Metabolic rates of prokaryotic microbes may inevitably rise with global warming

Thomas P. Smith, Thomas J. H. Thomas, Bernardo García-Carreras, Gabriel Yvon-Durocher, Thomas Bell, Samrāt Pawar

The collective respiration of prokaryotes is a major contributor to carbon efflux from terrestrial ecosystems. Therefore, understanding how the metabolic rates of these microorganisms respond to temperature is fundamental to our understanding of how ecosystem functioning will be altered by climate change[1] . Individuals of species living at higher temperatures may be expected to have higher metabolic rates than those in cooler niches due to thermodynamic constraints on rate-limiting enzymes[2,3,4,5]. However, this idea has never been tested thoroughly in prokaryotes. Here, using a new global dataset of thermal responses covering most major phylogenetic groups of bacteria and archaea, we find strong evidence that fitness increases with temperature for mesophiles (temperature optima $\lesssim$ 45°C), but not thermophiles ($\gtrsim$ 45°C). Given that climatic warming will mostly impact ecosystems in the mesophilic temperature range, we conclude that as microbes adapt to the higher temperatures, their metabolic rates, and therefore rates of many ecosystem functions such as carbon efflux will inevitably rise. Furthermore, we find that prokaryotes tend to have higher thermal sensitivities than autotrophic eukaryotes , suggesting that the short-term response of ecosystem carbon efflux due to prokaryotic respiration will also increase more rapidly than carbon fixation. Our results provide new insights and data...

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[6,7,8,9]. Because fundamental physiological rates of ectotherms are directly controlled by environmental temperature[3,4,8], climatic warming may be expected to lead to ectotherm communities with higher metabolic rates on average[8,10]. How environmental temperature drives metabolic rates of prokaryotes (bacteria and archaea) is of particular importance because they are globally ubiquitous, and estimated to comprise up to half of the planet's global biomass[11]. Therefore, climate-driven changes in prokaryotic metabolic rates may significantly alter ecosystem productivity , nutrient cycling[12], and carbon flux[13,14]. Indeed, increased carbon efflux has been observed in experimental measures of soil CO_2 loss to warming[15,16], as well as the responses of other microbial metabolic processes to increased temperature such as methanogenesis [17,18]. However, we lack a general understanding...

7: The next para below is disconnected from the first one. Need a sentence or two at the end of the first para that links the two and makes clear what the knowledge gaps are, especially in terms of short-term vs. long-term responses. I brought up and edited relevant parts of the old commented-out para to help re-establish this connection between intraspecific TPCs, HiB, and warming effects (see where and how you want to use it): **The characteristics of the intra-specific TPC, and any HiB constraints on it are crucial for understanding the the short- and long-term effects respectively, of climatic warming on ecosystem functioning. On the short term, a greater thermal sensitivity (higher E) would imply a faster increase in metabolic rates with warming. In the longer-term, if HiB holds true in general, warmer environments will inevitably have ectotherm communities with higher metabolic rates on average. This potential long-term effect is particularly important because of the the capacity for prokaryotes to rapidly evolve to new thermal regimes [19,20,21]. So if HiB were true, then ecosystems would be faced with a "double whammy" of long-term responses compounding the effect of short-term responses ecosystem function (e.g., carbon efflux) to global warming.**

The response of metabolic rates of individual organisms to changing temperature (the intra-specific thermal response) is typically unimodal, with the thermal performance curve (TPC) of the trait (e.g., respiration rate) increasing with temperature up to a peak value (T_{pk}), before decreasing as high temperature becomes detrimental to metabolic or cellular processes[2,7] (Fig. 1C). While T_{pk} for fitness and its underlying metabolic rates are expected to correspond to the typical thermal environment in which the species has evolved[2,22], whether trait performance at T_{pk} (henceforth denoted by P_{pk}) increases across organisms and environments is a question that is still debated[23,24,25]. This is the hotter is better (HiB) hypothesis, which is implicit in the universal temperature dependence concept of the MTE[3,4,5] (Fig 1A). Deviations from this pattern would indicate that thermodynamic constraints either do not play a role, or are compensated for by other mechanisms. An alternative hypothesis is that natural selection acts to override thermodynamic constraints, allowing peak trait performance and fitness to be, on average, equalized across different adaptation temperatures (Fig 1B)[23]. This pattern may also be seen if high temperature is detrimental, causing depressed fitness at higher temperatures. Intermediate scenarios are

1: Add a couple more refs here – ideally, recycle some that you have already used below

2: This explicit mmention of autrophs is confusing — shift focus to respiration of heterotrophs vs eukaryotes per se. See my comment above the modelling paragraph as well (comment # 24)

3: Need An ending statement on impacts/implications

4: needs reference, as other two items have references; or just combine all refs at end of sentence

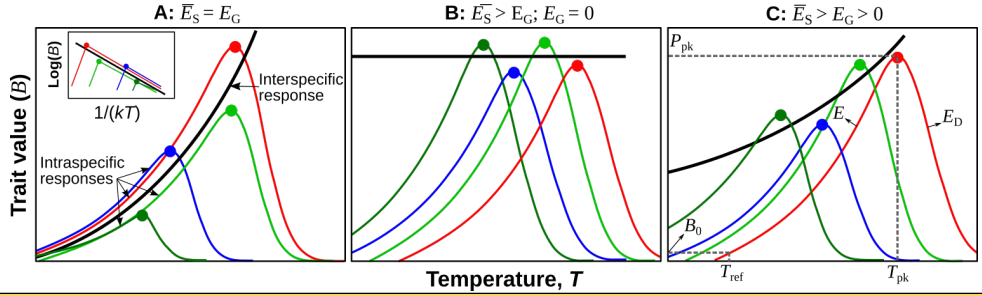
5: only one of these two papers by Gab is about methanogenesis, right?

6: See my next, inline comment

8: We use "metabolic rate", "metabolic trait", "performance", as well as "fitness". Let's make sure we stick with one or two of these consistently, using the others in specific contexts only if at all.

also possible, where adaptation of optimal trait performance or fitness is only partially constrained by thermodynamics (Fig 1C, e.g. metabolic cold adaptation [26]).

Here we build and analyse a global dataset of growth rates TPCs in bacteria and archaea to quantify general patterns in both short-term (intra-specific), and test whether HiB holds (optimal fitness increases) across taxonomic and functional groups adapted to different temperatures (long-term, inter-specific response; Fig. 1). These data go far beyond the scope of previous tests of the HiB hypothesis [23], covering practically the entire range of habitable global temperature niches (from bacteria isolated from Antarctic saline lakes at temperatures below 0°C, to a strain of methanogenic archaea able to proliferate at 122°C under high pressure) and the majority of the phylogenetic diversity of prokaryotes (spanning 9 bacterial phyla and the two major archael phyla, Euryarchaeota and Crenarchaeota - see supplementary figure S4).



12: How about saying "short-term" under "intraspecific responses" and "long-term" under "interspecific response".
Also, a hyphen is used in the main text in "intraspecific" and "interspecific", but not here. Either change it here, or throughout the main text to make it consistent.
Also, address short-term responses in the figure caption and legend — i.e., the intraspecific TPCs are the short-term response; so something like "Note that each intraspecific TPC represents the short-term thermal response of each species' population", or sth like that.

Figure 1: Three alternative hypotheses for evolution versus constraints on thermal performance curves of metabolic traits in response to environmental warming. 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 activation energy ($\bar{E}_S$) is statistically indistinguishable from the inter-specific activation energy of the group of species (E_G), and both are greater than zero. See methods for more details on the the definition and estimation of $\bar{E}_S$ and E_G, and the statistical methods used to differentiate between them. Inset panel illustrates how this would look on Arrhenius plot axes. **B.** Equalisation of fitness: selection overrides thermodynamic constraints, such that trait performance at T_{pk} is on average the same ($E_G = 0$). Alternatively the same effect of $E_G = 0$ may occur due to physiological constraints restricting potential growth at higher temperatures. **C.** Weak biochemical adaptation: an intermediate scenario where $E_G > 0$ but significantly less than $\bar{E}_S$. Panel C also illustrates the meaning of the parameters of the Sharpe-Schoolfield TPC model (eqn. 1) in the right-most (hottest) thermal performance curve (TPC).

First, we compared the peak growth temperature (T_{pk}) with lab culturing temperature (T_{lab}) each strain, to determine whether the TPCs reflect adaptation to growth conditions. For both bacteria and archaea we find a strong and significant association between T_{pk} and T_{lab} (supplementary Fig. S3) indicating that these strains are in general thermally well-adapted to their culturing conditions. Next, we tested the HiB hypothesis, by comparing the intra- and inter- specific thermal response curves (see Fig. 1; Methods). If there is a universal thermodynamic constraint, peak fitness (P_{pk}; r_{max} at T_{pk}) across species' TPCs would increase with their respective T_{pk}s (parameter E_G; Fig. 1) at the same rate as r_{max} would increase with temperature (parameter E_S), on average, within single species' TPCs. Analyzing this relationship across 416 bacterial and 82 archaeal TPCs, we find that Hotter is indeed Better (HiB holds) across mesophiles ($\bar{E}_S$ and E_G are > 0, and their 95% CIs overlap; Fig 2 and Table 1). However, this result does not extend to thermophiles, where instead fitness is on average invariant with respect to temperature. Thermophiles have evolved specific adaptations to extreme temperature stress, such as mechanisms to cope with increased membrane permeability at high temperatures [27] and thus adaptation to such niches may incur a fitness cost to thermophiles as seen in our results. This result is consistent with the "Biokinetic Spectrum for Temperature" proposed by Corkrey *et. al.* [28], which suggests a fundamental biological differences in growth rates between mesophiles and thermophiles.

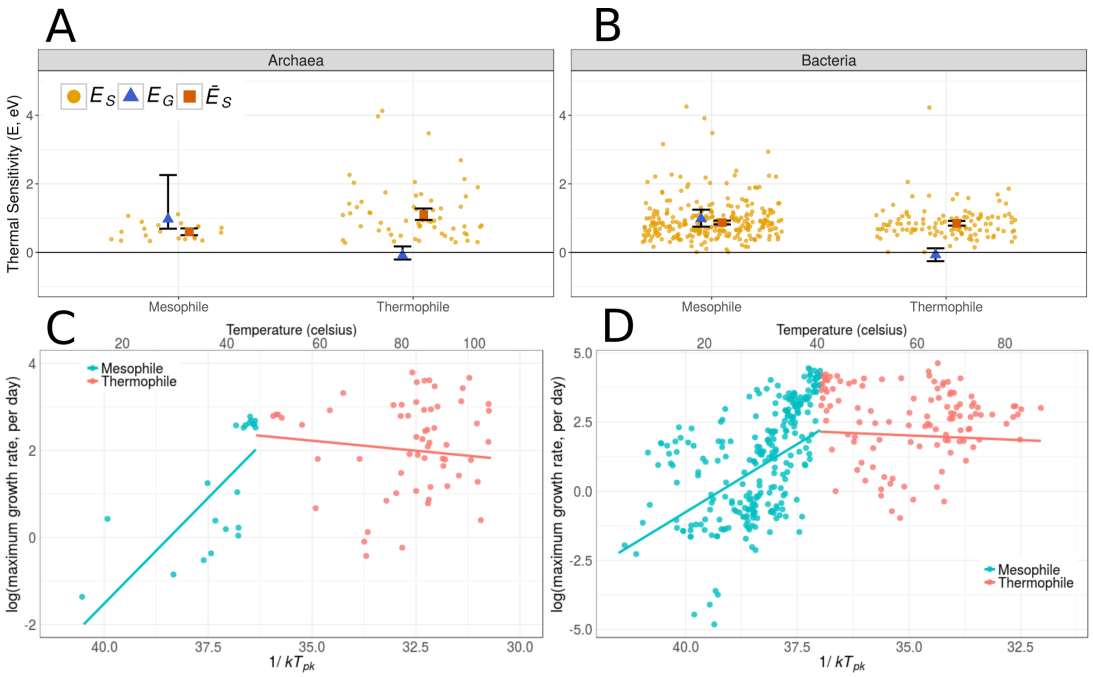
9: I edited this to say both fitness and performance — throughout this manuscript, make sure you are using mostly fitness, with trait performance only mentoned when relevant

10: Why would this not be a good example for Bichemical adapatation as well? Also, say what MCA is in a short phrase following the term.

11: revised this to be consistent with our short- and long-term theme now running throughout the manuscript...

13: Need stats; R-squared and p value, along with slope

14: move this and the next line to discussion, perhaps?



15: The regression lines in C and D above need prediction bounds. Also, the labels A, B, C, D are too big relative to the text size on the axes and in the subheadings etc. Make them a few pts larger than the axes text at most.

Figure 2: Test of the HiB hypothesis for archaea and bacteria, split by temperature preference, by comparison of inter-specific and intra-specific thermal sensitivity (E_G vs. $\bar{E}_S$). Panels **A** and **B** (archaea and bacteria respectively) show the bootstrapped confidence intervals for the activation energies from Boltzmann-Arrhenius model fits (E_G, blue triangle) compared to the intra-specific activation energies ($\bar{E}_S$, red square), with the distribution of E_S shown in the background (orange points). Panels **C** and **D** show Arrhenius plots for archaea and bacteria respectively, with the weighted Boltzmann-Arrhenius model (lines) fitted to mesophile and thermophile temperature groups seperately (points). Note that x-axes are inverted for visualisation purposes only and y-axes are not equal. For both mesophile groups the HiB hypothesis is best-supported, whilst in the thermophile groups equalisation of fitness is the observed result.

Kingdom	**Thermal Niche**	E_G	(E_G CI)	E_S	(E_S CI)	$E_G > 0$	$E_S \approx E_G$	HiB
Bacteria	Mesophile	0.98	(0.75-1.25)	0.87	(0.82-0.93)	TRUE	TRUE	TRUE
	Thermophile	-0.07	(-0.26-0.12)	0.84	(0.78-0.92)	FALSE	FALSE	FALSE
Archaea	Mesophile	0.97	(0.69-2.26)	0.60	(0.50-0.70)	TRUE	TRUE	TRUE
	Thermophile	-0.09	(-0.21-0.17)	1.11	(0.95-1.28)	FALSE	FALSE	FALSE

Table 1: Hypothesis testing. Calculated mean E_S and E_G values and the respective 95% CIs for bacteria and archaea split by temperature preference, as shown in Fig 2. HiB column indicates whether or not the hotter is better hypothesis can be accepted.

16: New construction - first our E results, then our E for metabol results, THEN explain why this is different to previous work (skew, medians, then other work, sofia, etc)
SP:That's good, but one more tweak needed: present the intra-specific (short term response) results first, and then the bits about checking wether growth rates reflect underlying flux rates from a ecosystem function perspective. Then the dicussion about ecosystem function and the model of carbon flux follow more naturally.

Whilst we focus on testing the HiB hypothesis using the activation energies of growth rates , it is often assumed that these reflect the activation energies of the underlying metabolic rates [29]. To investigate this assumption, we also assembled thermal response data from metabolic fluxes recorded in prokaryotes and asked whether, on average, thermal sensitivity is equivalent for growth rate and metabolic rates. We found that average intra-specific E values for growth rate TPCs (bacteria $\bar{E}_S = 0.88$eV; archaea $\bar{E}_S = 0.95$eV) were similar, and statistically indistinguishable from the mean activation energy for our metabolic flux data (bacteria $\bar{E}_S = 0.82$eV; archaea $\bar{E}_S = 1.01$eV; Fig. 3A, see supplementary table S1 for a list of fluxes

17: This should be stated more genrally, to remain faithful to our short- vs long-term response theme. So it's not just about testing the HiB, and it's not just about the activation energies but the TPCs. So you could say instead: "Whilst we focus on the TPCs (and activation energies) of population growth rates, it is necessary to test whether growth rates activation energies these reflect the activation ener-

analysed). This is important from an ecosystem perspective, as it strengthens the argument that the observation of HiB constraints on fitness also implies higher increases in metabolic rates, and therefore ecsystem function such as carbon flux, with global warming. Under MTE, the global inter-specific thermodynamic constraint is expected to centre on or around 0.65eV [3,4] and mean intra-specific thermal sensitivities have been found to be very similar to this value [7]. Our results deviate from this assumption, with average thermal sensitivities for both bacteria and archaea falling significantly above 0.65eV (Fig. 3). We observe the same right-skew in activation energies for prokaryotes as seen across other organisms and traits [7] and even accounting for this skew by taking the median instead of a mean, the activation energy still falls significantly above 0.65eV (bacteria median = 0.84, archaea median = 0.80; see supplementary figure S2). This is consistent with previous work on prokaryotic groups such as methanogenic archaea [18] and cyanobacteria [30] which have been shown to have intra-specific thermal sensitivities closer to 1eV. Additionally, we see a consistent pattern of mean thermal sensitivity >0.65eV throughout lower taxonomic groups (see supplementary figure S4). Taken together, these results imply that prokaryotic thermal sensitivity does not adhere to the MTE's 0.65eV generalization. Furthermore, we compare our prokaryotic E_S distributions to data on thermal sensitivity of respiration in autotrophic eukaryotes provide a visualisation and further support for the difference between eukaryotic and prokaryotic E_S as in this case $\bar{E}_S \approx 0.65$eV (autotroph respiration E_S median = 0.57, mean = 0.67, bootstrapped CI = 0.63-0.72; Fig 3D).

18: to tentative... say clearly it is an quantiative analysis to test something.

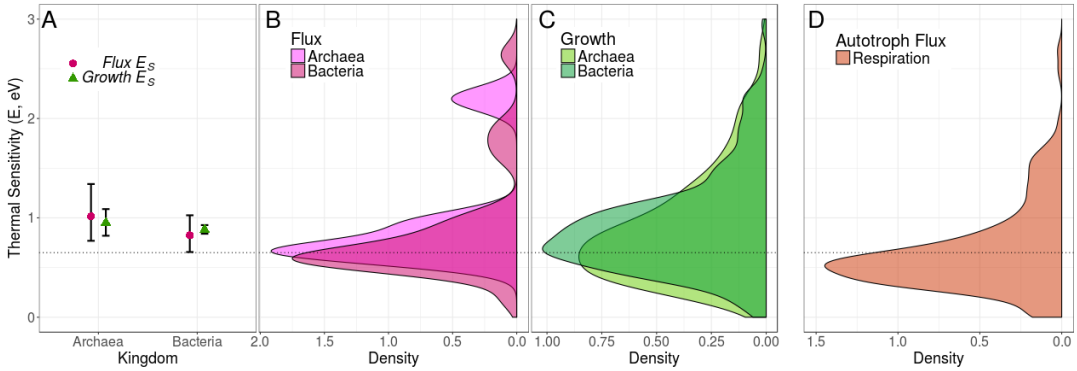


Figure 3: Differences in thermal sensitivity across traits and taxonomic groups. A. Comparison of the intra-specific thermal sensitivity ($\bar{E}_S$) for growth and metabolic fluxes. CIs for growth rate thermal sensitivity fall within those for metabolic fluxes and each sit above 0.65eV (dotted line) for both archaea and bacteria. **B.** Density plot of flux E_S values for archaea and bacteria. **C.** Density plot of growth rate E_S values for archaea and bacteria. **D.** Density plot of E_S values from a dataset of respiration rate TPCs in autotrophs showing comparatively lower thermal sensitivity than distributions for prokaryotes.

Most of the biomass on the planet exists within the mesophile temperature range of our analysis ($\lesssim$ 45°C), where we find the hotter is better hypothesis hold true. From an ecological perspective, the combination of conserved thermal constraints, hotter is better, and the comparatively high activation energies of prokaryotes leads to some important considerations. In addition to previous studies showing rapid adaptation to experimental warming conditions [19,20,21], here we have shown a strong association between T_{pk} and T_{lab} for each strain, implying adaptation of experimental strains to their lab environment. Due to this adaptive capacity, as global temperatures rise prokaryotes would be expected to adapt to new environmental temperatures rapidly, in effect pushing them further along the global HiB (inter-specific) curve. 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, as under MTE we would expect these prokaryotes also to effectively be further up the inter-specific temperature response curve. In either case, under HiB, we can expect global warming to result in prokaryotic communities with higher metabolic rates on average.

The implications of the effects of high E_S have been previously argued in the context of methanogenic archaea and increased methane production based on the high thermal sensitivities of these prokaryotes [18]. Here we find high values of E_S across prokaryotes more generally, and suggest that further production of greenhouse gases from the prokaryotic component of ecosystems via aerobic respiration is likely to increase at a greater rate compared to component eukaryotic organisms. Whilst in general, we see a tendency

19: Agin, make more consistent with our long-term vs short-term theme.

20: this is underselling our result. Bring up our result about adaptation to lab temps first. I would be cautious about the rapid evolution issue — as such, we don't have evidence for how fast the adaptition happened. So you should mention that though we don't know, but it is likely to have happened rapidly, citing these previous papers.

21: I added this - also cite fig 1

22: A bit ungainly - rephrase

towards high E_S in prokaryotes, there are distinct subgroups within our dataset for which this is not the case (see supplementary figure S4). Indeed, $\bar{E}_S$ for mesophilic archaea as a whole is not seen to deviate significantly from the MTE 0.65eV average (Table 1). Our mesophilic archaea data is primarily comprised of TPCs from the *Halobacteria* class of archaea, which are seen to generally have low E_S (significantly lower than the MTE 0.65eV average in fact; $\bar{E}_S = 0.46$; CI = 0.38-0.58; fig S4), likely a product of their extremophilic high salinity growth requirements (isolated only from saline lakes) . When we consider the impacts of deviations in $\bar{E}_S$ at an ecosystem level, we are not concerned with organisms adapted to such a specific extremophilic niche. The small number of other mesophilic archaea in our dataset are all methanogens, which are not relevant when discussing changes in aerobic respiration across ecosystems. It may be particularly difficult to derive generalisations for archaea, as these prokaryotes are typified by their adaptations to environments where they experience various types of extreme energy stresses [31], which may have a significant bearing on differences in thermal performance across taxa. Therefore when considering the potential broad ecosystem-level impacts of our findings, we exclude archaea and concern ourselves only with the mesophilic bacteria within our dataset. We note also that whilst the majority of heterotrophic bacteria in our dataset respire aerobically, there are also a number of anaerobic strains, the majority of which were grown under various fermentation conditions. When we consider these groups of bacteria separately however we see no significant difference between their mean intra-specific thermal sensitivities (aerobic $\bar{E}_S = 0.86$, CI = 0.81-0.91, n = 221; fermentation $\bar{E}_S = 0.86$, CI = 0.77-0.96, n = 62).

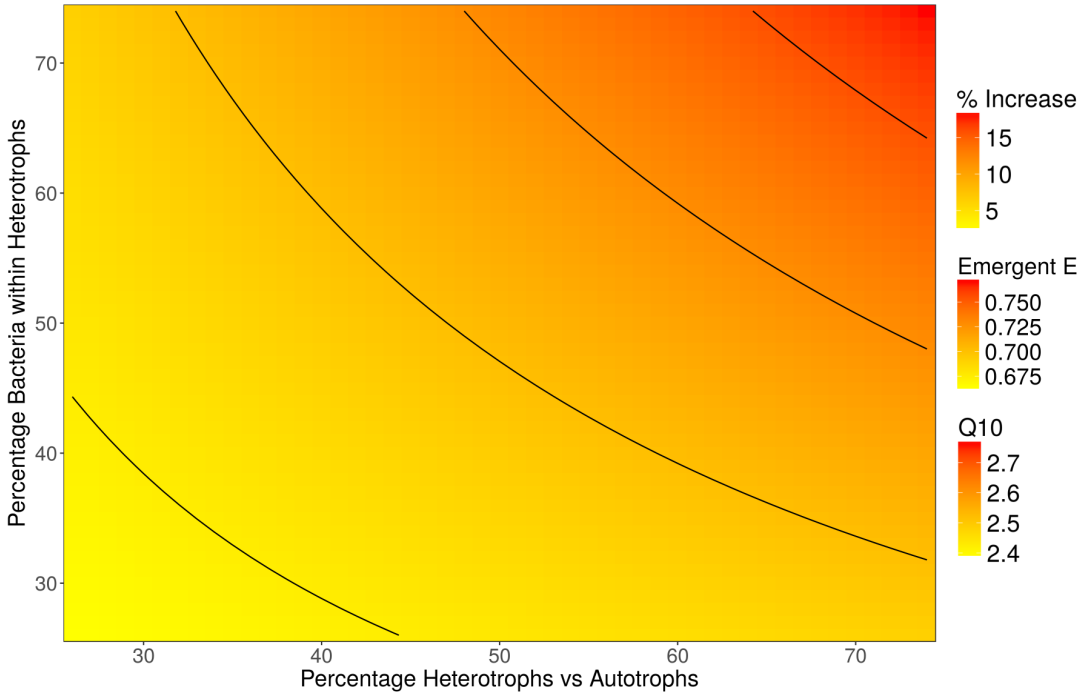
23: ugh! rephrase?

24: A lot of the details and justifications (e.g., the % of microbial/prokaryotic biomass) in the next two paragraphs should be in methods. Focus primarily on the result in the new figure, and explaining it. As such, this too much text, as it starts to read like we are going back to a results section somehow, and less like a discussion. So moving the details to methods will also allow you to combine the next two paras into one.
Also, you need to clearly justify why you are looking at autotroph respiration only (and not all other eukaryotes) – that's an easy one, as autotrophs are the biggest (and flux-wise, dominant) component of most ecosystems (put citations in Methods perhaps), and most global models only consider autotroph + heterotroph fluxes.
Finally,

To illustrate the potential global impacts of our findings on ecosystem carbon flux we present a simple model (Methods). Consider a typical forest ecosystem, where carbon flux at night is the sum of autotrophic and heterotrophic respiration. In such an ecosystem, the contribution of autotrophic to heterotrophic respiration has been estimated to be approximately 50% each [32]. 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 [14]. Calculating metabolic rates at two temperatures 10°C apart and then calculating the change in rate as a function of temperature change gives the Q_{10} temperature co-efficient. Q_{10} is a similar measure to activation energy, but reports the factor by which rate changes over the temperature interval, here we can consider it in terms of instantaneous flux equivalent to for instance the difference between day-time and night-time temperature. Here we compare changes in Q_{10} when using a 0.65eV thermal sensitivity for eukaryotes compared to a 0.87eV thermal sensitivity for prokaryotes ($\bar{E}_S$ for mesophilic bacteria) under different estimations of ecosystem heterotroph:autotroph and prokaryote:eukaryote compositions to investigate the potential impacts that our findings raise. We vary the percentage of heterotrophs within an ecosystem between 25-75% and the percentage of prokaryotes within heterotrophs between 25-75% and calculate Q_{10}, the emergent E of the ecosystem and potential percentage increase in flux compared to a baseline ecosystem E of 0.65eV (fig. 4). Given 50% heterotrophs and 50% prokaryotes within heterotrophs, we calculate a Q_{10} of 2.53, equivalent to $E = 0.71$, resulting in a flux increase of 8.32% over the baseline. At the higher extreme of 75% heterotrophs and 75% prokaryotes, $Q_{10} = 2.77$, $E = 0.77$, a potential flux increase of 18.72% (see fig. 4).

This calculation based on intra-specific thermal sensitivity is relevant to instantaneous increases in flux without evolution or acclimation. However, we can also consider the implications of long term warming on the prokaryotic sub-community using the inter-specific (evolutionary) thermal sensitivity, E_G. Calculating the Boltzmann-Arrhenius flux change over 4°C warming (IPCC predicted warming scenario [33]) using an activation energy of 0.65eV and comparing this to the flux change with 0.98eV activation energy (E_G for mesophilic bacteria) yields a relative flux increase of 19%, i.e. we may expect a 19% increase in prokaryotic carbon flux across ecosystems as these micro-organisms adapt to a global temperature rise of 4°C (see methods for full details of these calculations).

25: use short-term, to be consistent with the wording in the intro



26: some comments:

- Make the heatmap square
- I think a color map from white to black would work better, with flux values rescaled so that the minimum is white and maximum is black.
- Put numbers on the contour lines - otherwise, they are quite useless
- Change "% Increase" to "% Flux Increase"
- Italicize E
- Q10 should be Q_{10}
- The caption and legend need to be more explicit about timescales — using same "short-" vs "long-term" wording as elsewhere.
- Don't call it heat map – say directly what the color range signifies.
- Provide the take-home message(s).

Figure 4: Potential changes in instantaneous ecosystem carbon flux due to prokaryote thermal sensitivity. Heat map of calculated values for Q_{10}, E and % increase over a baseline 0.65eV E for a range of different ecosystem compositions.

27: We have quite rightly focused on the ecosystem consequences in the discussion, but we should have a short para on the evolutionary/biological implications - future studies should look into why prokaryotes differ systematucally in thermal sensitivity from Eukaryotes. I am no aware of any previous study that has shown this.

Here we have shown that the HiB hypothesis can be accepted for mesophilic prokaryotes, indicating that across-species biological rates inevitably rise with increasing temperature in accordance with their Boltzmann-Arrhenius inter-specific temperature dependence. We have also shown that intra-specific thermal sensitivity is significantly higher for prokaryotes than the 0.65eV MTE generalisation. This study has implications for predicting the effects of both short-term temperature fluctuations and longer-term responses to global warming. Ecosystem carbon efflux predictions at short timescales may be increased due to the increased activation energy of prokaryotes relative to eukaryotes. Additionally, a long-term 4°C global temperature rise may increase prokaryotic metabolic fluxes by 19% more than eukaryotic metabolic fluxes. This means that as the planet warms, prokaryotic respiration will provide a comparatively greater contribution towards ecosystem carbon flux than that contributed by eukaryotes. These are large deviations from current assumptions, and should be considered in future predictions of the impacts of climate change and included in efforts to model ecosystem respiratory fluxes. Our study shows that more work must be undertaken to address whether intra- and inter-specific thermal sensitivities are conserved across other groups of organisms. Note however that our comparisons for growth rate and metabolic flux E are simply averages across species and that direct within species comparisons of growth rate and metabolic TPCs will be needed in order to fully understand the implications of this work.

Methods

Data Collection

We compiled a new dataset of published prokaryotic thermal performance curves (TPCs) by searching the literature for papers with these data and using digitization software to collect the thermal performance point estimates. Candidate TPC data was 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 'characterization', 'isolation', 'nov.', 'novel', 'gen.', 'sp.', as it became clear that thermal responses were often tested in publications describing newly isolated species. When presented as a response curve figure, 'Plot Digitizer' software[34] was used to extract data points, including error bounds when reported. The 'Taxize' R package[35] 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 estimate 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, the raw data from which are displayed in supplementary figure S1.

Although we primarily collected growth rate data as a measure of fitness in order to test HiB, we additionally collected 78 TPCs covering various metabolic fluxes for comparison to growth rate TPCs. Our complete prokaryote dataset comprises 620 TPCs from 489 unique prokaryote strains across 242 published studies.

Finally we used TPC data collected for respiration rates in autotrophs that had previously been deposited in the "biotraits" database, as representative eukaryote data for cross-domain comparisons. In total this 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).

Model Fitting

To our dataset, we fitted a modified Sharpe-Schoolfield model[36] (eq. 1) to each growth rate TPC:

$$B(T) = B_0 \frac{e^{\frac{-E}{k}\cdot\left(\frac{1}{T}-\frac{1}{T_{\text{ref}}}\right)}}{1+\frac{E}{E_{\text{D}}-E}e^{\frac{E_{\text{D}}}{k}\left(\frac{1}{T_{\text{pk}}}-\frac{1}{T}\right)}} \tag{1}$$

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 is the activation energy in electron volts (eV) (a measure of "thermal sensitivity"), k is the Boltzmann constant (8.617×10^{-5} eV K^{-1}), T_{pk} is the the temperature where the rate peaks, and E_{D} the deactivation energy, which determines the rate of decline in the biological rate beyond T_{pk}. We fit this model to individual TPCs and solve for $T = T_{\text{pk}}$ to calculate the population growth rate at T_{pk} (P_{pk}) for each strain. Note that this has been reformulated from the model presented in the original paper, to include T_{pk} as an explicit parameter.

In contrast to these intra-specific TPCs, an inter-specific curve can be produced from a set of data points corresponding to different species. Each species has a potentially different T_{pk} and P_{pk}, and compiling these values across species yields an inter-specific thermal response curve (as shown in main text Fig 1). We fit the Boltzmann-Arrhenius equation (eq. 2, essentially the numerator in eq. 1) to these peak values to calculate inter-specific activation energy.

$$B = B_0 e^{-E/kT} \tag{2}$$

All Boltzmann-Arrhenius and Sharpe-Schoolfield model fitting was performed in Python with the NumPy package, using a least squares regression method to minimize the fits.

Testing whether Hotter is Better in prokaryotes

We determined whether hotter is better by testing whether the activation energies from intra- and inter-specific thermal responses were (statistically) significantly different. For each intra-specific curve we fitted the Sharpe-Schoolfield model (eq. 1) and extracted the intra-specific activation energy (E_{S}), peak temperature (T_{pk}) and corresponding growth rate (P_{pk}) for each curve.

To estimate E_G we fitted the Boltzmann-Arrhenius model (eq. 2) to the T_{pk} and P_{pk} values estimated from the intra-specific thermal responses. To account for uncertainty in the original Sharpe-Schoolfield model fits to the intra-specific curves, we weighted each P_{pk} using its standard error (see weightings section). In order to provide a comparison between intra- and inter-specific activation responses, we used bootstrapping to generate confidence intervals (CIs) around the mean in each case. To provide boostrapped CIs for E_G from the modified Boltzmann-Arrhenius fits, the data was re-sampled with replacement 1,000 times, with the model re-fitted to this data each time and the CIs defined as the 2.5^{th} and 97.5^{th} percentiles of E values extracted from these fits. $\bar{E}_S$ was calculated as the weighted mean E_S for the group (see supplementary methods), and CIs were taken as the 2.5^{th} and 97.5^{th} percentiles from the resultant distribution of E_S values from a bootstrap of the weighted mean.

We then determined whether the data was consistent with either of the three hypotheses (main text Fig. 1) by comparing the overlap of the (bootstrap-based) confidence intervals. First, we tested whether $\bar{E}_S$ was greater than zero (null hypothesis that the CI includes zero). Second, we tested whether E_G was greater than zero (null hypothesis that the CI includes zero). Finally, if both $\bar{E}_S$ and E_G were positive, we tested whether they were significantly different to each other (null hypothesis, that the CIs for $\bar{E}_S$ and E_G don't overlap). Under a hotter is better scenario, P_{pk} will increase with T_{pk} across species, 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 $\bar{E}_S$ and E_G should overlap each other, but not zero. Alternatively, if growth rates are not constrained by thermodynamics and P_{pk} does not increase with temperature, then E_G will be close to zero (CI for E_G includes zero), and HiB can be rejected. Finally, in scenarios where thermodynamic constraints may be partially evident but somewhat overcome by adaptation, $\bar{E}_S$ and E_G will both be positive, but with $\bar{E}_S$ being significantly greater than E_G (i.e. $\bar{E}_S > E_G > 0$)

Weightings

Weighted means were used to account for uncertainty in Sharpe-Schoolfield point estimates when calculating $\bar{E}_S$ and when fitting inter-specific Boltzmann-Arrhenius curves. After performing Sharpe-Schoolfield fits we extracted the E and μ_{pk} point estimates as well as the covariance matrix. We then sampled 1,000 times from a bivariate distribution accounting for the covariance, producing 1,000 model parameter combinations. We used these parameters to generate 1,000 different Sharpe-Schoolfield curves, providing a distribution of E and μ_{pk} from which we took the standard deviations (SD_E and SD_μ) as a measure of uncertainty. In some cases the Sharpe-Schoolfield fit did not produce a covariance matrix and these fits were excluded from further analysis.

When combining E values across species to calculate $\bar{E}_S$ we, took weighted arithmetic means of E to account for uncertainty in the original fits, where $Weight = 1/(SD_E + 1)$. Similarly, when fitting Boltzmann-Arrhenius, we apply a weighting to μ_{pk} where $Weight = 1/(SD_\mu + 1)$.

28: Have revised this notation - acceptable?

Applying these weightings does not alter the main results we obtain from this study in terms of whether the hotter is better hypothesis is accepted or not for different groupings, however we felt that it was important to acknowledge and account for error in the underlying Schoolfield fits so that our results were not skewed by poor parameter estimates from questionable fits, hence this step was included. Figure S2 illustrates the differences between $\bar{E}_S$ calculated with and without a weighting - applying a weighting pushes $\bar{E}_S$ down a little, likely due to high E values obtained from fits to lower quality data. In either case, with or without a weighting, $\bar{E}_S$ falls significantly above the 0.65eV MTE average activation energy for both Bacteria and Archaea.

Taxonomic and Physiological Groupings

Prokaryotes can be classified by the temperature niches they occupy. Pychrophiles and mesophiles inhabit low to medium temperature ranges, whilst thermophiles and hyperthermophiles grow at much higher temperatures[37]. In order to determine whether it was appropriate to consider mesophiles and thermophiles separately, we performed a break-point analysis on our dataset (see following section). In addition, archaea are typified by their adaptations to energetically demanding niches, whilst in contrast bacteria perform better in more "ambient" environments[31]. 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[31], 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.

The Mesophile-thermophile Break-point Analysis

A distinction between mesophiles and thermophiles is usually defined relatively arbitrarily, with mesophiles often considered species with thermal optima up to 45˚C and thermophiles those with thermal optima of 55˚C and above[37]. Corkrey *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[28].

We used the 'Segmented' R package[38] to look for break-points in our data when fitting Boltzmann-Arrhenius. Segmented is not compatible with non-linear least-squares (nls) fitting, so this was performed with a linearised version of Boltzmann-Arrhenius, i.e. $x \sim y$ where $x = 1/(k * T_{\mathrm{pk}})$ and $y = log(\mu_{\mathrm{pk}})$. As this process was merely to confirm whether it was appropriate to split the data into mesophiles and thermophiles as suggested by eye, it is not important that these 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 dataset at 40.48˚C and 46.21˚C respectively. These are similar to the ∼42˚C mesophile growth rate peak seen by Corkrey *et. al.* and were thus used as our cut-off points for defining mesophiles and thermophiles in our analysis.

Flux Calculations

29: This section, including the equations can use some editing for better presentation.

In order to illustrate the implications of prokaryotic thermal sensitivity deviating from the 0.65eV MTE average, we present some simple calculations. Given the standard 0.65eV thermal sensitivity, we can calculate an expected percentage increase in metabolic flux with 10˚C of warming (Q_{10} value) using Boltzmann-Arrhenius (eq. 2) as follows. First we calculate rate at two different temperatures, where E = 0.65 and C is a rate constraining constant (Eqs. 3 and 4).

$$N1 = C \times e^{-E/kT} \tag{3}$$

$$N2 = C \times e^{-E/k(T+10)} \tag{4}$$

The ratio of N2 to N1 gives the Q_{10}, in this case 2.34 - that is, a 234% increase in flux over 10˚C.

$$Q_{10} = N2/N1 \tag{5}$$

To understand how the inclusion of prokaryotic activation energies may change expectations of Q_{10}, we can extend this calculation to sum eukaryotic and prokaryotic fluxes, with the contribution of each defined by a new term (γ; eqs. 6 and 7).

$$N1 = \gamma \times (C \times e^{-E_{prok}/kT}) + (1-\gamma) \times (C \times e^{-E_{euk}/kT}) \tag{6}$$

$$N2 = \gamma \times (C \times e^{-E_{prok}/k(T+10)}) + (1-\gamma) \times (C \times e^{-E_{euk}/k(T+10)}) \tag{7}$$

30: This whole bit needs a quick re-write - TS
SP agrees!

Given a eukaryotic thermal sensitivity (E_{euk}) of 0.65eV and a prokaryotic thermal sensitivity (E_{prok}) of 0.87eV ($\bar{E}_{\mathrm{S}}$ for mesophilic bacteria), we can alter γ (and thus the contribution of prokaryotes to heterotrophic ecosystem respiration) and compare the resultant Q_{10} with the original 2.34 obtained when only considering the MTE 0.65eV thermal sensitivity. If we let $\gamma = 0.05$ (minimal 5% prokaryotic contribution to heterotroph component[14]), we calculate a Q_{10} of 2.38. This is only a 1.66% increase on our previous Q_{10} estimate, reduced to a 0.83% increase in ecosystem flux when we assume 50% of the carbon flux contributed by heterotrophs[11]. However, if we let $\gamma = 0.98$ (maximal 98% prokaryotic contribution from[14]), we calculate Q_{10} as 3.10, a relative increase in carbon flux of 16.3%.

The above outlines calculations for differences in Q_{10} estimates based upon our new estimate of intra-specific prokaryotic thermal sensitivity, however we can also use this method to understand the implications of climate warming on the prokaryotic sub-community. By substituting T+10 with T+4 in eq. 4 we can investigate the change in rate over 4˚C warming. Using an E value of 0.98eV ("evolutionary" thermal sensitivity, E_G calculated for mesophilic bacteria) yields a 19% increase in flux over 4˚C warming than rates calculated using 0.65eV thermal sensitivity.

Data Availability Statement

Code Availability Statement

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Author Contributions

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