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Effects of Temperature on Microbial Metabolic Rates

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Abstract

Prokaryotes (bacteria and archaea) are globally ubiquitous micro-organisms which play fundamental roles in biogeochemical cycles and ecosystem functioning. Understanding how these microbes are affected by temperature is key to our understanding of how ecosystem processes will be affected by and respond to climate change. This includes understanding both how temperature directly affects the biological rates of prokaryotes through to how community composition may change with temperature and the impacts that these responses have on overall community functioning. Combining meta-analyses with experimental work and mathematical modelling, I assessed the direct impacts of temperature on microbial biological rates, with the aim of understanding how these effects translate to community and ecosystem level changes. I performed a meta-analysis of prokaryotic growth and metabolic rates, spanning the entire temperature spectrum of life on earth, revealing that the metabolic rates of mesophilic prokaryotes are likely to rise with climate warming. I used experimental methods to reveal the phenotypic diversity of temperature fitness in microbial communities, suggesting that species sorting rather than direct thermal adaptation may play a major role in how ecosystems respond to climate change. This work also revealed a disparity in phylogenetic groups associated with cooler and warmer temperatures as well as a divergence in their growth strategies, with warmer adapted taxa tending towards growth specialism rather than yield specialism. I also tested how microbial carbon use efficiency — the proportion of carbon uptake allocated to growth — varies with temperature. I found a unimodal temperature dependence of this trait, a departure from previous understanding. This work shows that changes in the composition and function of microbial communities with global change are likely to have a profound impact on ecosystem responses to warming. I propose that through species sorting processes, microbial communities are likely to shift to warmer adapted taxa with higher metabolic rates on average, which tend to be less carbon efficient in their growth. Ultimately, this may lead to increased carbon efflux versus sequestration by the microbial components of ecosystems with climate warming.

Declaration of Originality

I declare that the work within this thesis was performed and prepared by myself under the supervision of Samraat Pawar and Thomas Bell. All sources of information used in the preparation of this thesis are appropriately acknowledged and cited.

Whilst I declare that this work is my own, it has also been shaped through many discussions with my supervisors, collaborators and colleagues. Science is, after all, a collaborative effort, and it would be disingenuous to suggest otherwise.

The prokaryote data for chapter 2 was collected in collaboration with Joss Thomas as part of his undergraduate dissertation ("Is hotter better? A meta-analysis of prokaryotic growth rates", 2016, Imperial College London). I have substantially changed the analysis pipeline from Joss' work, and the analyses presented in this chapter are my own. The autotroph data in chapter 2 was collated by Dr. Sofía Sal and is used with her permission.

A previous version of chapter 2 is available on the bioRXiV pre-print server as "Metabolic rates of prokaryotic microbes may inevitably rise with global warming", doi: 10.1101/524264.

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"I may not have gone where I intended to go,
but I think I have ended up where I needed to be."

— Douglas Adams

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Glossary and Abbreviations

Acclimation - physiological responses to a change in environmental variables, mediated through phenotypic plasticity.

Activation Energy - see thermal sensitivity.

Adapatation - evolutionary responses to change in environmental variables via selection processes.

Autotroph - primary producer, synthesises organic compounds from inorganic carbon, using energy from light (photo-autotroph) or chemical reactions (chemo-autotroph).

ALE - adaptive laboratory evolution, techniques to rapidly culture micro-organisms over large numbers of generations, in order to adapt their growth to different conditions.

ATP - adenosine triphosphate, the energy currency of cells.

Biodiversity - the variety and variability of organisms within a given habitat or ecosystem.

CI - (95%) confidence interval.

Community - groups of micro-organisms sharing the same living space, expected to interact with each other.

Copiotroph - an organism adapted to life in nutrient rich environments.

CUE - carbon use efficiency, the proportion of microbial carbon uptake allocated to growth.

Heterotroph - cannot fix inorganic carbon to produce their own food and therefore derive intake of organic carbon from other sources such as plant or animal matter.

HiB - 'hotter is better', the theory that warmer adapted taxa should outperform cooler adapted taxa due to the thermodynamic constraints of temperature on biological reactions.

Isolate - a culture of bacteria derived from a single pure colony isolated on solid agar.

MTE - Metabolic Theory of Ecology, an ecological framework positing that observed patterns in ecology are fundamentally driven by metabolic rate. Also termed 'Ecological Metabolic Theory' in this thesis.

Oligotroph - an organism adapted for life under low-nutrient conditions.

OTR - operational temperature range, the temperature range between an organism's minimum non-lethal temperature and it's peak performance T_{pk}, also termed the physiological temperature range (Pawar et al., 2016). This is the temperature range within which an organism is expected to generally live.

OTU - operational taxonomic unit, a cluster of organisms grouped by similar DNA sequences, a pragmatic proxy for "species" in microbes.

Prokaryote - collective term for the simplest micro-organisms, bacteria and archaea.

Species Sorting - a mechanism by which communities are assembled, whereby the abundance of species is determined by how well they are adapted to given environmental conditons.

Succession - the process of changes in community composition over time, via mechanisms such as species sorting.

Thermal Sensitivity - the rate of increase of a biological rate with temperature is described by it's thermal sensitivity or activation energy.

TPC - thermal performance curve, obtained by measuring a biological trait over a range of temperatures. Also termed 'thermal response curve' in this thesis.

Unimodal - a curve which rises to a single peak before dropping again.

Chapter 1

Introduction

1.1 Prokaryotes, biogeochemical cyles and climate change

The fifth Intergovernmental Panel on Climate Change (IPCC) assessment report predicts that without policies to mitigate climate change, global mean temperatures in 2100 will have increased by 3.7-4.8°C relative to pre-industrial levels (IPCC, 2014). Warming on this scale is likely to have a major impact on global biodiversity (Root et al., 2003; Thomas et al., 2004; Dillon et al., 2010; Stuart-Smith et al., 2015) and indeed is already causing discernible changes to animal and plant populations (Root et al., 2003; Parmesan, 2006), although increased temperature variation may also pose an important risk (Vasseur et al., 2014).

Prokaryotes are single-celled entities, first described according to the structure of their cells, which lack a nucleus and divide by fission rather than mitosis (Stanier and Niel, 1962; Sikorski, 2015). Prokaryotes are split into two domains, 'Bacteria' and 'Archaea' (Woese and Fox, 1977; Woese et al., 1990) which display dramatically different biology, such as divergent cell membrane structures and differing metabolic pathways (Valentine, 2007). These organisms are separate from all other life on Earth, 'Eukaryotes', which are themselves likely derived from the acquisition of a bacterial symbiont by an archaeal host cell (Eme et al., 2017). Prokaryotes have been estimated at contributing between 15% (Bar-On et al., 2018) and 50% (Whitman et al., 1998) of the planet's global biomass, albeit with a large amount of uncertainty around these

estimations. These microbes are fundamental to the Earth's biogeochemical cycles (Sikorski, 2015) and therefore understanding how they respond to temperature will be important for predictions of the future environmental effects of climate change (Bardgett et al., 2008). Micro-organisms in particular may be strongly affected by warming due to the basic physiological functions of ectotherms being strongly influenced by environmental temperatures (Deutsch et al., 2008).

Prokaryotes are particularly important in driving the global carbon (C), nitrogen (N) and phosphorous (P) cycles both in soils (Sikorski, 2015) and aquatic systems (Anderson, 2018; Kuypers et al., 2018). Indeed, prokaryotes have been estimated to store an equivalent amount of C as all plants on Earth, and up to 10 times more N and P (Whitman et al., 1998). Microbial carbon, nitrogen and phosphorous cycling are all inexorably linked – changes in one cycle are likely to impact the others (Anderson, 2018; Averill and Waring, 2018). Nitrogen is the main limiting nutrient for life on Earth (Kuypers et al., 2018). Whilst di-nitrogen (N_2) is abundant in the air, this is only accessible to particular bacteria and archaea, all other life relies upon fixation of nitrogen by these prokaryotes and its conversion in to more bio-available forms such as ammonium and nitrate (Kuypers et al., 2018). In soils N is often limited, however nitrogen fixing rhizobacteria (which colonise the roots of legumes) are able to convert N_2 to ammonium (NH^{4+}) which benefits both the host plant as well as the surrounding soil due to its leakage (Vacheron et al., 2013; Sikorski, 2015; Kuypers et al., 2018). In the oceans, ammonium is again provided by N-fixing bacteria. Subsequently this is assimilated and extensively recycled by *Cyanobacteria* before being released again in more complex forms such as urea when these bacteria are grazed upon by zooplankton (Kuypers et al., 2018). In a similar way to nitrogen, phosphorous is generally abundant in soils and is provided by the continuous weathering of rocks (Sikorski, 2015). However, P is mostly found in insoluble forms, unavailable to living organisms (Sharma et al., 2013). The conversion of P from its inorganic insoluble form into a bioavailable form (orthophosphate) is mediated by bacteria (Sharma et al., 2013; Sikorski, 2015), without whom P would be mostly unavailable to plants and other organisms. Finally, prokaryotes are heavily involved in the carbon cycle, broadly through pathways which affect ecosystem CO_2 and methane uptake, and those which affect ecosystem carbon efflux (Bardgett et al.,

2008). Alongside plants, *Cyanobacteria* and purple sulfur bacteria are able to fix atmospheric CO_2 into organic carbon via photosynthesis. Other microbes are able to fix carbon through more exotic methods such as chemosynthesis (Amend and Shock, 2001) and methanotrophy (Holmes et al., 1999). The arguably greater contribution of micro-organisms to the carbon cycle however is the re-mineralisation of organic carbon and its return to the atmosphere as CO_2 and methane (Trivedi et al., 2013; Sikorski, 2015). A large body of work has investigated the potential effects of climate warming on the microbial contribution to ecosystem carbon efflux, with particular focus on whether warming will cause increased carbon decomposition by microbes, thus inducing a positive feedback loop and further climate change (Davidson et al., 2006; Sikorski, 2015; Crowther et al., 2016).

The global respiratory efflux of carbon is balanced by CO_2 fixation, primarily by land plants and phytoplankton in the oceans. However, respiration rates have been shown to respond more positively to warming than rates of photosynthesis (Yvon-Durocher et al., 2010) and therefore warming may destabilise this balance leading to comparatively increased carbon efflux versus fixation (Bradford, 2013). In addition, rates of methane efflux (a more potent greenhouse gas than CO_2) by methanogenic archaea in wetland ecosystems have been shown to respond *even more* positively to warming than aerobic respiration. The net effects of these processes are proposed to be a redistribution of biologically sequestered carbon into atmospheric carbon (Bradford, 2013). This may be expected to cause a positive feedback loop as increased atmospheric carbon (as CO_2 and methane) further warms the climate, further enhancing ecosystem respiration, etc (Bradford, 2013). Figure 1.1 shows a simplified depiction of these feedback loops.

In reality ecosystem responses such as total soil carbon flux to warming are more complex than simply microbial respiration versus autotrophic carbon fixation. The soil carbon flux response to warming represents the combined activity of microbes, plants and animals and is heavily dependant upon substrate availability (Bradford, 2013). Given that there is more organic carbon stored globally in soils than within the atmosphere and living organisms combined (Köchy et al., 2015), fully understanding the temperature response of this carbon pool is key to our understanding of the impacts of climate change. There is some agreement with the theory

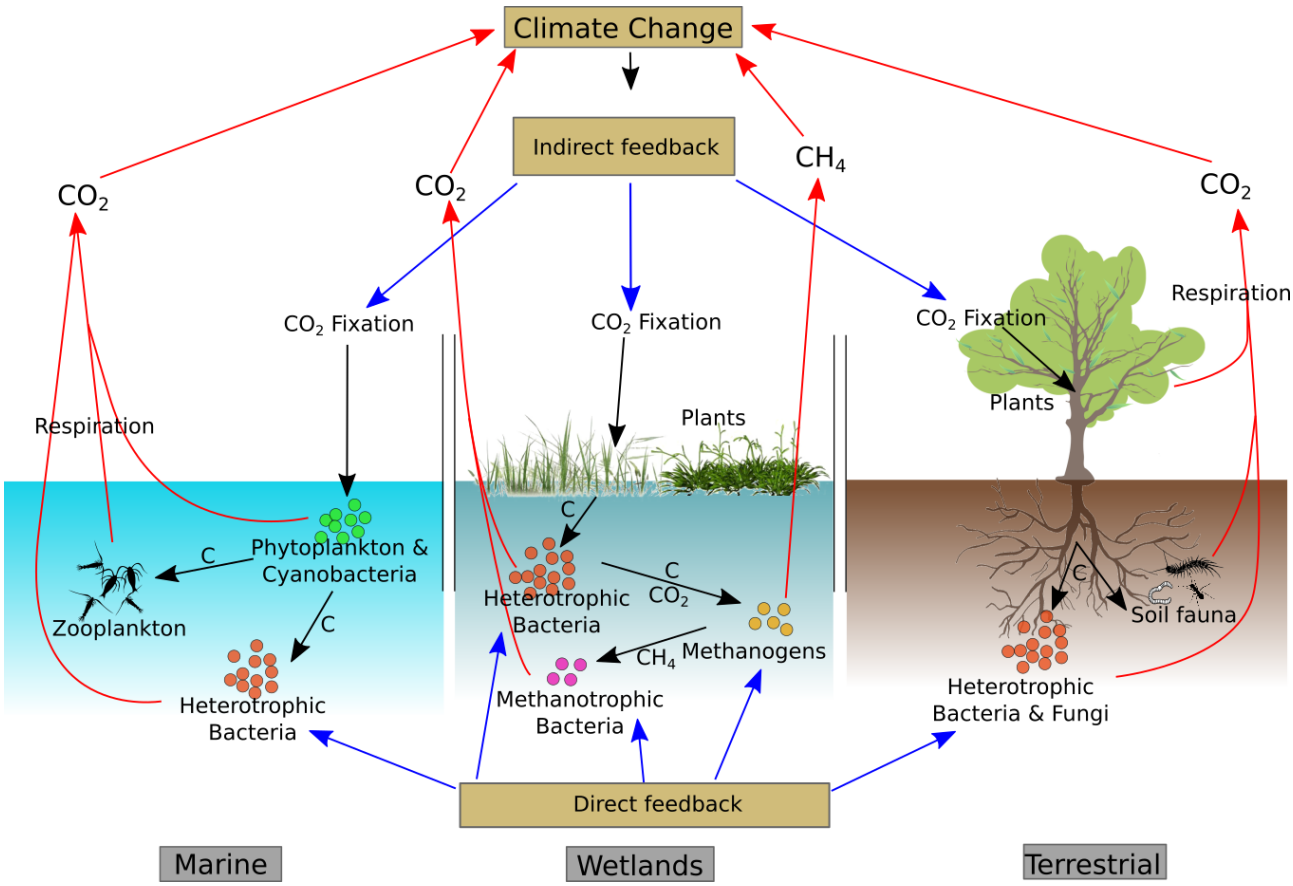

Figure 1.1: **Microbial carbon cycling feedback loops.**

This is a simplified depiction of microbial roles in the carbon cycle in marine, wetland and terrestrial (soil) systems, based on Bardgett et al. (2008); Trivedi et al. (2013); Buchan et al. (2014). Blue arrows indicate direct and indirect effects of climate change on microbial C cycling. Black arrows indicate transfer of carbon between trophic levels – 'C' simply indicates organic carbon. Red arrows indicate carbon efflux due to respiratory processes, which are likely to cause a feedback effect and enhance climate change. Direct feedbacks are those acting directly upon micro-organisms, whereas indirect feedbacks affect other organisms and trophic levels, the consequences of which trickle back to impact micro-organisms.

of a positive feedback loop, with many studies reporting increased soil respiration rates with experimental warming, e.g. Karhu et al. (2014); Crowther et al. (2016); Hicks Pries et al. (2017). The study of Crowther et al. (2016) is a particularly comprehensive analysis of 49 different field experiments across North America, Europe and Asia, showing a consistent direction of the soil carbon response across all scenarios. This is not generalised across all systems however, with some studies showing an attenuation of the respiration response over time, pointing towards acclimation or adaptation of the soil community (Luo et al., 2001). However, it has since been suggested that such attenuation of the soil respiration response is more likely due to alternative

effects such as changes in substrate availability, reduction of microbial biomass or changes to community composition (Frey et al., 2013; Karhu et al., 2014; Melillo et al., 2017), rather than direct thermal acclimation or rapid adaptation. This is further complicated by the proposition that increased soil respiration may actually be due to increased microbial growth and thus increased respiring biomass, rather than the direct physiological response of respiration rates (Zhang et al., 2013). Furthermore, counter to the data showing respiration increasing at a greater rate than photosynthesis, the meta-analysis of Lu et al. (2013) suggests that plant derived carbon influx may actually offset increased efflux, leading to terrestrial ecosystems being a weak carbon sink with climate warming. Clearly, whilst there may be a general trend in results of increased respiration with warming, more work is required to fully understand the impacts of warming on ecosystem carbon flux.

1.2 Microbial ecology, biogeography and ecophysiology

Microbial biodiversity is vast (Curtis et al., 2002). A single gram of soil is estimated to contain in the region of 10^{10} cells, comprising tens of thousands of operational taxnomic units, OTUs, the prokaryotic equivalent of "species' (Sogin et al., 2006; Sikorski, 2015). The majority of these OTUs are rare taxa, found only in very low abundance (Lynch and Neufeld, 2015). Whilst this genetic diversity is wide, much of the biology and physiology that it represents is unknown, as most OTUs remain uncultured (Steen et al., 2019). This leads to certain taxa being over over-represented in culture libraries (Floyd et al., 2005; Schloss et al., 2016) compared to their environmental abundance based on sequencing studies (Janssen, 2006; Delgado-Baquerizo et al., 2018). Therefore much of what is known about bacterial physiology and their ecological strategies is constrained to easily culturable taxa, not necessarily those things most abundant in the environment (Cobo-Simón and Tamames, 2017).

At the simplest level, there are two main factors which can contribute to patterns of microbial biogeography – environmental heterogeneity and dispersal limitation (Fierer, 2008). The control of environmental heterogeneity on dispersal unlimited micro-organisms is summarised by

the Baas-Becking hypothesis (Baas-Becking, 1934; O'Malley, 2007). This is a major tenet in microbial ecology, that for micro-organisms:

> "Everything is everywhere, but the environment selects."

This is the idea that due to global dispersal, all micro-organisms can theoretically be found anywhere, however the environment selects for which strains can proliferate in any given niche. This dispersal essentially erases the effects of previous evolutionary and ecological events and proposes that contemporary environments are entirely responsible for current patterns of microbial biogeography (Martiny et al., 2006). Hypotheses at the other end of the spectrum suggest that historical events do play a role in current microbial spacial variability via dispersal limitation and past environmental conditions (Martiny et al., 2006; Fierer, 2008). In this case, geographic distance would be a strong predictor of differences between microbial communities (Fierer, 2008).

There is DNA sequencing evidence for the propensity for global microbial dispersal, with a comparatively small proportion of bacterial phylotypes accounting for a large proportion of community biomass across all environments (Delgado-Baquerizo et al., 2018). This can also be putatively seen in the global dispersal of microbial phenotypes. Spores of thermophilic bacteria are found throughout cold ocean beds (Hubert et al., 2009), whilst benign soil samples harbour thermophiles, acidophiles and halophiles (Marchant et al., 2002, 2008; Sikorski, 2015). Similarly, functionally extremophilic communities have been isolated from freshwater ecosystems (Low-Decarie et al., 2016) and fresh-water communities have been shown to not be strongly affected by dispersal limitation (Van der Gucht et al., 2007). Despite this capacity for dispersal, there is substantial evidence that micro-organisms do indeed display distinct patterns of biogeography in terrestrial ecosystems (Martiny et al., 2006; Fierer, 2008; Delgado-Baquerizo et al., 2018; Ramirez et al., 2018). In soils there are innumerous micro-habitats caused by differences in soil structure on the micro-scale (such as porosity, particle size, nutrient levels, etc.) which dictate community composition (Ranjard and Richaume, 2001). At larger scales differences in microbial communities are driven by abiotic conditions such as carbon availability, moisture

content, pH and temperature (Griffiths et al., 2016; Hendershot et al., 2017; Delgado-Baquerizo et al., 2018).

The idea that this ecologically driven biogeography is independent of historical factors has been questioned however. Two intercontinental studies of prokaryotes in hot-springs for instance, have shown that community assemblages can be significantly differentiated by geographic distance, rather than environmental variables (Papke et al., 2003; Whitaker et al., 2003). Therefore, despite global dispersal, historical separation can still provide a strong effect on microbial community structure (Fierer, 2008). Furthermore, the traits of prokaryote communities, such as their carbon assimilation rates, are constrained by the evolutionary history of the taxa selected for by the environment (Morrissey et al., 2019). Therefore, patterns of microbial biogeography likely represent the influence of both environmental conditions and evolutionary history.

Microbial communities are not static through time and display consistent patterns of succession. This is true for long-term community assembly (Banning et al., 2011; Jangid et al., 2013) as well as repeated community assembly dynamics following environmental pertubations (Rivett et al., 2016, 2018). These assembly dynamics occur through species sorting, a mechanism whereby the abundance of taxa is related to their abilities to survive and proliferate in a given niche (Van der Gucht et al., 2007). Changes in taxa throughout succession can have profound effects on the ecological functioning of communities. In particular early succession appears to favour growth specialists which utilise abundant labile carbon sources (copiotrophs), whilst slower growing microbes which perform well under low nutrient conditions (oligotrophs) are late colonisers (Shrestha et al., 2007; Rivett et al., 2016, 2018). This is consistent with the r to K continuum and trade-off known in wider ecology (Andrews and Harris, 1986). The taxonomic groups of bacteria associated with different stages of succession are broadly consistent across timescales regardless of the sampled environment, with the phyla *Firmicutes* and *Bacteroidetes* associated with early succession, whilst other phyla such as *Actinobacteria* and *Acidobacteria* are more abundant at later stages of succession (Noll et al., 2005; Shrestha et al., 2007; Nemergut et al., 2007; Rui et al., 2009; Banning et al., 2011; Jangid et al., 2013). As such, these late-succession phyla also tend to be the more abundant constituents of the general community and play important roles in ecosystem functioning.

Climate change may have major impacts on the composition of microbial communities, driven both by temperature (Karhu et al., 2014; Melillo et al., 2017) and moisture (Hawkes et al., 2017). Allison and Martiny (2008) reviewed the literature and found that microbial composition often responds rapidly to experimental warming and that changes in community composition can affect the rates of ecosystem processes. Studies in arctic soils have also found community responses to warming, resulting in a significant change to soil nutrients over the duration (Rinnan et al., 2007, 2009). However, soil responses in these arctic studies occurred over a much longer time-scale than those of more moderate temperature systems. At a finer scale, different ecotypes of the same bacterial genus have been shown to have varied distributions across a climate gradient (Chase et al., 2018). In contrast, a study in alpine meadow soils found no overall shift of community with warming, however found that communities became more similar over time, potentially driven by increased distribution ranges of existing bacterial taxa (Li et al., 2016). Understanding changes in the microbial community with warming is important, as changes in abundance of taxa may affect ecosystem functioning (Melillo et al., 2017; Yu et al., 2018). This is important not only in soils, but also in the oceans, where the microscale community composition of prokaryotes on particle surfaces can significantly impact carbon turnover rates (Enke et al., 2018).

However, changes in ecosystem processes due to shifts in community composition are not a certainty, as microbial communities may display a large amount of functional redundancy (Wohl et al., 2004). Given the vast diversity of micro-organisms, an altered community may simply contain taxa that are functionally the same as the previous community. Alternatively, the new community may have taxa that function differently at lower levels, but result in the same overall function at the community level (Allison and Martiny, 2008). Therefore understanding not just how the composition of communities shifts in response to warming, but also the changes to ecosystem processes arising from these shifts, are key to our understanding of the impacts of climate change.

1.3 The effects of temperature on biological rates

The metabolic theory of ecology (MTE) provides a framework for understanding how temperature directly affects biological processes. MTE proposes that metabolic rate is a fundamental biological trait that governs patterns observed from organism, through population, to community and ecosystem scales. In MTE, the combined effects of body size, M and temperature, T (in K) on metabolic rate, I can be described using allometric scaling laws and the Boltzmann-Arrhenius equation (Gillooly et al., 2001; Savage et al., 2004; Brown et al., 2004):

$$I = I_0 M^{\alpha} e^{-E/kT}. \tag{1.1}$$

The allometric scaling portion of the model describes how biological traits vary with body size. I_0 is a normalisation constant, M is body mass and α is the scaling exponent. Since the 1930's, the scaling exponent for metabolic rate has generally been assumed to be conserved across organisms at $\alpha = 3/4$ (Kleiber, 1932), however more recent work suggests that scaling exponents may vary across different levels of biological organisation, with prokaryotes showing superlinear scaling of metabolic rate with size (DeLong et al., 2010). The effects of temperature are described by the Boltzmann-Arrhenius portion of the equation. Arrhenius temperature dependence describes the exponential increase of biological rates with temperature, within the operational temperature range of an organism (before temperature becomes detrimental to biological processes). Here, E is the activation energy (or thermal sensitivity) and k is the Boltzmann factor.

This theoretical framework (eq. 1.1) has been used to study how mass-specific rates vary with temperature across organisms. These have been found to vary fairly consistently with temperature across different groups of taxa, from unicellular fungi, to plants and animals (Brown et al., 2004) (as shown in fig. 1.2A) as well as alternative traits to metabolic rate such as developmental time (Gillooly et al., 2002) and maximum growth rate, r_{max} (Savage et al., 2004). Given this relationship, organisms operating at higher temperatures are expected to have higher mass-specific metabolic rates compared to those operating at lower temperatures

(Gillooly et al., 2001, 2002; Brown et al., 2004). These studies also find conserved activation energy when looking at inter-specific (across organism) responses, with a value of ∼0.65 eV for aerobic heterotrophs (Gillooly et al., 2001; Yvon-Durocher et al., 2012), although more recent work suggests that inter-specific temperature responses for prokaryotes may depart from this average (Yvon-Durocher et al., 2014; Huete-Stauffer et al., 2015; Chen and Laws, 2017).

Investigating how temperature affects an organism can be performed by simply measuring a biological trait across a range of temperatures for that species. This typically results in a unimodal response curve as performance rises with temperature to a peak before dropping due to detrimental effects of high temperature. These response curves can be used to understand the physiological temperature range of an organism as well as its thermal sensitivity. Organisms inhabiting more variable thermal environments will tend to have wider thermal niches (thermal generalists) whilst organisms inhabiting more constant niches will display a narrower temperature curve (thermal specialists), as depicted in fig. 1.2C (although Gilchrist (1995) shows that specialists may be favoured in niches with low inter- but high intra-generational variation). Although organisms tend to inhabit temperature environments below their biological peak (Deutsch et al., 2008; Martin and Huey, 2008), ongoing climate change may push organisms' habitats closer to their thermal peak, especially in the tropics where temperatures fluctuate less and thus organisms have adapted to be thermal specialists. Therefore a general understanding of how organisms are affected by temperature is necessary to understand and predict the direct impacts of climate warming on individual species as well as ecosystems as a whole.

Estimates of peak temperature, peak performance, baseline performance and activation energy can be derived by fitting models such as Sharpe-Schoolfield (Schoolfield et al., 1981), eq. 1.2, to thermal response curves:

$$B(T) = B_0 \cdot \frac{e^{\frac{-E_\mathrm{A}}{k}\cdot\left(\frac{1}{T}-\frac{1}{T_\mathrm{ref}}\right)}}{1+e^{\frac{E_\mathrm{D}}{k}\cdot\left(\frac{1}{T_\mathrm{h}}-\frac{1}{T}\right)}}. \tag{1.2}$$

This is an extension of the Boltzmann-Arrhenius equation, where simple Arrhenius temperature dependence is still used to describe the increase in rate with temperature within the operational temperature range (OTR), but extra parameters are added to describe a peak and fall in rate due to the detrimental effects of high temperatures. B is the biological rate at a given temperature, T (K), B_0 is the trait value at a low reference temperature (T_{ref}), E_{A} and E_{D} are the activation and deactivation energies (eV), which control the rise and fall of the curve respectively and T_{h} is the temperature (K) at which 50% of the enzyme units are inactive. Differentiating equation 1.2 with respect to T and solving for T allows calculation of the temperature where the peak of the curve lies (T_{pk}) and setting $T = T_{\text{pk}}$ allows peak performance of the biological trait (P_{pk}) to also be estimated. An example fit and depiction of the parameters is shown in fig. 1.2B. Equation 1.2 is a simplified formulation of the Schoolfield-Sharpe model, alternative formulations may include the effects of low-temperature enzyme inactivation (Schoolfield et al., 1981) and the simplified equation may also be reformulated to include T_{pk} as an explicit parameter (Kontopoulos et al., 2018).

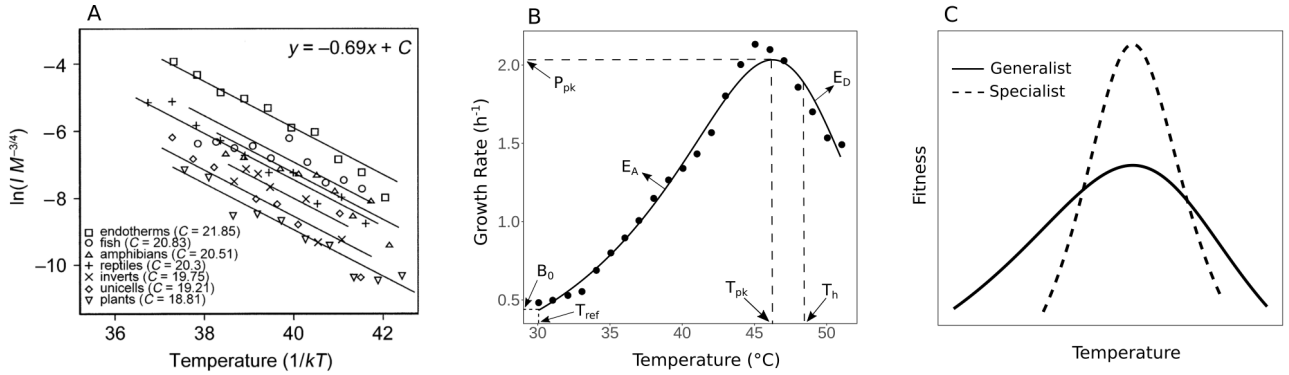

Figure 1.2: **Variation in biological rates with temperature.**

A. Figure 1A from Brown et al. (2004), regressions of logged mass-specific metabolic rates against the inverse of temperature multiplied by the Boltzmann constant, separated by taxonomic groups. This shows that across species whilst different groups of taxa may have different intercepts (B_0), their slopes (E_A) are very similar. **B.** Variation in growth rate of *Streptococcus salivarius* with temperature. Data points taken from Adamberg et al. (2003) and the Sharpe-Schoolfield model (eq. 1.2) fitted to them (line). The Sharpe-Schoolfield parameters are depicted on the plot. **C.** Depiction of differences between the TPCs of thermal generalists (solid line) and thermal specialists (dashed line).

Estimates of T_{pk}, P_{pk}, B_0 (considered a measure of baseline performance) and E_{A} are often cited through the literature in studies comparing thermal responses between species or experimental

treatments, e.g. Barneche et al. (2014); Kang et al. (2015); Molnár et al. (2013); Padfield et al. (2016). Comparative studies have also been made on large datasets comprising widely varied thermal response traits across diverse ranges of organisms, which highlight the generalities of thermal responses as well as their variation. For example, cold tolerances have been shown to be more variable between species than heat tolerance due to physiological boundaries constraining evolution of organisms to high temperature environments (Araújo et al., 2013). Other such meta-analyses have investigated the temperature responses of trophic interactions (Rall et al., 2012; Dell et al., 2014), predator-prey dynamics (Fussmann et al., 2014) and metabolic rate scaling (Ehnes et al., 2011). Average intra-specific activation energies have also been shown to be generally consistent with inter-specific activation energies as described by MTE (Dell et al., 2011). However whilst generalisations can be made across species, at the organism or population level variation is very much the norm rather than the exception (Dell et al., 2011). The generalised temperature dependencies across organisms implicit in the MTE framework have largely been derived from data from multicellular eukaryotic organisms. Testing whether the MTE predictions hold true in prokaryotic micro-organisms will lead to further insights into how these organisms respond to temperature. This may be crucial to our understanding of how ecosystems will be affected by our rapidly changing climate.

This is not to say that models based on reaction kinetics are the only viable way to quantify thermal responses. Many other models can be used to describe the shape of TPCs, based either on purely mathematical equations, or with different biological explanations (Low-Décarie et al., 2017). For example, Ratkowsky et al. (2005) showed using a thermodynamic rate model that the temperature behaviour of micro-organisms in solution closely resembles that of globular proteins. This indicates a common underlying mechanism and leads to the idea that protein stability is the key determinant of fitness at high temperatures in micro-organisms (Chen and Shakhnovich, 2010). In fact it has been suggested that protein stability governs biological growth rates at a fundamental level (Corkrey et al., 2012) and that protein thermal stability has been a unifying property in the evolution of both uni- and multi-cellular life (Corkrey et al., 2014). Molecular evolution may also be constrained by temperature, due to decreased mutational robustness at extreme temperatures (Puurtinen et al., 2015), leading to lower mutation

rates at temperatures where proteins are less stable. This is in contrast to the MTE framework which posits that rates of molecular evolution are controlled by temperature effects on metabolism (Gillooly et al., 2005), not protein stability. In either case, future rates of speciation may also be influenced by global temperature change.

1.4 Adaptation and acclimation of thermal performance

In addition to understanding how organisms are directly affected by their temperature environment, understanding how organisms adapt or acclimatise to differing thermal conditions may also yield important information on how organisms may respond to climate change (Hoffmann and Sgrò, 2011). Throughout this thesis, acclimation is defined as physiological responses to changes in environmental variables, via phenotypic plasticity, and adaptation as evolutionary responses to changes in environmental variables, i.e. selection for particular genotypes or selection for beneficial mutations. This is important, as adaptation or acclimation of microbes to new temperatures may play a role in how ecosystem processes respond to climate change (Bardgett et al., 2008; Hoffmann and Sgrò, 2011).

A major piece of work on bacterial temperature adaptation has been a long term evolutionary experiment led by Richard Lenski, where *E. coli* lines were evolutionarily adapted to different thermal regimes (Bennett et al., 1990). Many results from this experiment have shown that some bacteria can adapt to temperature in ways that improve evolutionary fitness at new temperatures over comparatively short absolute timescales (Bennett et al., 1990, 1992). Furthermore, similar experimental designs have shown that divergence in thermal optima of different groups has little effect on the limits of their thermal niches (Bennett and Lenski, 1993) and that thermal distance from an ancestral environment is not indicative of exceptionally high rates of evolutionary change (Mongold et al., 1996); i.e. growing bacteria in a very divergent thermal niche to their ancestral environment doesn't necessarily result in faster adaptation than growing them in a thermal niche closer to their ancestral ancestral environment.

Whilst adaptive laboratory evolution (ALE) like this has now been commonplace for over 25

years, the recent fall in cost of sequencing technologies has allowed for the identification of specific mutations in laboratory strains evolved to stresses in many recent studies (Dragosits and Mattanovich, 2013). Bacterial ALE experiments tend to have been restricted to work on *Escherichia coli* (see Dragosits and Mattanovich (2013)), however there have been recent studies on other bacterial species which include evolving them to higher temperature optima, e.g. *Lactococcus lactis* (Chen et al., 2015) and *Corynebacterium glutamicum* (Oide et al., 2015). In both cases, microbial genomics methods were used to identify genes and genomic regions with mutations affecting thermal tolerance in heat adapted strains. In summary then, whilst some strains of lab bacteria have been shown to evolve rapidly to temperature change, it is not known whether this rapid adaptation is a trait likely to be shared more generally across all bacteria.

The thermal responses of alternative metabolic traits to growth (fitness) may also be modified through adaptive processes. Padfield et al. (2016) evolutionarily adapted the phytoplankton *Chlorella vulgaris* to higher temperature and investigated trade-offs arising between respiration and photosynthesis from this adaptation. They found that the higher temperature adapted phytoplankton down-regulated respiration to more efficiently allocate fixed carbon to growth. A follow-up study however showed that up-regulation of photosynthesis can provide a similar outcome (Schaum et al., 2017). These adaptive responses show that emergent processes such as up- or down-regulation of traits in response to environmental conditions may play as important a part in defining thermal responses as the purely physical impacts of temperature on biochemical processes.

Acclimation through phenotypic plasticity is another important consideration in an organisms response to temperature. Leroi et al. (1994) used *E. coli* to test the beneficial acclimation hypothesis (BAH) - the assumption that acclimation to a particular environment always confers a fitness advantage in that environment - and determined the assumption to be false. Briefly, acclimation of *E. coli* to 32°C improved fitness at 32°C, relative to acclimation at 41.5°C, however acclimation to 41.5°C reduced fitness at 41.5°C, relative to acclimation at 32°C. Acclimation to 41.5°C did improve survival at 50°C (a lethal temperature) - associated with induction of stress hormones. Potentially induction of stress hormones during 41.5°C acclimation reduces

fitness when they are not required to prevent lethal damage. The BAH has been tested many times since, generally following the same standard procedures (Hoffmann, 1995; Huey et al., 1999; Angilletta Jr., 2009). In some cases, acclimation confers an advantage however in others no advantage is seen (Angilletta Jr., 2009). Overall, results show that BAH cannot be generalised, and a more complex understanding of acclimation is required (Angilletta Jr., 2009). For instance, beneficial acclimation may be seen in situations where the acclimation temperature coincides with the historical temperature in which the bacteria evolved (Bennett and Lenski, 1997), i.e. counter-intuitively a strain acclimated to its historical evolutionary temperature of 32°C may have a competitive advantage at 37°C compared to if it had been acclimated to 37°C. (Wilson and Franklin, 2002) however challenged these tests of beneficial acclimation, questioning whether they were actually analyses of the adaptive significance of developmental plasticity rather than the BAH and also suggesting that the inclusion of stressful conditions confounded analyses of the benefits of adaptation.

Furthermore, there remains a question over whether these sorts of experiments in bacterial populations can really be considered 'acclimation', and not something more like short-term adaptation. In the studies of Leroi et al. (1994) and Bennett and Lenski (1997), the acclimation time allowed for the bacteria to undergo a number of cell generations. Whilst this may be too few for a new beneficial mutation to arise and selection pressures to result in its fixation within the population, the fact that the population has undergone cell generations allows for selection for advantageous genotypes already present within the population (if the original population does not consist of identical genetic clones). Thus it is hard to consider that studies involving 'acclimation' periods in bacteria truly are such, and do not involve some level of population selection and thus 'adaptation'. This is a particularly important consideration with respect to generating TPCs for population level responses in bacteria. When culturing bacteria to test growth rate, they may undergo several rounds of division before the exponential growth measurements are actually made. This may lead to differing levels of acclimation or short-term adaptation between experiments and thus methods to standardise these experimental procedures are required for accurate comparisons of TPCs within and between studies.

1.5 Linking growth and metabolic traits

As microbial communities respond to climate change through adaptation, acclimation and species sorting processes, shifts in trade-offs that may occur between population growth, carbon efflux and carbon sequestration will have important impacts on future climate change feedbacks. Microbial turnover can be described through population growth rates (r or μ). In culture, this is an easily measurable and quantifiable trait, which can be used to produce a thermal response curve. Bacterial growth in batch culture follows a distinct set of phases; lag phase, exponential phase, stationary phase, death phase and long-term stationary phase (Rolfe et al., 2012) (fig. 1.3A). Upon inoculation into new media, bacteria quickly sense their surroundings and if conditions are favourable, prepare for exponential growth. During the lag phase, a suite of transcriptional machinery is activated, including genes involved in nutrient uptake and metabolic changes (Rolfe et al., 2012), as cells prepare for exponential growth. As well as taking up nutrients, bacteria have been shown to accumulate ATP during the lag phase, with cellular ATP levels peaking during the exponential growth phase (Haddix et al., 2008). During the exponential growth phase, bacteria divide at a constant rate, the population doubling with each generation. When the carrying capacity (K) of the media is reached, growth slows and the number of cells in the culture stabilises, beginning the stationary phase. Models have been devised to describe sigmoidal bacterial growth, based on the idea that in a closed habitat (i.e. batch culture), growth is controlled by two primary elements - the number of cells present and the remaining available resources (Peleg et al., 2007). Modelling of bacterial growth has been particularly prevalent in food microbiology studies for the purpose of food safety and a number of functions to describe the growth curve have been proposed (Zwietering et al., 1990; Baranyi and Roberts, 1994; Buchanan et al., 1997; Esser et al., 2015).

Microbes trade-off efficiency against growth rate, this is r vs K selection (Andrews and Harris, 1986). The fastest growing micro-organisms (r-specialists) supplement aerobic respiration with less efficient pathways to generate more ATP (the energy currency of cells) in the short-term, but with less total yield from the resources used (Pfeiffer et al., 2001). This can be seen in their carbon use efficiency (CUE), the proportion of total carbon uptake allocated to growth

(Keiblinger et al., 2010; Manzoni et al., 2012; Geyer et al., 2016), which for r specialists is expected to be low. The slower growing micro-organisms (K-specialists) are expected do so more efficiently (high CUE), and therefore achieve higher yield in the long term. This trade-off between growth and yield can be seen from single strains (Chakraborty et al., 2016) through to whole communities (Malik et al., 2019). These strategies can be partitioned further, with each associated with a number of different traits as well as growth efficiency (fig. 1.3B). K-specialists are associated with growth under nutrient limited conditions (oligotrophs) (Fierer et al., 2007; Ho et al., 2017), in spatially structured environments such as biofilms or soils (Lipson, 2015). At the other end of the spectrum, r-specialists tend to be adapted to high nutrient conditions (copiotrophs) and non-structured, planktonic environments, such as aquatic ecosystems (Lipson, 2015). Given the appropriate conditions, populations of both strategies can stably co-exist (Beardmore et al., 2011). Differences in temperature can also have an influence on these growth strategies, with higher temperature favouring growth strategists both within taxa and across communities (Lipson, 2015), implying a thermodynamic basis for the partitioning of these strategies.

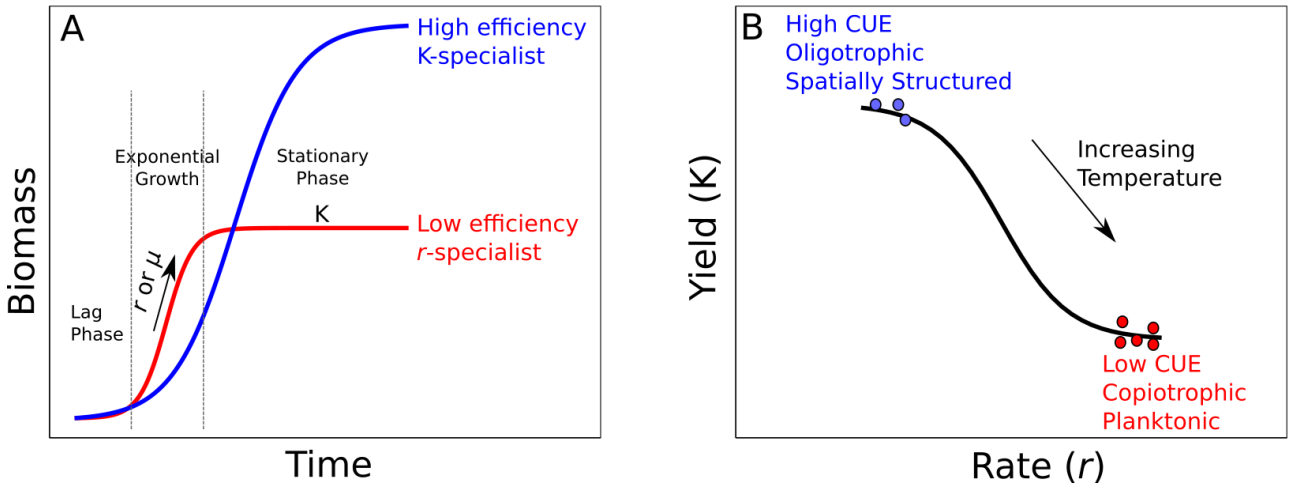

Figure 1.3: **Microbial growth/yield trade-offs.**

A. Example microbial growth curves, showing a growth specialist (red) and a yield specialist (blue). Phases of growth are shown for the growth specialist curve as well as the meaning of the r (or sometimes μ) and K parameters. **B.** Example partitioning of taxa (represented by coloured points) between growth and yield specialisms. Temperature can alter growth strategies both within taxa and across communities. Panel B adapted based on Lipson (2015).

Under MTE, the responses of more emergent traits (such as growth rate) to temperature, are

expected to be based upon the underlying thermal response of metabolic rate (Brown et al., 2004). Indeed, fitness is often used as a proxy for metabolic rate in the literature. However, whether the growth response to temperature is really a reflection of the underlying metabolic rate TPC is a question still debated (Lopez-Urrutia and Moran, 2007; Manzoni et al., 2012), with different traits showing potentially different distributions of thermal sensitivity (Dell et al., 2011). Despite this lack of certainty, many models for microbial population growth rate have been devised which are based on metabolic processes, e.g. Kempes et al. (2012); De Jong et al. (2017); Luhring and Delong (2017). This does seem reasonable from a theoretical perspective, as stated by Luhring and Delong (2017):

> "...(1) metabolic rate is a temperature-dependent process determined by the combined effects of temperature on enzyme stability and reactant kinetics (DeLong et al., 2017), (2) biomass production is fueled by metabolism (Verity, 1985; Ernest et al., 2003; Delong and Hanson, 2009), and (3) reproduction is limited by biomass production. Thus, population growth rate depends on biomass production, biomass production depends on metabolism, and metabolic rate depends on temperature-dependent processes..."

Luhring and DeLong go on to acknowledge however that organisms may vary the proportion of metabolism that is given to either maintenance or production of new biomass. If these proportions vary with temperature (or indeed other biotic or abiotic conditions), then the population level growth response may not be a simple reflection of the individual level metabolic response to temperature. This distinction is important because whilst growth rates are the commonly used metric to define a micro-organism's response to temperature, when assessing the impacts of climate change, we may be more concerned with changes to carbon flux and therefore the thermal sensitivity of respiration rate, not growth rate *per se* (although changes to growth rate may affect fluxes through changes in microbial abundance).

Thermal responses for metabolic fluxes can be found in the literature, however not to the same extent as growth rate TPCs, especially for population level responses. In order to understand

whether growth rate TPCs reflect those of the underlying metabolic rate, direct comparisons between these traits are required. However, studies measuring both of these thermal responses are largely lacking from the literature. That leaves comparison of these traits across different studies, however there is often a time-scale mismatch of thermal acclimation in these data. That is, growth rates by their nature require measurement over a reasonable time-period in which growth is observable, allowing a level of acclimation, whilst respiration rate is an acute response usually measured over shorter time where an acclimation period may or may not be given, e.g. Padfield et al. (2016).

Prokaryotic organisms display a wide range of diversity in metabolic processes utilised, which differ broadly in the Gibbs free energy yield of those reactions (Amend and Shock, 2001), with the Gibbs free energy of metabolic processes (and consequently ATP production) expected to directly affect the growth of bacteria (Andersen and von Meyenburg, 1980; Roden and Jin, 2011). Work by Yvon-Durocher et al. (2014) seems to agree with this, showing consistent thermal sensitivity between methanogenesis and growth rates in methanogenic archaea. Furthermore, these thermal sensitivities are considerably higher than the 0.65eV MTE generalisation based on aerobic respiration. This suggests a tight coupling between temperature sensitive metabolic rate and growth, however this has not been replicated for aerobically growing prokaryotic populations. Given that ATP may be considered an intermediate link between metabolism and growth, methods to directly quantify ATP may offer a measurement to understand the relationship between these traits. Simple assays can be used to measure ATP concentrations in microbial populations (e.g. see Haddix et al. (2008)) and combined with flow-cytometry methods, these can be used to infer the cellular content of ATP. Extending these measurements to thermal responses, alongside measures of growth and respiration, may yield powerful data to more fully understand growth rate thermal responses at a more fundamental metabolic level.

Work in phytoplankton has shown a temperature dependence for *allocation efficiency* — the proportion of net carbon flux (i.e. phytosynthesis minus respiration) which is allocated to growth (García-Carreras et al., 2018). Similarly, the CUE of heterotrophic bacteria is expected to vary with temperature (Schimel and Weintraub, 2003; Allison et al., 2010; Allison, 2014). Indeed, variation of CUE with temperature has been investigated numerous times in natural

systems (Rivkin et al., 2001; Apple et al., 2006; Dijkstra et al., 2011; Manzoni et al., 2012; Hagerty et al., 2014; Öquist et al., 2017; Qiao et al., 2019). Despite some variation in results, likely due to differences in methodology between studies (Sinsabaugh et al., 2013), these papers tend to converge on a decrease in CUE with temperature, although there is a question of whether the effects of temperature and nutrient availability can be disentangled in environmental data (Lopez-Urrutia and Moran, 2007). A similar result has also been shown in culture (Lehmeier et al., 2016), although the experimental design makes these results difficult to interpret, as growth remains at a fixed rate due to the chemostat method employed. For these efficiencies to vary with temperature, the implication is that growth and respiration must display different thermal responses (otherwise the efficiency would be invariant with temperature). A decrease in CUE with temperature for example is proposed to be due to microbial respiration displaying stronger thermal sensitivity than growth (Schimel and Weintraub, 2003; Allison et al., 2010; Allison, 2014). This observation has been suggested to be due to either increased maintenance energy demands (Manzoni et al., 2012), or increased energy spilling (waste metabolism) at higher temperatures (Bradford, 2013). This does not appear consistent with our expectations from MTE, however whilst metabolic rate may fundamentally depend on temperature, population growth is a more emergent process, whose thermal response may depend on many other factors. If organisms shift their allocation of resources between growth and maintenance depending on their environmental conditions, then the thermal response of this trait may appear uncoupled to that of respiration.

Overall, much remains unclear about the responses of metabolic traits to temperature and their relationship to the growth response. In particular aerobic respiratory TPCs are generally lacking from the prokaryotic literature, whilst tests of changes in efficiency with temperature have largely been constrained to natural systems rather than in culture, where other biotic and abiotic conditions can be more readily controlled and accounted for. Therefore further work is necessary to fully understand the link between growth and metabolic responses to temperature.

1.6 Linking organism responses to ecosystem processes

Measurements of individual (or in the case of microbes, population level) thermal performance are regularly made. Our capacity to predict ecosystem responses to climate change based on individual performance depends upon these processes scaling up in a simple manner (Enquist et al., 2003; Osmond et al., 2004). However, recent work suggests that temperature sensitivity may vary across levels of biological organisation, from the molecular level to the population level (Rezende and Bozinovic, 2019). Furthermore, the thermal sensitivity of microbial respiration may vary across scales, and with differing environmental conditions (Zhou et al., 2009; Zhou and Zhou, 2012). If thermal sensitivity is not consistent between levels of organisation, then the ecosystem flux response to temperature may not be as simple as a linear combination of each constituent species' thermal response.

Despite potential variation at different scales, it has been demonstrated that ecosystem fluxes thermal responses may indeed be consistent with the underlying temperature dependence of respiration at the individual level (Enquist et al., 2003; Yvon-Durocher et al., 2012). This has been the basis for various studies applying MTE principles to ecosystem flux (Enquist et al., 2003; Allen et al., 2005; Yvon-Durocher et al., 2012; Schramski et al., 2015). In these models, it is proposed that individual level metabolic rates control the flow of carbon through ecosystems, but the thermal sensitivity of these metabolic rates are usually parameterised by a single average value. We know however that species can differ broadly in the distribution of their thermal sensitivities (Dell et al., 2011), and that differences in thermal sensitivity can have major impacts on ecosystem flux (Yvon-Durocher et al., 2014). Extending MTE-based models of ecosystem flux to account for differences in thermal sensitivity between taxa based on their relative abundances may therefore be a valuable tool in predicting future responses to climate change. Furthermore, if thermal sensitivities vary systematically, then changes in taxa due to climate change may have profound impacts on the community or ecosystem thermal response. Therefore, understanding not just whether different ecological levels of biological traits respond similarly to temperature, but what the distributions of these thermal sensitivities are will be important for future models linking individual metabolism to ecosystem fluxes.

I propose therefore that in order to understand the responses of ecosystem processes to climate change, we must first understand the direct responses to temperature of the microbial constituents within those ecosystems. Thus far however, the responses of prokaryotes to temperature, whilst abundant in the literature, have been largely neglected in tests of metabolic theory generalisations. As well as the direct responses of microbes to temperature, I propose that in order to predict ecosystem responses we need to know how community composition may change through species sorting processes. This requires an understanding of the extent of functional redundancy present within microbial communities with respect to temperature, a subject which has not been tested. Finally, I propose that ecosystem function depends on the balance between carbon efflux and sequestration and thus the mechanistic link between growth and respiration. This balance can be incorporated into models as carbon use efficiency (CUE), however how CUE varies with temperature as well as the mechanistic basis for this variation remains in question. With a greater understanding of these issues, better predictions can be made about how ecosystems will respond to climate change.

In this thesis I aim tackle the issues raised here on multiple fronts. I investigate the direct responses of temperature on microbial rates through a meta-analysis of TPCs. I link these direct responses to changes in community responses through a species sorting experiment. I investigate the effects that these results may mean for community functioning by examining how microbial growth strategies may vary with temperature. This is further linked to ecosystem functioning via experimental tests of variation in carbon use efficiency with temperature.

1.7 Thesis Outline

This thesis combines laboratory experiments with meta-analyses and mathematical modelling to investigate how prokaryotes are affected by the temperature of their environment and how they respond to temperature change through a combination of acclimation, adaptation, and species sorting, resulting in community-level changes. For each of these processes, I address the implications for ecosystem responses in the face of global climate change.

Given the importance of prokaryotes to global biogeochemical cycles, their overwhelming biodiversity and the apparent ease of testing their thermal responses, it is surprising that most tests of questions arising from MTE have been constrained to small datasets of eukaryotes. The first piece of work presented (**Chapter 2**) is therefore a global meta-analysis to investigate the generalisations underpinning prokaryotic responses to temperature. I digitized hundreds of thermal performance curves from the literature and fitted the Sharpe-Schoolfield model (eq. 1.2) to compare TPC parameters across taxa. I ask whether biological rates rise with temperature across-species following a universal temperature dependence predicted by Metabolic Theory of Ecology and whether the thermal responses of prokaryotes differ systematically from those of eukaryotes. Subsequently, I make comparisons between thermal sensitivities of growth rates and metabolic fluxes, and using a simple model consider the implications for ecosystem carbon flux predictions arising from these results.

Data collection for the meta-analysis revealed that there are very few cases where TPCs from multiple different prokaryotic taxa have been experimentally measured in the same study. This is a potential caveat of the meta-analysis approach, as growth conditions are not identical across studies, potentially affecting TPCs from different studies. I also found that studies directly comparing TPCs of population growth rate to metabolic traits (such as respiration rate) are almost entirely lacking from the literature. Furthermore, the experimental methods employed for studies which do include multiple measurements preclude these traits from being measured at the same time-scale, potentially affecting TPC comparisons through a mismatch in acclimation times. In **Chapter 3** I therefore develop high-throughput methodology for generating TPCs for different traits concurrently, for multiple strains of bacteria all under equivalent growth conditions. This method development allowed me to produce potentially the largest prokaryote TPC dataset from a single experimental study (**Chapter 4**), which I use to answer further questions in **Chapters 4** and **5**.

Microbial communities are expected to display a large amount of functional redundancy (Wohl et al., 2004), however I could find no studies which have directly investigated redundancy of temperature fitness – i.e. whether communities contain taxa specifically adapted to different temperatures. This may have impacts for climate change and whether warming will promote

shifts in communities to already extant warmer-adapted taxa. In **Chapter 4** I therefore design a species sorting experiment to isolate different strains of bacteria under different temperature conditions, from the same ecological patch (soil at Silwood Park). Using the methods developed in **Chapter 3**, I measure growth rate, respiration rate and ATP content across a range of temperatures for > 50 of these environmental isolates, in order to determine their TPC parameters. I use this data to ask whether latent functional diversity in soils extends to phenotypic variation in temperature fitness and whether community change is likely to be mediated by thermal specialists or generalists. Subsequently I look more deeply at differences in ecology between isolated strains, in particular focussing on differences between r and K selected taxa. Based on these results, I discuss the effects of climate warming on potential shifts in taxa and community function.

In **Chapter 5** I use the data generated in **Chapter 4** in order to make direct comparisons between the thermal responses of microbial growth and respiration rates. I ask whether these traits tend to respond to temperature in the same way, or whether they differ systematically and why. Subsequently, I use this data to investigate how bacterial carbon use efficiency (CUE) may vary with temperature. This plugs an important gap in the literature with respect to how the thermal response of emergent traits such as growth rate are related to temperature effects on more fundamental metabolic processes. I discuss what the impacts of variation in these traits, and thus the thermal response of CUE, may have with respect to climate warming.

Finally, in **Chapter 6** I tie these results together and discuss why linking the responses of population growth, community composition and metabolic functioning to temperature is important for our understanding of how ecosystem processes may respond to climate change.

1.8 Publication Status

Chapter 2 has undergone a second round of reviews for publication in *Nature Communications* and now requires minor revisions before resubmission.

Chapter 2

Metabolic rates of prokaryotes may inevitably rise with climate warming

2.1 Abstract

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, I find that maximal growth rate at thermal optimum increases with temperature for mesophiles (temperature optima $\lesssim 45$°C), but not thermophiles ($\gtrsim 45$°C). Furthermore, short-term (within-day) thermal responses of prokaryotic metabolic rates are typically more sensitive to warming than those of eukaryotes. Given that climatic warming will mostly impact ecosystems in the mesophilic temperature range, I conclude that as microbial communities adapt to higher temperatures, their metabolic rates and therefore, CO_2 production, will inevitably rise. Using a mathematical model, I illustrate the potential global impacts of these findings.

2.2 Introduction

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 (Williams et al., 2008; Dell et al., 2011; Huey et al., 2012; Pawar et al., 2015). Because fundamental physiological rates of ectotherms are directly affected by environmental temperature (Huey et al., 2012; Gillooly et al., 2001; Brown et al., 2004), climatic warming may be expected to lead to ectotherm communities with higher metabolic rates on average (Deutsch et al., 2008; Huey et al., 2012). 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 (Whitman et al., 1998), and are the primary end-users of the majority of net primary production (Rivkin et al., 2001; Raich and Potter, 1995). Therefore, climate-driven changes in prokaryotic metabolic rates are expected to significantly alter ecosystem productivity, nutrient cycling, and carbon flux (Raich and Potter, 1995; Paerl and Pinckney, 1996; Bano et al., 1997; Rivkin et al., 2001; Joergensen and Wichern, 2008; Bardgett et al., 2008). Indeed, increased carbon efflux (CO_2 emission) has been observed in experimental measures of soil CO_2 loss to warming (Crowther et al., 2016; Hicks Pries et al., 2017), as well as the responses of other microbial metabolic processes to increased temperature such as methanogenesis (Yvon-Durocher et al., 2014). 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 years or months, years or longer) evolutionary adaptation (Hoffmann and Sgrò, 2011; Huey et al., 1999; Visser, 2008) 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 (Yvon-Durocher et al., 2012). 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 prokaryotyes *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 (T_{pk}), before decreasing as high temperature becomes detrimental to metabolic or cellular processes (Huey and Kingsolver, 1989; Dell et al., 2011) (Fig. 2.1C). The T_{pk} for maximal population growth rate (r_{max}, 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) (Huey and Kingsolver, 1989; Angilletta Jr., 2009). The Hotter-is-Better (HiB) hypothesis posits that trait performance at T_{pk} (henceforth denoted by P_{pk}) 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. P_{pk} increases with T_{pk} (Fig 2.1A) (Angilletta Jr., 2009; Huey and Kingsolver, 1989; Angilletta et al., 2010). Thus 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) (Gillooly et al., 2001; Brown et al., 2004; Savage et al., 2004). However, whether the HiB hypothesis holds across organisms and environments is a question that is still debated (Angilletta et al., 2010; Phillips et al., 2014; Kutcherov, 2016; DeLong et al., 2018). 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 (Fig 2.1B) (Angilletta et al., 2010). Intermediate scenarios are also possible, where adaptation of optimal trait performance or fitness is only partially constrained thermodynamically (Fig 2.1C). Moreover, trade-offs between protein rigidity and activity at high temperatures may in fact cause hot-adapted organisms to display depressed maximal fitness (P_{pk} decreasing with T_{pk}) (Pucci and Rooman, 2017). Indeed, the existence of thermal constraints leading to an upper limit of prokaryotic growth rates has been shown recently (Corkrey et al., 2016, 2017). However a comparison of short- and long-term (HiB) responses of prokaryotic populations has never been made, nor the potential effects of responses at different time-scales on ecosystem fluxes studied.

Under MTE, the global thermodynamic constraint is expected to center around 0.65eV (Gillooly et al., 2001; Brown et al., 2004). This is the long-term, inter-specific (across-species) thermal sensitivity around which species are expected to evolve, here termed E_L (see Fig. 2.1A). Mean intra-specific thermal sensitivities (i.e. acute organism or species-level responses, here termed E_S) have been found to be very similar to this value, although the distribution is right-skewed with a median value of ~0.55eV (Dell et al., 2011). However, these values are derived from datasets which have largely or entirely excluded prokaryotes. Previous work on sub-groups of prokaryotes — cyanobacteria (Chen and Laws, 2017) and methanogenic archaea (Yvon-Durocher et al., 2014) — 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 (Schramski et al., 2015).

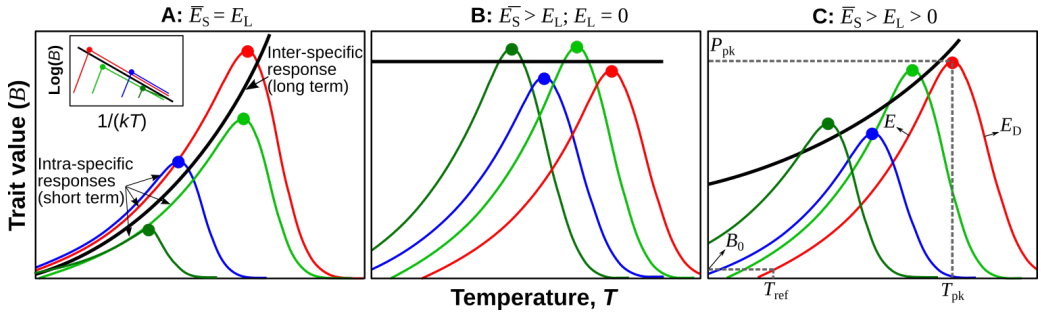

Figure 2.1: **Three alternative hypotheses for the short- vs. long-term responses of thermal performance curves of a fitness-related metabolic trait in response to environmental warming.**

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 (short-term) activation energy ($\bar{E}_S$) is statistically indistinguishable from the inter-specific (long-term) activation energy of the group of organisms (E_L), and both are greater than zero. See methods for more details on the the definition and estimation of $\bar{E}_S$ and E_L, 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 T_{pk} is on average the same ($E_L = 0$). Alternatively the same effect of $E_L = 0$ 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 $E_L > 0$ but significantly less than $\bar{E}_S$. Panel C also illustrates the the Sharpe-Schoolfield TPC model parameters (eqn. 2.1, Methods).

Here I build and analyse a global dataset of TPCs in bacteria and archaea to quantify general patterns in both the short-term (intra-specific) response, and to test whether the HiB hypothesis holds (long-term, inter-specific response) within and across taxonomic and functional groups adapted to different temperatures (Fig. 2.1). These data go far beyond the scope of previous tests of the HiB hypothesis with or without microbes (Angilletta et al., 2010), 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 archaeal phyla, Euryarchaeota and Crenarchaeota; Fig. 2.4). In total I compare 542 growth rate TPCs and an additional 54 metabolic flux TPCs, spanning 482 unique prokaryotic strains. In order to test the generalisations around global thermodynamic constraints I also assemble a dataset of 381 eukaryote TPCs spanning aquatic and terrestrial autotrophs (Appendix Figure A.4), for direct comparison to the prokaryote TPCs. I find that HiB holds for mesophilic bacteria, but not thermophiles, and that mesophilic archaea also show long-term increases in rate with temperature, although the coupling of short- and long-term responses is less clear for these prokaryotes. I also show that prokaryotes tend to have generally higher thermal sensitivities than eukaryotes and conclude that as global temperatures increase, carbon efflux from the prokaryotic components of ecosystems is likely to increase at a greater rate than carbon efflux from eukaryotes.

2.3 Methods

2.3.1 Data Collection

I compiled a dataset 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. 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 'characterisation', 'isolation', 'nov.', '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 (Huwaldt, 2015) was used to extract data points, including error bounds when reported. The 'Taxize' R package (Chamberlain and Szöcs, 2013) 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, doubling time $t_d = \ln(2)/\mu$ was used 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 542 prokaryotic growth rate TPCs were collected.

Although growth rate data as a measure of fitness was primarily collected in order to test HiB, 54 TPCs covering various metabolic fluxes were also collected for comparison to growth rate TPCs. The complete prokaryote dataset comprises 596 TPCs from 482 unique prokaryote strains across 239 published studies.

Finally, thermal response data for respiration rates in autotrophs were complied from the literature using the same methods for digitisation and data collation as for the prokaryote dataset. In total this autotroph dataset 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).

2.3.2 Biological replicates and pseudoreplicates

I 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-Schoolfield 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 the dataset. 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 dataset did contain organisms identified as the same species grown under similar conditions. Given the slower generation times of eukaryotes and thus slower species divergence I do not consider these as representative of different strains, as I do for prokaryotes, and thus consider them pseudoreplicates. To remove pseudoreplicates, I compared Sharpe-Schoolfield 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.

2.3.3 Model Fitting

To each TPC in the dataset, I fitted a modified Sharpe-Schoolfield model (Schoolfield et al., 1981) (eq. 2.1):

$$B(T) = B_0 \frac{e^{\frac{-E}{k}\cdot\left(\frac{1}{T}-\frac{1}{T_{\text{ref}}}\right)}}{1+\frac{E}{E_{\text{D}}-E}e^{\frac{E_{\text{D}}}{k}\left(\frac{1}{T_{\text{pk}}}-\frac{1}{T}\right)}} \tag{2.1}$$

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}. I 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 (Kontopoulos et al., 2018).

Each strain's TPC has a potentially different T_{pk} and P_{pk}. Compiling these values across strains yields an inter-specific thermal response curve (Fig 2.1). TPCs without a peak are thus excluded from this analysis. I fit the Boltzmann-Arrhenius equation (eq. 2.2, essentially the numerator in eq. 2.1) to these peak values to calculate inter-specific activation energy. To account for uncertainty in the original Sharpe-Schoolfield model fits to the intra-specific curves, I fitted Boltzmann-Arrhenius using a weighted regression (see accounting for uncertainty).

$$B = B_0 e^{-E/kT} \tag{2.2}$$

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

2.3.4 Testing the Association Between Thermal Optimum and Culturing Temperature

From the information collected for each thermal response curve, I extracted data on the routine culturing conditions of each strain prior to the experiment. When organisms were provided by a cell culture bank, this temperature is simply the temperature that the strains were cultured at by the experimentalists prior to use, however in studies of novel organisms, this temperature was often also the temperature that was used during isolation of the organism. I test whether the slopes for bacteria and archaea deviate from a 1:1 line by asking whether the confidence intervals of the slopes include 1.

2.3.5 Comparing Short- and Long-term thermal responses

I determined whether Hotter-is-Better by testing whether the activation energies from intra- (short-term) and inter-specific (long-term) TPCs were (statistically) significantly different. In order to provide a comparison between intra- (E_{S}) and inter-specific (E_{L}) activation energies, I used bootstrapping to generate confidence intervals (CIs) around the mean in each case. To

provide boostrapped CIs for E_L 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 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.

I then determined whether the data was consistent with either of the three hypotheses (Fig. 2.1) by comparing the overlap of confidence intervals of the relevant E estimates. First, I tested whether $\bar{E}_S$ was greater than zero (null hypothesis that the CI includes zero). Second, I tested whether E_L was greater than zero (null hypothesis that the CI includes zero). Finally, if both $\bar{E}_S$ and E_L were positive, I tested whether they were significantly different to each other (null hypothesis, that the CIs for $\bar{E}_S$ and E_L don't overlap). Under a HiB scenario, P_{pk} will increase with T_{pk} 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 $\bar{E}_S$ and E_L 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_L will be close to zero (CI for E_L 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_L will both be positive, but with $\bar{E}_S$ being significantly greater than E_L (i.e. $\bar{E}_S > E_L > 0$)

Accounting for Statistical Uncertainty

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 I extracted the E and P_{pk} point estimates as well as the covariance matrix. I then sampled 1,000 times from a bivariate distribution accounting for the covariance, producing 1,000 model parameter combinations. I used these parameters to gener-

ate 1,000 different Sharpe-Schoolfield curves, providing a distribution of E and μ_{pk} from which I 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 strains to calculate $\bar{E}_{\mathrm{S}}$, I took weighted arithmetic means of E to account for uncertainty in the original fits, where $Weight = 1/(SD_{\mathrm{E}}+1)$. Similarly, when fitting Boltzmann-Arrhenius, I apply a weighting to μ_{pk} where $Weight = 1/(SD_{\mu}+1)$.

Applying these weightings does not alter the main results I obtain from this study in terms of whether the HiB hypothesis is accepted or not for different groupings, however I felt that it was important to acknowledge and account for error in the underlying Schoolfield fits so that the results were not skewed by poor parameter estimates from questionable fits, hence this step was included. Appendix Figure A.2 illustrates the differences between $\bar{E}_{\mathrm{S}}$ calculated with and without a weighting – applying a weighting pushes $\bar{E}_{\mathrm{S}}$ down a little, likely due to high E values obtained from fits to lower quality data. In either case, with or without a weighting, $\bar{E}_{\mathrm{S}}$ falls significantly above the 0.65eV MTE average activation energy for both Bacteria and Archaea.

2.3.6 Taxonomic and Physiological Groupings

Psychrophiles and mesophiles inhabit low to medium temperature ranges, while thermophiles and hyperthermophiles grow at much higher temperatures (Willey et al., 2008). 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 (Willey et al., 2008). Corkrey et al. (2016) 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, I performed a break-point analysis on the dataset using the 'Segmented' R package

(Muggeo, 2008). 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 $x = 1/(kT_{\mathrm{pk}})$ 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 I determined significant break-points for bacteria and archaea within the 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. (2016) and were thus used as cut-off points for defining mesophiles and thermophiles in the analysis. For this work I provide no lower limit for mesophiles, essentially grouping psychrophiles and mesophiles together. Were psychrophiles to display a pattern different to mesophiles, this would be expected 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 (Valentine, 2007). 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 (Valentine, 2007), a difference that may be particularly important for thermal performance. As such, bacteria and archaea are separated in the analysis as disparate organisms with divergent evolutionary histories.

In order to classify prokaryotes by the energy generating metabolic processes that they use, I took note of the growth conditions used when initially digitizing 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, sulfur reduction, etc. In these cases I matched taxa against those able to utilise certain metabolic reactions according to Amend and Shock (2001) before manually checking the culture conditions in each study for the metabolites required for certain metabolic processes.

I also categorised taxa by their status as potential pathogens. I matched taxon names against the database of host-pathogen interactions provided in Wardeh et al. (2015) to understand whether each strain was potentially pathogenic, and what taxa they were known to infect.

2.3.7 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 carbon efflux (F_x) of an ecosystem as,

$$F_x = \frac{F_{T+x}}{F_T},$$

where x is the temperature increase (at the end of a warming scenario), and F_T and F_{T+x} 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 biomasses of these compartments, I can re-write F_x as:

$$F_x = \frac{(1-\delta)c_{\mathrm{ae}}e^{\frac{-E_{\mathrm{ae}}}{k(T+x)}} + \delta\left(\beta c_{\mathrm{hp}}e^{\frac{-E_{\mathrm{hp}}}{k(T+x)}} + (1-\beta)c_{\mathrm{he}}e^{\frac{-E_{\mathrm{he}}}{k(T+x)}}\right)}{(1-\delta)c_{\mathrm{ae}}e^{\frac{-E_{\mathrm{ae}}}{kT}} + \delta\left(\beta c_{\mathrm{hp}}e^{\frac{-E_{\mathrm{hp}}}{kT}} + (1-\beta)c_{\mathrm{he}}e^{\frac{-E_{\mathrm{he}}}{kT}}\right)}. \tag{2.3}$$

Here, each compartment's total flux contribution (identified by a subscript: autotrophic eukaryotes = ae, heterotrophic prokaryotes = hp; heterotrophic eukaryotes = he) is modelled as a Boltzmann-Arrhenius equation, with c a 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 heterotrophic component (so $1-\beta$ is the proportion of non-prokaryotic heterotrophs such as fungi or insects). I do not use the Sharpe-Schoolfield model here because it does not apply to long-term thermal responses (Fig. 2.1), 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_{pk} (the heat-stress region) (Pawar et al., 2016). I 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.

I then use eqn. 2.3 to calculate the percent change in ecosystem flux due to differences in activation energies of the three compartments (E_{ae}, E_{hp}, and E_{he}):

$$\left(\frac{F_{x,2}}{F_{x,1}} - 1\right) * 100,$$

where $F_{x,2}$ and $F_{x,1}$ 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. 2.6). That is, for $F_{x,1}$, all E values, i.e. E_{au}, E_{hp} and E_{he} in Eq. 2.3 = 0.65eV. This is the assumption made by most current ecosystem carbon flux models (Cramer et al., 1999; Friedlingstein et al., 2006; Zhou and Zhou, 2012). For $F_{x,2}$, the differing activation energies were parameterised using either the mean of the estimated Es for the short-term (intra-specific) or the long-term (inter-specific) TPCs (Table 2.1; Fig 2.3). For this I used estimates of E_{L} and $\bar{E}_{\mathrm{S}}$ from mesophilic bacteria (long-term "evolutionary" $E_{\mathrm{L}} = 0.98$eV, short-term "instantaneous" $\bar{E}_{\mathrm{S}} = 0.87$eV) only, because the archaea in the data are largely composed of strains adapted to ecologically extreme niches, which are largely irrelevant from a global warming perspective. Archaea 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 the model could be parameterised with their inclusion. Alternative model formulations to include archaea are described in the Supplementary Methods.

I calculated the emergent E of the $F_{x,2}$ 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:

$$E = (1 - \delta)E_{ae} + \delta(\beta E_{hp} + (1 - \beta)E_{he}).$$

I also calculated the emergent Q_{10} of the $F_{x,2}$ ecosystems, as it is a widely used measure in climate change models of carbon flux (Lenton and Huntingford, 2003; Friedlingstein et al., 2006):

$$Q_{10} = (F_{x,2})^{\frac{10}{x}}.$$

I chose a warming magnitude $x = 10$°C for short-term responses because this at the upper end (e.g., generally, at higher latitudes) of the range of daily (over 24 hrs) fluctuations that organisms experience (Vasseur et al., 2014). For long-term warming scenarios, I used $x = 4$°C, the approximate upper end of the range for the year 2100 projected by the IPCC (IPCC, 2014).

The biomass proportions δ and β 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 (Hermle et al., 2010). 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 (Joergensen and Wichern, 2008). Here I vary the percentage of heterotrophs within an ecosystem (δ) between 25 – 75% and the percentage of prokaryotes within heterotrophs (β) between 25 – 75% to generate a range of potential scenarios in Fig. 2.6.

2.3.8 Code and Data Availability

All code and associated raw data is available at the following git repository:

https://github.com/smithtp/hotterbetterprokaryotes

2.4 Results

2.4.1 Adaptation To Culture Conditions

First, I compared each strain's thermal optimum (T_{pk}) with the temperature at which it was cultured (T_{lab}) to determine whether the TPCs reflect adaptation to growth temperature. For both bacteria and archaea, I find a strong and significant ($p < 0.00001$) association between T_{pk} and T_{lab} (Fig. 2.2; bacteria $R^2 = 0.91$, 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 $= 0.87 \pm 0.04$, archaea slope $= 0.93 \pm 0.05$). This is because T_{pk} tends to be higher than culturing temperature at low temperatures, whereas T_{pk} and T_{lab} are generally more similar at high culturing temperatures (Fig. 2.2).

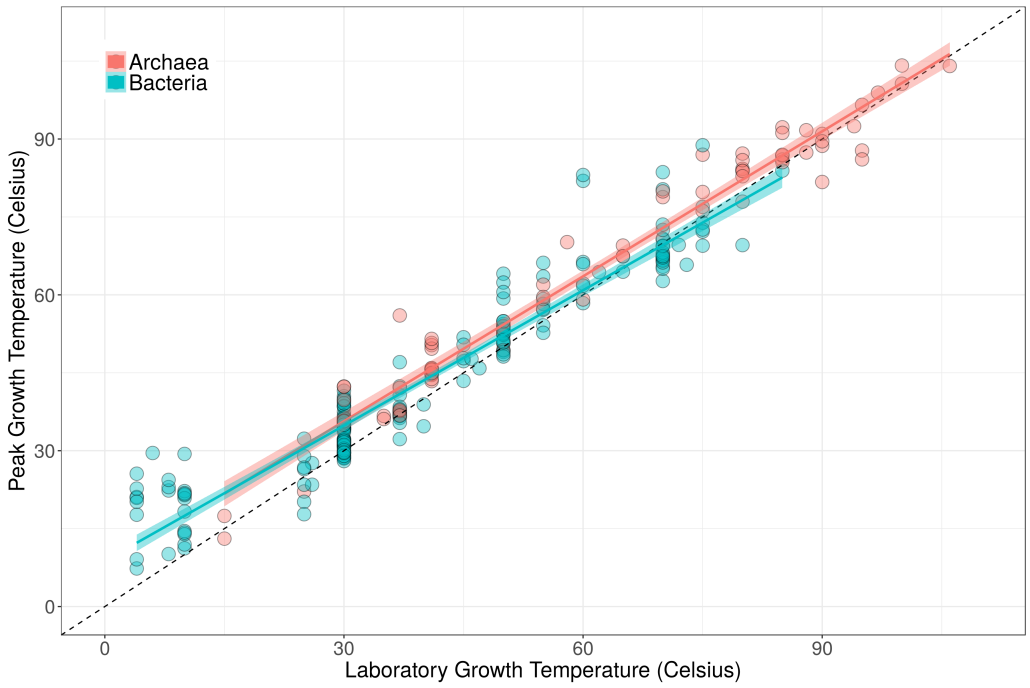

Figure 2.2: **Adaptation of strains to laboratory growth conditions.**

Peak growth temperature (T_{pk}) plotted against the laboratory growth temperature (T_{lab}), with linear models fit for bacteria and archaea (bacteria intercept $= 8.79$, slope $= 0.87 \pm 0.04$, $R^2 = 0.91$, $p < 0.00001$; archaea intercept $= 7.77$, slope $= 0.93 \pm 0.05$, $R^2 = 0.96$, $p < 0.00001$) and a 1:1 line shown (black dashes). In general there is a strong association of T_{pk} with T_{lab}. The confidence intervals for the slopes do not include 1 however, indicating that there are stronger constraints on T_{pk} for prokaryotes operating at very high temperatures.

2.4.2 Comparison of Short- and Long-term Thermal Responses

Next, I tested the HiB hypothesis by comparing the short-term (intra-specific) and long-term (inter-specific) thermal responses (see Fig. 2.1; 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. 2.1) at the same rate as $r_{\max}$ would increase with temperature (parameter E_{S}), on average, within single a strain's TPC. The analysis relies on the comparison of P_{pk} and T_{pk} 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) (Angilletta et al., 2010; Frazier et al., 2006). Analysing this relationship across 416 bacterial and 82 archaeal strains, I find that hotter is indeed better (HiB holds) across mesophilic bacteria ($\bar{E}_{\mathrm{S}}$ and E_{L} are > 0, and there is significant overlap of their 95% CIs; Fig 2.3 and Table 2.1). 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 $E_{\mathrm{L}} > 0$, there is less than 50% overlap of E_{L} and $\bar{E}_{\mathrm{S}}$ CIs.

Table 2.1: **Estimated mean short-term and long-term activation energies across archaea and bacteria, and test of the HiB hypothesis.**

Estimated mean intra-specific (short-term, $\bar{E}_{\mathrm{S}}$) and inter-specific (long-term, E_{L}) thermal sensitivities (95% CI ranges in parentheses) for bacteria and archaea split by thermal niche (also see Fig 2.3). As the same data is used to calculate $\bar{E}_{\mathrm{S}}$ and E_{L}, the number of datapoints (given in parentheses) applies to both. The last column indicates whether or not the HiB hypothesis is supported.

Kingdom	**Thermal Niche**	$E_{\mathbf{L}}$	$\bar{E}_{\mathbf{S}}$	$E_{\mathbf{L}} > 0$	$\bar{E}_{\mathbf{S}} \approx E_{\mathbf{L}}$	HiB
Bacteria	Mesophile (n = 264)	0.98 (0.75 – 1.25)	0.87 (0.82 – 0.93)	TRUE	TRUE	TRUE
	Thermophile (n = 114)	-0.07 (-0.26 – 0.12)	0.85 (0.78 – 0.92)	FALSE	FALSE	FALSE
Archaea	Mesophile (n = 21)	0.97 (0.69 – 2.26)	0.60 (0.50 – 0.70)	TRUE	FALSE	FALSE?
	Thermophile (n = 60)	-0.09 (-0.21 – 0.17)	1.11 (0.95 – 1.28)	FALSE	FALSE	FALSE

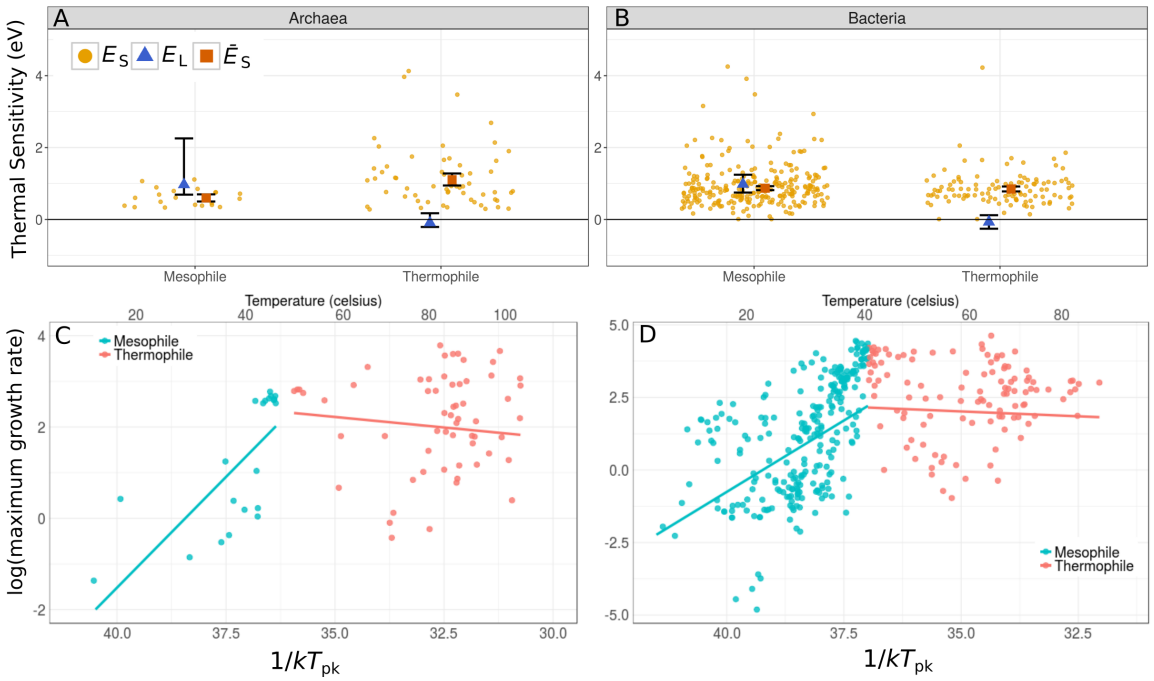

Figure 2.3: **Patterns of short- and long-term thermal responses of growth rate (fitness) for archaea and bacteria.**

A and **B**: Activation energies (with 95% Confidence Intervals) from Boltzmann-Arrhenius model fits (E_L, blue triangle) compared to mean activation energy ($\bar{E}_S$, red square) of the intra-specific (short-term) thermal responses. The distribution of E_S is also shown (orange points). **C** and **D**: Arrhenius plots (x-axes inverted to aid visualisation) fitted to mesophile and thermophile sub-groups separately within bacteria and archaea, respectively. That is, the points are the natural log of P_{pk} (r_{max}, per hour) plotted against $1/kT_{pk}$ (where k is the Boltzmann constant) for each TPC, whilst the lines (the long-term thermal responses) are the Boltzmann-Arrhenius model fitted using weighted regression to mesophile and thermophile data separately, after determining the breakpoint (Methods). The HiB hypothesis is best-supported for the mesophilic bacteria, while equalisation of fitness is best supported in both thermophile sub-groups. The result for mesophilic archaea is less clear (Table 2.1).

2.4.3 Variation in Thermal Sensitivity

I find mean thermal sensitivity ($\bar{E}_S$) for bacteria = 0.88eV and $\bar{E}_S$ for archaea = 0.95eV (Fig. 2.5). These are significantly greater than the 0.65eV global inter-specific constraint expected under MTE (Gillooly et al., 2001; Brown et al., 2004) and previously observed mean intra-specific values (calculated primarily from eukaryote data) (Dell et al., 2011). The data is right-skewed (as observed by Dell et al. (2011)), but even after accounting for this skew by taking the median, activation energy still falls significantly above 0.65eV (bacteria median = 0.84eV, archaea median = 0.80eV; Appendix Figure A.2). Furthermore, I see a consistent pattern of

median thermal sensitivity >0.65eV throughout the lower taxonomic groupings (Fig. 2.4A).

To further understand these findings I investigated differences between groups of strains sharing functional traits, such as their pathogenicity and their main energy-generating metabolic pathway (Fig. 2.4B). Again I find mean and median thermal sensitivity >0.65eV in the majority of functional groups, suggesting that this high E is a trait generally conserved across prokaryotic organisms.

Here I 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, I assembled another thermal response dataset (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. I 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 $\bar{E}_S$ = 0.82eV; archaea flux $\bar{E}_S$ = 1.01eV; Fig. 2.5A, see Appendix Table A.1 for a list of fluxes analysed). Furthermore, I compared both the prokaryotic growth rate and flux E_S distributions, with a new dataset (Methods) on thermal sensitivity of respiration in autotrophic eukaryotes. The results (Fig. 2.5D) further support a lower thermal sensitivity of short term responses for eukaryotes than prokaryotes ($\bar{E}_S$ = 0.67eV with CI = 0.63 – 0.72, median = 0.57).

2.4.4 Potential Ecosystem-level Impacts

These results suggest that higher sensitivity of both short- (higher intraspecific activation energies) and long-term (higher interspecific 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, I built a simple mathematical model to calculate the potential change in the contribution of heterotrophs to ecosystem carbon efflux (based on

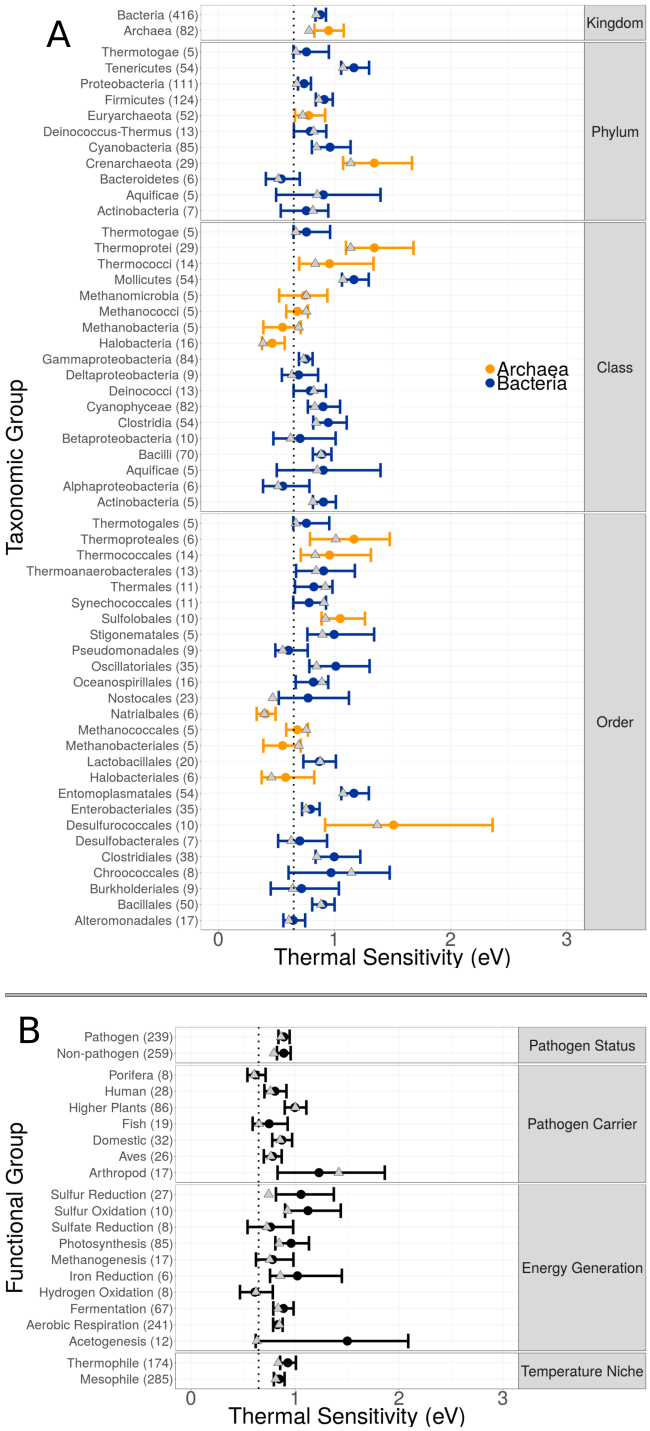

Figure 2.4: **Variation in thermal sensitivity among prokaryotic groups.**

Comparison of intra-specific population growth rate activation energies ($\bar{E}_S$) across taxonomic levels **(A)** and functional trait groupings **(B)**. 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 $\bar{E}_S$ was calculated for each grouping. The dotted line marks 0.65eV, the mean E previously reported within the MTE framework. Grey triangles mark the median E_S for each group.

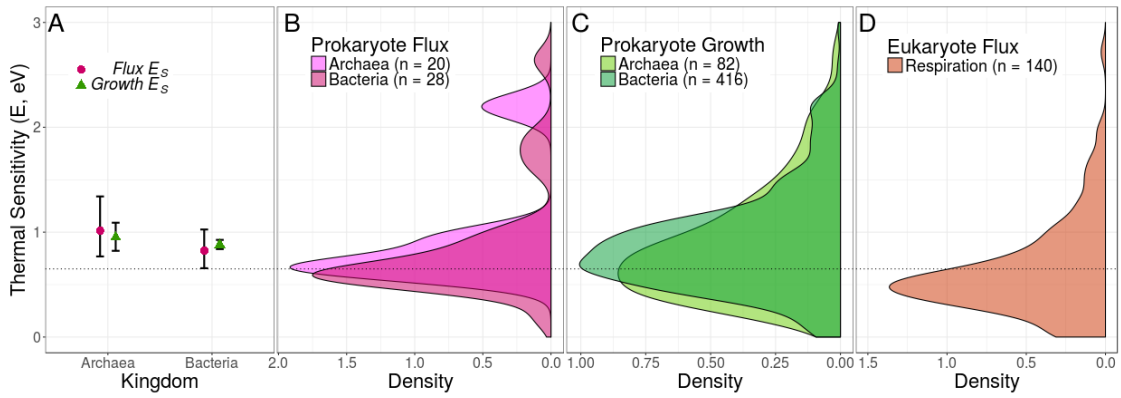

Figure 2.5: **Differences in sensitivity (activation energy) of short-term thermal responses across taxonomic groups.**

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 (bacteria growth $\bar{E}_S$ = 0.88eV, n = 416; bacteria flux $\bar{E}_S$ = 0.82eV, n = 28; archaea growth $\bar{E}_S$ = 0.95eV, n = 82; archaea flux $\bar{E}_S$ = 1.01eV, n = 20). **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 for respiration rate TPCs in autotrophic eukaryotes, showing comparatively lower mean thermal sensitivity than those of the distributions for prokaryotes ($\bar{E}_S$ = 0.67eV, n = 381).

biomass distributions typical of terrestrial forest ecosystems, see Methods). Using the new estimates of $\bar{E}_S$ and E_L to parameterise this model, I calculate the impact of short- and long-term warming on the thermal response of carbon flux of model ecosystems that differ in composition of autotroph vs. heterotroph and eukaryote vs. prokaryote biomass. The results (Fig. 2.6) show that the difference in prokaryotic vs eukaryotic thermal sensitivities can substantially change the predicted increase in carbon efflux due to warming on the short- as well as long-term. For example, compared to the case where both prokaryotes and eukaryotes have the same short-term thermal sensitivity of 0.65eV (the assumption made by most current ecosystem carbon flux models (Cramer et al., 1999; Friedlingstein et al., 2006; Zhou and Zhou, 2012)), using the actual difference in sensitivity that I have found ($\bar{E}_S$ 0.65eV for eukaryotes vs. 0.87 for mesophilic bacteria; Table 2.1; Figure 2.3), the flux increases by ∼8% with 10°C short-term warming for a ecosystem composition of 50% heterotrophs (50% of which in turn are bacteria). This calculation based on the average intra-specific activation energy is relevant to short-term increases in ecosystem fluxes without evolution or acclimation in response to, for example, temperature fluctuations from time-scales of minutes to days (10°C is at the upper end of daily

temperature fluctuations that organisms may typically experience (Vasseur et al., 2014)). When I consider the effects of longer-term warming (such as through gradual global climate change) on the prokaryotic sub-community using the inter-specific (evolutionary) thermal sensitivity, E_{L} (0.98eV), I find that modelled ecosystem flux increases by ∼5% with 4°C warming (again with 50% heterotrophs of which 50% are bacteria) compared to a baseline where the long-term thermal sensitivity is 0.65eV 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 (Whitman et al., 1998; Joergensen and Wichern, 2008; Hermle et al., 2010; Bar-On et al., 2018; Johnston and Sibly, 2018). In the 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.

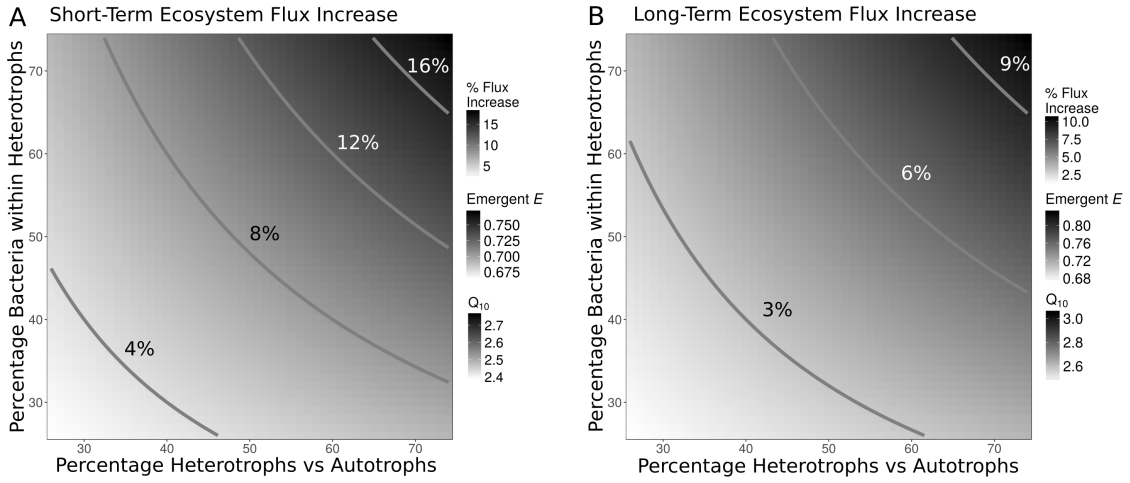

Figure 2.6: **Potential changes in short- and long-term ecosystem carbon flux due to differences in sensitivity between prokaryotic and eukaryotic thermal responses.**

A. Heat map 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 (0.65eV) 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}s 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 the estimated $\bar{E}_{\mathrm{S}}$ and E_{L} respectively for mesophilic bacteria (Table 2.1). The mathematical model is described in Methods.

2.5 Discussion

These results demonstrate that mean thermal sensitivities for both bacteria and archaea fall significantly above 0.65eV (Fig. 2.5, bacteria $\bar{E}_S = 0.88$eV; archaea $\bar{E}_S = 0.95$eV), suggesting that prokaryotes operate under different thermal constraints to more complex eukaryotes. Indeed, I also present a new dataset of autotrophic eukaryotes which show thermal sensitivity consistent with the 0.65eV MTE generalisation. These findings of high (relative to eukaryotes) intra-specific thermal sensitivities in prokaryotes are consistent with previous work on methanogenic archaea (Yvon-Durocher et al., 2014) and cyanobacteria (Chen and Laws, 2017), but have never been demonstrated across all major lineages of prokaryotes. In particular, Yvon-Durocher et al. (2014) 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. The results suggest how these two different scales of response may be related — the short-term responses may be coupled with a Hotter-is-Better 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 time-scale of the adaptation resulting in the HiB pattern, but numerous previous studies have shown rapid adaptation of prokaryotes to experimental warming conditions (Bennett et al., 1990; Kishimoto et al., 2010; Blaby et al., 2012). 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. 2.1A). 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. 2.3). In either case, under HiB, I 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 (Rhee and Gotham, 1981). This can been 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 (Melillo et al., 2017). Thus overall, these results suggest that further production of greenhouse gases from the prokaryotic component of ecosystems is likely to increase at a greater rate than that by component eukaryotic organisms (Fig. 2.6), albeit mediated by other biotic and abiotic factors. The 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 understanding ecosystem functioning (Rivett et al., 2018). Therefore the empirical results and 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, I see a tendency towards high thermal sensitivity (E_{S}) in prokaryotes, there are taxonomic subgroups within the dataset for which this is not the case (Fig. 2.4). For example, $\bar{E}_{\mathrm{S}}$ for mesophilic archaea as a whole does not deviate significantly from the MTE 0.65eV average (Table 2.1). This is largely because this subgroup is primarily comprised of strains from *Halobacteria*, which have thermal sensitivities significantly lower than 0.65eV (Halobacteria $\bar{E}_{\mathrm{S}}$ = 0.46; CI = 0.38 – 0.58; Fig. 2.4A). 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). Additionally, despite mesophilic archaea displaying a clear long-term increase in rate with temperature, I found only a very small overlap in CIs between $\bar{E}_{\mathrm{S}}$ and E_{L} for these strains which may not be enough to infer equivalence (Cumming, 2009), although mapping p-values onto % overlap of bootstrapped CIs is not a trivial task (Afshartous and Preston, 2010). 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 the dataset 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 (Valentine, 2007). That is not to say that archaea do not contribute sig-

nificantly to ecosystem functioning in benign environments, however (as demonstrated through the 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.

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

For simplicity, when parameterising the ecosystem model I used $\bar{E}_{\mathrm{S}}$ and E_{G} calculated from all of the mesophilic bacteria in the dataset, as I expect a huge amount of variation in the taxa present at the ecosystem scale. To expand this work further it may be possible to consider more specific situations where certain prokaryotes may dominate in certain environments based on global biogeographic studies (Delgado-Baquerizo et al., 2018). However, the majority of microbial taxa are known only from sequencing data (Lynch and Neufeld, 2015), for example *Acidobacteria* are thought to make up in the region of 50% of soil biodiversity (Janssen, 2006), yet very few strains have actually been cultured and therefore have TPCs available (Sikorski, 2015). 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), where Boltzmann-Arrhenius is an appropriate model (Pawar et al., 2016). If these fluctuations were frequent enough to have strong effects on the community, using Sharpe-Schoolfield models 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 data on the specific species present in a given ecosystem and their individual TPCs,

which is outside of the scope of the current study.

I have focused on the ecosystem consequences in the face of global change, but these results also have implications for understanding of prokaryotic physiology. I 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 (DeLong et al., 2010; Kempes et al., 2012). Also, the 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 I have found here.

These results are also important for understanding differences in thermal physiology between taxa, given my findings of HiB for mesophilic bacteria, but invariant inter-specific fitness with temperature for thermophiles (Fig. 2.3). Thermophiles have evolved specific adaptations to extreme temperature stress, such as mechanisms to cope with increased membrane permeability at high temperatures (van de Vossenberg et al., 1995) and thus adaptation to such niches may incur a fitness cost to thermophiles as seen in the 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 (Corkrey et al., 2016, 2017). These results are also important for our understanding of the limits to thermal adaptation. I find that strains cultured at very high temperatures tend to display thermal optima very close to their culturing conditions, whilst for strains cultured at low temperatures the constraints on thermal optima appear to be relaxed (Fig. 2.2). These results have implications for theoretical models of the thermal limits for life.

In summary, these 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.

Chapter 3

High-Throughput Growth and Respiration Methods

3.1 Abstract

Understanding the physiological basis for microbial responses to temperature requires an understanding of how the thermal responses of different traits are linked. In order to compare the thermal responses of different traits, is important to have consistent experimental methods to accurately quantify those traits. Microbial growth rate is an common measure of fitness which can be quantified across temperatures to investigate a strain's thermal performance. However, extending this to multiple strains with replication can be time-consuming. Respiration rate is a measurement of metabolic rate, which has been regularly quantified to test microbial growth efficiency. However, thermal response data for respiration rates are largely lacking from the literature. Respiration rates of populations in exponential growth are expected to be substantially higher than respiration during stationary phase. Meaningful comparison of growth and respiration therefore requires respiration to be measured during growth — the "active" respiration rate. Where active respiration rates have been published however, these are likely to be over-estimates of the true per-capita respiration rate. This is because rates tend to be normalised to the starting population size, failing to correct for population size changes during

growth. Here I propose a closed-system approach for accurate quantification of per-capita respiration rates for exponentially growing microbial cultures. I also propose new methodology for high-throughput measurements of growth rate, which allow TPCs to be measured over shorter absolute timescales. Finally, I show how these methods can be to be coupled to produce TPCs of growth and metabolic rate which can be directly compared to one another. This methodology has the potential to plug a substantial gap in the literature, with respect to accurate comparisons of TPC parameters for different traits.

3.2 Introduction

Understanding how microbes respond to temperature is important for improving our general understanding of microbial physiology. Much is known about how microbial growth varies with temperature, however how the thermal responses of more fundamental metabolic traits such as respiration determine the more emergent response of growth is less well known from a physiological perspective. In **Chapter 2** I show that, whilst microbial growth rate thermal performance curves (TPCs) are found throughout the literature, there is a lack of data to directly compare these with respiration rate. In theory, multiple bacterial growth curves can be produced easily, by measuring the optical density (OD) of batch cultures in a continuous manner with a multiple-well plate reader (fig. 3.1A). Alternatively growth curves can be produced through manually taking repeated measurements at different time-points of either OD, or potentially more accurate measurements of cell numbers through plate counts or flow cytometry. Maximum growth rate can then be calculated by fitting models to the resulting sigmoidal growth curve (Zwietering et al., 1990; Baranyi et al., 1993; Buchanan et al., 1997), or more traditionally by simply measuring the slope of log-transformed OD measurements against time (Monod, 1949; Hall et al., 2014). Expanding these measurements to produce TPCs can be problematic however. Continuous OD measurements, whilst easy at room temperature, would require a plate-reader with the capacity for refrigeration and incubation in order to generate growth curves across multiple temperatures. Refrigeration in particular is not standard for most mass-market models. An additional problem with such continuous procedures is that condensation

will often form on the plate cover during incubation, obscuring OD measurements. Plate reader models which specifically avoid condensation are again not industry standard (although some models such as the Bioscreen C™ purportedly address these problems, ref. GrowthCurvesUSA (2015)). These issues can of course be avoided via manual growth measurements at different time points during temperature incubation. This is labour-intensive however, as a full bacterial growth curve may take in the region of 24 hours to proceed from initial inoculation. Accurate maximum growth rate calculations would require multiple data points at regular intervals within the exponential growth phase. Extending this to multiple temperatures is possible for a single strain, however attempting to scale this up to even tens of strains, with replication, may not be feasible without an unacceptably high time investment. These difficulties can be seen in the meta-analysis dataset in **Chapter 2**, where most studies only provide a handful of TPCs at most (65% of all data sources contained only 1 TPC, 92.5% of source papers contained a maximum of 5 TPCs, as shown in Appendix Fig. A.1).

Attempting to measure a secondary trait (such as respiration rate) simultaneously adds yet another level of complexity to the problem. As such, studies including measurements of both growth rate and respiration rate (even without the inclusion of temperature) are generally missing from the microbial literature, see for example a meta-analysis by Sinsabaugh et al. (2015) – "We could not find enough studies that included measures of bacterial respiration and biomass for a regression analysis". This leaves the alternative of trying to compare measures from different studies, however when considering TPCs there is often a time-scale problem with this approach, due to thermal acclimation. This is because growth rates by their nature require measurement over a reasonable time-period in which growth is observable, whilst respiration rate is an acute response usually measured over shorter time, e.g. Padfield et al. (2016). During the lag phase of growth cells will acclimate to the experimental temperature, before proceeding to exponential growth, where the rate is measured. In contrast, respiration rates are often measured in stationary phase cells (often termed "passive" or "endogenous" respiration rates) without an explicit period of acclimation to the experimental temperature. Realised growth rates in experiments at temperatures outside of the operational temperature range may be expected to be increased via acclimation during the lag phase, whilst populations used

for respiration rates measured without a comparable acclimation period do not receive this benefit. Comparison of TPCs for growth and passive respiration are therefore unlikely to yield meaningful results due to these mismatches in acclimation.

The acclimation mismatch may be avoidable by measuring respiration during growth. Such "active" respiration rates (also termed "exogenous") have been measured (see Makarieva et al. (2005)), however the way in which they are often calculated may lead to uninformative comparisons across the literature as I now describe. To measure respiration, experimentalists use either "instantanous" flux, or closed-system cumulative flux approaches. Instantanous flux approaches use probes which constantly measure the consumption of O_2 or production of CO_2 over the duration of an experiment, giving a time-series of flux measurements (Briand et al., 2004; Warkentin et al., 2007). Although in name these methods are instantaneous, experimentalists will typically take measurements over a time period (usually of hours) in order to produce a rate of respiration over time (Briand et al., 2004; Warkentin et al., 2007). In comparison, closed-system approaches capture the total cumulative flux produced over the course of an experiment, such as the MicroResp™ system which has been used to measure CO_2 production in various culture studies (Lawrence et al., 2012; Foster and Bell, 2012; Rivett et al., 2017). Again, this requires measurement over a number of hours in order to capture a measurable change in CO_2 to quantify the rate of respiration.

Using the MicroResp closed system example, the specific (i.e. *per capita*) respiration rate (R) of a stationary phase population can be calculated by diving the total cumulative respiratory output (R_{tot}) by the duration of the experiment (t) and the starting population size (N), i.e:

$$R = \frac{R_{tot}}{tN}. \tag{3.1}$$

When the population is growing however, changes in population size must be considered, as CO_2 production is dependant upon the biomass of cells respiring (fig 3.1B). The same is true for instantaneous rate approaches, as although measured continuously, respiration is still measured over a time-period in which growing populations will show significant changes in biomass.

However, many "exogenous" or "active" respiration rates are normalised only to the starting mass of the growing population, i.e. using equation 3.1, and neglect to include changes in the growing population size over time (e.g. Khanna et al. (1984); Bauer et al. (2000); Berg et al. (2002)). This is likely to result in exaggerated active respiration rates reported and may be partly responsible for the orders of magnitude differences seen in meta-analyses comparing active and passive microbial metabolic rates, e.g. Makarieva et al. (2005); DeLong et al. (2010).

I aim to address the lack of direct growth and respiration rate comparison data by producing a dataset of experimentally derived rates across a range of environmentally isolated bacteria. I resolve the difficulties of up-scaling this type of work by describing liquid culture methodology to generate high throughput growth rates. The time-scale issues around growth and respiration rate TPCs are reconciled through the ability to measure these traits simultaneously, and the derivation of an equation for specific respiration rate (eq. 3.10), which is directly comparable to specific growth rate.

3.3 Methods

3.3.1 Origin of Bacterial Strains

For method development, I used 11 strains of bacteria isolated from environmental soil samples at Silwood Park (Ascot, UK). The strains used for this trial are detailed in table 3.1, see **Chapter 4** for full isolation details. I selected these strains as they display fairly similar growth rates at room temperature in continuous culture and also provide a range of different taxa and isolation conditions.

3.3.2 High Throughput Growth Rate Methodology

The aim of this method was to quantify maximum growth rate in a high throughput manner without measuring the entire growth curve. Batch cultures in exponential growth phase are

Table 3.1: **Bacterial strains used for this methods trial.**

Full details of strains and isolation conditions can be found in **Chapter 4** and Appendix table C.1.

Strain	**Genus**
04_04_02	*Pseudomonas*
04_RT_01	*Bacillus*
04_RT_02	*Pseudomonas*
10_RT_01	*Pseudomonas*
10_RT_02	*Pseudomonas*
21_21_01	*Bacillus*
21_21_02	*Pseudomonas*
21_RT_01	*Collimonas*
21_RT_02	*Bacillus*
30_30_04	*Bacillus*
40_40_01	*Bacillus*

expected to continue in exponential growth with little to no lag after inoculation into fresh media (Baranyi et al., 1993). I therefore aimed to skip the lag phase by priming cells for growth, providing them with fresh media, then immediately commencing measurements.

Initially I revived frozen stocks in LB broth and grew them to carrying capacity over 3 days. Pilot experiments showed that after a 1 in 100 dilution in fresh media at room temperature, these isolates display lag times between ∼6-8 hours then proceed to grow for a further ∼8-14 hours before approaching carrying capacity. I therefore diluted the cultures in fresh media and allowed growth to proceed overnight for ∼12 hours, ensuring that each strain would have entered exponential growth, but have not yet reached carrying capacity. Following overnight culture, 100μl was taken from each strain and optical density at 600nm wavelength (OD_{600}) was read, to check that culture density was indeed demonstrably above a negative, but below carrying capacity – i.e. still in growth phase.

I then took 800μl of these growing cells, centrifuged them at 8,000rpm for 5 minutes (i.e. gently to prevent cell rupture), removed the supernatant and re-suspended them in 400μl of fresh media in a 96-well plate to over-concentrate them (as a proportion of cells will inevitably be lost during centrifugation and re-suspension). I then made 50% serial dilutions seven times for each strain, taking 200μl of cell suspension into 200μl of fresh media (and discarding 200μl from the final suspension), to give a range of eight initial starting culture densities. Growth

was then recorded by measuring optical density at $\lambda = 600$nm (OD_{600}) every 30 minutes for 3 hours, using a Synergy HT microplate reader (BioTek Instruments, USA).

This method was repeated three times independently.

3.3.3 Comparative Growth Rate Method

Using the same strains, I generated growth rate data using the standard methodology of continuous OD_{600} measurements in a plate reader. As with the high throughput methods, strains were initially grown to carrying capacity in LB over a 3 day period. I then diluted 2μl of bacterial culture into 198μl fresh LB (for a 1 in 100 dilution) and set a plate-reader to measure OD_{600} every 30 minutes (with shaking) for 24 hours to produce a full growth curve from lag phase through to stationary phase. This method was repeated three times independently. The first trial used 8 replicates of each bacterial strain, the second and third trials used 4 replicates each.

3.3.4 Growth Rate Calculations

Given measurements of optical density (OD_{600}, a proxy for biomass) at two time-points (t_1 and t_2), assuming the population size is in exponential growth, the specific growth rate (μ) can be calculated as the slope of a linear regression of the logged population size (N; the OD_{600}) against time, i.e.:

$$\log(N(t_2)) = \log(N(t_1)) + \mu(t_2 - t_1). \tag{3.2}$$

This is the approach used to calculate growth rates from the serial dilution data. For the comparative data which spans the whole growth curve, I applied different methodology to calculate maximum growth rate. I approximate the classical method (i.e. eq. 3.2) using a sliding-window approach. I fit linear models to $\log(OD_{600})$ against time in 6 data-point windows and calculate the slopes, the maximum growth rate is calculated from the window

with the highest slope. Six data points is three hours - the same time window measured by the serial dilution method. This is similar to the approach taken by Hall et al. (2014) and provides a direct comparison to the rates calculated from the serial dilution method.

3.3.5 Statistical Tests

To test whether mean growth rates were consistent between methodologies, I performed Welch's t-tests for each strain.

To test whether the variability of the growth rates was equivalent between methods, I tested for significant differences between coefficients of variance for each method using both the asymptotic test described by Feltz and Miller (1996) and the modified signed-likelihood ratio test described by Krishnamoorthy and Lee (2014).

All growth rate calculations and statistical tests were performed in R, version 3.2.2 (R Core Team, 2015). The variability tests used are implemented in the cvequality R package (Marwick and Krishnamoorthy, 2019).

3.3.6 Specific Respiration Rate Calculation

To measure respiration rate, I use the MicroResp™ system. This is a colorimetric assay originally devised for measuring soil respiration (Campbell et al., 2003), but since used for bacterial cultures (Lawrence et al., 2012; Foster and Bell, 2012; Rivett et al., 2017). A light sensitive dye is fixed within an agarose gel containing Sodium Bicarbonate ($NaHCO_3$), set into a clear plastic "detection plate". A rubber sealing plate is attached to a deep well plate containing the bacterial cultures and the detection plate clamped into place above, ensuring that the wells are sealed together. A hole in the sealing plate allows CO_2 evolved from the culture to reach the detection well, where it reacts with the bicarbonate, changing the pH of the gel and thereby causing a colour change in the indicator dye. The colour change is measured spectrophotometrically, by measuring the absorbance of the detection plate wells at 572nm. This can be compared to

a calibration curve of known CO_2 concentrations, and the quantity of CO_2 produced by the culture determined (as described in Appendix B.1). Therefore, this method measures the total cumulative respired CO_2 over time. In stationary phase it is simple to calculate the respiration rate as this is simply the total accumulated CO_2 divided by the product of the biomass and time duration of the experiment (eq. 3.1). However when the population is growing, the constant change in population size will affect how much CO_2 is accumulated over time and therefore must be incorporated into the respiration rate calculation.

Assuming that respiration is a mass-specific rate that is constant across the experiment and that CO_2 accumulates under the assumption of negligible escape or re-absorption, the rate of change of cumulative total CO_2 respired (i.e. captured by the MicroResp™ system) (R_{tot}) can be written:

$$\frac{dR_{tot}}{dt} = RN(t); \tag{3.3}$$

where the change in total respired CO_2 (R_{tot}) at time t is given by the product of the mass-specific rate of respiration R and the population biomass at time t, $N(t)$, i.e. the rate of change of accumulated CO_2 is proportional to the population size. Given the exponential growth equation 3.2, population size at any given time point is:

$$N(t) = N_0 e^{\mu t}, \tag{3.4}$$

where N_0 is the initial population size. Substituting eq. 3.4 into eq. 3.3 gives:

$$\frac{dR_{tot}}{dt} = RN_0 e^{\mu t}; \tag{3.5}$$

We can integrate this expression across time to get the predicted total cumulative respiration at any time point. This gives:

$$R_{tot}(t) = c + \frac{N_0 R e^{\mu t}}{\mu}, \tag{3.6}$$

where c is the constant of integration. Setting $t = 0$ and solving for c we get:

$$c = R_{tot}(0) - \frac{N_0 R e^{\mu(0)}}{\mu} = R_{tot}(0) - \frac{N_0 R}{\mu}. \tag{3.7}$$

We can substitute this back into eq. 3.6 in place of c, to get:

$$R_{tot}(t) = R_{tot}(0) + \frac{N_0 R e^{\mu t}}{\mu} - \frac{N_0 R}{\mu}, \tag{3.8}$$

which we can re-arrange to give R, the biomass specific respiration rate:

$$R = \frac{\mu(R_{tot}(t) - R_{tot}(0))}{N_0 e^{\mu t} - N_0}. \tag{3.9}$$

Given that the total respiration at $t(0) = 0$, we can simplify this in the form of eq. 3.10:

$$R = \frac{\mu R_{tot}}{N_0 e^{\mu t} - N_0}. \tag{3.10}$$

This measure of respiration rate is directly comparable to the respiration rate of stationary phase populations because it incorporates the change in population size over time. This is necessary to accurately quantify per-capita respiration rates in this closed system experiment where cumulative accumulation of CO_2 is measured, while biomass increases exponentially over time.

3.3.7 Comparative Respiration Rate Data

In order to show the potential differences in respiration rate calculations, I use respiration data obtained for the trial strains in **Chapter 4**. I calculated respiration rate for these strains based

on the calculation including population growth (eq. 3.10), as well as calculation based on the starting population size only (i.e. eq. 3.1) and visualise the differences.

3.4 Results

Using the newly derived respiration rate equation, I visualise how a growing population would produce more cumulative CO_2 than a stationary phase population (fig 3.1B). The per-capita respiration rate and starting population sizes are kept constant, however the population represented by dotted lines is given a growth rate whilst the population represented by solid lines remains stationary. By the end of the experiment, the growing population has produced more cumulative CO_2, despite the specific respiration rates of the populations being equal.

I show the difference that this can make in figure 3.1C, where empirical respiration rates from **Chapter 4** are calculated with population growth incorporated (eq. 3.10, red - the true per-capita respiration rate), versus calculated based only on the starting population size (eq. 3.1, blue - resulting in an over estimation of respiration rate).

I then test whether my newly described high-throughput serial dilution method produces comparable growth rates to a standard technique (measuring the full growth curve). For each strain, I compared mean growth rates derived from both the standard methodology and the high throughput method. I find no significant differences at the 95% confidence level in the mean rates calculated from each method for any of the strains used in the trial (t-tests, fig. 3.2, table 3.2).

I also tested whether the variability between repeated measurements was equivalent for the two experimental methods. Using the signed likelihood ratio test, I find no difference in variability of growth rate results between each method ($p > 0.05$ in all cases, table 3.3). Using the asymptotic test, I do find a significant difference in variability for the growth rates of strain 30_30_04 ($p = 0.049$, table 3.3), however this is due to the new method in fact producing less variable results between replicates than the continuous method.

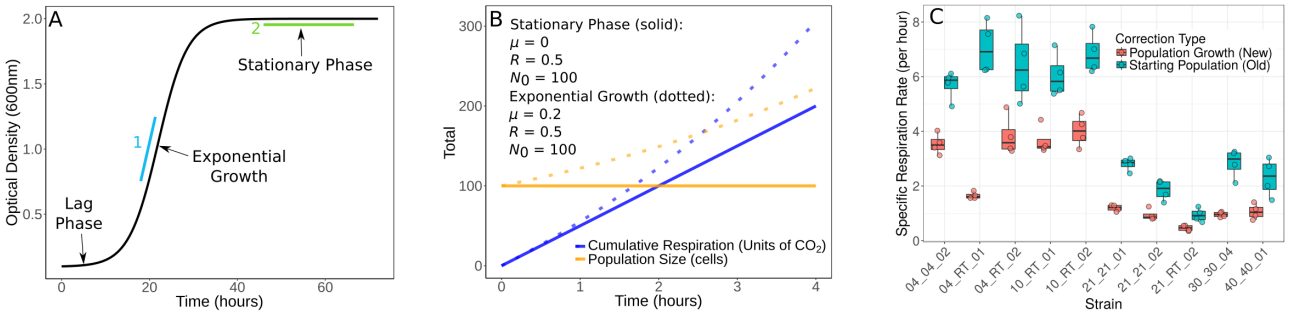

Figure 3.1: **Miscalculation of "active" respiration rates.**

A. An example growth curve using optical density measured over time. Inoculating stationary phase cells into fresh media results in a lag phase, followed by exponential growth, until carrying capacity is reached and the culture enters a stationary phase (Rolfe et al., 2012). The length of this lag phase is dependent upon the initial state of the inoculated cell population - previously growing cells will display shorter lag phases than cells inoculated from stationary phase (Baranyi and Roberts, 1994). Growth rate is by definition measured during exponential growth (depicted in blue at point 1), whilst respiration rate can be measured either with growing cells for "active" rates, or with stationary phase cells (depicted in green at point 2) for "passive" rates. **B.** The respiration rate of a stable population (blue, solid line) yields a linear output of CO_2 over time, however a growing population (yellow, dotted) would yield an exponential output of CO_2 (blue, dotted), proportional to the growth rate. **C.** Comparison of respiration rates calculated via the equation presented here (new method, red) versus a calculation without accounting for population growth (old method, blue). Failing to incorporate growth of the population into "active" respiration rate calculations leads to overestimation of respiration rate, especially at high growth rates.

3.5 Discussion

Here I have shown that exponential phase bacterial populations can be sub-sampled, provided with fresh media, and then continued exponential growth is observed. I have shown that this growth proceeds at the same rate as their maximum growth rates as calculated from full growth curve experiments. This is important as it allows this method to be used to assume constant exponential growth in experiments (over short time-scales) which do not measure growth rate continuously. I propose that this can be used in combination with other trait measurements in order to achieve accurate "active" rates which can be directly compared to growth rate.

I have also shown that correcting for population growth substantially changes estimates of respiration rate in closed systems. I can obtain the population-growth corrected respiration rate using the new method developed here. A work-flow for achieving this is presented in figure 3.3. Briefly, exponentially growing bacterial populations are prepared as described in the methods

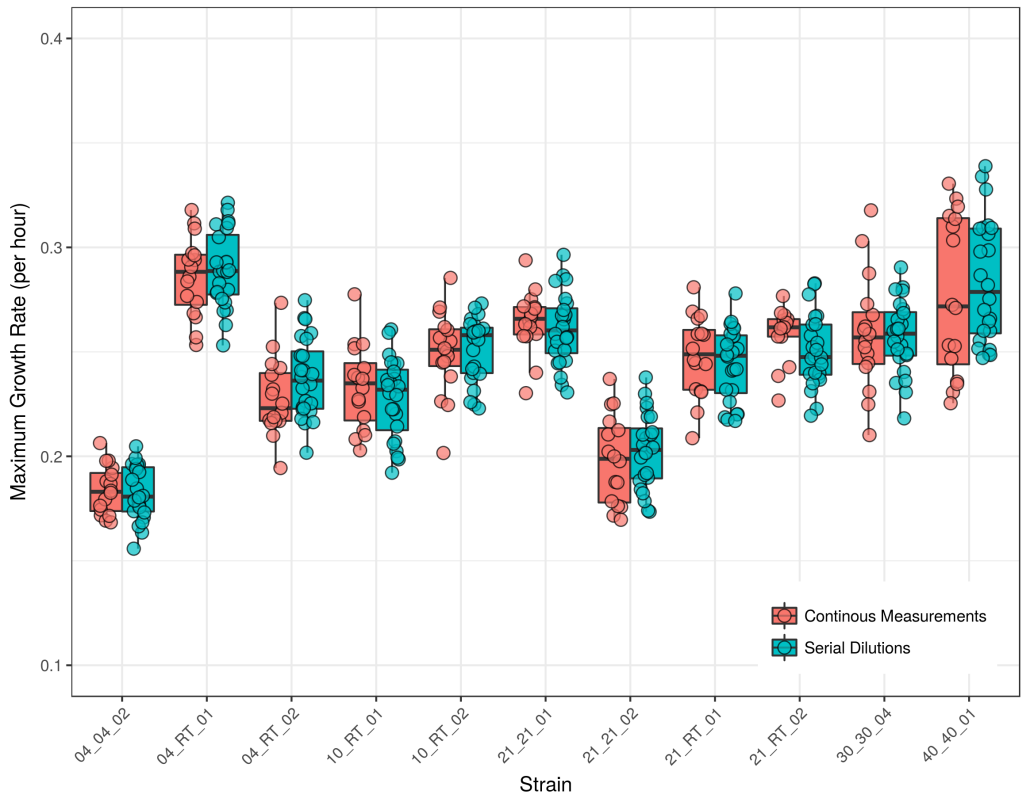

Figure 3.2: **Comparison of growth rates derived from different experimental methods.**

Growth rates of 11 strains measured at 20°C, measured using the two different methods. Growth rates from the "standard" method (red) were derived by measuring OD_{600} every 30 minutes for the entire growth curve, and then using a sliding window approach to calculate the maximum growth rate within a 5 data-point window. Growth using our new method (blue) was measured over a short time-period (3 hours) and rates then calculated as the slope of a linear model of logged OD_{600} against time.

section. The starting population size after re-suspension is measured, before respiratory flux determined over a given time period. Finally, the finishing cell population size is measured. This yields a respiratory flux over time, coupled with starting and ending population sizes. Given these results showing that the bacterial cultures will remain in exponential growth, the growth rate (μ) can be calculated using equation 3.2. Given this growth rate, the mass-specific or cell number-specific respiration rate can be calculated, whilst accounting for the given change in population size over the duration of the respiration rate measurements (eq. 3.10). This provides a new level of accuracy, as changes in population size have been largely overlooked in previous estimates of active bacterial metabolic rates.

The method shown here is specific to closed system approaches, where respiration is measured

Table 3.2: **Comparison of mean growth rates derived from different experimental methods.**

Results from t-tests to compare mean growth rates from the standard continuous measurements method against our serial dilutions method for each strain used in the trial. P-values show that there is no significant difference between methods for any of the strains at the 95% confidence level.

Strain	Continuous Mean	Serial Dilutions Mean	t value	df	p-value
04_04_02	0.184	0.180	0.854	33.5	0.399
04_RT_01	0.286	0.287	-0.221	31.6	0.827
04_RT_02	0.228	0.235	-1.105	30.9	0.278
10_RT_01	0.233	0.226	1.168	32.1	0.251
10_RT_02	0.249	0.249	0.135	26.6	0.894
21_21_01	0.264	0.259	1.094	35.6	0.281
21_21_02	0.198	0.199	-0.133	28.3	0.895
21_RT_01	0.248	0.243	0.787	31.3	0.437
21_RT_02	0.258	0.248	1.75	31.3	0.089
30_30_04	0.259	0.256	0.345	22.9	0.733
40_40_01	0.277	0.282	-0.415	25.3	0.682

only as a cumulative total over a period of time. In theory, many of these issues around population growth affecting the quantification of active rates could be avoided through rapid measurements of instantaneous fluxes. Here, measurements could be taken over a very short time-period where growth of the population would not significantly alter the results. In reality though, in order to generate significant signal, these measurements have historically been taken continuously over long time periods (of many hours) in which population size changes are likely to have a large bearing on the respiratory output (Briand et al., 2004). Faster methods have been developed, e.g. Warkentin et al. (2007), however these are still usually performed over a time-scale in which meaningful changes in biomass will occur in exponentially growing populations. This isn't necessarily a problem in studies which use respiration to calculate other metrics such as growth efficiency, e.g. Briand et al. (2004). Here, both active respiration and abundance are tracked per volume and turned into a growth efficiency - this does not rely on an accurate quantification of the respiration *rate*. However, many previous studies have indeed calculated active respiration rates but normalised to the starting population size, omitting changes in population over time (Khanna et al., 1984; Bauer et al., 2000; Berg et al., 2002). This leads to an over-estimation of the true per-capita rate, as shown in Fig. 3.1C.

Table 3.3: **Comparison of variation between experimental replicates.**

Results from two tests of differences between coefficients of variance. Asym denotes results from the asymptotic test (Feltz and Miller, 1996), M-SLR denotes results from the modified signed likelihood ratio test (Krishnamoorthy and Lee, 2014).

Strain	Asym Test Stat	Asym p-value	M-SLR Test Stat	M-SLR p-value
04_04_02	0.093	0.760	0.115	0.735
04_RT_01	0.020	0.887	-0.017	1.00
04_RT_02	0.141	0.708	0.104	0.747
10_RT_01	0.012	0.912	0.009	0.923
10_RT_02	1.207	0.272	1.042	0.307
21_21_01	0.580	0.446	0.641	0.423
21_21_02	0.560	0.439	0.513	0.474
21_RT_01	0.010	0.922	-0.003	1.00
21_RT_02	1.528	0.216	1.716	0.190
30_30_04	3.878	0.049*	3.450	0.063
40_40_01	2.134	0.144	1.925	0.165

* significant difference in variability at the 95% confidence level.

Measuring these traits simultaneously is particularly important for comparisons of their thermal performance (and indeed the thermal responses of other measures derived from these traits, such as growth efficiency). This is because it ensures that the acclimation period is equivalent when measuring each trait. Differences in acclimation, i.e. holding organisms at different temperatures prior to measurement, can cause major deviations in TPC parameters within species (Schulte et al., 2011; Luhring and Delong, 2017). Specifically for micro-organisms, temperature shock can halt growth whilst cells acclimatise to the new temperature, affecting key cellular processes, before growth can continue (Van Derlinden and Van Impe, 2012). In growth measurements, this acclimation is included by definition, as cells will automatically go into a temperature induced lag before growth proceeds and is measured. For acute measurements of respiration however, if no acclimation period is directly given by the experimentalist then the respiration response of micro-organisms at temperatures away from their normal operating range is likely to reflect their shock response. This may differ widely from their acclimatised response. Therefore, methods which ensure equivalent acclimation (as described here) are required for meaningful comparison of TPC parameters between traits. Whilst I have used aerobic respiration as an example, this methodology could conceivably be coupled to any metabolic trait which can be measured as a rate over time.

As well as the possibility for coupling metabolic rate and growth rate measurements, the method that I have described here allows for higher throughput measurements than may normally be obtained, with potentially less hands-on time for the experimentalist. This is due to not measuring the entire growth curve, and only two population size estimates being required for growth rate calculation (due to the assumption of exponential growth). In **Chapter 4** I utilise this methodology to produce TPCs for growth rate coupled to respiration rate for multiple strains of bacteria concurrently, demonstrating the potential applications of this method.

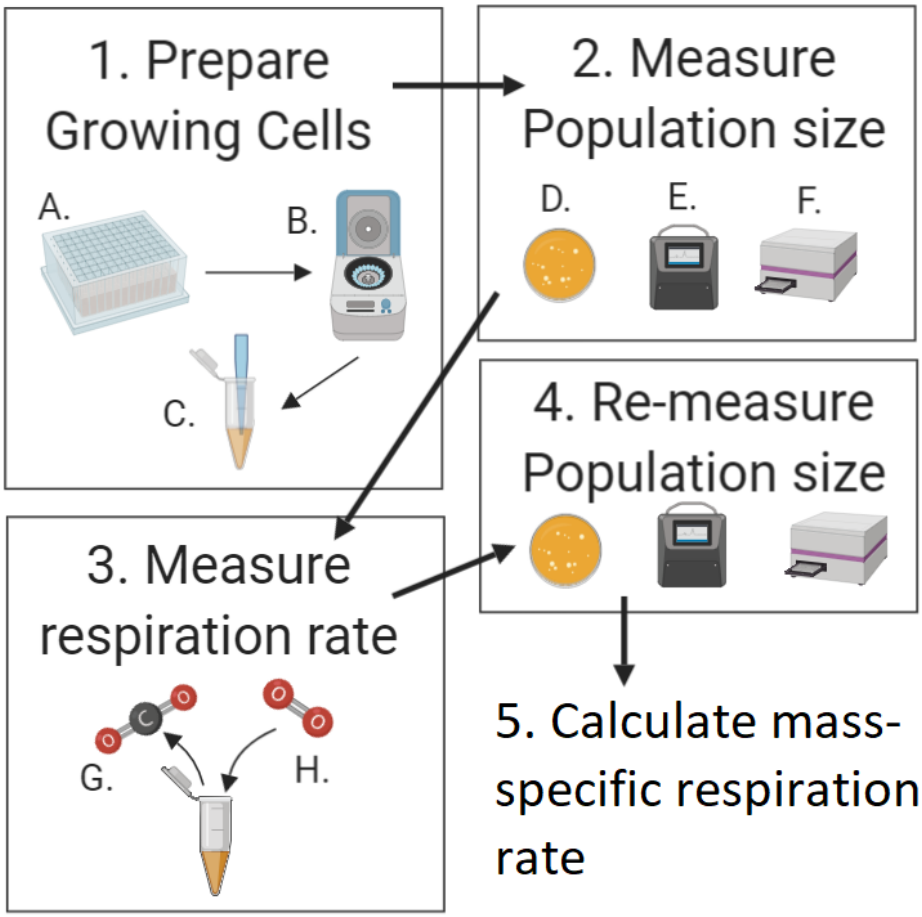

Figure 3.3: **Active metabolic rates method work-flow diagram.**

1. First, exponentially growing bacterial populations are prepared: A. cells grown to exponential phase, B. culture centrifuged to pellet cells, C. cells re-suspended in fresh media. **2.** Population size measured via colony counts (D.), flow cytometry cell counts (E.) or optical density biomass estimates (F.). **3.** Respiration rate measured over short time-period via either CO_2 production (G.) or O_2 use (H.). **4.** Population size measured again at the end of the respiration rate measurements. **5.** Finally, the respiration rate and the change in population size over time can be used to calculate mass-specific respiration rate, assuming exponential growth of the culture.

Chapter 4

Species sorting may accelerate microbial community responses to warming

4.1 Abstract

There has been much discussion about the role of acclimation and adaptation with respect to the potential ecosystem responses to warming. However, given enough latent diversity of temperature fitness, species sorting may allow different species with environment-specific optimal temperatures to emerge, mimicking "adaptation" of the community to maintain function. Sequencing methods have shown that thousands of distinct microbial taxonomic units can be found in small environmental samples, with a high proportion being low abundance, "rare", taxa. Whether this diversity extends to an equivalent abundance of phenotypes, which are continuously selected for by species sorting mechanisms, remains in question. Here I test this idea through a species sorting experiment designed to select strains of bacteria with differing thermal optima, from the same environmental patch of UK soil. I find that the thermal optima of isolated strains is strongly dependent upon the conditions under which they were isolated. This shows that there is a wide pool of taxa with differing thermal optima for species sort-

ing processes to act upon. With climate warming, microbial communities are therefore likely to utilise the wide latent thermal diversity and shift towards taxa better adapted to warmer temperatures. I also propose that community change with gradual warming is likely to be mediated by thermal specialists, rather than thermal generalists. Furthermore, I find that the abundances of taxa utilising different growth strategies are likely to change. Overall, these results show that the relative abundances of different taxa within bacterial communities are likely to shift with changing temperature, and that this is expected to impact community function. In particular, warming may promote a reduction in oligotrophs versus increased abundance of copiotrophs, leading to more rapid depletion of labile carbon sources in soils.

4.2 Introduction

Micro-organisms play an important role in ecosystem functioning, particularly through their contribution to heterotrophic respiration (Trivedi et al., 2013; Sikorski, 2015). Changes to environmental temperature, from short timescales (such as daily temperature fluctuations) through to longer term changes (climate warming), affect ecosystem functioning via changes in metabolic activity of microbial communities (Dijkstra et al., 2011). How the metabolic activity and thus functioning of ecosystems varies with temperature is itself heavily dependent upon the composition of microbial communities (Balser and Firestone, 2005; Melillo et al., 2017; Yu et al., 2018). Many investigations into changes in ecosystem function with temperature change have focussed on how microbial communities may acclimate or adapt to climate warming (Luo et al., 2001; Bradford, 2013; Karhu et al., 2014; Melillo et al., 2017). However, alongside direct thermal acclimation and adaptation of community constituents, microbial community composition can respond rapidly to warming (Allison and Martiny, 2008; Aydogan et al., 2018). Such changes in community composition are likely to have an important impact on how ecosystem processes respond to climate change (Karhu et al., 2014; Melillo et al., 2017; Yu et al., 2018).

Changes in community composition arise through assembly mechanisms such as species sorting at the local level, or processes related to dispersal (Székely and Langenheder, 2014). Many

changes to bacterial communities can be due to immigration resulting from wide dispersal of these organisms (Van der Gucht et al., 2007; Lawrence et al., 2015). This can also take place at the local level however, if a large amount of hidden phenotypic diversity is generally present at smaller scales. This is species sorting, a process which can mimic adaptation of the community, provided a suitable immigration pool is available and accessible (Loeuille and Leibold, 2008; Pawar, 2009). Where this is due to changes in the abiotic conditions preventing the establishment or persistence of particular species, it can be termed environmental filtering (Kraft et al., 2015). Community assembly processes are expected to differ between habitat specialists and generalists, with species sorting having a large impact upon the distribution of specialists taxa, but neutral processes being more important for generalists (Langenheder and Székely, 2011; Székely and Langenheder, 2014). This is likely due to taxa with large niche widths not being 'sorted' by environmental conditions and persisting within the community regardless of environmental change (Langenheder and Székely, 2011). In order to understand the effects of climate change on community composition and thus ecosystem processes, it is therefore important to understand the processes driving community assembly and succession. This involves understanding the amount of phenotypic diversity present at local levels and thus whether species sorting and environmental filtering are likely to drive community change with or without the effects of dispersal and immigration.

The diversity of micro-organisms is undeniably vast (Curtis et al., 2002). Sequencing methods have shown thousands of distinct microbial taxa present in small environmental samples (Sogin et al., 2006), with the majority being "rare" taxa found in low abundance (Lynch and Neufeld, 2015). Whether this diversity is largely due to the capacity of microbes for wide dispersal (i.e. "everything is everywhere"), or whether it is representative of adaptation of microbes to innumerous micro-habitats at the microscopic scale, remains in question. Work on the occurrence of extremophiles in benign environments has widened the debate, as we should not normally expect ecosystems to contain organisms unable to proliferate in the conditions provided by that environment. Spores of thermophilic bacteria are found throughout cold ocean beds (Hubert et al., 2009) hinting at the wide dispersal of these micro-organisms. Similarly, thermophilic bacteria are prevalent in cool soils (Marchant et al., 2002), likely deposited by rainwater (Marchant

et al., 2008). Functionally extermophilic (acidic, basic and saline) bacterial communities have been isolated from a freshwater lake (Low-Decarie et al., 2016), however these communities were found to be composed of generalist species rather than obligate extremophiles. Acidophilic and halophilic bacteria are also often isolated from benign soil samples, but potentially this is due to the highly mosaic nature of the soil environment (Sikorski, 2015). We may therefore expect the genetic diversity of environmental samples to reflect an equally wide phenotypic diversity of microbial strains. There is however a major discrepancy between genetic microbial diversity and the numbers of cultured isolates (Sikorski, 2015). Recent censuses of 16S rRNA sequence data suggests that prokaryotic species may number in the millions (Amann and Rosselló-Móra, 2016; Schloss et al., 2016; Louca et al., 2019), many orders of magnitude lower than the numbers suggested by scaling laws (Locey and Lennon, 2016), but significantly higher than the numbers of described, cultivated taxa, numbering in the thousands (Sikorski, 2015). Likely this discrepancy is due to culture conditions not adequately reflecting the physiological requirements of these "unculturable" species. Therefore in order to access the phenotypic diversity of bacteria, culturing conditions need to be expanded in order to isolate "unculturable" strains which, according to genetic evidence, are present in high number.

Performance curves can be used to describe how biological rates vary with changes in environmental variables. Generalist species that perform well across a range of conditions will have performance curves with a greater breadth than specialists, which would have narrow performance curves (Seebacher et al., 2015). Generalism however may come with the trade-off of increased metabolic costs, causing generalists to show decreased maximum growth rates compared to specialists (Huey and Hertz, 1984; Angilletta Jr., 2009). Organisms are also expected to trade-off growth rates against carrying capacity (r vs K selection, Andrews and Harris (1986)). This trade-off can be seen in whole communities, with those investing more in resource acquisition producing lower overall growth yield (Malik et al., 2019). One mechanism for this trade-off in bacteria is via increasing the rate of ATP production (and thus cellular energy) through supplementing aerobic respiration with fermentation (Pfeiffer et al., 2001). This increases the rate of ATP production, at the cost of lower total yield, allowing populations to reach higher growth rates but lower carrying capacity from the same resource input. In

microbes, these different strategists can be thought of as copiotrophs and oligotrophs (Fierer et al., 2007; Ho et al., 2017). Here, copiotrophs are growth specialists which perform well under nutrient rich conditions, whereas oligotrophs grow under lower nutrient conditions, utilising substrate more efficiently to produce higher biomass yield per unit of substrate consumed (K-specialists). This difference in strategies can be seen in studies of bacterial succession, where growth specialists utilising labile carbon sources are first to colonise new niches, whilst slower growing K-specialists colonise later, utilising less favourable resource supplies (Rivett et al., 2016, 2018). Copiotrophs are therefore the opportunist taxa which proliferate after an environmental perturbation when resources are in good supply, whereas oligotrophs are the more standard constituents of the community with more stable populations through time. Understanding differences in growth strategies present in bacterial communities is important for understanding community change due to species sorting. Temperature change may drive increased abundance of specialists adapted to different temperatures, or alternatively the community response may be mediated by generalists which perform well across a range of environmental conditions. Furthermore, whether temperature affects the ratio of r to K specialists is likely to have important impacts on the community response to climate change.

The dynamics of bacterial succession are strongly influenced by incubation temperature (Rui et al., 2009). This may expected, given the association between growth rate and succession stage (Shrestha et al., 2007) and the influence of temperature on maximum growth rate (Savage et al., 2004). The temperature where a species' growth is at it's maximum ($\mu_{\max}$) is the 'optimal' or 'peak' temperature (here termed T_{pk}). T_{pk} is expected to correspond to the environmental conditions usually experienced by an organism (Huey and Kingsolver, 1989; Angilletta Jr., 2009), within some tolerance margins to account for temperature fluctuations. This parameter is expected to evolve gradually through evolutionary time (Kontopoulos et al., 2019), potentially through structural changes to enzymes which alter their melting temperatures over time (Pucci and Rooman, 2017). In **Chapter 2** I show that evolution of T_{pk} necessitates equivalent changes in $\mu_{\max}$ due to thermodynamic constraints. Bacteria are also known to be able to rapidly adapt to new temperatures in adaptive evolution experiments (through shifts in T_{pk}) over much shorter timescales (Bennett et al., 1990; Kishimoto et al., 2010; Blaby et al., 2012), however it is not

known whether these types of rapid responses are common in nature, or are restricted to lab environments. If adaptation in the environment is possible at such short absolute timescales, then this is also likely to be an important factor in community responses to environmental change.

Here I investigate whether and how species sorting and latent functional diversity can result in reorganization of bacterial communities to cope with changes in environmental temperature. I perform a species sorting experiment to assess the phenotypic breadth of bacterial temperature-fitness in a single soil patch. I compare thermal optima of cultivated taxa to their isolation conditions, to understand whether species sorting processes are likely to lead to changes in the relative abundances of taxa based on their thermal adaptations. I also test the thermal niche widths of cultivated taxa, to understand the relative importance of temperature generalists versus specialists in mediating community assembly processes with warming. By comparing measures of growth, respiration and ATP, I investigate differences in growth strategies between taxa. I use these results to understand the mechanistic, individual-level basis of potential community level changes with temperature. Finally, I investigate the importance of adaptation versus species sorting in defining the community response to warming via a phylogenetic analysis of the evolutionary history of T_{pk} for isolated strains.

4.3 Methods

4.3.1 Methods Overview

I performed a species sorting experiment to investigate the level of functional redundancy present in soil bacterial communities with respect to temperature (Fig. 4.1). I took soil samples and incubated them at various temperatures to allow for community assembly (or, potentially, adaptation) processes to progress. I then isolated bacteria across the same temperature range, as well as at room temperature from each incubation condition. I characterise the thermal performance curves (TPCs) of each isolate and investigate whether peak temperature

T_{pk} varies with isolation conditions, i.e. whether there are species present ready to colonise a new temperature niche should it become available. I also compare the temperature niche widths (a measure of specialism or generalism) of strains isolated under different conditions to understand the relative importance of generalists in mediating the community response to temperature change. I compare measurements of growth rate, respiration rate and ATP content to investigate differences in growth strategies between taxa. Growth specialists (copiotrophs, *r*-specialists) are expected to have high ATP content in combination with high growth rates, but low overall yield (carrying capacity). Yield specialists (oligotrophs, K-specialists) should display the opposite, comparatively low growth rates and ATP content, but high yield. In order to understand whether differences in temperature fitness are due to the effects of species sorting and environmental filtering constraining the taxa isolated at each temperature, or whether strains isolated are likely to be those with the capacity to adapt to the isolation conditions over the course of the experiment, I perform a phylogenetic analysis. If strains have rapidly adapted to experimental conditions, I expect to see little or no phylogenetic signal in T_{pk}, however if the strains are isolated largely due to species sorting processes I expect to see a distinct pattern of phylogenetic heritability in T_{pk}.

4.3.2 Sampling and Isolation

Soil cores were taken from Nash's field (Silwood Park, Berkshire, UK), ambient soil temperature at time of sampling was 19.4°C. Soil cores were incubated at different lab temperatures (4°C, 10°C, 21°C, 30°C, 40°C, 50°C) for four weeks to potentially allow microbial communities to assemble based on temperature preference, via species sorting and environmental filtering effects. Soil was re-hydrated periodically with autoclaved Milli-Q® water to prevent desiccation during incubation. To isolate bacterial strains, soil from each core was then washed with PBS, plated onto R2 agar and incubated again at both their respective temperature treatments and room temperature (22°C, climate controlled lab). Morphologically different colonies were then isolated on R2 agar before being frozen as glycerol stocks (fig. 4.1). In total, 74 strains were isolated.

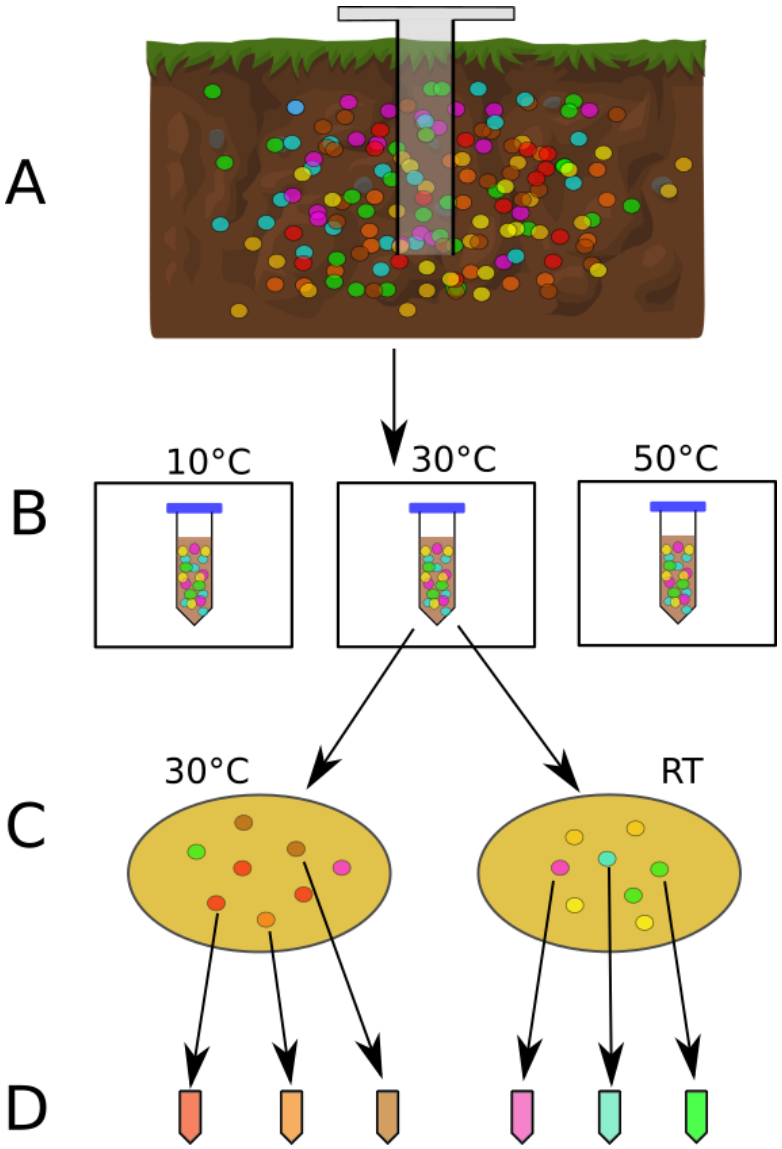

Figure 4.1: **Species sorting work-flow.**

A. Soil community sampled. Different bacterial taxa represented by coloured circles. **B.** Samples incubated at different temperatures (only three examples shown, the full experiment used 4, 10, 21, 30, 40 and 50°C), allowing community assembly via species sorting and environmental filtering processes. **C.** Soil washes from each core plated out onto agar and grown at both the previous incubation temperature, and room temperature (RT). **D.** Various colonies from each plate picked, streaked and isolated, before storage at −80°C.

4.3.3 Taxonomic Identification

16S rDNA sequences were used to identify the isolates. Raw sequences were first trimmed using Geneious 10.2.2 (https://www.geneious.com), BLAST searches were then used to assign taxonomy to each trimmed sequence. Where all BLAST hits above 97% sequence similarity

were to the same species, that species was assigned to the isolate. In cases where no species hits were above 97% sequence similarity, or there were multiple different species hits above 97% similarity, taxonomy was assigned only to the genus level.

Amongst the isolates were number of strains belonging to the *Bacillus cereus* group, which consists of several genetically similar species - *B. anthracis*, *B. cereus*, *B. mycoides*, *B. thuringiensis* and *B. weihenstephanensis* (Logan and Vos, 2015). Their close genetic relatedness prevents 16S rDNA sequences from being a good tool for accurate delemitation of these species (Ash et al., 1991), so taxonomy was therefore assigned for these strains based on phenotypic characteristics. *B. mycoides* were differentiated from other *Bacillus cereus* groups strains by rhizoid colony architecture (Logan and Vos, 2015). Strains displaying cold tolerance (growth at 5°C were designated as *B. weihenstephanensis* (Lechner et al., 1998; Logan and Vos, 2015). Finally, two strains with highest BLAST matches to *B. cereus*, *B. mycoides* and *B. weihenstephanensis* but displaying neither cold tolerance, nor rhizoid colonies, were designated *B. cereus*. Due to the high similarity of 16S sequences across this group, these strains do not cluster into monophyletic species groups.

4.3.4 Quantifying Thermal Performance Curves

Rate Measurements

To test the thermal response of each isolate, the methodology described in **Chapter 3** was used to measure growth and respiration rates simultaneously. Additionally, the ATP content of the cell cultures was measured at the start and end of growth. These are static snap-shots of ATP at a given time-point and will not produce rates of ATP production or use *per se*. Briefly, strains were revived from glycerol stocks into fresh LB broth and incubated to carrying capacity (determined by optical density measurements - OD_{600}, a proxy for biomass) at the temperature of the subsequent experiment (the acclimation period, typically 72hrs, but longer for cold temperatures). Prior to experimental testing the strains were then diluted 1:100 in LB, pushing them into a short lag before exponential growth starts again. This was allowed

to proceed for a number of hours, for the cultures to have begun exponential growth but not yet have reached carrying capacity (∼12hrs for 30-40°C growth, longer at colder temperatures, tracked by OD_{600} measures). The cultures were subsequently spun down at 8,000rpm for 5 minutes to pellet the cells and then re-suspended in fresh LB to obtain 400μl culture at a final OD_{600} of ∼0.2-0.3. These cultures were serially diluted in LB, producing a range of starting densities of growing cells. Sub-cultures of each strain were taken in order to track OD_{600} to ensure that cells were in exponential growth. Initial ATP and cell count (using a BD Accuri C6 flow cytometer) measurements were taken, before cells were incubated with a MicroResp™ plate at the experimental temperature and allowed to continue growth for a short p1eriod of time (typically 3-4hrs). After growth, the MicroResp plate was read, and final cell count and ATP measurements were taken.

Rate Calculations

From the flow cytometry cell diameter measurements I estimate average cell volumes at each point and estimate the cellular carbon per cell using:

$$\text{fgC cell}^{-1} = 133.754V^{0.438},$$

as proposed by Romanova and Sazhin (2010). Multiplying this by the cell counts gives an estimate of the carbon biomass of the culture at the starting and ending points.

The difference between the initial biomass and biomass at the end of the experiment gives the total carbon sequestered through growth. Given an initial biomass (C_0) that grows over time (t) to reach a final biomass (C_{tot}), assuming the population is in exponential growth, the mass specific growth rate (μ) is given by:

$$\mu = \frac{log(\frac{C_{tot}}{C_0})}{t}. \tag{4.1}$$

Respiration rates of cultures were measured using the MicroResp™ system (Campbell et al.,

2003). This is a colorimetric assay initially developed to measure CO_2 production from soil samples, which has since been used to measure respiration of bacterial cultures (Rivett, 2013). In short, a light sensitive dye is fixed within an agarose gel containing Sodium Bicarbonate ($NaHCO_3$), set into a clear plastic "detection plate". A rubber sealing plate is attached to a deep well plate containing the bacterial cultures and the detection plate clamped into place above, ensuring that the wells are sealed together. A hole in the sealing plate allows CO_2 evolved from the culture to reach the detection well, where it reacts with the bicarbonate, changing the pH of the gel and thereby causing a colour change in the indicator dye. The colour change is measured spectrophotometrically, by measuring the absorbance of the detection plate wells at 572nm. This can be compared to a calibration curve of known CO_2 concentrations, and the quantity of CO_2 produced by the culture determined (as described in Appendix B.1). As the molecular mass of carbon within CO_2 is known, the mass (in μg) of carbon produced can be calculated.

The specific respiration rate is then calculated according to the equation presented in **Chapter 3** (eq. 4.2). The derivation is reproduced here:

Assuming that respiration is a simple mass-specific rate that is constant across the experiment, the change in total carbon respired (R_{tot}), as measured via MicroResp, can be written:

$$\frac{dR_{tot}}{dt} = RC(t) = RC(0)e^{\mu t};$$

where the change in total respired carbon (R_{tot}) at time t is given by the product of the mass-specific rate of respiration R and the population biomass at time t, $C(t)$. We can integrate this expression across time to get the predicted total respiration at any time point. This gives:

$$R_{tot}(t) = C_1 + \frac{C_0 R e^{\mu t}}{\mu},$$

where C_1 is the constant of integration. Setting t = 0 and solving for C_1 we get:

$$R_{tot}(t) = R_{tot}(0) + \frac{C_0 R e^{\mu t}}{\mu} - \frac{C_0 R}{\mu},$$

which we can re-arrange to give R, the biomass specific respiration rate:

$$R = \frac{\mu(R_{tot}(t) - R_{tot}(0))}{C_0 e^{\mu t} - C_0}.$$

Given that the total respiration at $t(0) = 0$, we can simplify this in the form of eq. 4.2:

$$R = \frac{\mu R_{tot}}{C_0 e^{\mu t} - C_0}. \tag{4.2}$$

Here, R_{tot} is the total mass of carbon produced according to the MicroResp™ measurements, C_0 is the initial population biomass, μ is the previously calculated growth rate and t is the duration of the experiment.

ATP content of the cultures was measured using the Promega BacTiter-Glo reagent, which produces luminescence in the presence of ATP, proportional to the concentration of ATP. 50μl of culture (diluted 1:100) was incubated with 25μl reagent. Luminescence was measured over a 6 minute period to allow the reaction to develop completely, and measurements of luminescence recorded at the 0, 2, 4 and 6 minute timepoints. The highest relative light units (RLU) measurement for each culture was taken and used to calculate the quantity of ATP, using:

$$log(nMATP) = 1.21 \cdot log(RLU) - 4.69 \tag{4.3}$$

This was then normalised by the flow cytometry measurements to calculate a value of ATP/biomass. Although I produce values for ATP content at the start and end of the experiment, this cannot give a rate *per se*, as ATP is being constantly produced and used. These are snap-shots of the total ATP present in the cultures at the given times.

TPC Model Fitting

The Sharpe-Schoolfield model (Schoolfield et al., 1981) (eq. 4.4) was fitted to the experimentally derived temperature-dependent growth rate and respiration rate data for each isolate in order to determine the thermal response parameters.

$$B(T) = B_0 \frac{e^{\frac{-E}{k}\cdot\left(\frac{1}{T}-\frac{1}{T_{\text{ref}}}\right)}}{1+\frac{E}{E_{\text{D}}-E}e^{\frac{E_{\text{D}}}{k}\left(\frac{1}{T_{\text{pk}}}-\frac{1}{T}\right)}} \tag{4.4}$$

Here, T is temperature in Kelvin (K), B is a biological rate (in this case, growth rate, μ), 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}. Note that this has been reformulated from the model presented in the original paper, to include T_{pk} as an explicit parameter (Kontopoulos et al., 2018).

T_{pk} from the growth rate fits was used as the optimum growth temperature for each isolate. By solving eq. 4.4 for $T = T_{\text{pk}}$ the maximum growth rate at T_{pk} can be calculated (μ_{pk}). Thereafter, the niche width was calculated as the temperature range between the minimum growth temperature, and T_{pk}. This measure is commonly used to describe an organism's "operational temperature range", OTR (Pawar et al., 2016), within which it is normally expected to live.

All rate calculations and model fitting were performed in R.

4.3.5 Phylogenetic Reconstruction

The 16S sequences were used to build a phylogeny in order to investigate the evolution of thermal performance. Sequences were aligned in MAFFT (v7.205) using the default settings. From this alignment 100 trees were inferred in RAxML (v8.1.1), using a GTR-gamma nucleotide substitution model. The tree with the highest log-likelihood was taken and time-

calibrated using PLL-DPPDiv (Heath et al., 2012), with calibration nodes derived from TimeTree (http://www.timetree.org/), to produce the final phylogeny.

4.3.6 Phylogenetic Analysis

To test whether there was evidence of T_{pk} as an evolutionary trait, Blomberg's K and Pagel's λ were calculated, two quantitative measures of phylogenetic signal which both assume a Brownian motion (BM) model of trait evolution (Pagel, 1999; Blomberg et al., 2003). Blomberg's K calculates the phylogenetic signal strength as the ratio of the mean squared error of the tip data and the mean squared error of the variance-covariance matrix of the given phylogeny, under the assumption of BM (Münkemüller et al., 2012). $\mathrm{K} = 1$ indicates relatives resembling each other as closely as would be expected under a BM model, $\mathrm{K} < 1$ indicates less phylogenetic signal than expected under BM and $\mathrm{K} > 1$ indicates more phylogenetic signal than expected and thus a substantial degree of trait conservatism (Blomberg et al., 2003). Pagel's λ is a scaling parameter that defines a weighting of factors unrelated to phylogenetic history which impact trait evolution. λ is the transformation of the phylogeny which best fits a BM model of trait evolution (Münkemüller et al., 2012). $\lambda = 0$ indicates no phylogenetic signal, $\lambda = 1$ indicates that the phylogenetic signal is equivalent to that expected under a BM model, and $0 < \lambda < 1$ is in-between. The two metrics differ in their approach and thus both were used to provide a comparison of methods to understand the evolution of T_{pk}. If adaptation of strains to new temperatures during the incubation period were entirely responsible for any variation in T_{pk} with incubation temperature (as opposed to species sorting), then no phylogenetic signal in T_{pk} would be expected.

To map the evolution of T_{pk} onto this tree the ancestral characteristics at each internal node were estimated using maximum likelihood, assuming a Brownian motion model for trait evolution. In most cases, T_{pk} was derived directly from the Sharpe-Schoolfield flow-cytometry growth rate fits. Four strains of *Streptomyces* proved a special case where the flow-cytometry method did not provide reliable estimates of cell counts (see discussion), so for these strains growth rates derived from optical density measurements were used to estimate T_{pk}. Finally, for a small

number of strains which for which growth was recorded at too few temperatures to fit Sharpe-Schoolfield, the temperature with the highest directly measured growth rate was taken as the estimate for T_{pk}.

The estimates of phylogenetic signal and the visualisation of trait evolution were performed using tools from the R packages "ape" and "phytools" (Revell, 2012, 2013).

4.4 Results

4.4.1 Effects of temperature-driven species sorting

In total, 74 strains of bacteria were isolated; six from each temperature regime (either incubation followed by isolation at the same temperature - "incubation temperature isolates", or incubation followed by isolation at room temperature - "room temperature isolates"), with the exception of the 30°C incubation/isolation regime, from which I isolated 8 strains. Of these isolates, 62 were reliably revivable in liquid culture, from which 53 grew across a wide enough temperature range to produce enough data points for us to fit the Sharpe-Schoolfield model for thermal performance to. The revivable strains represented 14 genera within 3 bacterial phyla (Appendix, table C.1 and fig. 4.5A). Whilst there is diversity in the strains isolated, there were also genetically similar strains (tentatively the same "species" or "ecotypes") which were isolated more than once. In particular, strains from the *Bacillus cereus* group (including *B. cereus*, *B. mycoides* and *B. weihenstephanensis*) were commonly isolated across many temperature treatments. Similar strains were therefore grouped together as representative of the same ecotypes, as detailed in Appendix table C.1.

The incubation temperature isolates were in general well adapted to their isolation temperatures - fitness peaks near the isolation temperature (fig. 4.2A). A straight-line regression shows a significant fit with slope 0.63 ($p < 0.0001$), however a quadratic linear model also provides a significant fit ($p < 0.0001$, shown in fig. 4.2A) and is preferred to the straight-line model (ANOVA, $p < 0.0001$). The quadratic regression model fits better because the T_{pk}'s of strains

isolated at low (4°C and 10°C) temperatures are unexpectedly higher than their isolation temperature (fig. 4.2A). In comparison, peak growth temperatures of room temperature isolates appeared to be largely unaffected by the temperature that they were previously incubated at (fig. 4.2B).

To test the effects of temperature incubations on the isolation of thermal generalists versus specialists, niche width was compared to the deviation between incubation temperature and isolation temperature (fig. 4.2C). That is, when strains were isolated at room temperature, the number of degrees difference between room temperature (22°C) and their incubation temperatures. If abundant generalism were the driver for finding different taxa at different temperatures, rather than cryptic diversity of specialists only able to survive limited temperature conditions, then there would be no overall difference between the niche widths of strains from the isolation temperature or room temperature conditions. However, if temperature specialists are abundant but excluded via environmental filtering when conditions are not favourable, then that would be evident if isolates from temperatures far away from incubation temperatures are more generalist. A significant increase in niche width (generalism) was found as strains were isolated further away from the sample's incubation temperature, implying environmental filtering of specialists under these conditions (linear regression slope = 0.210, $p = 0.011$, fig. 4.2C).

4.4.2 Differences in growth strategies

To investigate whether different taxa isolated employ different growth strategies, I compared the raw growth and respiration rates across phyla. I found that the *Firmicutes* isolates have significantly higher growth rates than the *Actinobacteria* and *Proteobacteria* isolates, *on average* (Kruskal-Wallis test, $p < 0.0001$, fig. 4.3A). There is a different pattern in respiration rate data, with *Proteobacteria* tending to have higher respiration rates than *Firmicutes*, which in turn have higher respiration rates than *Actinobacteria* ($p < 0.0001$, fig. 4.3B). As well as growth and respiration, ATP content of the cultures was measured at the start and end of growth. Similar to the growth rates, *Firmicutes* isolates have significantly higher standing ATP content than the *Actinobacteria* and *Proteobacteria* isolates ($p < 0.0001$, fig. 4.3C).

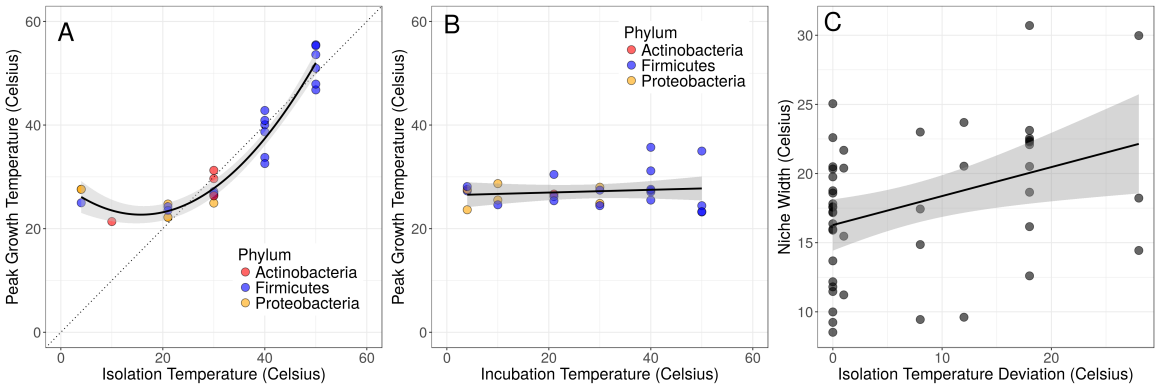

Figure 4.2: **Comparison of strains isolated at room temperature and strains isolated at their respective incubation temperatures.**

A. Peak temperature closely matches isolation temperature for IT strains (black line: polynomial model fit $p < 0.0001$). Dotted line is 1:1. **B.** No significant association between incubation and peak temperature for those strains isolated at room temperature (linear regression $p = 0.74$). **C.** Strains isolated at temperatures far away from their incubation temperatures have wider niche widths (more generalist) than strains isolated at or close to their incubation temperature (linear regression $p = 0.011$). These results support species sorting over direct thermal adaptation as the basis for differences in T_{pk} at different isolation conditions and also show the effects of environmental filtering on the room temperature isoates.

Using the OD_{600} values obtained at carrying capacity across each experimental temperature as a proxy for biomass and the maximum growth rates estimated from the Sharpe-Schoolfield fits, the phyla were compared in terms of r vs K selection. I found that *Firmicutes* have a tendency towards high growth rates but low carrying capacity (r specialists), whilst *Proteobacteria* display comparatively higher carrying capacity but lower growth rates (K specialists, fig. 4.3D). As reliable Sharpe-Schoolfield fits were produced for only 2 strains of *Actinobacteria*, conclusions were unable to be drawn for this phylum.

Given the results of different metabolic rates between taxa, I asked whether these differences have direct effects upon the accessible energy content of the cells (ATP measurements). Due to the small number of *Actinobacteria* strains, this investigation was constrained to the more numerous *Proteobacteria* and *Firmicutes*. I found that across the entire dataset, respiration rate and ATP content display a power-law relationship in both phyla (fig. 4.3E). Whilst the *Firmicutes* have generally higher ATP levels overall (as shown in fig. 4.3C), they display a sub-linear scaling relationship of ATP levels with respiration rate (scaling exponent = 0.60 $\pm$0.07). In comparison, whist *Proteobacteria* have less standing ATP content on average, they

show an approximately linear scaling relationship between ATP and respiration rate (scaling exponent = 0.99 ±0.06).

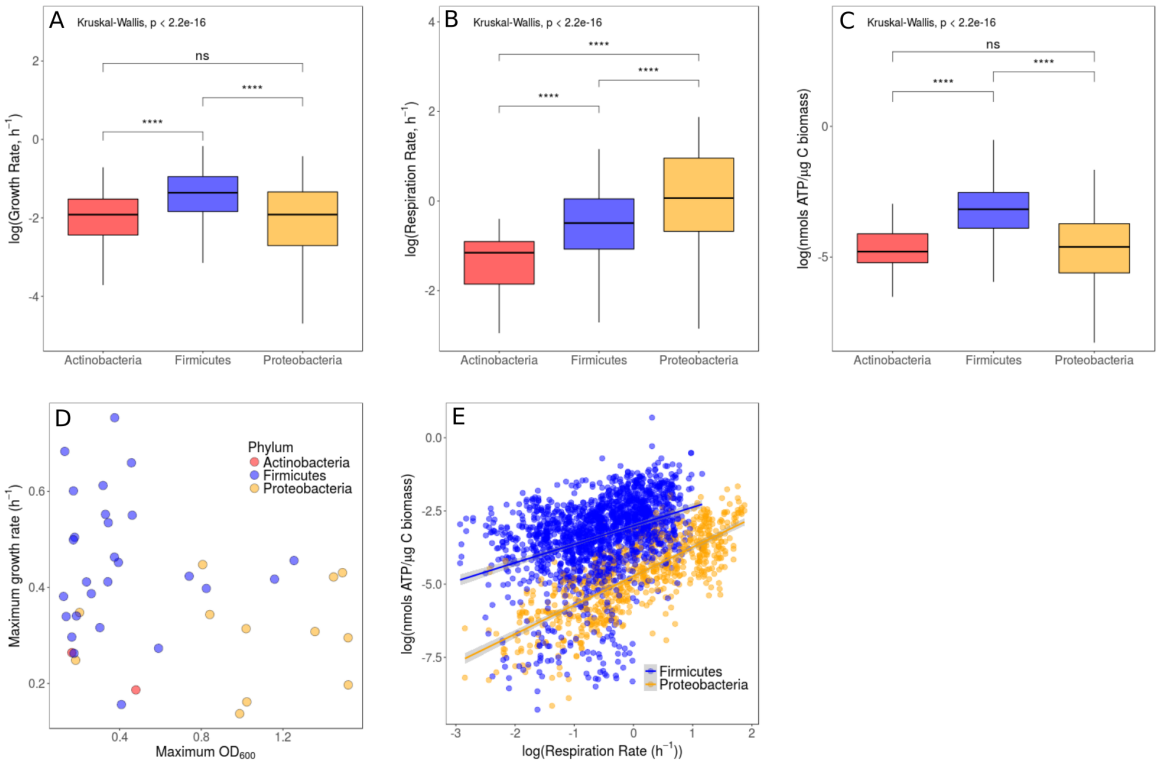

Figure 4.3: **Comparison of trait values between phyla.**

All recorded trait values plotted for growth rate **(A.)**, respiration rate **(B.)** and ATP content **(C.)**. Significant differences between phyla shown as tested by Kruskal-Wallis one-way ANOVA on ranks. ns $p > 0.05$; * $p \leqslant 0.05$; ** $p \leqslant 0.01$; *** $p \leqslant 0.001$; **** $p \leqslant 0.0001$. **D.** Maximum optical density (proxy for carrying capacity, K) versus maximum growth rate (r), coloured by phylum. *Firmicutes* tend to be r specialists, *Proteobacteria* tend to be K specialists. **E.** ATP content of cultures is a reflection of the respiration rate. *Firmicutes* show a sub-linear scaling relationship of ATP with respiration rate (scaling exponent = 0.60 ±0.07), whilst *Proteobacteria* display an approximately linear scaling relationship (scaling exponent = 0.99 ±0.06).

4.4.3 Comparison to other datasets

To ask whether the higher growth rates and lower respiration rates of *Firmicutes* comparative to *Proteobacteria* was a phenomenon constrained to this small dataset, or whether it was a general trend observed between the two phyla, I compared this to the data compiled in **Chapter 2** as well as a secondary meta-analysis by DeLong et al. (2010). DeLong et al. (2010) compiled data on both active (growth phase) and passive (stationary phase) metabolic rates across a range of bacteria (mainly from Makarieva et al. (2005)), which were corrected to

20°C using an activation energy of 0.61eV. In this dataset, *Proteobacteria* have higher active and passive metabolic rates than *Firmicutes* (active rates Wilcoxon rank-sum test p-value = 0.0017; passive rates Wilcoxon rank-sum test p-value = 0.0098, fig. 4.4A), consistent with the work presented here. The DeLong *et. al.* dataset also contains maximum growth rate data (also corrected to 20°C using an activation energy of 0.61eV). However, there is no significant difference between the growth rates of the two phyla in these data (Wilcoxon rank-sum test p-value = 0.66,fig. 4.4B). Additionally, I investigated differences in the growth rates of data compiled in **Chapter 2**. Given the findings in **Chapter 2** of hotter-is-better for mesophiles but not thermophiles, and the small number of thermophilic *Proteobacteria*, these data were restricted to mesophiles ($T_{\mathrm{pk}} < 40.5$°C) and temperature corrected to 20°C based on each strain's individual TPC parameters. In this dataset, there is a significant difference between growth rates, with *Firmicutes* on average higher than *Proteobacteria* (Wilcoxon rank-sum test p-value = 0.00035, fig. 4.4C). I also compared the distribution of T_{pk} for both phyla in the data from **Chapter 2** and find that *Proteobacteria* account for much more of the low-temperature strains, whilst *Firmicutes* are more associated with high temperatures (fig. 4.4D), this is consistent with the temperature isolation findings (fig. 4.2A).

4.4.4 T_{pk} as an evolutionary trait

Using either Blombergs K or Pagel's λ, T_{pk} displays a signal of phylogenetic heritability (Blomberg's $K = 0.32$, $p - value = 0.005$; Pagel's $\lambda = 0.93$, $p - value < 0.001$), i.e. closely related species have more similar T_{pk} than random pairs of species. The estimated ancestral states of T_{pk} were mapped onto a phylogeny, where it can be seen that colder- or hotter-adapted strains tend to cluster together (fig. 4.5A). The estimated evolution of T_{pk} through time is also shown, where it can seen that a large amount of the trait space is explored by *Firmicutes*, but not the other phyla that were isolated, which are constrained to a much narrower range of peak temperatures (fig. 4.5B). These results support the interpretation of species sorting of strains already adapted to different temperatures being the primary driver for differences in T_{pk}, over thermal adaptation during the incubation period.

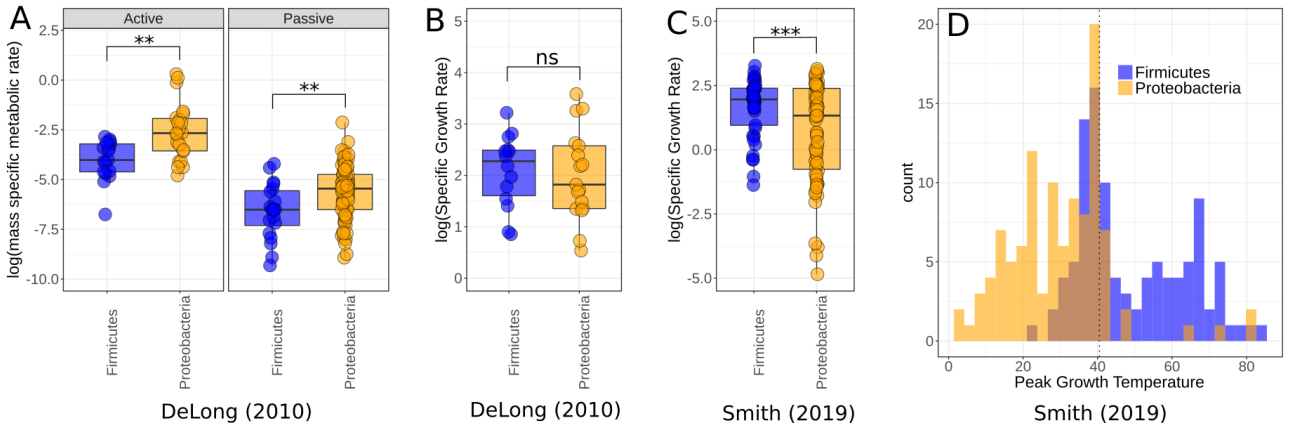

Figure 4.4: **Comparison of *Firmicutes* and *Proteobacteria* meta-analysis datasets.**

A. Dataset used by DeLong et al. (2010) shows significantly higher active and passive metabolic rates for *Proteobacteria* than *Firmicutes*. Significance determined by Wilcoxon rank-sum tests – ns $p > 0.05$; * $p \leqslant 0.05$; ** $p \leqslant 0.01$; *** $p \leqslant 0.001$; **** $p \leqslant 0.0001$. **B.** The growth rate data used by DeLong et al. (2010) shows no significant difference between the phyla. **C.** The meta-analysis data from **Chapter 2** does show significantly increased growth rates for *Firmicutes* over *Proteobacteria* however. **D.** Distribution of *Firmicutes* and *Proteobacteria* T_{pk} from meta-analysis **Chapter 2**. *Proteobacteria* account for a large proportion of the low temperature strains, whilst *Firmicutes* dominate the high temperatures. Dotted line marks 40.5°C, a cut-off between mesophiles and thermophiles.

4.5 Discussion

Here, I used a species sorting experiment to understand the extent of latent functional diversity present within bacterial communities with respect to temperature fitness. The isolation treatments resulted in the successful isolation of bacterial strains with differing thermal performance. There was a striking similarity between the peak growth temperatures and isolation temperatures of incubation temperature isolates from the 21°C treatment and above (fig. 4.2A) showing that, through species sorting, isolation temperature can determine the thermal physiology of taxa cultivated. There was an uneven distribution in the taxa isolated across the temperature range, as well as a significant phylogenetic signature in T_{pk} (fig. 4.5). This indicates that the differences in T_{pk} were driven by differences in the taxa isolated, rather than any adaptation to temperature over the course of the incubation period. Were rapid adaptation during the incubation period a driver for differences in T_{pk}, one would also expect to see a relationship between isolation and incubation temperatures in the room temperature strains. This was not the case, with room temperature isolates showing no correlation between T_{pk} and their previous

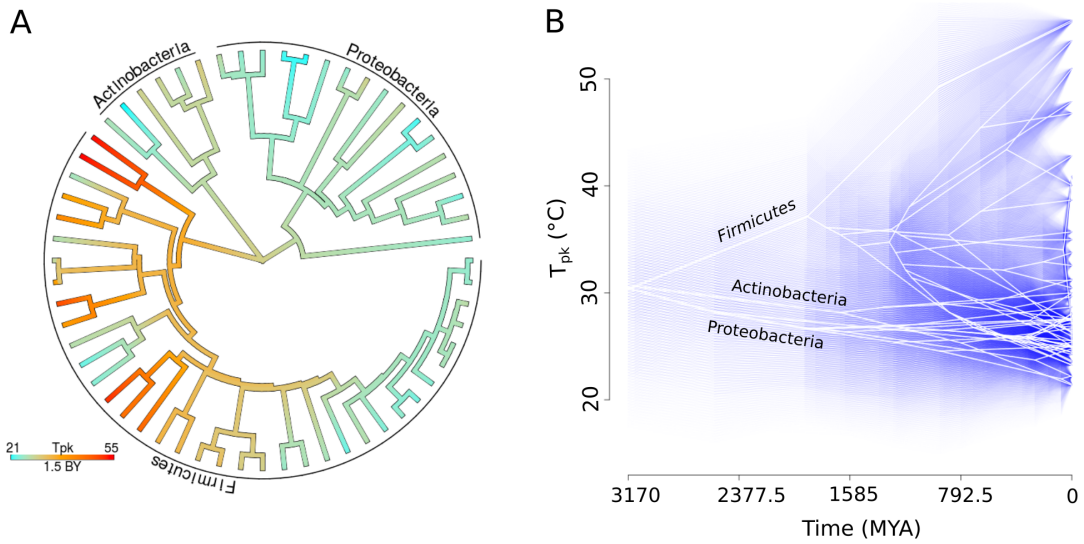

Figure 4.5: **Evolution of Tpk.**

A. Ancestral trait reconstruction of T_{pk} visualised on a tree, from lower temperatures in blue, to higher temperatures in red. All of the higher temperature (40-50°C) isolates belong to *Firmicutes*. **B.** Projection of the phylogenetic tree into the T_{pk} trait space (y-axis), over time in millions of years (MYA, x-axis). The increasing transparency of blue lines reflects the uncertainty in the estimation of T_{pk} at each node.

incubation temperatures (fig. 4.2B). Together, these results show that species sorting processes are likely to have a much greater impact on how communities respond to changing climate, than direct adaptation of microbial strains.

A potentially important question with regard to climate-driven species sorting, is whether community responses are likely to be mediated by generalists which perform well over a range of conditions. Here, I find a significant increase in thermal generalism (niche width) with increased deviation between incubation and isolation temperature (fig. 4.2C). In other words, communities incubated at temperatures far away from room temperature tended to produce more temperature-generalist taxa when isolated. Thermal specialist taxa unlikely to survive at temperatures far from their optimum are excluded and more generalist strains were preferentially isolated, an example of environmental filtering (Kraft et al., 2015). However, given that this relationship is fairly weak (slope = 0.21, i.e. a 0.21°C increase in niche width for every 1°C increase in deviation between isolation and incubation conditions), generalists may only be favoured when there is environmental filtering through *severe* fluctuations in abiotic

conditions. Thermal specialists may therefore be more likely to mediate community change via species sorting with gradual changes in environmental conditions.

There were differences in the taxa isolated at different temperatures, with all of the high temperature strains (T_{pk} 40°C+) coming from the *Firmicutes* phylum. As well as differences in thermal physiology, the *Firmicutes* appear to employ a divergent strategy of carbon use from the *Proteobacteria*, the other main phylum represented in this experiment. These phyla diverged early in bacterial evolution, estimated to last share a common ancestor over 3 billion years ago (Battistuzzi et al., 2004) (cf. fig. 4.5). As such, there are many physiological differences which typify their species. *Firmicutes* are largely gram-positive and produce endospores in nutrient poor conditions, allowing them go into a dormant state until conditions are more favourable. *Proteobacteria* are gram-negative, generally motile, do not form spores and tend to be associated with growth under low-nutrient conditions. Indeed, many of the room temperature isolates which had been incubated at high temperatures but retained mesophilic T_{pk} were *Bacilli* (*Firmicutes*), a potential reflection of their propensity to form endospores and remain dormant until temperatures allow for growth (fig. 4.5 and Appendix Table C.1). This is a possible explanation for not seeing a more pronounced relationship between temperature generalism (niche width) and deviation between isolation and incubation temperatures — the ability to form spores allowed these taxa to escape the effects of environmental filtering.

Here, *Proteobacteria* were typified by low growth rates, high respiration rates and low levels of ATP in culture. Conversely, *Firmicutes* show high growth rates, low respiration rates and high ATP content. Comparatively increased growth rates and lower respiration rates of *Firmicutes* relative to *Proteobacteria* are findings consistent with data from meta-analyses of bacterial rates (see fig. 4.4). Additionally, previously reported values for cellular ATP content have generally been higher for *Firmicutes* than *Proteobacteria* with Hattori et al. (2003) reporting more than 10-fold greater intracellular ATP content for *Bacillus* versus *Pseudomonas* strains, some of the major representatives of *Firmicutes* and *Proteobacteria* in this experiment respectively. This suggests that these phyla tend to allocate resources to growth and respiration in fundamentally different ways (fig. 4.4). One explanation for these seemingly divergent strategies may found in *Firmicutes* deriving extra energy though fermentation pathways. There is a trade-off between

rate and yield whereby bacteria may increase their rate of ATP production through supplementing aerobic respiration with fermentation (Pfeiffer et al., 2001). This increases the rate of ATP production, at the cost of lower total yield, allowing populations to reach higher growth rates but lower carrying capacity from the same resource input (r vs K selection, Andrews and Harris (1986)). This trade-off between growth rate and yield has been experimentally shown between a strain of *Bacillus* and a strain of *Pseudomonas* (Chakraborty et al., 2016). Indeed, the *Firmicutes* in my dataset tend to have lower carrying capacity than the *Proteobacteria* strains (fig. 4.3D). The differences in scaling relationship between ATP content and respiration rate may provide further evidence of differences in metabolic pathways utilised. Across *Proteobacteria*, ATP content has a scaling exponent of approximately 1 with respiration rate, indicating that theses strains are deriving ATP solely from aerobic respiration. The fact that *Firmicutes* have a lower scaling exponent may be indicative of their derivation of ATP from alternative pathways alongside aerobic respiration (i.e. fermentation).

This is generally in fitting with what is known about the ecology of these bacteria in the environment. The fast growth, high metabolic rate, but low yield of *Firmicutes* is consistent with their spore-forming nature. The strategy of these bacteria is to form an endospore until nutrient conditions are good and then utilise those nutrients in the quickest way possible to gain a competitive advantage over low nutrient specialists. This is reflected in the fact that *Firmicutes* tend to be over-represented in culture libraries (Floyd et al., 2005; Schloss et al., 2016) compared to their environmental abundance based on sequencing studies (Janssen, 2006; Delgado-Baquerizo et al., 2018). In contrast, the *Proteobacteria* in this experiment were generally more suited to oligotrophic environments (e.g. *Collimonas*, Leveau et al. (2010)), where constituent species are expected to present low growth rate and high carry capacity (K specialists, Fierer et al. (2007)) as well as increased respiration (Keiblinger et al., 2010). *Proteobacteria* are abundant in soils, play key roles in ecosystem functioning (Kersters et al., 2006) and have been shown to be associated with "healthy" soils (Wang et al., 2017). The high yield strategy seems to be particularly representative of co-operative populations, in environments where they can be insulated from r-selected populations (Lipson, 2015). Both strategies can coexist however in well mixed environments, if there are differences in mutation rates within the

community, leading to a trade-off between 'survival-of-the-fittest' and 'survival-of-the-flattest' (Beardmore et al., 2011).

Patterns of succession reflect these differences in growth strategy between taxa. Studies have revealed taxonomic groups associated with different stages of microbial succession, with patterns broadly consistent across timescales of days (Noll et al., 2005; Shrestha et al., 2007; Rui et al., 2009) and years (Nemergut et al., 2007; Banning et al., 2011) through to upwards of thousands and tens of thousands of years, as revealed through sequencing of soil sediments (Jangid et al., 2013). Generally, across these studies the phyla *Firmicutes* and *Bacteroidetes* are associated with early succession, whilst other phyla such as *Actinobacteria* and *Acidobacteria* are more abundant at later stages of succession. *Proteobacteria* are less consistent at the phylum level, with *Alphaproteobacteria* associated with late succession, *Betaproteobacteria* associated with early succession and *Gammaproteobacteria* variously associated with different stages of succession in different studies. Isolated taxa reveal a strong association between early succession and high growth rates (Shrestha et al., 2007) as well as rRNA operon copy numbers, a key determinant of bacterial growth rate (Klappenbach et al., 2000). The K-selected *Proteobacteria* can therefore be thought of as general constituents of soil, associated with standard low turnover of carbon, whilst the *r*-selected *Firmicutes* can been seen as more opportunistic from their involvement in early succession. This idea is reinforced by the fact that the soil from which these strains were isolated from is sandy and acidic (i.e. oligotrophic) (Fornara et al., 2013) and sequencing studies reveal *Proteobacteria* as the most abundant phylum (Macdonald et al., 2015). I do not suggest that this *r* vs K specialism is general to all *Firmicutes* or *Proteobacteria*, indeed meta-analysis reveals little consistency in the phyla associated with copiotrophy or oligotrophy (Ho et al., 2017), however the results presented here are consistent with phylum-specific traits for the majority of isolates.

Amongst the higher temperature isolates were obligate thermophiles; the lowest growth temperature that was recorded for the 50°C IT strains was 35°C. As the growth of these strains is constrained to temperatures largely outside of the temperatures usually experienced by the sampling environment, it can be concluded that these species are present due to dispersal and are not active members of the soil community. In comparison no obligate psychrophiles were

isolated in this experiment; the lowest incubation temperature isolates still displayed T_{pk}s in the 20-25°C range (figs. 4.2A and 4.5). The sampling was carried out in June, when ambient soil temperature was recorded as 19.4°C, therefore one possibility is that only the "summer community" was sampled and that psychrophiles may be found were the sampling to be carried out in winter under colder ambient conditions. However, it may be simply that true psychrophiles are not present in the soil in great enough quantities to reliably isolate; this is reflected in thermal performance data from bacteria isolated from very cold environments (e.g. Arctic or Antarctic samples), where growth rate is often seen to peak at more mesophilic temperatures ($\sim$ 20°C) (Bowman et al., 1998; Knoblauch and Jorgensen, 1999). Indeed, this result is similar to the results seen in **Chapter 2** where strains routinely cultured at low temperatures are shown to have higher peak temperatures than may be expected (fig. 2.2).

Whilst bacteria can rapidly adapt to new temperature regimes in the lab through adaptive evolutionary methods (Bennett et al., 1990), the absolute changes in experimental temperature regime tend to be small ($<$ 10°C, e.g. Bennett et al. (1992); Kishimoto et al. (2010); Blaby et al. (2012)) and the methods involve gradual temperature increase and regular turn-over of media to maintain exponential growth (Dragosits and Mattanovich, 2013). In comparison, this incubation was performed on whole soil cores (no turn-over of media, thus population growth rate expected to be low) with wide changes from the sampling temperature and no ramping step — conditions not conducive to adaptation, but to allow species sorting based on diversity in temperature fitness already present within the community.

In summary, I observe that the thermal fitness of bacterial isolates is largely determined by the conditions under which they were isolated. I have shown that this result can be interpreted as due to species sorting mechanisms, rather than direct adaptation of strains to experimental temperatures over the duration of the experiment. With climate warming, we may therefore expect changes in communities, driven by species sorting. In particular, we may expect shifts in some communities away from cooler adapted *Proteobacteria* towards warmer adapted *Firmicutes*. There is some evidence for this sort of community shift both from experimental warming (Aydogan et al., 2018) and natural climate gradients (Morrissey et al., 2019). This is not definitive though and the opposite may be true if warming promotes changes in lifestyle from

copiotrophy to oligotrophy due to the depletion of labile C sources (Lladó et al., 2017) and reduction in N supply (Craine et al., 2018). However, whether ecosystems will trend towards oligotrophy or copiotrophy with climate warming is a question very much still in current debate (Craine et al., 2019; Hiltbrunner et al., 2019). Community function is expected to be driven by interactions between the most abundant taxa (Rivett and Bell, 2018) and therefore changes in the abundance of taxa with warming are likely to drive profound changes in overall community function. Overall, these results show that temperature induced species sorting processes are likely to accelerate changes in microbial community function with climate warming.

Chapter 5

Variation in the efficiency of microbial growth with temperature

5.1 Abstract

The efficiency of microbial allocation of carbon to growth is a commonly quantified measure, termed carbon use efficiency (CUE). Understanding the temperature dependence of microbial CUE is critical both for predictions of how ecosystems will respond to climate change, as well as understanding microbial population dynamics. Here I present a mathematical model using Ecological Metabolic Theory, based on the thermal performance curves of growth and respiration, that challenges current assumptions about the variation of CUE with temperature. Using experimentally derived thermal performance curves from a diverse range of environmental bacterial isolates, I show that microbial CUE typically responds unimodally to temperature. This is a departure from current understanding, where efficiency is generally assumed to decrease linearly with temperature. In particular, I find that microbial growth rates tend to respond more strongly to temperature and peak earlier than respiration rates. I propose that the temperature dependence of carbon use efficiency, driving the difference in thermal sensitivity of respiration and growth, is due to differences in maintenance requirements at different temperatures. The results linking temperature dependent respiration to growth via an efficiency are

important for physiologists aiming to understand the processes underlying growth. The result of unimodal temperature dependence is important for ecosystem carbon cycle models, which have previously assumed linear responses of microbial carbon use efficiency to temperature.

5.2 Background

Micro-organisms play a major role in the soil carbon cycle by decomposing organic carbon substrates and releasing CO_2 (Manzoni et al., 2012). The efficiency with which micro-organisms convert organic carbon into biomass is therefore a critical determinant of global carbon cycling (Geyer et al., 2016, 2019). This efficiency is quantified as the ratio of carbon allocated to new biomass production relative to the total carbon consumed (del Giorgio and Cole, 1998) and is termed "carbon use efficiency" (Manzoni et al., 2012) (CUE, fig. 5.1A). By definition, high CUE values describe increased biomass production over CO_2 release due to respiration, whilst low CUE values indicate relatively greater CO_2 losses to the environment and thus lower carbon sequestration (Manzoni et al., 2012). In theory maximal values of CUE can be calculated based on thermodynamic principles (Roels, 1980; Gommers et al., 1988), however these are not likely to represent realistic efficiencies that microbes operate at in the environment. In reality, microbial CUE varies with environmental conditions such as resource stoichiometry and availability (Keiblinger et al., 2010) or physical parameters such as temperature (Apple et al., 2006). CUE values reported from environmental samples are therefore generally much lower than may be expected from theoretical calculations (del Giorgio and Cole, 1998), as microbial communities are very rarely operating under conditions for optimal efficiency. Microbial CUE is incorporated into various carbon cycle models, such as those modelling soil biogeochemical processes (Allison et al., 2010; Frey et al., 2013) or marine particle export (Dunne et al., 2005). These models often differ in how CUE is parameterised however, with many models of terrestrial biogeochemistry assuming a constant value for CUE (Manzoni et al., 2012, 2018), whilst others allow CUE to vary with other parameters such as temperature (Manzoni et al., 2012, 2018).

Where temperature has been included as an effect on CUE in studies attempting to model

soil-carbon responses, CUE has typically been assumed to decrease with temperature, based on the premise of microbial respiration displaying stronger thermal sensitivity than growth (Schimel and Weintraub, 2003; Allison et al., 2010; Allison, 2014), as shown in fig. 5.1B. Results from some empirical studies in both soil (Steinweg et al., 2008; Qiao et al., 2019) and aquatic systems (Rivkin et al., 2001; Apple et al., 2006; Manzoni et al., 2012) have indeed shown decreases in CUE with temperature, however others have shown little to no change with temperature (Dijkstra et al., 2011; Hagerty et al., 2014) whilst recently Öquist et al. (2017) have shown a potentially unimodal soil CUE response to temperature. Some of this variability may be accounted for by differences in methodology between studies (Sinsabaugh et al., 2013) and additionally in in natural systems there may be difficulties in disentangling the effects of nutrient availability from temperature (Lopez-Urrutia and Moran, 2007). Furthermore, in microbial communities the relative proportion of carbon flux from different metabolic pathways has been shown to vary with temperature (Dijkstra et al., 2011). This can also lead to variation in CUE, adding further difficulties in understanding the mechanisms behind empirical results from environmental data. CUE has also been seen to decrease with temperature in culture (Lehmeier et al., 2016), however due to the nature of fixed growth in chemostat, this experiment was not a direct comparison of active growth and respiration rates. Therefore, there is still much uncertainty surrounding how microbial CUE varies with temperature. Given the ubiquitous nature of micro-organisms and their role in biogeochemical cycles, this is crucial to our understanding of the future trajectories of global carbon cycling (Keiblinger et al., 2010; Manzoni et al., 2012; Sinsabaugh et al., 2013; Geyer et al., 2016, 2019). In particular, understanding how CUE varies with temperature will be integral to future predictions of the impacts of climate change on climate-soil feedbacks.

In order to investigate microbial CUE, measurements of population growth rate and rates of carbon uptake from the environment must be taken. However, direct measurements of uptake cannot be made in all experimental systems and so uptake is usually estimated as the total carbon accumulated in growth, plus the total carbon respired (Keiblinger et al., 2010; Manzoni et al., 2012; Sinsabaugh et al., 2013; Geyer et al., 2016) (fig. 5.1A), which means

$$\text{CUE} = \frac{\text{Growth}}{\text{Growth} + \text{Respiration}}. \tag{5.1}$$

While the temperature response of CUE is not clearly understood, the responses of growth and respiration to temperature have been well studied in isolation. These fundamental biological traits typically display a unimodal response to temperature, rising to a peak before decreasing as temperature becomes detrimental to biological function (Huey and Kingsolver, 1989; Gillooly et al., 2001; Brown et al., 2004; Dell et al., 2011). These thermal performance curves (TPCs) can be well described by mathematical models such as Sharpe-Schoolfield (Schoolfield et al., 1981), based on the idea that biological rates mirror the activity of rate-limiting enzymatic reactions (fig. 5.5B). In the operational temperature range (OTR) below peak temperature, where species are typically expected to live (Huey and Kingsolver, 1989; Angilletta Jr., 2009; Pawar et al., 2016), these responses follow a Boltzmann-Arrhenius increase of rate with temperature (Savage et al., 2004; Brown et al., 2004; Dell et al., 2011). For CUE to display thermal sensitivity, there must be differences in the thermal responses of these traits (Lopez-Urrutia and Moran, 2007) (fig. 5.5B). This however is a question that remains in debate (Lopez-Urrutia and Moran, 2007; Dell et al., 2011; Manzoni et al., 2012; Smith et al., 2019), indeed in **Chapter 2** I show that *on average* thermal sensitivities for growth rates and metabolic traits appear to be similar across species. This is reasonable from a metabolic theory of ecology perspective where metabolic rate is modelled as a function of temperature. If biomass production is fuelled by metabolism, then it follows that temperature dependent growth rate must depend upon the underlying thermal sensitivity of metabolic rate (Luhring and Delong, 2017). This is reflected in the close relationship between the temperature-size-dependence of biomass production and metabolic rate (Ernest et al., 2003) and is the basis for models linking microbial metabolism to population growth rate (Kempes et al., 2012; De Jong et al., 2017; Luhring and Delong, 2017). However, whilst energetics would suggest a link between temperature dependent growth and metabolism, this may not result in a direct relationship between these traits due to incomplete coupling between anabolism and catabolism (Russell and Cook, 1995). Changes in maintenance requirements with temperature as well as variation in overflow metabolism (energy

spilling) can lead to this lack of direct coupling (Russell and Cook, 1995), leading to variation in CUE with temperature. Aside from the impacts for climate change models then, understanding the variation of CUE with temperature is fundamentally important for understanding the physiology and evolutionary biology of prokaryotes. In order to develop mechanistic models for temperature-dependent microbial growth, an understanding of the underlying temperature dependence of metabolic rates and CUE is required.

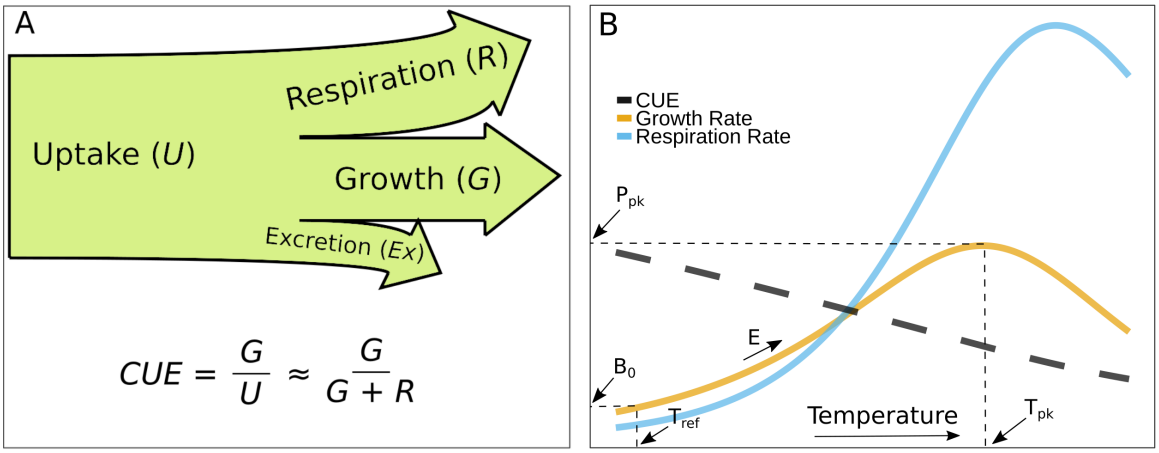

Figure 5.1: **Carbon use efficiency.**

A. All carbon uptake is either incorporated during growth, respired (including respiration due to maintenance, growth and overflow metabolism), or excreted. In practice, it is commonly assumed that excretion represents a negligible loss of carbon compared to respiratory processes, and so uptake is calculated post-hoc as the total carbon used for growth plus respiration (as these are the processes measured). **B.** Growth (orange) and respiration (blue) show a unimodal relationship with temperature, which can be adequately modelled using the Sharpe-Schoolfield function (eq. 5.3, parameters labelled on growth rate curve). The difference in the Sharpe-Schoolfield parameters between growth and respiration describe how CUE (black) changes with temperature. The Sharpe-Schoolfield parameters are indicated on the growth rate curve.

Direct comparisons of the thermal responses of growth and respiration are largely lacking from the literature so it is difficult to link these underlying processes to temperature dependent CUE. One important consideration is that there is often a time-scale mismatch of thermal acclimation in these data, in that growth rates by their nature require measurement over a reasonable time-period in which growth is observable, allowing a level of thermal acclimation, whilst respiration rate is an acute response usually measured over shorter time where an acclimation period may or may not be given, e.g. Padfield et al. (2016). Additionally, respiration rate measurements are usually made during stationary phases when populations are not growing, making direct

comparisons to growth rate inappropriate. Finally, where microbial metabolic rates have been measured during growth, the calculated mass-specific rates tend to be normalised to an initial starting population biomass and omit any changes in biomass due to growth over the course of the experiment. That is, given two populations with the same initial population size and same mass specific respiration rate, one of which is in growth and the other is in stationary phase, the growing population will inevitably produce more total respiratory output, by virtue of increased population size over the duration of the experiment. This has generally not be accounted for and therefore "active" rates reported in the literature may often be an over-estimate of the true specific respiration rate. **Chapter 3** discusses this problem in more detail. Here, by measuring growth and respiration rate concurrently and calculating specific respiration rate as described in **Chapter 3**, I am able to reconcile the time-scale differences usually apparent when comparing these responses. As I am looking at exponentially growing populations in a rich culture medium, the effects of nutrient limitation do not apply here. This may be considered as potentially an inaccurate representation of processes underlying carbon fluxes at the ecosystem or community level, as these systems are often assumed to be at steady state. However, microbial systems may be more dynamic in nature, with repeated successional changes following environmental pertubations (Rivett et al., 2018). Furthermore, environments variously contain 'hot-spots' of microbial activity with much higher process rates than average conditions (Kuzyakov and Blagodatskaya, 2015), where exponential growth is relevant.

Given that growth rate and respiration rate show a unimodal relationship to temperature with a form that can be encapsulated with the Sharpe-Schoolfield equation, then differences in the Sharpe-Schoolfield parameters between growth and respiration can describe how CUE varies with temperature. Using these traits' TPCs, I propose a mathematical model for how CUE is expected to vary with temperature. This is similar to the autotroph allocation efficiency model of García-Carreras et al. (2018), here applied to heterotrophs. Using a dataset of empirical growth and respiration rates from 47 bacterial isolates, spanning a wide range of tanxomic groups and growth strategies, I compare the TPC parameters for these traits to understand whether they vary in a systematic manner. I use these results to inform an expectation for how CUE varies with temperature, and ask whether CUE displays a consistent thermal response.

Subsequently, I show through the CUE model that the assumption of a linear reduction in CUE with temperature, as it is often parameterised (Schimel and Weintraub, 2003; Allison et al., 2010; Allison, 2014), is unlikely to be met and that unimodal CUE is a more plausible outcome generally.

5.3 Methods

5.3.1 Modelling CUE

To understand how cellular carbon is allocated to growth in a bacterial population, I start with a simple model:

$$\frac{1}{C}\frac{dC}{dt} = \mu = \epsilon U;$$

where the change in population biomass, C, over time, t, (the growth rate, μ) is determined by the product of the carbon uptake rate, U, and an efficiency, ϵ. This is the nutrient unlimited version of a more general growth equation (García-Carreras et al., 2018), appropriate for exponential growth measurements. Although there may be other sources of carbon loss to a growing bacterial population such as metabolite excretion, I assume that the majority of carbon uptake is allocated to growth and respiration, i.e. $U \approx (R + \mu)$. Given this approximation, I estimate ϵ as:

$$\epsilon = \frac{\mu}{\mu + R}. \tag{5.2}$$

which is analogous to the carbon use efficiency (CUE) measure found throughout the bacterial literature (Keiblinger et al., 2010; Manzoni et al., 2012; Sinsabaugh et al., 2013; Allison, 2014; Geyer et al., 2016). This is effectively a measure of the proportion of carbon taken up by the cell, which is allocated to growth (see fig. 5.5A).

The effects of temperature on biological rates (such as growth and respiration) across the full TPC can be approximated using the Sharpe-Schoolfield model (Schoolfield et al., 1981) (eq. 5.3), which describes a Boltzmann-Arrhenius increase in rate with temperature to a peak, before a decrease in rate when temperature becomes detrimental.

$$B(T) = B_0 \frac{e^{\frac{-E}{k}\cdot\left(\frac{1}{T}-\frac{1}{T_{\text{ref}}}\right)}}{1+\frac{E}{E_{\text{D}}-E}e^{\frac{E_{\text{D}}}{k}\left(\frac{1}{T_{\text{pk}}}-\frac{1}{T}\right)}} \tag{5.3}$$

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"), E_{D} the deactivation energy, which determines the rate of decline in the biological rate beyond the peak temperature (T_{pk}, sometimes termed the optimum temperature, T_{opt}) and k is the Boltzmann constant (8.617×10^{-5} eV K^{-1}). Solving the equation for $T = T_{\text{pk}}$ gives the peak performance for the trait (P_{pk}). A visualisation of these parameters is shown in fig. 5.1B. Note that this has been reformulated from the model presented in the original paper, to include T_{pk} as an explicit parameter (Kontopoulos et al., 2018).

To understand how CUE may vary with temperature, I substitute the SS equation defined for growth rate (μ) and respiration rate (R) into eq. 5.2, to give:

$$CUE = \frac{B_{0,\mu}\frac{e^{\frac{-E_\mu}{k}\cdot\left(\frac{1}{T}-\frac{1}{T_{\text{ref}}}\right)}}{1+\frac{E_\mu}{E_{D,\mu}-E_\mu}e^{\frac{E_{D,\mu}}{k}\left(\frac{1}{T_{\text{pk}}}-\frac{1}{T}\right)}}}{B_{0,\mu}\frac{e^{\frac{-E_\mu}{k}\cdot\left(\frac{1}{T}-\frac{1}{T_{\text{ref}}}\right)}}{1+\frac{E_\mu}{E_{D,\mu}-E_\mu}e^{\frac{E_{D,\mu}}{k}\left(\frac{1}{T_{\text{pk}}}-\frac{1}{T}\right)}} + B_0\frac{e^{\frac{-E_R}{k}\cdot\left(\frac{1}{T}-\frac{1}{T_{\text{ref}}}\right)}}{1+\frac{E_R}{E_{D,R}-E_R}e^{\frac{E_{D,R}}{k}\left(\frac{1}{T_{\text{pk}}}-\frac{1}{T}\right)}}}. \tag{5.4}$$

I explore the possible outcomes of different parameterisations of this model to understand how CUE may vary with temperature.

5.3.2 Experimental Measurements

This chapter uses the temperature dependent growth and respiration data generated in **Chapter 4**, using the methodology described in **Chapter 3**. Briefly, cells were revived from frozen and grown to carrying capacity at experimental temperature (acclimation period - to restrict influence of temperature stress on TPC, or equalise it across experimental points). Carrying-capacity cultures were diluted into new media and grown until in exponential growth (as determined by optical density). Growing cells were then centrifuged and supernatant removed. Cells were then re-suspended in fresh media to $OD_{600} \approx 0.2$ and serially diluted to give 4 starting cell densities for each culture. This yields cells primed for growth, unlimited by nutrients, with different cell number starting points for replication. Growth and respiration were tracked by flow cytometry and MicroResp™ respectively. This was repeated across a range of temperatures spanning the full TPC of each isolate.

5.3.3 Empirical Rates

From the flow cytometry cell diameter measurements I estimate average cell volumes at each point and estimate the cellular carbon per cell with

$$\text{fgC cell}^{-1} = 133.754V^{0.438},$$

as described by Romanova and Sazhin (2010). Multiplying this by the cell counts gives an estimate of the carbon biomass of the culture at the starting and ending points.

The difference between the initial biomass and biomass at the end of the experiment gives the total carbon sequestered through growth. Given total biomass (C_{tot}) produced from a starting biomass (C_0) over a given time period ($t_{tot} - t_0$, or Δt), assuming the population is in exponential growth, the mass specific growth rate (μ) is given by:

$$\mu = \frac{\log(\frac{C_{tot}}{C_0})}{\Delta t}. \tag{5.5}$$

The MicroResp™ system measures the total CO_2 evolved from the culture. This is a measurement of the total cumulative CO_2 produced over the course of the experiment and because the molecular mass of carbon within CO_2 is known, I can calculate the mass (in μg) of carbon produced. This is directly comparable to the total biomass produced over the course of the experiment, but not to the growth rate, as the CO_2 production is dependant upon the cellular biomass which changes across the experiment.

I thus calculate the specific respiration rate as derived in **Chapter 3**:

$$R = \frac{\mu R_{tot}}{C_0 e^{\mu \Delta t} - C_0}. \tag{5.6}$$

Here, R_{tot} is the total mass of C produced according to the MicroResp™ measurements, C_0 is the initial population biomass, μ is the previously calculated growth rate and t is the duration of the experiment. This measure of respiration rate is directly comparable to the specific growth rate, μ.

I calculate CUE from these rates according to eq. 5.2.

5.3.4 TPC Fitting

By growing the bacterial populations at different temperatures I generated TPCs for specific growth rate and specific respiration rate based on eqs. 5.5 and 5.6. To each TPC I fitted a modified Sharpe-Schoolfield model (Kontopoulos et al., 2018) (SS, eq. 5.3) in order to compare the thermal response parameters for theses traits within and across bacterial strains.

Given the mathematical model (eq. 5.4), I can predict how CUE is expected to vary with temperature, however I allow for alternative outcomes. To test my predictions, I fit SS (5.3 to the empirical CUE TPCs and also fit the simpler Boltzmann-Arrhenius function (BA, eq. 5.7):

$$B(T) = B_0 e^{\frac{-E}{k}\cdot\left(\frac{1}{T}-\frac{1}{T_{\text{ref}}}\right)} \tag{5.7}$$

This is used to describe a monotonic increase in rate within the operational temperature range (OTR), but can also describe a decrease in rate with temperature when E is negative. Here I make no assumption of the directionality of the BA fit and allow fits with positive or negative E. I then use model selection to see whether SS or BA fits are preferred for each strain and thus whether the CUE response is unimodal, or monotonic with a positive or negative slope.

All model fitting was performed in R using the "minpack.lm" package for non-linear least squares regression.

5.4 Results

5.4.1 Empirical variation in TPC parameters

By fitting the Schoolfield model to the empirical data, I can compare the TPC parameters for growth and respiration to understand how these are linked across different microbial strains, this is shown in fig. 5.2. Here I find that whilst there is a very strong association between T_{pk} for respiration rate and growth rate (fig. 5.2A, Pearson's correlation $t = 16.952$, df $= 39$, $p < 0.0001$), respiration rate T_{pk} is consistently higher than growth rate T_{pk} (paired t-test $t = 6.0665$, df $= 40$, $p < 0.0001$). In contrast, whilst growth rate and respiration rate thermal sensitivity, E, are also strongly correlated (fig. 5.2B, Pearson's correlation $t = 3.8587$, df $= 41$, $p < 0.001$), E for growth rate tends to be higher than that of respiration rate (paired t-test, $t = -2.7097$, df $= 42$, $p = 0.0049$).

Given the lack of consensus on whether thermal sensitivity is expected to differ between growth and respiration, I explore the observed variation in more detail (fig. 5.2C). I find a clear difference in the distribution of E between growth and respiration, with growth rate E extending to higher values, where respiration rate E displays a sharper cut-off.

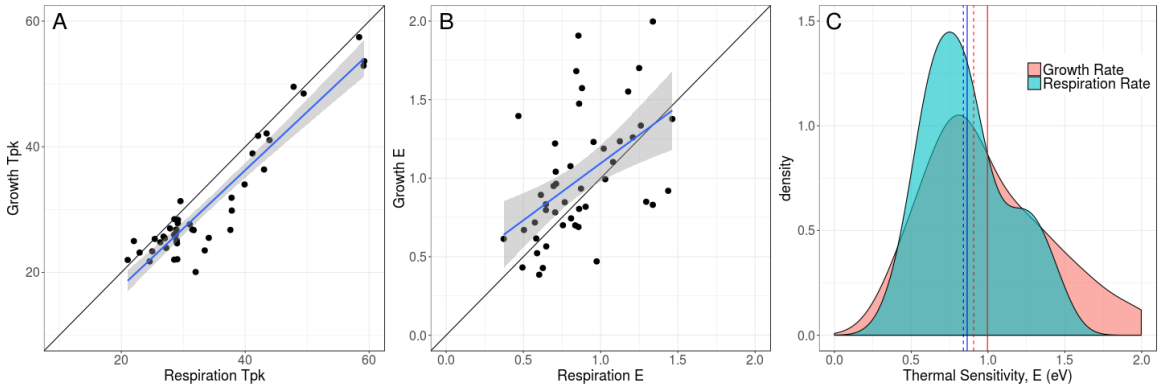

Figure 5.2: **Variation in TPC parameters.**

Plots of growth rate against respiration rate for T_{pk} (**A**, n = 40) and E (**B**, n = 43) from the experimental data. Black lines are 1:1 lines, blue lines represent linear fits to the data with associated confidence intervals (grey). Note that there are three fewer points for the T_{pk} comparison; in these cases one or both of the responses did not display a peak and thus T_{pk} could not be compared, however the thermal sensitivity, E, can still be estimated. **C.** Distribution of E for growth rate and respiration rate. Red (growth rate) and blue (respiration rate) lines show mean (solid) and median (dashed) values.

5.4.2 Empirical variation in CUE with temperature

Given the results from the microbial data that growth rates tend to display higher thermal sensitivity than respiration rate and that respiration rate tends to peak at higher temperature (fig. 5.2), I consider how allocation of carbon to growth versus respiration may vary with temperature. An example of this type of response seen in the empirical data is shown for strain 04_RT_01, a strain of *Bacillus weihenstephanensis* (fig. 5.3). The basal respiration rate for this strain is higher than it's growth rate, however growth shows a higher thermal sensitivity, so CUE increases with temperature to a peak at ~ 25°C before decreasing as growth rate drops at higher temperatures, but respiration rate continues to rise. This CUE response may take different forms for different taxa however, if the variation in TPC parameters is not consistent across all strains (see fig. 5.5D).

To formally test how consistently this CUE thermal response is conserved across taxa, I fit both SS and BA to the CUE data and investigate the resulting distribution of parameters (fig. 5.4). Five strains showed no variation of CUE with temperature (fig. 5.4A), largely due these strains having very similar growth and respiration rate TPCs. 14 strains' CUE TPC was best modelled by a monotonic BA equation. Most of these (12/14) display a decrease in CUE with

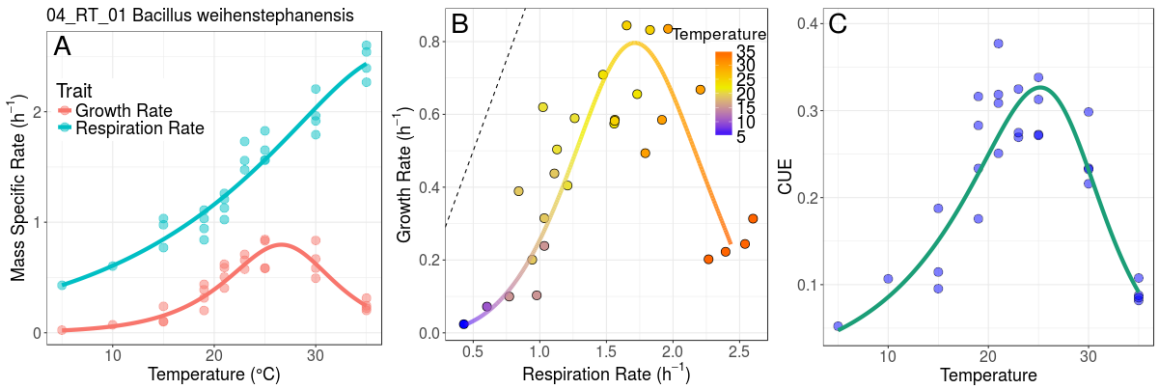

Figure 5.3: **Example empirical CUE temperature response for strain 04_RT_01, *B. weihenstephanensis*.**

A. Growth rate and respiration rate plotted on the same axis against temperature. Growth is seen to peak within the experimental temperature range, however respiration rate continues to increase. **B.** Growth and respiration points plotted against each other, coloured by temperature. Line represents the SS fits for growth and respiration plotted together, coloured by temperature. Dotted line is 1:1, where growth and respiration are equal, equivalent to CUE= 0.5. **C.** CUE plotted against temperature, line is the unimodal SS fit for CUE.

temperature shown by their negative E (fig. 5.4B). Two strains display an apparent positive temperature dependence of CUE, however these can be considered outliers due to noise in the underlying data, as by eye these two strains' CUE TPCs appear uni-modal. For the majority of strains (28/47) the unimodal SS model provides the best fit to the data, in these strains I see that CUE peaks at lower temperature than either growth or respiration rate (fig 5.4C). In general this is due to there being an inflection in the growth rate TPC (which occurs before the TPC peaks), at a temperature where the respiration rate TPC remains exponential. This is predictable from the underlying variation in parameters (fig. 5.2). All empirical TPCs for CUE with their respective best fitting model are shown in Appendix Fig. C.1.

5.4.3 Parameters driving the CUE thermal response

Using the CUE model (eq. 5.4), I simulate CUE thermal responses using different parameter combinations to investigate the importance of the parameters driving the TPC. I show that differences in B_0 between growth and respiration ("mismatches") control the overall magnitude of CUE (fig. 5.5A). Mismatches in E control the initial rise or fall of the response (fig. 5.5B), and mismatches in T_{pk} cause an inflection of the curve (fig. 5.5C). Therefore, whilst B_0 mismatches

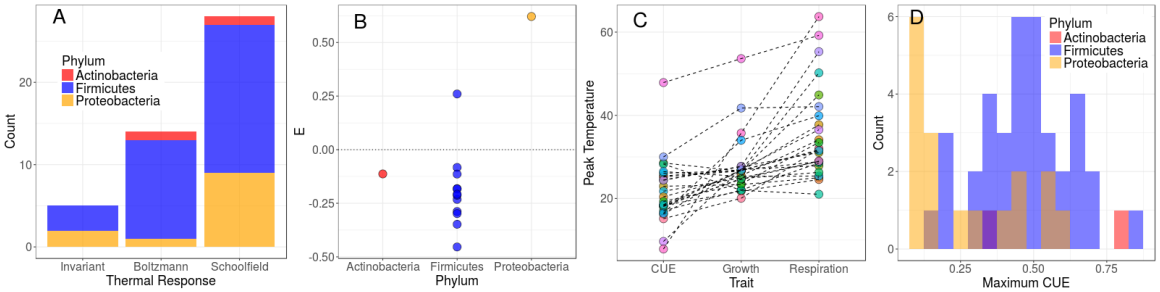

Figure 5.4: **Temperature dependent CUE model fitting.**

A. Model selection for the CUE thermal responses based on AIC. In cases where there was no significant fit for either model, CUE was deemed invariant with temperature. **B.** Distribution of E for cases where the monotonic BA model was preferred. Positive or negative E values signify an increase or decrease in CUE with temperature respectively. **C.** Peak CUE temperature for strains best represented by the SS model compared to their respective peak growth and respiration temperatures. CUE tends to peak at lower temperatures than growth or respiration. Colours in panel C denote different individual strains. **D.** Distribution of maximum CUE values obtained for each strain.

are important for determining the offset of the curve, mismatches in E and T_{pk} are more important in determining the overall *shape* of the response curve. I do not consider differences in E_{D} as this will largely affect the CUE response only at high temperatures, outside of the temperature range where organisms are expected to normally operate. I then investigate the potential different shapes that the overall CUE TPC may take based on mismatches in E and T_{pk}. I find that mismatches in these parameters cause the CUE curve to produce an initial increase or decrease in CUE with temperature (due to mismatches in E, fig. 5.5B), followed by an inflection either upwards or downwards (due to mismatches in T_{pk}, fig. 5.5C). The results of these mismatches may therefore not always result in unimodal CUE (as found in the empirical data) but may take different forms based on how consistently growth and respiration show differences in thermal performance (fig. 5.5D). This extends to the possibility of CUE showing an approximately linear decrease with temperature within the OTR, as has been previously assumed in some models, e.g. Schimel and Weintraub (2003); Allison et al. (2010); Allison (2014). For CUE to display a linear decrease with temperature, E_R must be greater than E_μ for CUE to initially decrease with temperature. Secondly $T_{pk,R}$ must be greater than $T_{pk,\mu}$ so that there is no inflection within the OTR. When $B_{0,\mu}$ is greater than $B_{0,R}$, E_R must be *much* greater than E_μ to produce an approximately linear response across the whole OTR. This is the condition shown in fig. 5.1B, using the following parameter combination: $E_\mu = 0.7$;

$B_{0,\mu} = 0.1$; $T_{pk,\mu} = 25°C$; $E_{D,\mu} = 1.8$; $E_R = 1.1$; $B_{0,R} = 0.04$; $T_{pk,R} = 30°C$; $E_{D,R} = 1.8$. However when $B_{0,\mu}$ is less than $B_{0,R}$, E_R must only be *slightly* greater than E_μ for CUE to decrease approximately linearly with temperature (condition not shown).

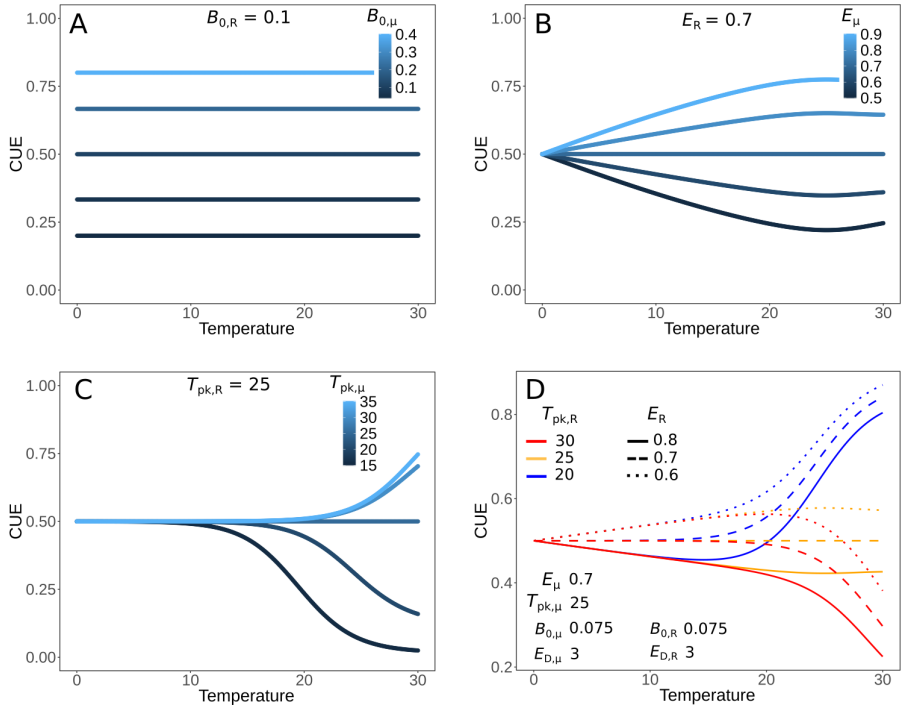

Figure 5.5: **Effects of parameter mismatches on CUE TPC.**

A. Differences in B_0 between growth (subscript μ) and respiration (subscript R) control the offset of the CUE thermal response when all other parameters are equal. **B.** Differences in T_{pk} cause an inflection in the CUE TPC. If $T_{pk,R} > T_{pk,\mu}$ the inflection is upward whilst if $T_{pk,R} < T_{pk,\mu}$ the inflection is downward. **C.** Differences in E control the direction of the initial slope of the CUE TPC. When $E_R > E_\mu$, CUE initially decreases with temperature and when $E_R < E_\mu$, CUE increases with temperature, when all other parameters are equal. In panels A - C all parameter values for growth and respiration remain constant ($E_\mu = 0.7$; $E_R = 0.7$; $B_{0,\mu} = 0.1$; $B_{0,R} = 0.1$; $T_{pk,\mu} = 25°C$; $T_{pk,R} = 25°C$), aside from the parameter explored in the panel. **D.** Combining variation in E and T_{pk} I can see the varied shapes that a CUE TPC may take. Here, the parameters for growth rate remain constant, whilst the respiration E and T_{pk} parameters are allowed to vary above and below those of growth.

5.5 Discussion

Here, using a combination of empirical data and mathematical modelling, I have presented a new understanding of how microbial carbon use efficiency varies with temperature. I generated thermal performance curves for growth rate and respiration rate from 47 strains of environ-

mentally isolated bacteria and used these to derive the carbon use efficiency TPCs. I find that in most cases growth rate responds more strongly to temperature ($E_\mu > E_R$), but peaks earlier ($T_{pk,\mu} < T_{pk,R}$). Following these generalisations, as temperature increases there is an initial allocation towards growth following it's increased thermal sensitivity, before an allocation towards respiration after growth rate peaks at lower temperature. Expressing this in terms of carbon use efficiency, I would expect CUE to display a uni-modal response, initially rising with temperature before peaking and then dropping with temperature as comparatively more resources are allocated to maintenance processes than growth at higher temperatures. This is indeed what I find — that for the majority of strains CUE displays a unimodal response to temperature, in line with my findings of systematic differences in thermal performance of growth rate and respiration rate. The differences found in T_{pk} between growth rate and respiration rate are in agreement with expectations that growth may be expected to peak when respiration continues to increase with temperature (Manzoni et al., 2012). Indeed, this effect has been seen in the whole soil response to temperature (Pietikäinen et al., 2005), as well as culture studies of bacteria respiring both aerobically (Christian and Wiebe, 1974; Kusnetsov et al., 1996) and anaerobically (Knoblauch and Jorgensen, 1999). I also found a difference in thermal sensitivity (E) between growth and respiration rate, with growth tending to be more temperature sensitive, however there is less precedent for this in other studies. Community respiration rates have in fact been reported to display higher thermal sensitivity than growth rate in aquatic systems (Rivkin et al., 2001; Apple et al., 2006), however it has since been suggested that this may be accounted for due to resource limitation of growth but not respiration (Lopez-Urrutia and Moran, 2007). This is the basis for many modelling approaches using CUE as a decreasing function of temperature (eg. Schimel and Weintraub (2003); Allison et al. (2010); Allison (2014)). It is not clear whether the growth and respiration responses should display differences in thermal sensitivity without the effects of nutrient limitation.

If metabolic rate is a temperature dependant process, and biomass production is fuelled by metabolism, it would seem to follow that the temperature sensitivities of each should match (Luhring and Delong, 2017). However, although the responses of growth and respiration to temperature can be modelled on the basis of their responses being similar to a single rate-

limiting enzymatic reaction (Schoolfield et al., 1981), these rates are in reality the end result of numerous complex biochemical and physiological processes, each with their own independent thermal sensitivities (Brown et al., 2004; Apple et al., 2006). For aerobic heterotrophs, we may consider respiration rate to be equivalent to their "metabolic rate", a process fundamentally dependant upon the temperature (Brown et al., 2004). Growth (or production) however is a more emergent trait based on the fraction of metabolism allocated to it (Brown et al., 2004). Given that the efficiency of allocation of carbon to growth can vary with temperature in autotrophs (García-Carreras et al., 2018), it may follow that growth can also display a thermal sensitivity distinct from respiration in heterotrophs. Theoretically the differences in observed E may be explained by bacteria having to allocate comparatively more resources to maintenance versus growth at temperatures further away from their optimum. Proteins display distinct stability curves with respect to temperature, being generally most stable at temperatures in which the organism lives (Pucci and Rooman, 2017). In microbes, protein repair is expected to dominate maintenance costs (Kempes et al., 2017). Therefore, growth at temperatures away from the optimum is likely to incur high maintenance demands through increased requirements for protein repair. This may lead to comparatively increased respiration at low temperatures compared to growth, resulting in differences in observed thermal sensitivity. I additionally found a narrower distribution of E_R values than E_μ (fig. 5.2C). This may indicate stronger evolutionary constraints acting upon the thermal sensitivity of respiration (the more fundamental metabolic process) than growth rate (the more emergent process), which has the potential to explore more of the parameter space.

I have also shown theoretically that the unimodal temperature dependence of CUE is generally a likely outcome, given the underlying unimodality of growth rate and respiration rate temperature responses. I find that whilst an approximately linear decrease in CUE with temperature (as used in previous ecosystem modelling studies (Allison et al., 2010; Allison, 2014)) is technically *possible* within the parameter space, there are many other potential outcomes that result in non-linear responses. The assumption of a linear decrease in CUE with temperature is therefore likely to be unreasonable if the combination of $E_\mu < E_R$ and $T_{pk,\mu} < T_{pk,R}$ is not normally evident in nature. For the empirical bacterial dataset, these conditions are not

met, however this may not be consistent across different taxa. In contrast to these results for example, phytoplankton *do* consistently display $E_\mu < E_R$ (Kontopoulos et al., 2019), although for these microbes allocation of carbon to growth is likely to depend upon the balance between respiration and photosynthesis (García-Carreras et al., 2018). These processes are different to those of carbon allocation to heterotrophic growth and therefore my results are not likely to be generally tractable to autotrophs.

Theoretical calculations place maximum CUE at about 0.6 (Roels, 1980; Sinsabaugh et al., 2013), and similar values have been reported from pure culture experiments (CUE = 0.6-0.85) (Gommers et al., 1988; Babel, 2009; Geyer et al., 2016). In natural systems there are a wide range of reported CUE values, from as low as 0.01 in the most dilute systems, to nearer 0.5 in eutrophic systems (del Giorgio and Cole, 1998). The experimental data shown here fall very much within this range (fig. 5.4D), although the *Proteobacteria* tended to display lower CUE than the other phyla. This may be expected on the basis of these strains tending to be adapted for growth under oligotrophic conditions (see **Chapter 4**), as K-specialists tend to have comparatively high respiration and low growth rates and hence lower CUE (Keiblinger et al., 2010). The overall magnitude of these CUE values are likely to be an over-estimate compared to the "real" growth efficiency calculated as the total carbon uptake allocated to growth. This is due to the (widely used) methodology of estimating that all carbon is allocated to either growth or respiration. In reality, there may be other avenues of carbon loss that are not visible to this experiment, such as excretion of metabolites. Whether this would cause a significant difference to these results of temperature dependent CUE would depend on whether excretion displays a pattern of temperature sensitivity distinct from respiration. The release of carbon by excretion is commonly assumed to be insignificant in models of bacterial growth (Touratier et al., 1999), however bacteria do excrete or leak metabolic by-products into the culture medium (Russell and Cook, 1995; Touratier et al., 1999; Manzoni et al., 2012). In particular, with high levels of excess carbon in the substrate, some heterotrophic bacteria will excrete partially oxidised carbon into the environment in order to drain reducing power (Braakman et al., 2017). When nitrogen or phosphorous are the limiting nutrients and carbon levels are high, carbon excretion levels are high (Dauner et al., 2001). When carbon is the limiting nutrient however, levels of

carbon excretion are much lower — Dauner et al. (2001) report in the region of 3-6% of carbon uptake for *B. subtilis*. My experimental data is derived from growth in LB medium. This is rich medium designed for exponential growth under essentially nutrient-unlimited conditions. This was used to avoid the limitations of studies from natural systems, where nutrient limitation is likely to play a major role in the CUE response (Lopez-Urrutia and Moran, 2007). The most likely nutrient limiting growth in LB however is carbon (Sezonov et al., 2007) and therefore excretion is expected to account for only a small percentage of carbon loss. The results shown here are thus likely a good approximation for the temperature dependence of CUE.

Despite the empirical data being derived from lab experiments under nutrient saturated conditions, I used a variety of strains isolated from environmental soil samples grown in a complex culture medium. This may make these results more tractable to real-world scenarios than previous culture-based experiments, which have tended to use lab-adapted strains grown on single carbon substrates, e.g. glucose. My data is derived from cultures in exponential growth and therefore may provide a poor comparison to natural environments, where CUE may be driven by maintenance metabolism of much lower turnover populations more generally. However, these results are applicable to "hot-spots" of intense microbial activity which occur in soils (Kuzyakov and Blagodatskaya, 2015), as well as ecosystems undergoing intermittent perturbations, which is likely to result in repeated invasions and colonisations of strains under rapid growth (Rivett et al., 2018).

In conclusion I have shown that, in contrast to current assumptions, a set of taxonomically and functionally diverse environmentally isolated bacteria tend to display a unimodal relationship between temperature and CUE. This is evidenced by differences in the thermal sensitivity parameters of growth rate and respiration rate. This is of importance both for physiologists aiming to understand abiotic effects on bacterial growth efficiency, as well as for parameterising ecosystem models which consider environmental effects on variation in microbial carbon sequestration and efflux. In terms of physiology, this is a major step in understanding the underlying mechanisms behind growth efficiency. In terms of ecosystem modelling, parameterising microbial CUE as a unimodal response to temperature is likely to have a major effect on the resulting predictions of short-term ecosystem fluxes (i.e. in the absence of longer term

acclimation, adaptation or species sorting processes).

Chapter 6

Synthesis

Micro-organisms play a critical role in global biogeochemical cycles (Sikorski, 2015; Anderson, 2018; Kuypers et al., 2018) and ecosystem functioning (Balser and Firestone, 2005; Van Der Heijden et al., 2008). Understanding their responses to warming, from physical changes and adaptation at the individual and population level, through to shifts in community composition, is important for future predictions of the impacts of climate change (Bardgett et al., 2008). This thesis aimed to investigate the fundamental effects of temperature on microbial biological rates, and then use modelling approaches to link these to more emergent processes at larger scales, such as the effects of temperature on ecosystem carbon flux. The main questions addressed were:

1. Are prokaryotic temperature responses consistent across taxa and time-scales and are they therefore evolutionarily conserved?

2. What is the physiological basis for these responses to temperature, and do the emergent responses of growth rate (fitness) to temperature reflect those of underlying metabolic rates?

3. How diverse is the thermal ecology of microbial communities and is there a large amount of functional redundancy with respect to temperature fitness which may contribute to species sorting processes?

4. What effects may climate change therefore have on microbial community composition and carbon fluxes?

6.1 Thermal constraints across taxa and timescales

In **Chapter 2** using a meta-analysis approach, I have shown that a diverse range of hundreds of prokaryotic taxa produce unimodal thermal performance curves for growth rates, with peak growth temperatures approximating the conditions under which those strains were routinely cultured. The thermal sensitivity (E) of strains produced a right-skewed distribution, as expected from previous intra-specific studies (Dell et al., 2011), however with a mean significantly higher than that previously reported (bacteria mean $E = 0.88$eV, archaea mean $E = 0.95$eV, as opposed to 0.66eV mean intra-specific E reported by Dell et al. (2011)). Previous meta-analyses have been constrained to datasets almost exclusively composed of Eukaryotes. Therefore the work presented here, in combination with other smaller-scale studies producing intra-specific E data for *Cyanobacteria* (Chen and Laws, 2017) and methanogenic archaea (Yvon-Durocher et al., 2014), suggests that prokaryotes may operate under different thermal constraints to more complex eukaryotes.

Chapter 2 also linked short-term intra-specific thermal responses, to the long-term inter-specific thermal response. I showed that mesophilic prokaryotes display thermal sensitivity consistent with a 'hotter-is-better' thermal constraint, meaning that on average hotter adapted prokaryotes have higher peak growth rates. This does not extend to thermophiles however, for whom adaptation to high temperatures is likely to incur a fitness cost not seen in mesophiles. Although I found that E is generally closely distributed around a mean value, I also found that different lower-level taxonomic groups show variation in intra-specific thermal sensitivity. This implies that, whilst there is evidence for a global thermal constraint, different clades adapt around this constraint.

In **Chapter 4** I take a step further and, using experimentally derived TPCs, show that peak growth temperature shows a phylogenetic signature. There are limits to thermal evolution

however, as shown by certain clades evolving to utilise different regions of the temperature spectrum. Interestingly, in the strains collected for **Chapter 4**, no obligate psychrophiles were found. The T_{pk} of strains isolated under cold conditions generally fell in the 20-25°C range. The lack of psychrophiles may not be unusual, as isolates even from very cold environments such as the Arctic or Antarctic are often reported as having mesophilic growth ranges (Bowman et al., 1998; Knoblauch and Jorgensen, 1999). In many respects this fits with the universal temperature dependence results, as specific adaptation to cold temperature may not confer a fitness advantage, if fitness is globally constrained by temperature and there is no cost to being able to also grow at more moderate temperatures.

In summary, it is likely that intra-specific thermal responses are dependent upon the same physical constraint as inter-specific thermal responses. The inter-specific constraint implies that peak growth temperature and peak growth rate increase together across species, however the phylogenetic results suggest that only particular clades have the underlying physiology necessary to adapt to particular temperatures.

6.2 Physiological basis for thermal responses of microbial growth rates

In **Chapter 2**, I compared average thermal sensitivity from growth rate data and respiration rate data, and found that they average around the same value. This is consistent with MTE-based models which assume a direct relationship between metabolic rate and emergent traits such as growth rate (Luhring and Delong, 2017). However, in order to fully test this, direct comparisons of growth and active respiration rates are required. These are generally lacking from the literature. Where these data are available, there are various issues with their comparison, due to differences in acclimation time and inconsistencies with *per capita* rate calculations. In **Chapter 3** I resolve these issues through new methodology enabling me to directly compare the thermal responses these traits. In **Chapter 5** this method is utilised to compare growth and respiration rate thermal responses across 47 strains of environmentally

isolated bacteria. I find that, counter to expectations from the meta-analysis data, there are systematic differences in the thermal performance curves of these traits. Specifically, in these data, the growth rate curves are consistently steeper (higher E) and peak at lower temperature (lower T_{pk}) than respiration rate curves. These differences in thermal response can be encapsulated in the unimodal relationship of carbon use efficiency with temperature. That is, the relative proportion of carbon allocated to growth versus respiration varies with temperature. I propose that this temperature dependence may be due to differences in maintenance costs depending upon variation in protein stability with temperature.

To date no other study has captured the relationship with temperature for both population growth and respiration across such a wide range of bacterial strains. These results are therefore an important development for understanding the physiological processes underlying growth. Various models have previously been developed to describe population growth rates, based on underlying metabolic rates, with the simplification of assuming that maintenance remains constant with respect to temperature (Kempes et al., 2012; Wang and Post, 2012; Luhring and Delong, 2017; De Jong et al., 2017). It has been known for decades however that maintenance requirements can shift with changes in abiotic factors (Pirt, 1965; Lipson, 2015). Furthermore, maintenance has been shown to vary non-linearly with growth rate (Van Bodegom, 2007), depending upon a complex range of processes (Lipson, 2015). Therefore, including temperature effects within the maintenance terms of growth models such as Kempes et al. (2012) or Wang and Post (2012) and applying these models to this new data may provide a new avenue for further understanding the physiological responses of microbial growth to temperature.

6.3 Thermal ecology and functional diversity of bacterial communities

In **Chapter 4** I presented an experiment to investigate whether species sorting and latent functional diversity is likely to result in reorganisation of bacterial communities to cope with changes in temperature. The experiment was designed to capture (if present) a diverse range

of bacteria adapted to different temperatures, all from the same environmental patch. I found that bacteria captured generally had thermal optima close to the temperature conditions under which they were isolated. This is important in terms of the effects of climate change on community composition, showing that species sorting is likely to drive increased abundance of hotter-adapted strains compared to cooler-adapter strains with climate warming, as the dynamics of bacterial succession are strongly affected by temperature (Rui et al., 2009).

In **Chapter 4** I also investigated the functional diversity of isolated strains. I found that the two main phyla isolated could be broadly partitioned into differing growth strategies, with *Proteobacteria* largely composed of K-strategists (a hallmark of cooperative populations (Lipson, 2015)) and *Firmicutes* largely composed of *r*-strategists (growth rate strategists). Growth rate specialists are expected to be early colonisers of new niches, utilising labile carbon sources, whilst K-specialists colonise later, utilising less favourable resources (Rivett et al., 2016, 2018). This effect can be seen in patterns of succession in soils, with *r*-selected taxa tending towards early colonisation and K-selected taxa colonising later (Shrestha et al., 2007).

A similar partitioning is also evident in the thermal biology of these strains, with *Proteobacteria* generally displaying lower thermal optima than the warmer-adapted *Firmicutes*. Given these results, with warming communities may be likely to shift away from cooler adapted *Proteobacteria* to warmer adapted *Firmicutes*. Indeed, similar changes in community composition are evident in warming experiments (Aydogan et al., 2018). If this is a general pattern, it may have profound impacts on the functioning of ecosystems, given the partitioning of these phyla between *r*- and K- strategies.

6.4 Effects of climate change on microbial ecosystem fluxes

Through these pieces of work, I have shown that changes in environmental temperature are likely to impact microbially-driven ecosystem carbon fluxes in a variety of ways. **In chapter 2**

I showed that, consistent with MTE, mesophilic bacteria adapt around a global thermodynamic constraint, with hotter adapted taxa inevitably displaying higher metabolic rates than cooler adapted taxa. Bacteria have been shown to have the capacity for rapid adaptation to new temperatures in lab settings. Such adaptation would be likely to increase metabolic rates in line with peak temperature increases, although it is not known whether this happens in nature at the same timescales as has been shown in the lab. In the environment however, it may be expected for species sorting to be more important in structuring communities than local adaptation (Loeuille and Leibold, 2008). Given my results in **Chapter 4**, suggesting a large amount of redundancy in temperature fitness of bacterial communities, species sorting is likely to mimic adaptation, utilising the open pool of warmer-adapted taxa already available, resulting in shifts in community composition (Pawar, 2009).

In **Chapter 2** I also showed that for prokaryotic communities these increased metabolic rates are likely to be even greater than potential increases of eukaryotic rates, due to the generally increased thermal sensitivities of prokaryotes. I used a toy model to illustrate the impacts of these findings, showing that the greater the contribution of prokaryotes to a community, the greater the likely increase in ecosystem respiration and therefore carbon efflux. These results are important for future parameterisations of more complex carbon flux models, where thermal sensitivity could be allowed to vary with different ecosystem compositions.

Finally, in **Chapter 5**, I show that temperature has a consistent effect on bacterial carbon use efficiency. More than simply changes to respiratory output, understanding the balance between efflux and sequestration (carbon use efficiency) will be important in determining how communities respond to climate change. This links back to temperature controls of microbial growth yield and how the growth strategies defined by r and K selection (**Chapter 4**) will be affected by climate change. Understanding how changes in these characteristics within communities feeds back to changes in ecosystem carbon efflux and sequestration will be important in understanding overall ecosystem responses to climate change (Lipson, 2015).

6.5 Future work

I have shown that mesophilic prokaryotic thermal responses adapt around a global constraint and that warmer adapted taxa necessarily have faster metabolic rates than colder adapted taxa. Furthermore, I have shown that prokaryotic communities contain a large amount of functional redundancy with respect to temperature. I postulate that with climate change we can therefore expect communities to shift towards strains adapted to higher temperatures, which will inevitably have higher metabolic rates, increasing carbon flux at the ecosystem level. I have also shown that population growth and respiration rate do not follow a simple relationship, due to the temperature dependence of carbon use efficiency. Therefore, future models will also need to take into account this non-linear variation of CUE with temperature in order to predict the effects of climate change on ecosystem processes.

This depends however on these results at the population level being fully applicable to ecosystem processes. Modelling has thus far been fairly constrained to MTE-based upscaling approaches (e.g. Schramski et al. (2015)), which generally exclude microbial interactions and population dynamics. Little has been done to experimentally verify whether these simplifications produce results that reasonably approximate real-world processes. Future experiments will be required to test whether community responses to temperature are a refection of the temperature dependencies of their constituent taxa, i.e. does a community composed of species with high thermal sensitivity of metabolic rates inevitably produce a high community response to temperature, or are interactions between species more important drivers of the community response? This could be tested via microcosm experiments in which artificial communities are constructed from components with already known TPCs, with the aim of understanding how the community response to temperature is linked to it's constituent taxa.

Furthermore, future work is required to understand how changes in communities with warming may actually correspond to changes in community function, or whether functional redundancy is likely to extend to all ecosystem processes such that changes in taxa do not change overall community function. Although functional redundancy is an important aspect of ecology, there is little agreement on how to measure the level of redundancy in a community (Ricotta et al.,

2016). Gene-centric methods can be used to disentangle metabolic function from taxonomy in microbial samples (Louca et al., 2018). Advancements in sequencing technologies are likely to play an important role in allowing researchers to understand whether community gene content is related to taxonomy, or remains conserved across communities with different taxa. Emerging metabolomics methods may also play a role in answering these questions. Similar to gene-centric methods focussing on the meta-genome as a whole, metabolomics can be used to understand the specific metabolites produced and used by a community. This may be a more specific approach than meta-genomics, which requires detailed knowledge of gene function to truly understand community function. This approach has already begun to be incorporated into questions of gut microbiome functional redundancy (Moya and Ferrer, 2016; Choo et al., 2017), and should thus also be applied to environmental community samples in the future.

My results of mismatches in thermal performance curve parameters of population growth and respiration show that more work is necessary to fully understand the link between these processes. I have proposed a mechanism for this result, with increased maintenance costs at temperatures away from a strain's thermal optimum due to increased protein repair accounting for decreased growth compared to respiration. A full mechanistic model to test this idea could be developed and then fit to these data. A potential starting point is the model of Kempes et al. (2012) in which growth is described via partitioning of energy between biosynthesis and maintenance. Incorporating temperature dependence component into the fractioning of growth and maintenance in this model may be a potential route towards understanding the mechanistic basis of the thermal responses of growth and growth efficiency.

6.6 Conclusion

The overall aim of this thesis was to investigate the effects of temperature on microbial metabolic rates. Prokaryotic metabolic rates are constrained by a universal temperature dependence and their thermal sensitivity deviates systematically from those of eukaryotes. Prokaryote communities contain taxa adapted to a wide variety of temperatures, with warming species sorting

processes are likely to shift communities towards taxa better adapted to warmer temperatures. These combined results are likely to lead to prokaryotic communities with higher metabolic rates on average and thus increase ecosystem carbon efflux. I demonstrate this process through a simple model. Furthermore, less efficient r-specialist taxa are favoured in warmer temperature environments compared to more efficient K-specialists. Species sorting may therefore drive communities towards being more r-adapted in general, resulting in major changes to community functioning. The situation is more complex however, as microbial allocation of carbon to growth versus respiration also depends on temperature. A small amount of warming may actually induce some taxa to become more efficient, however a large amount of warming (past the CUE T_{pk}) is likely to result in very low carbon efficiency of micro-organisms. This means that the balance between ecosystem carbon sequestration and efflux may vary with warming. Adaptation and species sorting process are likely to mediate this response over longer timescales however. Overall, climate warming is likely to have a large impact on the species composition, function and metabolic processes of microbial communities and thus ecosystems, potentially resulting in increased carbon efflux versus sequestration.

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Appendix A

Meta-analysis supplementary information

A.1 Number of TPCs per data-source paper

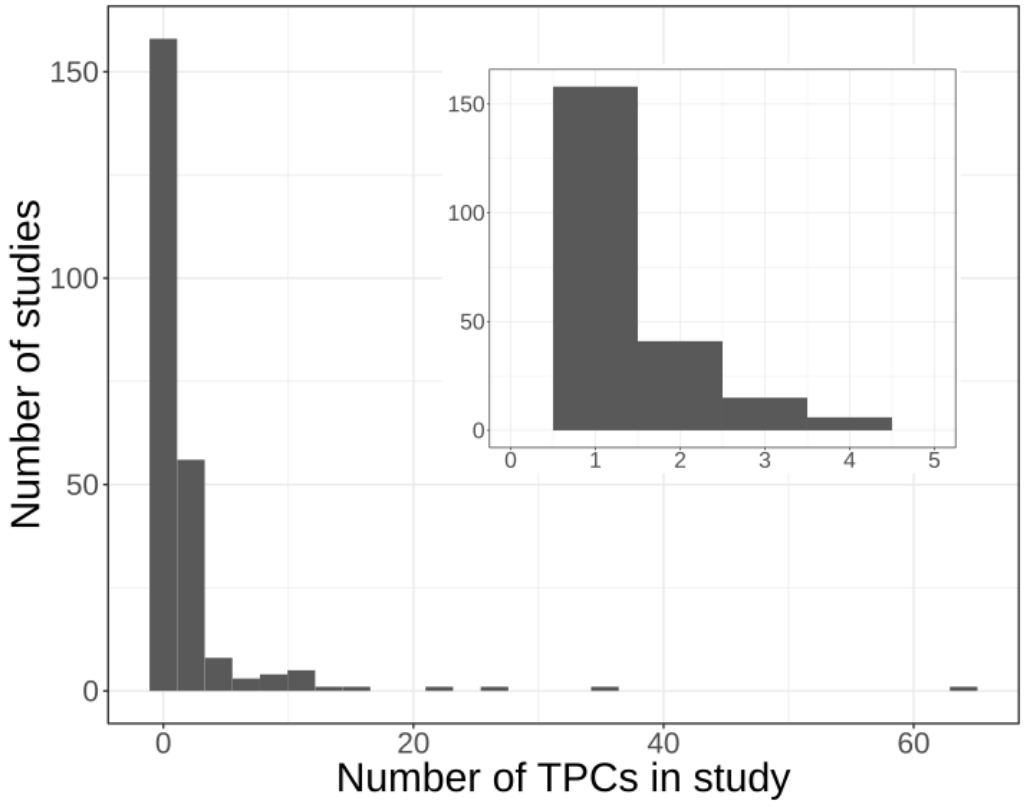

Figure A.1: **Histogram of TPCs collected from each data source.**

Main figure shows a breakdown of counts of the number of TPCs taken from each source, bins = 30. Inset shows data for papers with up to five TPCs, one bin per TPC count.

A.2 Comparison of Intraspecific Thermal Sensitivities

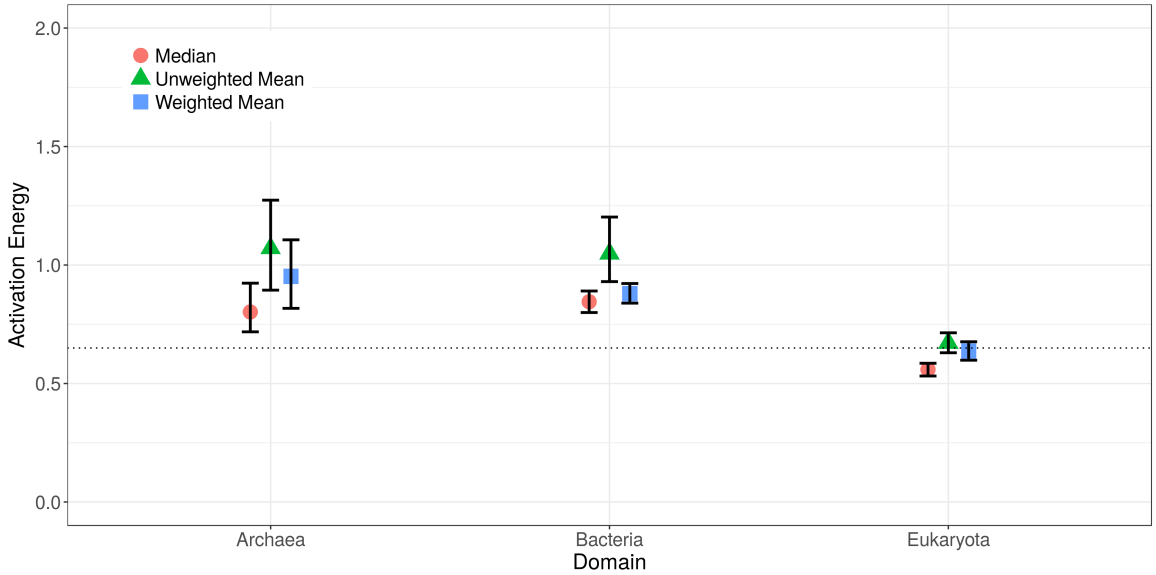

Figure A.2: **Comparison of intra-specific thermal sensitivities (E_S) calculated as the median, unweighted and weighted means respectively.**

The median is lower than the mean E_S for both Bacteria and Archaea as expected due to the right-skew of the data (Dell et al., 2011), however in all cases E_S is above the 0.65eV average (dotted line) expected from the Metabolic Theory of Ecology, which I find only holds for Eukaryotes (Main text Fig. 2.5).

A.3 Microbial Metabolic Fluxes

To test whether thermal sensitivity for growth rate (fitness) reflects the thermal sensitivity of underlying metabolic fluxes, I assembled prokaryotic flux data for comparison. Metabolic flux data is sparse in the literature in comparison to growth rate data, however I was able to find some temperature-dependent flux data from a number of different studies. The various metabolic flux data that were compiled for this analysis and their respective sources are detailed in Table A.1.

Table A.1: **Metabolic fluxes which TPCs were fitted to for comparison to growth rate TPCs.**

Metabolic Flux	**References**
Sulfur oxidation rate	Franzmann et al. (2005)
Fe3+ oxidation rate	Franzmann et al. (2005)
Fe2+ oxidation rate	Franzmann et al. (2005); Golyshina et al. (2000)
Sulfide production rate	Boyd et al. (2007)
Methanogenesis	Zeikus and Wolfe (1972); Zehnder and Wuhrmann (1977); Zinder and Mah (1979); Huser et al. (1982); Ferguson and Mah (1983); Zinder et al. (1984); Westermann et al. (1989)
Nitrate production rate	Mével and Prieur (2000)
Nitrate removal rate	Oshiki et al. (2011)
Fe2+ production rate	Slobodkin et al. (1997)
Dehalogenation rate	Utkin et al. (1994)
Sulfate reduction rate	Isaksen and Jørgensen (1996); Knoblauch and Jorgensen (1999); Mori et al. (2003)
Caffeine degradation rate	Ibrahim et al. (2016)
Hydrogen sulfide production rate	Oda et al. (1997)

A.4 Biomass Distributions and Alternative Parameterisations for Ecosystem Flux Modelling

A.4.1 Aquatic Ecosystems

In the main text I provide a simple model for changes in ecosystem respiratory carbon efflux with temperature change, based on differences in thermal sensitivity between taxa. I parameterised this for a terrestrial forest ecosystem, where the major sources of respiratory flux would be represented above-ground by plants (autotrophic eukaryotes) and below-ground by fungi (heterotrophic eukaryotes) and bacteria (heterotrophic prokaryotes). However, it is possible to extend this simple model to other ecosystem types, comprised of different taxa.

As a secondary example, I consider the short and long-term flux of marine ecosystems. Here, the major sources of biomass include heterotrophic bacteria (prokaryotes), phytoplankton (including both autotrophic eukaryotes - algae, and autotrophic prokaryotes - *Cyanobacteria*), zooplankton (heterotrophic eukaryotes) and larger animals (also heterotrophic eukaryotes)Bar-On et al. (2018). Similarly to the main text, I invoke a model based on ratios of different ecosystem components to one-another:

$$\text{Ecosystem Flux} = \delta(\beta F_{\text{hp}} + (1-\beta)F_{\text{he}}) + (1-\delta)(\gamma F_{\text{ap}} + (1-\gamma)F_{\text{ae}}). \tag{A.1}$$

Here, total flux is the combination of fluxes (F) produced by each component of the ecosystem (hp = heterotrophic prokaryotes, he = heteretrophic eukaryotes, ap = autotrophic prokaryotes, ae = autotrophic eukaryotes), where each flux is represented by a Boltzmann-Arrhenius function. With the proportions of each component represented by δ (overall proportion of heterotrophs to auototrophs), β (proportion of prokaryotes to eukaryotes within heterotrophic component) and γ (proportion of prokaryotes to eukaryotes within autotrophic component).

This model is parameterised using specific E values for each component and, as in the main text, the differences in flux with rising temperature are calculated, compared to a baseline model where all ecosystem components have the MTE derived 0.65eV E. I first parameterise this for short-term fluxes, based on $\bar{E}_S$ values with a 10°C temperature rise. For the hp component I used $E = 0.87$ ($\bar{E}_S$ for aerobic bacteria), for the he component I used $E = 0.65$eV ($\bar{E}_S$ expected under MTE, observed across many eukaryotic organismsDell et al. (2011)), for ap I used $E = 0.96$ ($\bar{E}_S$ observed for *Cyanobacteria* in my results, see main text Fig. 2.5) and for the ae component I used $E = 0.65$ ($\bar{E}_S$ for eukaryotic aquatic autotrophs is not significantly different from 0.65eV, Fig. A.4). I used the same parameterisation for long-term responses, given the results linking short- and long- term thermal sensitivity in the main text ($E_G \approx \bar{E}_S$) and calculate flux differences for a 4°C temperature rise. Although I don't directly test this for autotrophic eukaryotes in the main text, previous work has shown inter-specific E in plants to be consistent with 0.65eVGillooly et al. (2001).

As with terrestrial systems, there is considerable variation in estimates for the potential biomass contributions of different marine taxa. A meta-analysis by Bar-On et al. (2018) gives a total estimate of 1.6 Gt of carbon biomass from prokaryotes within 6 Gt total marine carbon (∼26%), see Bar-On et al. (2018) supplementary table S23. A meta-analysis by Gasol et al. (1997) suggests differences in biomass contributions between coastal and open ocean systems, with more heterotrophic bacterial biomass in the open ocean compared to coastal regions (∼28% heterotrophic plankton carbon in coastal systems provided by bacteria, ∼46% heterotrophic plankton carbon in oceanic systems provided by bacteria, calculated from Gasol et al. (1997) table 1), however this study does not assess the contribution of autotrophic bacteria. Additionally, Gasol et al. (1997) compile estimates for the overall ratios of autotroph to heterotroph biomass in these systems, giving an average ratio of 0.98:1 heterotrophs:autotrophs in coastal systems and 1.85:1 heterotrophs:autotrophs in oceanic systems. Given these estimates I allow both β and γ to vary between 0.1 and 0.5 to provide a range of potential ecosystem biomass combinations within realistic bounds. I fix δ at 0.495 for coastal ecosystems and 0.65 for oceanic ecosystems given the ratio estimates from Gasol et al. (1997) and show a heat-map of potential flux changes for both types of marine ecosystem (Fig. A.3).

Parameterising the model for aquatic systems shows an even greater disparity between temperature induced short-term flux estimations based on a global 0.65eV activation energy versus estimations using the thermal sensitivity averages for major groups (Fig. A.3). This is due to the autotrophic component of aquatic systems also containing prokaryotes and these *Cyanobacteria* having on average even greater thermal sensitivities than heterotrophic bacteria. The accuracy of the ecosystem modelling approaches taken here and in the main text are dependant upon reliable estimates of biomass distributions in different environments. Various meta-analyses have attempted to estimate the distributions of different taxa in different ecosystems, however each has significant sources of error and give very wide ranges of possible distributions (see del Giorgio and Gasol (1995); Gasol et al. (1997); Whitman et al. (1998); Joergensen and Wichern (2008); Bar-On et al. (2018)). Additionally the biomass distributions of heterotrophs to au-

totrophs have been shown to vary with warming in freshwater ecosystemsYvon-Durocher et al. (2011), further complicating the parameterisation of these models under climate change. I have thus taken a simplistic, global approach to this modelling in order to illustrate the impacts of these findings.

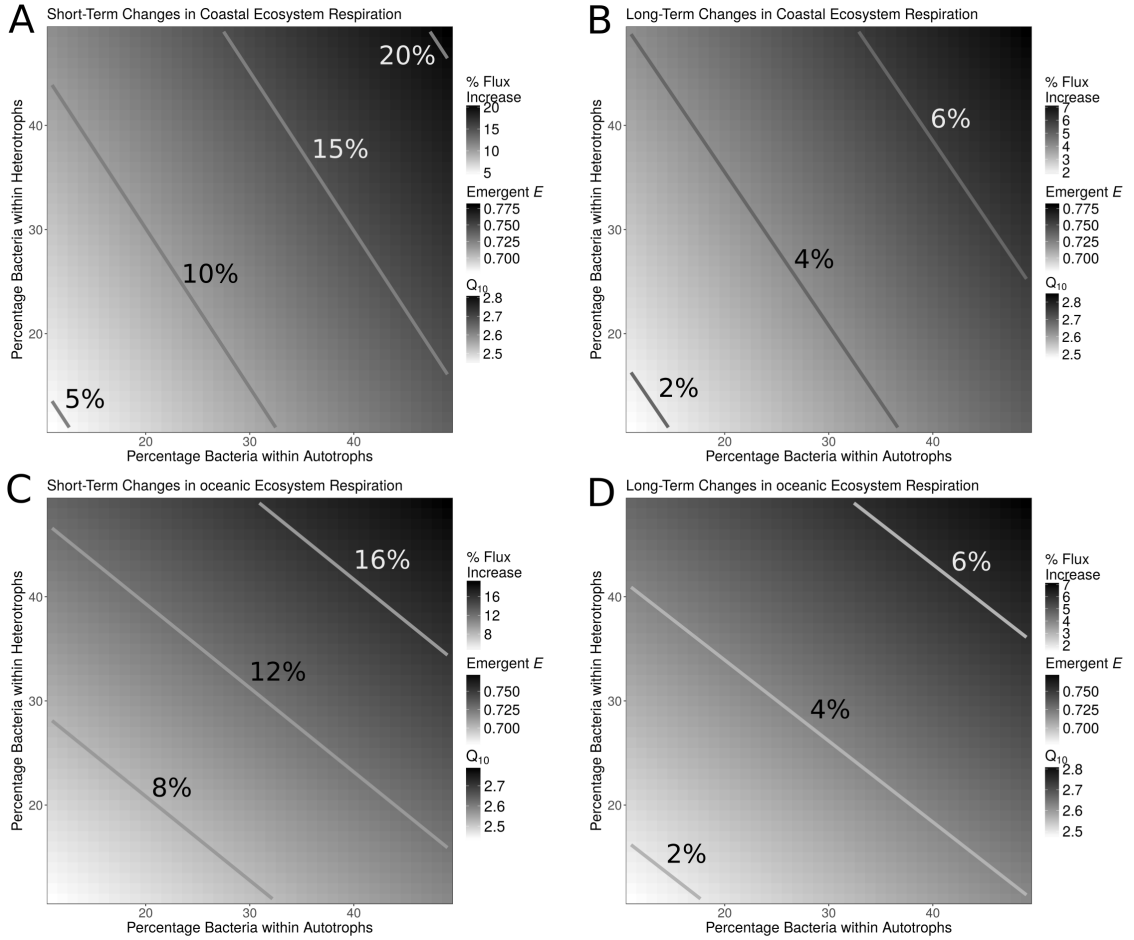

Figure A.3: **Potential changes in climate-driven aquatic ecosystem carbon flux due to differences in sensitivity between prokaryotic and eukaryotic thermal responses.**

A. Heat map of % short-term increase in flux with 10°C temperature increase of model coastal ecosystems with bacteria having a different activation energy on average than eukaryotes, relative to ecosystems with all components having the same (0.65eV) average activation energy. The flux change is shown over a range of ecosystem biomass compositions in terms of bacteria vs eukaryotes within autotrophic and heterotrophic ecosystem components. The x and y axes represent γ and β respectively, expressed as percentages. The overall δ is set to 0.495 based on ref. Gasol et al. (1997). The scale of emergent activation energies and Q_{10}s for the ecosystems with amplified flux are also shown. **B.** As A, but for long-term flux changes with a 4°C temperature increase due to climate change. **C.** and **D.** as A and B (short- and long-term) respectively, but for oceanic ecosystems with $\delta = 0.65$. As I parameterised these assuming $\bar{E}_S \approx E_G$, emergent ecosystem E is constant between short- and long-term plots, with only the difference in temperature change influencing then flux increase.

A.4.2 Archaea

In the main text *Archaea* are excluded from the ecosystem flux modelling as the data for mesophillic archaea were very restricted, leading to potentially unreliable estimates for $\bar{E}_S$ and E_G. However, were I to have reliable estimates for mesophilic, aerobic archaea, it would be trivial to also include these in such an ecosystem flux model. Extending the previous model (eq. A.1), may be done as follows:

$$\text{Ecosystem Flux} = \delta(\beta(\epsilon F_{hb} + (1-\epsilon)F_{ha}) + (1-\beta)F_{he}) + (1-\delta)(\gamma F_{ap} + (1-\gamma)F_{ae}). \quad \text{(A.2)}$$

Here, heterotrophic prokaryote flux (previously F_{hp}) is broken down further into heterotrophic bacteria flux (F_{hb}) and heterotrophic archaea flux (F_{ha}), the comparative ratios of which are controlled by the new parameter, ϵ. In marine ecosystems, bacteria may overall represent in the order of $\sim$4 times more biomass than archaea (from the estimates given in ref. Bar-On et al. (2018)), in which case the model may be parameterised with $\epsilon = 0.8$. In terrestrial ecosystems the weighting towards bacteria may be even greaterBar-On et al. (2018), however there are certain more specific ecosystems where archaea are known to be more abundant such as wetlandsStoeva et al. (2014).

A.5 Variation in autotroph thermal sensitivity

In order to further interpret the potential ecological impacts of these results, I investigated variation of thermal sensitivity within the autotroph respiration dataset. I find that whilst mean E_S may be marginally higher for aquatic autotrophs (0.67eV, CI 0.54-0.80) compared to terrestrial autotrophs (0.61eV, CI 0.53-0.69), there is a significant overlap of confidence intervals, which include 0.65eV in both cases (Fig. **??**). Therefore I can not report a statistically significant difference in $\bar{E}_S$ for terrestrial and aquatic autotrophs. Differences in how organisms from different habitats respond to temperature may be driven by differences in the phylogenetic composition of those habitats. I find that the thermal sensitivity of vascular plants in this dataset is consistent with the MTE 0.65 observation (vascular plant $\bar{E}_S = 0.62$, CI 0.54-0.71) and that CIs for both green and red algae also include 0.65eV (red algae $\bar{E}_S = 0.77$, CI 0.57-0.98; green algae $\bar{E}_S = 0.50$, CI 0.33-0.68). CIs for mosses and brown algae fall significantly outside of this range (moss $\bar{E}_S = 0.92$, CI 0.78-1.03; brown algae $\bar{E}_S = 0.37$ CI 0.23 - 0.49). However, the wide confidence intervals for green and red algae and the small number of observations for mosses and brown algae suggest that these data are of too limited scope to draw general conclusions for these autotroph groups.

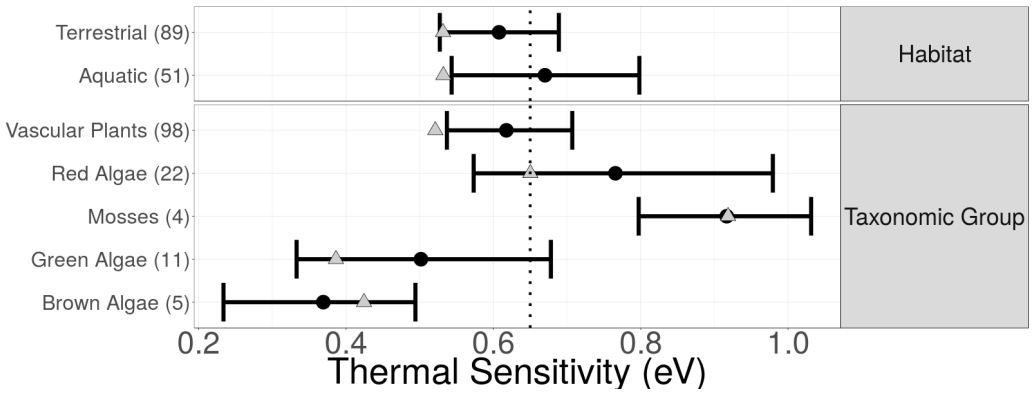

Figure A.4: **Comparison of intra-specific thermal sensitivities ($\bar{E}_S$) for autotroph respiration across taxonomic and ecological divisions.**

As in main text Fig. 2.4, points and error bars represent weighted mean and 95% CIs of E_S for each group. The number in brackets indicates the number of data points $\bar{E}_S$ was calculated from for each grouping, the dotted line marks 0.65eV and grey triangles mark the median E_S for each group.

Appendix B

Experimental methods calibrations

B.1 Temperature calibration of MicroResp system

CO_2 production measurements were performed using the MicroResp™ system (Campbell et al., 2003), as previously adapted for culture experiments (Rivett, 2013; Rivett et al., 2017). As the gel-based system may be affected by temperature and my experiment involved measurements across a wide temperature range, temperature calibration was required.

MicroResp gels were made up according to the manual, with final indicator plate wells containing 1% w/v agarose, 150mM KCl, 2.5mM $NaHCO_3$, 12.5μg ml^{-1} Cresol Red, made up with deionised water. All indicator plate absorbance readings (A_{572}; $\lambda = 572$ nm) were taken using a Synergy HT plate spectrophotometer (BioTek Instruments Inc., VT, USA).

In order to calibrate the indicator system, reactions of $NaHCO_3$ with HCl were run to completion to produce known quantities of CO_2. 50% serial dilutions of 0.1M $NaHCO_3$ and a water control plated out to 500uL, with final concentrations of 0.1M, 0.05M, 0.025M, 0.0125M, 0.00625M, 0.003125M, 0.0015625M. A H_2O negative was also included with each round of serial dilutions. 500μl of HCl was added to each well, the MicroResp plate was assembled, and the reaction was allowed to run to completion. This was a large over-dose of HCl for the quantities of $NaHCO_3$, so that the reaction swiftly ran to completion. This was run for 5, 10, 21, 30, 40 and 60°C temperatures and repeated twice. The mass of CO_2 produced was calculated according to the reaction:

$$NaHCO_3 + HCl \longrightarrow NaCl + CO_2 + H_2O$$

The mass of CO_2 produced can then be calculated based on its molecular mass the quantity of $NaHCO_3$ added. A calibration curve of log transformed A_{572} values against masses of CO_2 across different temperature was produced. The detection limits of the gel were reached at the highest and lowest CO_2 values, so the calibration model was constrained to the 0.05M, 0.025M, 0.0125M and 0.00625M $NaHCO_3$ concentrations. Linear regression was performed on the logged A_{572} and CO_2 values, with temperature as an interaction term (Fig. B.1). The resulting model (eq. B.1) was compared to a model without temperature included as an effect, and the temperature model shown to be a significant improvement (ANOVA, $p = 0.023$).

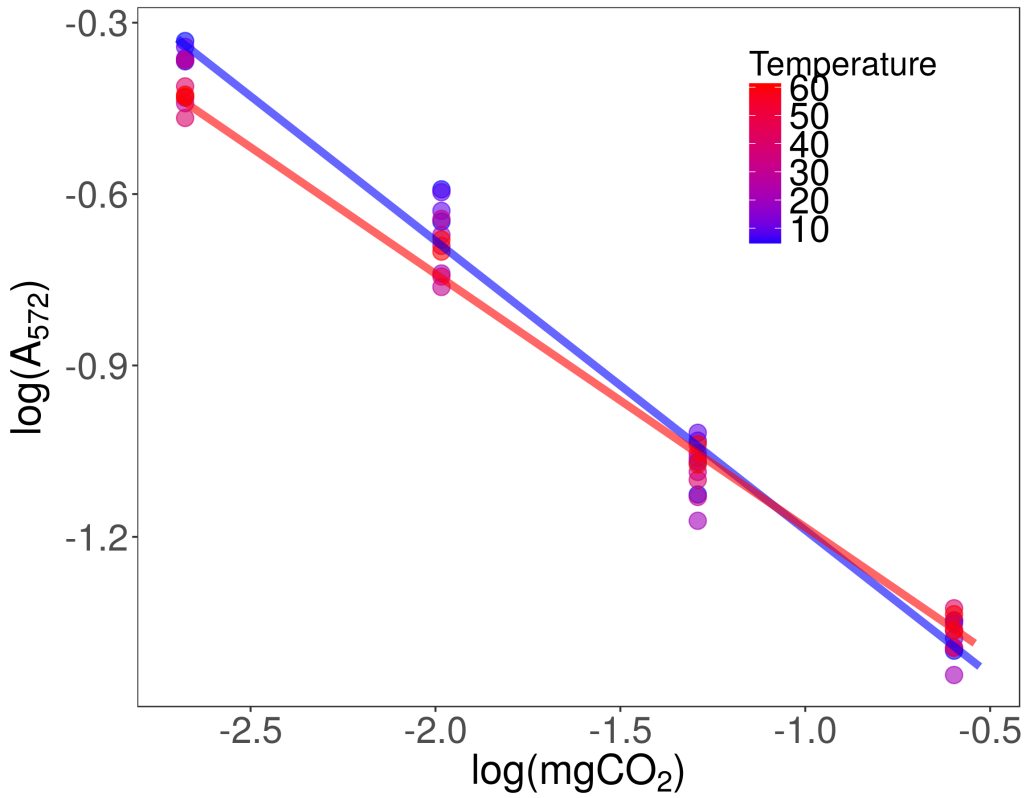

Figure B.1: **Temperature calibration of MicroResp system.**

Log-transformed A_{572} values regressed against log transformed CO_2 masses. Fitted model (eq. B.1) shown for 5°C (blue) and 60°C (red).

The regression model produced the following equation:

$$\text{mgCO}_2 = e^{\frac{log(\text{A}_{572})-(-1.700785+0.001239228T)}{-0.5120839+0.001145753T}} \tag{B.1}$$

where T is the temperature in °C. This was used to calculate CO_2 masses from absorbance values in chapters 3, 4 and 5 of this thesis.

B.2 Flow cytometry cell size calibration

In order to estimate cell sizes from flow cytometry measurements, micro-beads with known sizes were measured. Spherical beads with three diameters (1.1μm, 5.7μm, 14.8μm) were measured on a Accuri™ C6 flow cytometer (BD Biosciences, CA, USA). The forward scatter (FSC, arbitrary units) was regressed against the known diameters, to produce a calibration line (Fig. B.2), giving:

$$\text{FSC} = -76026 + 152457D, \tag{B.2}$$

where D is the diameter in μm. After using this to calculate average cell diameters from flow cytometry measurements, cell volumes were estimated using the approximation of spherical cells.

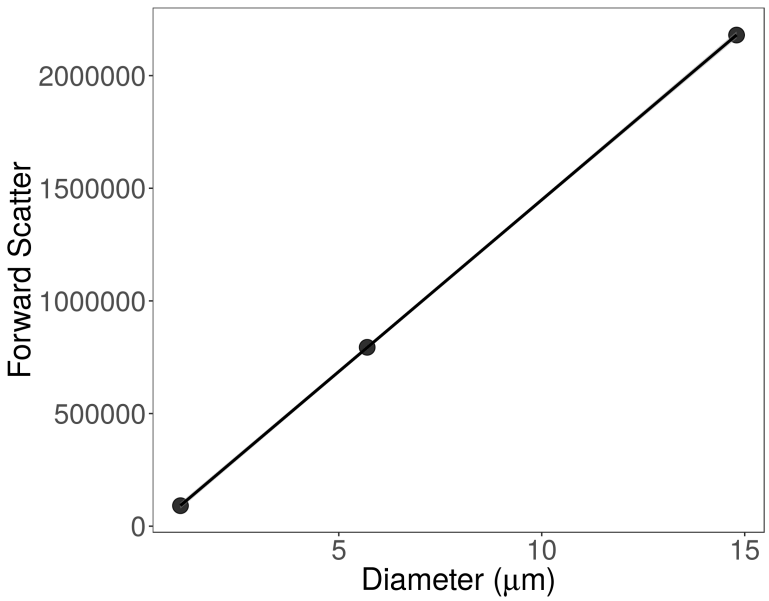

Figure B.2: **Flow cytometry size calibration.**

Spherical beads with known sizes were ran through the flow cytometer and their forward scatter measured.

Appendix C

Isolate experiments supplementary information

Full details of all the isolated strains and taxonomy as determined through 16S sequencing is shown in Table C.1.

The variation in carbon use efficiency (CUE) with temperature across all strains with measurements for $\geqq 5$ temperatures are shown in Fig. C.1.

Table C.1: **List of strains and ecotypes isolated.**

Strain codes follow XX_YY_ZZ naming convention, where XX is the incubation temperature, YY is the isolation temperature and ZZ is a numeric designator for the specific isolate. RT = room temperature.

Ecotype	Associated Strains	Phylum	Class	Order	Family
Anoxybacillus caldiproteolyticus	50_50_06	Firmicutes	Bacilli	Bacillales	Bacillaceae
Anoxybacillus tepidamans	50_50_02	Firmicutes	Bacilli	Bacillales	Bacillaceae
Arthrobacter sp.	10_10_06	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae
Bacillus bataviensis	40_RT_02	Firmicutes	Bacilli	Bacillales	Bacillaceae
Bacillus megaterium	40_40_01; 40_40_03; 21_RT_02; 40_RT_05	Firmicutes	Bacilli	Bacillales	Bacillaceae
Bacillus mycoides	21_21_05; 30_30_05; 30_RT_03	Firmicutes	Bacilli	Bacillales	Bacillaceae
Bacillus cereus	21_21_01; 21_R_06	Firmicutes	Bacilli	Bacillales	Bacillaceae
Bacillus weihenstephanensis	04_04_05; 30_30_02; 30_30_04; 04_RT_01; 04_RT_03; 10_RT_03; 30_RT_06; 40_RT_06; 50_RT_02; 50_RT_06	Firmicutes	Bacilli	Bacillales	Bacillaceae
Bacillus simplex	40_RT_01; 50_RT_01	Firmicutes	Bacilli	Bacillales	Bacillaceae
Bacillus sp. bataviensis-like	50_RT_04	Firmicutes	Bacilli	Bacillales	Bacillaceae
Bacillus sp. niacini-like	50_RT_03	Firmicutes	Bacilli	Bacillales	Bacillaceae
Bacillus sp. Shackletonii-like	50_50_05	Firmicutes	Bacilli	Bacillales	Bacillaceae
Brevibacillus thermoruber	50_50_03; 50_50_04	Firmicutes	Bacilli	Bacillales	Bacillaceae
Cohnella sp.	40_40_02; 40_40_05	Firmicutes	Bacilli	Bacillales	Paenibacillaceae
Collimonas sp.	04_04_04; 21_21_04; 21_RT_01	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae
Dyella japonica	30_RT_04	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae
Dyella marensis	21_RT_04	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae
Labrys methylaminiphilus	30_30_08	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae
Nocardia coeliaca	21_RT_05	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae
Paenibacillus sp.	21_RT_03	Firmicutes	Bacilli	Bacillales	Paenibacillaceae
Pseudomonas helmanticensis	04_04_02; 04_04_06; 04_RT_02; 04_RT_05	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
Pseudomonas protegens	10_RT_01; 10_RT_02	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
Pseudomonas rhodesiae-like	21_21_02; 21_21_06	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
Rummeliibacillus pycnus	40_40_04	Firmicutes	Bacilli	Bacillales	Planococcaceae
Rummeliibacillus stabekisii	50_50_01	Firmicutes	Bacilli	Bacillales	Planococcaceae
Streptomyces lactacystinicus	30_30_01; 30_30_06; 30_30_07	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae
Streptomyces mirabilis	30_30_03	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae
Variovorax boronicumulans	30_RT_01; 30_RT_02	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
Variovorax soli	30_RT_05	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
Viridibacillus arenosi	40_RT_03; 40_RT_04	Firmicutes	Bacilli	Bacillales	Planococcaceae
Viridibacillus sp.	40_40_06	Firmicutes	Bacilli	Bacillales	Planococcaceae

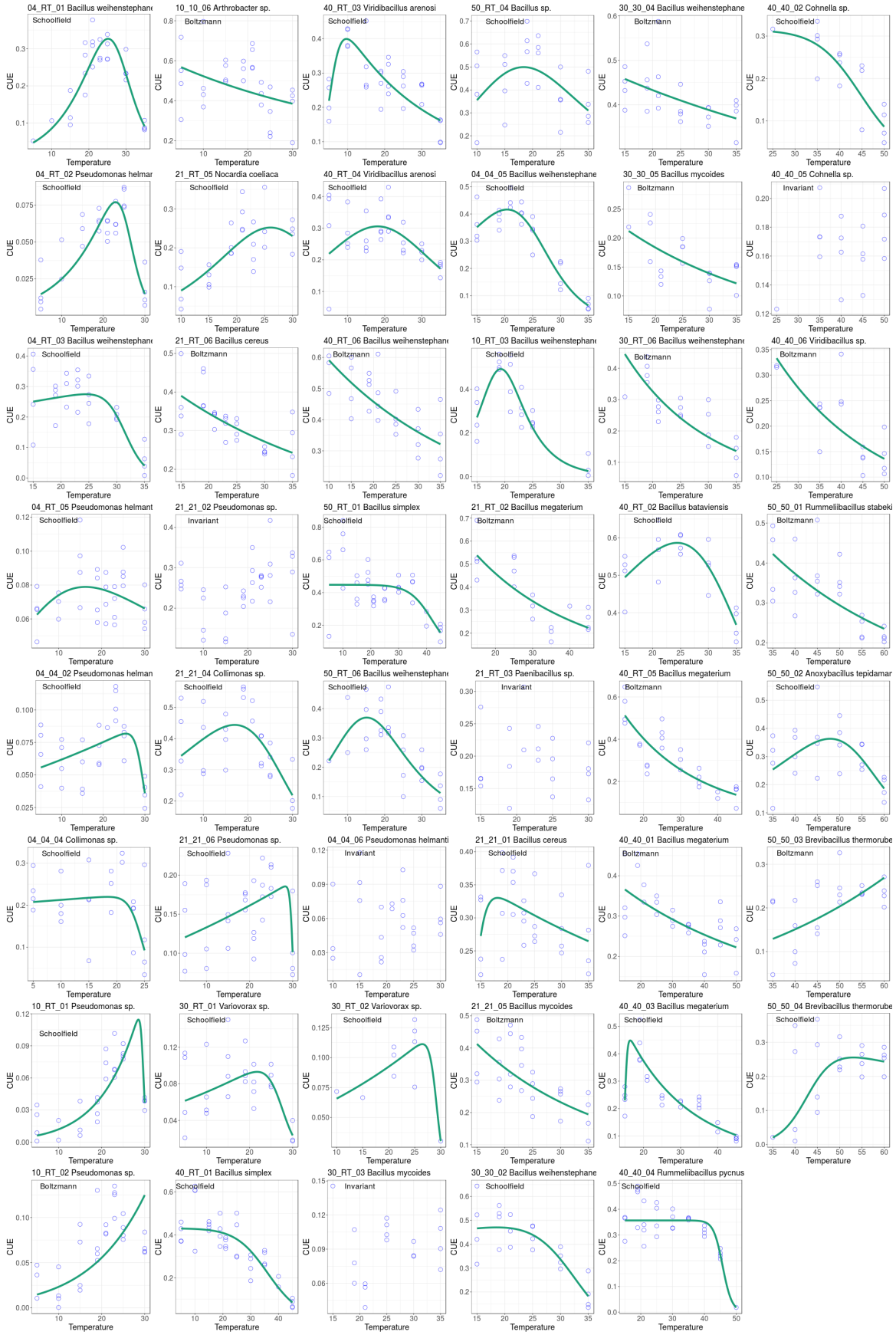

Figure C.1: **Variation of CUE with temperature for all strains.**

The Sharpe-Schoolfield and Boltzmann-Arrhenius models were fit to the CUE vs temperature data for each strain. The best fitting model is shown (line) and labelled on each plot. If neither model produced a significant fit, no fit is shown and strains are labelled "invariant" with temperature.