.

Our results are also important for understanding differences in thermal physiology between taxa, given our findings of HIB for mesopholic bacteria, but invariant inter-specific fitness with temperature for thermophiles (Fig. 3). Thermophiles have evolved specific adaptations to extreme temperature stress, such as mechanisms to cope with increased membrane permeability at high temperatures and thus adaptation to such niches may incur a fitness cost to thermophiles as seen in our results. This result is in concurrence with an investigation of the maximum growth rates of life on Earth, which found increases in microbial growth up to a peak before an attenuation of growth rates in warmer adapted organisms. Our results also suggest a limit to thermal adaptation as we find that strains cultured at very high temperature tend to display lower than expected thermal optima (Fig. 2). These results have implications for theoretical models of the thermal limits for life.

In summary, our results significantly deviate from current assumptions about the thermal sensitivity of heterotrophic respiration in ecosystems, and should be considered in ongoing efforts to model the impacts of climate change on ecosystem fluxes. More work needs to be undertaken to address whether intra- (short-term) and inter- (long-term)-specific thermal responses are similarly conserved across other groups of organisms that are important for ecosystem function, such as fungi and insects in terrestrial, and phytoplankton and zooplankton in marine ecosystems.

Candidate TPC data were identified via manual searches of google scholar and pubmed databases. Search terms, such as ‘bacteria’, ‘bacterium’, ‘archaea’, ‘archaeon’, ‘temperature’, ‘temperature response’, ‘thermal response’, ‘growth’, ‘adaptation’, were used to find papers with response data particularly for growth rates. Later searches included terms such as ‘characterisation’, ‘isolation’, ‘novel’, ‘gen’, ‘sp’. as it became clear that thermal responses were often tested in publications describing newly isolated species/strains. When presented as a response curve figure, Plot Digitizer software was used to extract data points, including error bounds when reported. The Taxize R package was used to standardise taxonomy of extracted data to the NCBI database. The papers were also manually searched to collect data on growth conditions as well as other metadata where possible (historical lab growth conditions, sampling location). In instances where doubling rates or doubling times were reported, we used Doubling time $t_d = \ln(2)/\mu$ to calculate the maximum specific growth rate. Raw data were normalised to rates per second and degrees Celsius for use in modelling comparisons. In total, we collected 542 prokaryotic growth rate TPCs.

Although we primarily collected growth rate data as a measure of fitness in order to test HIB, we additionally collected 54 TPC covering various metabolic fluxes for comparison to growth rate TPCs. Our complete prokaryote data set comprises 596 TPCs from 482 unique prokaryote strains across 239 published studies.

Finally, we compiled thermal response data for respiration rates in autotrophs from the literature using the same methods for digitisation and data collation as for the prokaryote data set. In total, this autotroph data set comprises 381 respiration rate TPCs from 140 unique autotroph species (98 vascular plants, 4 mosses, 11 green algae, 22 red algae and 5 brown algae species).

**Biological replicates and pseudoreplicates.** We use prokaryotic strains to designate separate prokaryotic taxonomic entities with potentially differing TPCs.

If a single study provided multiple TPCs from the same prokaryotic strain under the same conditions, these were considered pseudoreplicates. In these cases, all data were collected and a single Sharpe–Schofield fit (see Model fitting) was computed for the combined set of points, yielding a single set of TPC parameters. Where multiple TPCs were provided for the same strain under different growth conditions, these were considered as separate biological replicates, however in practice this is only the case for two replicates in each of two different strains in our data set. Where TPCs were obtained from prokaryotes identified only to the species level (or higher), these were considered biological replicates as likely representing different strains of those species.

The eukaryotic autotroph data set did contain organisms identified as the same species grown under similar conditions. Given the slower generation times of eukaryotes and thus slower species divergence, we do not consider these as representative of different strains, as we do for prokaryotes, and thus consider them pseudoreplicates. To remove pseudoreplicates, we compared Sharpe–Schofield model fits (see Model fitting) from each replicate and chose the one with the best fit as most representative of thermal performance for that species, discarding data with worse fits.

**Model fitting.** To each TPC in the data set, we fitted a modified Shear–Schofield model, Eq. (1):

$$B(T) = \frac{B_0}{1 + e^{\frac{-E_k}{T - T_0}}}$$

Here, $T$ is temperature in Kelvin (K), $B$ is a biological rate, $B_0$ is a temperature-independent metabolic rate constant approximated at some (low) reference temperature $T_{ref}$, $E_k$ is the activation energy in electron volts (eV) (a measure of thermal sensitivity), $k$ is the Boltzmann constant ($8.617 \times 10^{-5}$ eV K$^{-1}$), $T_{ref}$ is the the temperature where the rate peaks, and $E_k$ is the deactivation energy, which determines the rate of decline in the biological rate beyond $T_{ref}$. We fit this model to individual TPCs and solve for $T = T_{ref}$ to calculate the population growth rate at $T_{ref}$ ($\mu_{max}$) for each strain. Note that this has been extracted from the model presented in the original paper, to include $T_{ref}$ as an explicit parameter.

Each strain’s TPC has a potentially different $T_{ref}$ and $\mu_{max}$. Compiling these values across strains yields an inter-specific thermal response curve (Fig. 1a). TPCs without a peak are thus excluded from this analysis. We fit the Boltzmann–Arrhenius equation (Eq. (2), essentially the numerator in Eq. (1)) to these peak values to calculate inter-specific activation energy. To account for uncertainty in the original Sharpe–Schofield model fits to the inter-specific curves, we fitted Boltzmann–Arrhenius using a weighted regression (see accounting for uncertainty).

$$B = B_0 e^{\frac{-E}{T_{ref}}}$$

All Boltzmann–Arrhenius and Sharpe–Schofield model fitting were performed in Python with the NumPy package, using the Levenberg–Marquardt ordinary non-linear least-squares regression method.

**Methods**

**Data collection.** We compiled a data set of published prokaryotic thermal performance curves (TPCs) by searching the literature for papers with these data and using digitisation software to collect the thermal performance point estimates.
Comparing short- and long-term thermal responses. We determined whether HBi by testing whether the activation energies from intra- (short-term) and inter-
specific (long-term) temperature experiments (see (statistically) differential 
activation energies, we used bootstrapping to generate confidence intervals (CIs) around 
the mean in each case. To provide bootstrapped CIs for Ei from the modified 
Boltzmann–Arrhenius fits, the data were re-sampled with replacement 1000 times, 
with this number based on the default number of times and the CIs defined as the 2.5th and 
97.5th percentiles of E values extracted from these fits. E was calculated as the 
weighted mean Ei for the group (see methods), and CIs were taken as the 2.5th and 
97.5th percentiles from the resultant distribution of E values from a bootstrap of 
the weighted mean.

We then determined whether the data were consistent with either of the three 
hypotheses (Fig. 1) by comparing the overlap of confidence intervals of the relevant 
E estimates. First, we tested whether Ei was greater than zero (null hypothesis that 
the CI includes zero). Second, we tested whether Ei was greater than zero (null 
hyothesis that the CI includes zero). Finally, if both Ei and E were positive, we 
tested whether they were significantly different to each other (null hypothesis, that 
the CIs for Ei and E do not overlap). Under a HBi scenario, Ei will increase with 
T across strains, and according to MTE this is best quantified by a 
Boltzmann–Arrhenius model. As a result, the Boltzmann–Arrhenius activation 
energies from the intra- and inter-specific responses should be positive and any 
differences between them not statistically significant, i.e., the confidence intervals of 
E and Ei should overlap each other, but not zero. Alternatively, if growth rates are 
not constrained by thermodynamics and Fa does not increase with temperature, 
then Ei will not be Ei includes zero), and HBi is included. 

Finally, in scenarios where thermodynamic constraints may be partially evident but 
somewhat overcome by adaptation, Ei and Ei will both be positive, but with Ei 
being significantly greater than Ei (i.e., Ei > Ei > 0).

Accounting for statistical uncertainty. Weighted means were used to account for 
uncertainty in Sharpe–Schofield point estimates when calculating Ei and when fitting 
the Boltzmann–Arrhenius curve during performance analysis. Sharpe–Schofield fits, 
we extracted the E and Fa point estimates as well as the 
coefficient matrix. We then sampled 1000 times from a bivariate distribution accounting 
for the covariance, producing 1000 model parameter combinations. We 
used these parameters to generate 1000 different Sharpe–Schofield curves, 
providing a distribution of E and Fa from which we took the standard deviations (SD2 
and SD1) as a measure of uncertainty. In some cases theSharpe–Schofield fit did 
not produce a covariance matrix and these fits were excluded from further analysis. 
When combining E values across strains to calculate Ei, we took a 
arithmetical mean of E to account for uncertainty in the original fits, where 
Weight = 1/(SD2 + 1). Similarly, when fitting Boltzmann–Arrhenius, we applied a 
weighting to Fa where Weight = 1/(SD + 1).

Applying these weightings does not alter the main results we obtain from this 
study in terms of whether the HBi hypothesis is accepted or not for different 
groups, however, we felt that it was important to acknowledge and account for 
error in the underlying Schofield fits so that our results were not skewed by poor 
parameter estimates from questionable fits, hence this step was included. 

Supplementary Fig. 1 illustrates the differences between Ei calculated with and 
without a weighting – applying a weighting pushes Ei down a little, likely due to high 
E values obtained from fits to lower quality data. In either case, with or 
without a weighting, Ei falls significantly above the 0.65 eV MTE average activation 
energy for both Bacteria and Archaea.

Taxonomic and physiological groups. Psychrophiles and mesophiles inhabit 
low to medium temperature ranges, while thermophiles and hyperthermophiles 
grow at much higher temperatures. The distinction between these groups is 
usually defined relatively arbitrarily, with mesophiles often considered strains with 
thermal optima up to 45 °C, and thermophiles those with thermal optima of 55 °C 
and above. Corke et al. found a peak in microbial growth rates at ~42 °C 
(mesophile peak) followed by an attenuation of maximum growth rates until a 
second peak at ~67 °C (thermophile peak), suggesting a biological transition between 
mesophiles and thermophiles.

In order to determine whether it was appropriate to consider mesophiles and 
thermophiles separately, we performed a break-point analysis on our data set using 
the ‘Segmented’ R package. Segmented is not compatible with non-linear least-
squares (nls) fitting, so this was performed with a linearised version of 
Boltzmann–Arrhenius, i.e., x = y where x = 1/(4T) and y = log(μT ). As this 
process was merely to confirm whether it is appropriate to split the data into 
mesophiles and thermophiles as suggested by and is not important that 
linearised fits may give slightly different slope and intercepts to the weighted nls fits. 
Using this methodology, we determined significant break-points for bacteria 
and archaea within our growth rates data set at 40.48 °C and 46.21 °C, respectively. 
These are similar to the ~42 °C mesophile growth rate peak seen by Corke et al. and were thus cut-off points for defining mesophiles and 
thermophiles in our analysis. For this work, we provide no lower limit for 
mesophiles, essentially grouping psychrophiles and mesophiles together. Were 
psychrophiles to display a pattern different to mesophiles, we would expect this to 
have shown up in the break-point analysis, but only one break point (separating 
mesophiles and thermophiles) was found for both bacteria and archaea.

In addition, archaea are typified by their adaptations to energetically demanding 
niches, while in contrast bacteria perform better in more ambient environments. 
A major physiological difference between these taxa lies in their fundamentally 
divergent membrane structures. This affects these organisms’ abilities to maintain 
proton gradients and thus drive metabolism under different conditions, a 
difference that may be particularly important for thermal performance. As such, 
we separate bacteria and archaea in our analysis as disparate organisms with divergent 
evolutionary histories.

In order to classify prokaryotes by the energy generating metabolic processes 
that they use, we took note of the growth conditions used when initially digitising 
the TPC data. For the majority of heterotrophic bacteria and archaea, this was 
simply whether they were grown under aerobic or anaerobic (fermentative) 
conditions. However, there are also a number of strains utilising more exotic 
metabolic processes such, as methanogenesis, sulphur reduction, etc. In these cases, 
we matched taxa against those able to utilise certain metabolic reactions according 
to Amend and Shock before manually checking the culture conditions in each 
study for the metabolites required for certain metabolic processes.

We also categorised taxa by their status as potential pathogens. We matched 
taxon names against the database of host-pathogen interactions provided in 
(Pandey et al.) to understand whether each strain was potentially pathogenic, 
and what taxa they were known to infect.

Ecosystem carbon flux model. To quantify the effect of differences in activation 
energy of respiration between prokaryotes and eukaryotes on carbon flux, one can 
calculate the fold increase in (F) of an ecosystem as:

\[ F = \frac{F_{\text{i} \rightarrow \text{s} \rightarrow \text{f}}}{F_{\text{e} \rightarrow \text{i} \rightarrow \text{f}}} \]  

where x is the temperature increase (at the end of a warming scenario), and F and 
F are the fluxes at the two temperatures. Because ecosystem carbon flux at night 
(i.e., without photosynthesis) is the sum of autotrophic and heterotrophic 
respiration rates weighted by the biomass of these compartments, we can re-write 
F as:

\[ F_{\text{i} \rightarrow \text{s} \rightarrow \text{f}} = \frac{(1 - \delta) c T^{c} + \delta \beta c T^{c} + (1 - \beta) c T^{c}}{(1 - \delta) c T^{c} + \delta \beta c T^{c} + (1 - \beta) c T^{c}} \]  

Here, each compartment’s total flux contribution (identified by a subscript: 
autotrophic eukaryotes = a, heterotrophic prokaryotes = h; heterotrophic 
eukaryotes = he) is modelled as a Boltzmann–Arrhenius equation, with a c 
normalisation constant. Each compartment’s contribution is weighted by the 
biomass proportionality constants: δ is the proportion of heterotrophic biomass in 
the ecosystem, while β is the proportion of prokaryotic biomass within the 
the heterotrophic component (so 1 – β is the proportion of non-prokaryotic 
archaebacteria, such as fungi or insects). We do not use the Sharpe–Schofield 
model here because it does not apply to long-term thermal responses (Fig. 1), 
whilst for short-term responses most warming as well as temperature fluctuations 
are expected to occur within an operational temperature range, which excludes 
temperatures greater than T (the heat-stress region). We do not include any 
potential contribution of autotrophic prokaryotes (such as cyanobacteria), as these 
are not expected to provide a significant flux contribution to a typical terrestrial 
ecosystem. Alternative models and parameterisations for ecosystems which include 
cyanobacteria (i.e., aquatic ecosystems) are described in the Supplementary 
Methods and further discussed in the Supplementary Discussion.

We then use (Eq. 4) to calculate the percent change in ecosystem flux due to 
differences in activation energies of the three compartments (E, E, and E):

\[ \frac{F_{\text{i} \rightarrow \text{s} \rightarrow \text{f}}}{F_{\text{e} \rightarrow \text{i} \rightarrow \text{f}}} = 100 \]  

where F and F are the warming-induced flux changes in ecosystems with 
and without differences in activation energies of the compartments, respectively 
(the value of the heat map in Fig. 8). That is, for F, all E values, i.e., E, E, and E 
in Eq. (4) are 0.65 eV. This is the assumption made by most current ecosystem 
carbon flux models. For F, the differing activation energies were 
parameterised using either the mean of the estimated Es for the short-term (intra-
specific) or long-term (inter-specific) TPCs (Table 1; Table 3). For this, we 
used estimates of E and E from mesophilic bacteria (long-term evolutionary E = 0.98 eV, short-term instantaneous E = 0.87 eV) only, because the archaean 
our data are largely composed of strains adapted to ecologically extreme niches, which 
are largely irrelevant from a global warming perspective. Archaeae are known to 
play important roles in carbon cycling across various ecosystems however, so were 
more data available for the thermal performance of non-extremophilic archaea our 
model could be parameterised with their inclusion. Alternative model formulations 
to include archaean are described in the Supplementary Methods.
We calculated the emergent $E$ of the $F_{12}$ ecosystems (flux response to warming when prokaryotic and eukaryotic thermal sensitivities differ), which is the the average of activation energies for each ecosystem compartment weighted by its biomass proportion:

$$E = (1 - \delta)E_w + \delta(E_{ty} + (1/\beta)E_{sy}).$$

We also calculated the emergent $Q_{10}$ of the $F_{12}$ ecosystems, as it is a widely used measure in climate change models of carbon flux:

$$Q_{10} = (F_{10})^2.$$  

We chose a warming magnitude $x = 10^\circ C$ for short-term responses because this at the upper end (e.g., generally, at higher latitudes) of the range of daily (over 24 h) fluctuations that organisms experience. For long-term warming scenarios, we used $x = 4^\circ C$, the approximate upper end of the range for the year 2100 projected by the IPCC.

The biomass proportions $\delta$ and $\beta$ were varied to capture the effect of different ecosystem compositions. In a typical forest ecosystem, the contribution of autotrophic to heterotrophic (mostly soil) respiration has been estimated to be approximately 50% each. This heterotrophic component would be comprised largely of prokaryotes and soil fungi biomass, the ratios of which have shown to vary widely depending on soil type and the experimental methodology used.

Here, we vary the percentage of heterotrophs within an ecosystem ($\delta$) between 25 and 75%, and the percentage of prokaryotes within heterotrophs ($\beta$) between 25 and 75% to generate a range of potential scenarios in Fig. 6. For simplicity, and consistent with current approaches, this model does not include changes in the relative biomass of ecosystem components with warming.

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**Acknowledgements**

We would like to thank Rebecca Kordas for helpful comments on an earlier version of the paper. T.P.S. was supported by a BBSRC DTP scholarship (BB1014575/1). B.G.C., S.S. and S.P. were supported by a NERC grant awarded to S.P. (NE/M004740/1). T.B. was supported by an ERC starting grant (311399-Redundancy). G.Y.-D. was supported by an ERC starting grant (677278 TEMPEDEP).

**Author contributions**

T.P.S. and S.P. conceived the study. T.P.S., T.J.H.T., S.S. and G.Y.-D. compiled the data. T.J.H.T. wrote the thermal model fitting code. T.P.S. and T.J.H.T. wrote the analysis code, with advice and expertise from B.G.C. T.P.S., T.J.H.T. and S.P. analysed the data. T.P.S., T.B. and S.P. wrote the first draft of the paper, and all authors contributed to the revisions.

**Competing interests**

The authors declare no conflicts of interest.

**Additional information**

Supplementary information is available for this paper at https://doi.org/10.1038/s41467-019-13109-1.

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**Peer review information**

*Nature Communications* thanks Martina Doblin, Matthew Wallenstein and the other, anonymous, reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

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